W-Pos81

KINESIN DECORATION OF THE MICROTUBULE SURFACE.

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The interaction of the kinesin motor domain with the microtubule surface lattice was examined by electron microscopy of negatively stained and frozen-hydrated specimens. The N-terminal 401 amino acids of the Drosophila kinesin heavy chain (K401) which contains both the ATP and microtubule binding domains, were expressed in E. coli and purified to homogeneity as soluble, fully active, protein. This truncated monomeric form of kinesin made it possible to decorate individual microtubules heavily without cross-linking them into bundles. Complexes were formed by mixing taxol-stabilized microtubules and K401 at 1 to 3 times the molar concentration of tubulin, pelleted, and analyzed by gel electrophoresis. Saturating binding was found to correspond to one molecule of K401 per tubulin dimer. According to both conventional negative staining and cryo-electron microscopy, the complexes were coated with regular patterns of bound K401 molecules with an axial repeat of 8nm. Optical diffraction of decorated microtubules showed a strong layer-line at this spacing, confirming that one kinesin head binds per tubulin heterodimer. The addition of ATP to the K401-microtubule complex led to complete dissociation of kinesin from the microtubule surface.

W-Pos82

FUNCTIONAL AND STRUCTURAL ASPECTS OF ASSOCIATION OF A REGULATORY LIGHT CHAIN WITH 22S DYNEIN

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A 29kD polypeptide that copurifies with 22S dynein of *Paramecium tetraurella* is phosphorylated or thiophosphorylated in a cAMP-dependent, Ca^{2+}-sensitive manner. This phosphorylation regulates the speed of microtubule translocation by 22S dynein *in vitro* (PNAS, 88:7918-7922, 1991). The 29kD polypeptide may be considered a dynein regulatory light chain (dLC_r). After thiophosphorylation within the axoneme we isolated the dLC_r away from the heavy chains and partially purified the protein. Isolated 22S dynein not otherwise treated retains substoichiometric amounts of the 29 kD polypeptide. The partially purified 29kD dLC_r specifically rebinds to this 22S dynein but not to 14S dynein (a single headed molecule) nor BSA. The specific association with 22S dynein can be competed away by using either partially purified thiophosphorylated or a corresponding unphosphorylated dLC_r. A standard *in vitro* microtubule motility assay was used to test if this rebinding is functional. When 22S dynein with reassociated dLC_r was used as a substratum microtubule translocation velocity increased significantly over controls. Velocity increased further when the reassociated dLC_r was thiophosphorylated. *Paramecium* 22S dynein can be proteolytically digested with chymotrypsin to yield one headed and two headed structures, as previously shown in *Tetrahymena* by Y. Y. Toyoshima (JCB 105:887-895, 1987). The dLC_r binds preferentially to the one headed fraction. Based on these results the 29 kD dLC_r is probably part of a single unique subunit of the 22S dynein arm that includes one specific heavy chain. Furthermore this association is apparently functional in that it modifies the translocation rate of microtubules by 22S dynein according to the phosphorylation state of the 29kD dLC_r, thereby regulating ciliary beat frequency.

W-Pos83

SINGLE CELL MEASUREMENT OF CILIARY BEAT FREQUENCY AND INTRACELLULAR CALCIUM IN TRACHEAL EPITHELIAL CELLS

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Mucociliary clearance is the primary mechanism by which inhaled foreign particles, including bacteria, are removed from the airways and lung. This clearance depends upon both the mucus secreted by the goblet cells and the microtubule-based ciliary beat frequency (CBF) of airway epithelial cells. To determine changes in CBF and $[Ca^{2+}]_i$ of the same cell, tracheal epithelial cells were obtained from sheep by dissociation with protease and grown in culture for 2-14 days. Cells were imaged with a 100X Fluor DL oil objective, enabling single cilia to be clearly observed. CBF was measured by online FFT analysis of intensity changes of single pixels from digitized phase contrast microscopy images. Using a video camera with RS-170 timing, frequency response is limited to <15Hz since each pixel represents a 1/30s sampling interval. With 128 samples per FFT (=4.26s), the magnitude spectra usually showed a clear single peak. At 20°C, this peak frequency was between 5Hz and 10 Hz and was stable (±1Hz) for >30minutes. This measure of CBF increased with increasing temperature, and often surpassed the 15Hz limit at >30°C. Cholinergic stimulation with 10μM ACh produced a reversible increase in CBF at 20°C by ~30% above baseline. By switching the light path to an intensified CCD camera, we could measure Fura-2 fluorescence of the same ciliated cell. Using the ratio of emitted light with alternating 340/380nm excitation, we found ACh reversibly increased $[Ca^{2+}]_i$. This $[Ca^{2+}]_i$ increase was likely due to Ca from internal stores, since Ca-free medium did not prevent the rise in $[Ca^{2+}]_i$. This demonstrates that $[Ca^{2+}]_i$ and CBF can be measured in a single cell and that ACh produces temporally correlated increases in CBF and $[Ca^{2+}]_i$, thus setting the stage for an exploration of the role of $[Ca^{2+}]_i$ in the regulation of CBF. (Supported in part by the Swiss National Science Foundation).

NUCLEIC ACID STUDIES

W-Pos84

EQUILIBRIUM ELECTROPHORESIS OF SS PD(A)$_{20}$, SS PD(T)$_{20}$, DS PD(AT)$_{20}$.

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Equilibrium electrophoresis is a method to determine the apparent charge on macroions in aqueous solution. A second generation instrument has been used to study DNA oligonucleotides with a length of 20 bases or base pairs. In 20 mM Tris, 20mM KCl, pH 8.00 buffer, either form of ss DNA had an apparent charge from 5.97 to 6.17 electron equivalents, 31% of its titrable charge. In the same buffer, the ds DNA had an apparent charge of 7.57 electron equivalents, 18.9% of its titrable charge. Condensed ion theory predicts 30% of the titrable charge will be expressed for the ss DNA, in excellent agreement with these measurements. However, the agreement between predicted (12%) and measured (18.9%) charge is not as good for the ds DNA. The theory, operation and limitations of this device are discussed. Supported by NSF DIR 8914571.

W-Pos85

RMS AMPLITUDE OF LOCAL ANGULAR MOTION OF PURINES IN DNA

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Deuterium NMR and time-resolved fluorescence polarization anisotropy (FPA) measurements were made to determine the rms amplitude of local angular motion of purines in a 12 bp duplex DNA (CGCGAATTCGCG) which is deuterated at the H8 positions of the adenines and guanines. FPA measurements of this sample made in dilute solution yield the hydrodynamic radius of the DNA, $R_H = 9.94 \pm 0.2$ Å. FPA measurements of the sample at the NMR concentration are employed to characterize the collective motions of the DNA in terms of either an enhanced viscosity or end-to-end dimer formation. Expressions we have derived for R_2^Q and the results of the FPA measurements are used to analyze the linewidth of the deuterium NMR spectrum. When the principal-axis frame of the electric field gradient tensor is assumed to undergo overdamped libration around each of its three body-fixed axes in an isotropic deflection potential, then the rms amplitude of local motion around any single axis is found to lie in the range 10 to 11° provided the high DNA concentration acts to enhance the viscosity, and about 9° if it acts to produce end-to-end dimers. The proton NMR data of Eimer *et al.* are reanalyzed and shown to yield an rms amplitude of angular motion of the cytosine H5-H6 internuclear vector of 9 to 10° depending upon its orientation with respect to the helix-axis. Within experimental error these results lie in the same range (8 to 10°) inferred for base motions at low and intermediate hydration levels in the solid state.

W-Pos86

INTERNAL DYNAMICS OF DNA AS MONITORED BY A 2-ATOM-TETHERED NITROXIDE SPIN LABEL. ((R.S. Keyes and A.M. Bobst)) Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221.

Over the past decade, we have reported on a series of spin-labeled nucleic acids containing nitroxide rings attached to thymidine and cytidine bases via tethers of various lengths. The present study focuses on a double-stranded DNA 15mer, 30mer, and 45mer singly-labeled with the 2-atom tethered nitroxide DUMTA [1]. Analysis of the experimental data has been accomplished in a manner similar to that of Hustedt et al. [2] in their implementation of the model-free approach of Lipari and Szabo [3]. The dynamics are separated into a length dependent global motion arising from overall tumbling and a length independent local motion resulting from base and probe oscillation. The oligonucleotides are modeled as cylinders and the internal motion is accounted for by averaging the g and A magnetic tensors. Currently, we have not employed a model for tensor averaging. Simulations have been fit to the experimental spectra empirically. Results with the DUMTA-labeled oligomers indicate that there is a high degree of tensor averaging indicative of rapid internal motion. This is in contrast to results obtained with a 2-atom acetylenic-tethered nitroxide (ACET) where much less tensor averaging was observed [2]. Molecular dynamics simulations performed with Biosym's InsightII and Discover software do not indicate a significant difference in mobility between DUMTA- and ACET-labeled bases. Supported in part by NIH GM 27002.

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W-Pos87

tRNA ROTATIONAL TIME AND KERR CONSTAT IN $MgCl_2$. ((Kerwin Ng, Martin Fuchs, and Don Eden)) San Francisco State Univ., San Francisco, CA 94132

The rotational time of yeast tRNA has been determined by observing its birefringence in optical Kerr effect (OKE) and transient electric birefringence (TEB) experiments. tRNA was studied in Tris-NaCl buffers containing $MgCl_2$. OKE experiments were performed at 22°C in D_2O buffers and TEB in H_2O buffers at 4°C. Birefringence comes from the orientation due to an induced dipole moment of tRNA molecule. In OKE, the induced dipole moment arises from the strong optical electric field from a Q-switched YAG laser. In TEB, the dipole moment arises from an applied electric field. In OKE, the $MgCl_2$ sample has a rotational time of 22 ns, which corresponds to 38 ns corrected to 4°C. This time constant agrees with TEB experiments. The volume specific Kerr constant was extracted from OKE. It is found to be $1.12X10^{-20}$ m^2/V^2.

An approximate optical Kerr constant was calculated using the X-ray crystallographic structure of tRNA and the optical polarizability tensor of individual bases, calculated by MOPAC, a molecular simulation software. The result for tRNA is 1.7 - $2.7X10^{-20}$ m^2/V^2, depending on the method of calculation and type of tRNA used.

W-Pos88

A PROTON NUCLEAR MAGNETIC RESONANCE INVESTIGATION OF STRAND ASSOCIATION OF THE DODECAMER 5'-d(CGCGAATTCGCG)-3' ((E. J. Folta-Stogniew and I. M. Russu)) Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459-0175

Proton nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the dependence on salt of the equilibria and kinetics of strand association of the self-complementary dodecamer 5'-d(CGCGAATTCGCG)-3'. The helix-coil transition is monitored using the proton methyl resonances of the two thymines, as a function of the concentration of sodium ions (from 0.05 M to 0.26 M). The equilibrium constant for helix formation (K_a) is obtained from chemical shift measurements and the rate constants for strand association (k_a) and duplex dissociation (k_d) from line width measurements. The number of counterions released in the helix-coil transition and their distribution between the association and dissociation processes are calculated from the salt dependence of K_a, and k_a and k_d, respectively. The results indicate that the standard enthalpy change (90 ± 10 kcal/mol of duplex) is independent of counterion concentration and, most of the temperature dependence of the equilibrium constant can be attributed to the activation energy for the dissociation process (80 ± 20 kcal/mol of duplex). A total of 2.1 ± 0.3 sodium ions are found to be thermodynamically released in the helix-coil transition. Moreover, the number of sodium ions that associate with the DNA during strand association is found to be, within experimental errors, the same as the number of sodium ions released during helix dissociation. The relationship between these results and previous experimental and theoretical findings on this and related DNA oligomers will be discussed.

W-Pos89

A MULTIPLE QUANTUM FILTRATION NMR STUDY OF COUNTERION QUADRUPOLAR RELAXATION IN POLYMERIC DNA SOLUTION ((H. Deng, W. H. Braunlin)) Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304

Many biological metal ions have quadrupolar nuclei with spin greater than 1/2. When they bind to biopolymers, these cations tumble slowly, resulting in non-Lorentzian NMR lineshapes. Filtration of the NMR signal through a state of multiple quantum coherence can make the multiple exponential decay apparent. Here we present our ^{25}Mg (I=5/2) NMR study of Mg^{2+} binding to core length DNA using three-quantum and five-quantum filtration techniques. Consistent with the work of Chung and Wimperis (1990, Chem. Phys. Lett., 172, 94) in protein solution, the excitation and observation of multiple-quantum coherence is possible in the absence of scalar, dipole, or quadrupolar splitting in DNA water solution. Preliminary multiple quantum filtration experiments on ^{59}Co (I=7/2) NMR will also be presented.

W-Pos90

HYDRATION OF THE MINOR GROOVE OF POLYNUCLEOTIDES ((Dionisios Rentzeperis[1], Luis A. Marky[1] and Donald W. Kupke[2]))
[1]Department of Chemistry, New York University, New York, NY 10003; and [2]Department of Biochemistry, University of Virginia, Charlottesville, VA 22908.

The minor groove ligand distamycin A has been used to probe the relative hydration of the minor groove of eight synthetic polynucleotide duplexes of known sequence and composition. A combination of densimetric, calorimetric, and temperature-dependent spectroscopic techniques have been used to obtain complete thermodynamic profiles ($\Delta G°$, $\Delta H°$, $\Delta S°$ and $\Delta V°$) for the association of distamycin A to all polymer duplexes. In 10 mM phosphate buffer, pH 7 and 20°C, binding of this ligand to each of the polymeric duplexes resulted in characteristic negative changes in both the volume and enthalpy. Although the binding affinities were found to be similar for pairs of isomer polynucleotides, the values of $\Delta H°$, $\Delta S°$ and $\Delta V°$ of each such pair were remarkably different. The heteropolymer duplexes generated much larger exothermic contributions, less favorable entropies and larger volume contractions than did the corresponding homopolymer duplexes of identical composition, and strongly suggest that polynucleotides with homopurine sequences are more hydrated than polynucleotides with alternating purine/pyrimidine sequences. We found an apparent linear correlation between $\Delta S°$ and $\Delta V°$. The slope of the fitted line, $\Delta S°$ *vs* $\Delta V°$, is about one half that reported for the protonation of small organic acids. This reflects a smaller compression of the water dipoles in the minor groove of the unligated duplexes. The amount of releasable spine water is believed to be a function of the DNA sequence, thus giving rise to the observed linear correlation because other entropic effects appear nearly identical for these polynucleotides. This work was supported by Grants GM-42223 (L.A.M.) and GM-34938 (D.W.K.) from the National Institutes of Health.

W-Pos91

ELECTRON MIGRATION IN GAMMA-IRRADIATED SOLUTIONS OF DNA. ((A.F. Fuciarelli, E.C. Sisk, J.H. Miller, and J.D. Zimbrick)) Pacific Northwest Laboratory, Richland, WA 99352 (Spon. by J.D. Zimbrick)

Solid state studies of irradiated DNA reveal that the initial site of radical, or radical ion, formation may not necessarily be the site at which radiation damage becomes localized. Although in solid state studies maximum distances for electron migration were reported to be on the order of 5 to 110 bases, electron migration in irradiated aqueous solutions of DNA has been technically more difficult to study. However, interaction of electrons generated from exposure to ionizing radiation is known to result in a quantitative reduction of 5-bromouracil (BU) to bromide ions and uracil in the presence of a suitable hydrogen donor. These reactions are being used as molecular indicators of charge migration in aqueous solutions of DNA by selectively incorporating 5-bromouracil into short oligonucleotides using automated DNA synthesis techniques. Following irradiations, the yield of uracil is measured using gas chromatography-mass spectrometry methodology. Our data suggest that base composition plays a significant role in electron migration processes which, in turn, can affect migration distances in irradiated DNA. Potential mechanisms of electron migration from the site of initial energy deposition will be discussed with reference to our experimental observations.

This work is supported by the U.S. Department of Energy under contract DE-AC06-76RLO 1830.

W-Pos92
CHARACTERISTICS OF N^2-dG-OLIGONUCLEOTIDE ADDUCTS DERIVED FROM ENANTIOMERIC ANTI-BENZO[A]PYRENE DIOL EPOXIDES. ((B. Mao, B. Li and N. E. Geacintov)) *Chemistry Department, New York University, New York, NY 10003.*

The mutagenic and tumorigenic characteristics of the (+)- and (-)-enantiomers of *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrohydrobenzo[a]pyrene (*anti*-BPDE) are strikingly different from one another. Therefore, differences in the structural and biochemical characteristics of the BPDE-DNA adducts are of great interest. We have synthesized covalent adducts in which *anti*-BPDE is linked via its 10-position to N^2-dG in an oligonucleotide 16 bases long. Stereospecific BPDE-oligonucleotide adducts, (+)-*trans* and (-)-*trans*, were used in snake venom phosphodiesterase I (SVPD) and spleen phosphodiesterase II (SPD) enzyme digestion experiments. The (+)-*trans* adduct partially inhibits SPD activity which hydrolyses DNA from the 5'-side to the 3'-side, while the (-)-*trans* adduct partially inhibits SVPD activity (3' -> 5' hydrolysis). These results are consistent with recent NMR data[1], which show that the covalently bound BPDE residues tend to be oriented in opposite directions relative to the 5'-> 3' strand polarity in duplex DNA. Similar stereospecific BPDE-oligonucleotide adducts 16 bases long with cohesive ends were synthesized as well. Local DNA distortions at the site of the lesion were amplified by polymerization (using ligase) and quantitatively evaluated by using gel electrophoresis. Prominent hinge joints at the site of BPDE lesions are observed in the (+)-*trans*, but not in the (-)-*trans* adducts. (Work supported by the Department of Energy and NIH/NCI).

[1] de los Santos, C. et al., (1992) *Biochemistry* 31, 5245-5252.

W-Pos93
RECOGNITION OF DNA STRUCTURES BY COBALT(III) AMMINE CATIONS. ((Q.-W. Xu & W. H. Braunlin)) Chemistry Department, University of Nebraska-Lincoln, Lincoln, NE 68588-0304

^{59}Co NMR provides a convenient probe of the rotational mobility of cobalt(III) ammine cations. Upon binding to DNA, the rotational mobility of cobalt(III) ammine cations is perturbed in a manner which depends on the base sequence of the DNA. DNAs which strongly reduce the mobility of cobalt(III) ammine cations usually contain a run of consecutive guanines and cytosines. Although the rotational mobility of $(\pm)Co(en)_3^{3+}$ are reduced to a similar extent in solutions of these DNAs, the binding environments are stereo-selective. Both ^{59}Co NMR and equilibrium dialysis experiments indicate that D-$Co(en)_3^{3+}$ preferentially binds to right-handed DNAs, while L-$Co(en)_3^{3+}$ prefers left-handed DNAs. NOESY spectra show that the binding sites are in the middle of DNA octamers. In contrast, DNAs which are rich in adenines and thymines hardly affect the mobility of cobalt(III) ammines cations. ^{59}Co NMR shows that these DNAs do not discriminate toward binding the two enantiomers of $Co(en)_3^{3+}$. It appears that cobalt(III) ammine cations bind these DNAs in a diffuse way.

Strong interactions between DNAs and cobalt(III) ammine cations correlate with DNA conformational transitions, including B-A and B-Z transitions. For the same DNA sequence, the two enantiomers of $Co(en)_3^{3+}$ appear to induce distinctly different conformations upon binding.

W-Pos94
THERMAL DENATURATION OF DA/DT POLYMERS AT HIGH PRESSURE. ((J. Q. Wu & R. B. Macgregor, Jr.)) Department of Medicinal Chemistry, University of Illinois at Chicago, Chicago, IL 60612.

Numerous studies have pointed to the differences in the physical properties of poly(dA)·poly(dT) and poly[d(A-T)], we have measured spectroscopically the thermal denaturation of these two polymers at 260 nm as a function of sodium ion concentration and hydrostatic pressure between 0.1 and 200 MPa (0.1 MPa = 1 bar). Both polymers exhibit increased stability at elevated pressure. Using the Clausius-Clapeyron equation, the slope of the change in the melting temperature (Tm) as a function of pressure was used to find the molar volume change ($\Delta V°$) for the helix-coil transition at each [Na^+]. The $\Delta V°$ for poly[d(A-T)] varied linearly with log[Na^+] from +0.3 cm^3/(mol bp) at 20 mM Na^+ to +3.5 cm^3/(mol bp) at 1 M Na^+. For poly(dA)·poly(dT), $\Delta V°$ = +3.0 cm^3/(mol bp) at 20 mM Na^+ and +3.6 cm^3/(mol bp) at 50 mM Na^+. These data imply that differences in the extent of hydration persist in denatured polymers. Analysis of the Tm of poly[d(A-T)] as a function of [Na^+] at different pressures reveals that the number of Na^+ released during the transition increases with pressure from an average of 0.20/bp at 0.1 MPa to 0.26/bp at 200 MPa. The design, construction, and performance of the Temperature-Regulated Iso-Hyper-Baric Spectrophotometer (TRIHBS), used for these measurements will also be presented.

W-Pos95
EFFECTS OF Ni^{2+} AND Cd^{2+} ON DNA MELTING
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Differential scanning calorimetry (DSC), laser Raman spectroscopy, pH potentiometry, and optical densitometry have been used to investigate DNA melting profiles in the presence of Ni^{2+} and Cd^{2+}. The destabilizing effects of these metals on the DNA duplex are seen through lower melting temperatures and spectroscopic indications of metal-base binding, decreased backbone order, base unstacking, and ruptured hydrogen bonds. pH potentiometry shows that interactions of Ni^{2+} and Cd^{2+} with DNA cause a pH drop by more than 2.5 units in weakly buffered solutions. This effect, which is partially reversible upon DNA melting, provides information about probable metal binding sites. From these results, a mechanism is proposed in which the metal cations enhance the carcinogenic effects of certain compounds that require acid catalysis for activation. Optical densitometry, which was used to detect DNA aggregation, shows increased turbidity in the range of temperatures where the melting transition is detected by Raman spectroscopy and DSC. We employ a statistical theory to describe this phenomenon in which the configurational entropy of the aggregate is the driving force for DNA aggregation at elevated temperatures in the presence of divalent metal ions.

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W-Pos96
THERMAL STABILIZATION OF A DRUG-DNA COMPLEX BY LIPOSOME ENCAPSULATION.
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Liposome encapsulation of oligonucleotides is of interest due to the potential use of such constructs as carriers of and delivery systems for antisense DNA/RNA as well as other pharmacologically active ligands. Consequently, it is important to assess the impact of such encapsulation on the properties of the included ligand. To this end, we have used temperature-dependent fluorescence spectroscopy to measure the melting of a drug-DNA complex, both free in solution and encapsulated in large unilamellar vesicles. Using reverse-phase evaporation or extrusion techniques, we prepared vesicles containing egg-phosphatidylcholine, cholesterol, and dipalmitoyl phosphatidylserine, with a 50:41:9 molar ratio, respectively. We used bisbenzimide (BB), an AT-specific minor groove binding drug, as the fluorophore in these studies. Our preliminary results reveal a significant increase (greater than 7°C), in the thermal stability of the encapsulated drug-DNA complex relative to either the complex free in solution or the complex in the external aqueous phase of the vesicles. We are initiating parallel differential scanning calorimetric measurements on these same systems to evaluate the thermodynamic origins of the measured ΔT_m data. These studies should allow us to define better the impact of encapsulation on the properties of DNA in both its free and drug-bound states. Supported by NIH grants GM34469 and GM23509.

W-Pos97
KINETIC STUDIES OF OLIGONUCLEOTIDE-DNA HYBRIDIZATION IN SOLUTION BY FLUORESCENCE RESONANCE ENERGY TRANSFER. ((K. M. Parkhurst and L. J. Parkhurst)) Dept. of Chemistry, Univ. of Nebraska, Lincoln, NE 68588-0304.

A 16-mer 5'R-GTAAAACGACGGCCAG-3'F (O16), where R is Rhodamine X, and F is fluorescein was prepared for energy transfer studies. When F was excited at 488nm, emission was observed from both the fluorescein and rhodamine. Most of the rhodamine fluorescein derived from fluorescence energy transfer from the fluorescein, and the ratio of acceptor fluorescence to donor fluorescence provided a sensitive measure of hybridization of the oligo probe. The 3'-5' distance distribution was explored using steady-state methods with I^- as the quencher to vary the Förster R_0. In 1M NaCl, data for the free probe could be fit well to a random coil, whereas upon hybridization, a shifted Gaussian was used. The average (RMS) end-to-end distances were respectively 36Å and 66Å. The value of σ following hybridization, 17Å, appears too large for a rigid helix. The kinetics of hybridization of O16 to the complementary region in M13mp18(+) followed simple monophasic second-order kinetics with a rate constant 0.1 that for a 16-mer complement. The kinetics are consistent with rapid secondary structural fluctuations around the target site. (Grant Support NIH DK 36288, Univ. of Nebraska Biotechnology Postdoctoral Fellowship.)

W-Pos98

Investigating Differences in the Interaction of Cations with a 160 BasePair Calf Thymus DNA and a 20 Base Pair Oligonucleotide Using ^{23}Na NMR

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The interaction of cations with NaDNA is examined using ^{23}Na NMR relaxation rates. Previous studies showed that the relaxation rates of ^{23}Na decrease as NaDNA is titrated with NaCl or a competitive ion, such as putrescine.[1] We previously quantified the interaction of Na^+ with sonicated calf thymus DNA using a simple two state model to describe the exchange of various cations with Na^+.[1] The "bound" state is defined as those sodium ions radially localized close enough to the surface of DNA so that their relaxation rates are affected. The "free" state encompasses all other environments of Na^+. The two states are in fast exchange on the NMR time scale (milliseconds).[2] A comparison of ^{23}Na NMR relaxation rates in solutions of mononucleosomal calf thymus DNA (160 ± 5 base pairs (bp)) and of a 20 bp oligonucleotide shows a greater enhancement of the initial observed ^{23}Na relaxation rates for the longer segment of DNA. The difference in the initial relaxation rates may result from differences in r°, the fractional neutralization of DNA phosphates by "bound" Na^+, and/or R_B, the relaxation rate of the "bound" Na^+. Competitive titrations with putrescine (2+) are being used to determine which of these parameters is (are) affected. The results of this study are being compared with predictions of oligoelectolyte theory[3] and polyelectrolyte theory[4] in order to investigate the effects of oligomer length on cation-DNA interactions.

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W-Pos99

PHASE TRANSITIONS OF HIGH MOLECULAR WEIGHT DNA IN SIMPLE SALINE SOLUTION. ((Kunal Merchant and Randolph L. Rill))

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Theories of phase transitions in solutions of rigid rod-like polymers predict the formation of liquid crystalline phases above a critical concentration that decreases with increasing polymer length. Recent extensions of the theory include the effects of polymer charge and flexibility, and predict a large increase in the critical concentration mainly due to flexibility. A semi-rigid polyelectrolyte such as DNA exhibits phase transitions in aqueous solution dependent on the length and the ionic strength. The critical concentration of high molecular weight DNA (>13,000 Å or 4000 base pairs long) at an ionic strength of 0.1 M (Na^+,X-) was obtained by solid state ^{31}P NMR methods. The appearance of a broad resonance superimposed on the sharp resonance of isotropic DNA was used to detect and quantitate the ordered phase. Anisotropy was confirmed and analyzed using the polarizing microscope. The anisotropic phase of this very long DNA exists beyond a DNA concentration of 9.6 mg/ml, a concentration well in the physiological range and much lower than the critical concentration (> 100 mg/ml) observed for nucleosome core length (146 bp) DNA . The critical concentration for anisotropic phase formation for large DNA is surprisingly lower than predicted by theory for a semi-flexible polymer with the persistence length of DNA. As observed for shorter DNA, a second anisotropic phase forms as the DNA concentration is increased, but at a lower concentration than required for total disappearance of the isotropic phase (60 mg/ml). (Supported by NIH grant GM37098 and the FSU Center for Materials Research and Technology.)

W-Pos100

ETHIDIUM BINDING TO DNA HAIRPIN LOOPS

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We have used a combination calorimetric and spectroscopic techniques to study the association of ethidium to a set of DNA hairpins, with the sequence d(GCGCT$_n$GCGC) where n = 3, 5 and 7. In 10 mM phosphate buffer at pH 7, all three hairpins melt in a two-state monophasic transition, with similar transition enthalpies of +38.5 kcal/mol, and T_m's ranging from 79.1°C (for n=3) to 57.5°C (for n=7). Thus, the addition of a dT residue to the loop destabilizes the hairpin (at 20°C) by an average $\Delta G°$ of +0.5 kcal/mol. Deconvolution of the ethidium-hairpin calorimetric titration curves, indicate two sets of binding sites that correspond to one ligand in the stem and two ligands in the loop of these hairpins. We obtained an average binding affinitiy, K_b, of 1.8 x 10^6 M^{-1} and 4.3 x 10^4 M^{-1} for the stem and loop site respectively. However, the binding enthalpy, ΔH_b, for both sites is more exothermic when more dT residues are in the loop, ranging from -8.7 (for n=3) to -11.6 kcal/mol (for n=7) for the stem site, and -6.5 (for n=3) to -12.7 kcal/mol (for n=7) for the loop site. Relative to the n=3 hairpin, we obtained an overall thermodynamic contribution (per dT residue) of $\Delta\Delta H_b = \Delta(T\Delta S_b) =$ -0.6 kcal/mol for the stem sites, and $\Delta\Delta H_b = \Delta(T\Delta S_b) =$ -1.3 kcal/mol for the loop sites. This suggests that ethidium binding induces structural perturbations, that results in a differential compensation of favorable stacking interactions and unfavorable ordering of the ligands.
This work was supported by NIH Grant GM-42223.

W-Pos101

THERMODYNAMIC INVESTIGATION OF NETROPSIN BINDING TO DNA OLIGOMER DUPLEXES WITH A CENTRAL CORE OF d(A$_5$T$_5$)

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Netropsin binds in the minor groove of B-DNA with high specificity for A•T stretches, and without inducing conformational changes. The complex is stabilized by contributions from peptide- nucleotide hydrogen bonding, van der Waals and electrostatic interactions. To further our understanding of the molecular forces that control the affinity and specificity of netropsin to A•T base pairs, we have thermodynamically characterized the interaction of this ligand to a series of self-complementary oligonucleotide duplexes with sequence d(G$_n$A$_5$T$_5$C$_n$), for n = 0-2. Standard thermodynamic profiles for the helix- coil transition of these duplexes show an increase in the duplex thermal stability, ΔT_m, of 12-22°C with the addition of flanking G•C base pairs. Thefavorable differential free energy terms are enthalpic driven, with a $\Delta\Delta H$ of 27-50 kcal/mol, and correspond to the contributions from the closing of frayed A•T base pairs and from the formation of additional nearest-neighbors base-pair stacks. These duplexes contain two identical binding sites for netropsin. Thermodynamic profiles for the association of this ligand to each duplex reveal similar binding affinities of ~10^8, and average binding enthalpies of -0.5 kcal/mol and -5.7 kcal/mol for the first and second site, respectively. These lower enthalpic values are consistent with the endothermic contribution of the release of water molecules from the minor groove of these sites, as seen previously in similar binding studies with poly dA•poly dT. Therefore, the local hydration of dA$_5$•dT$_5$ stretches in these oligomers is similar to that of the homopolymer. Supported by NIH Grant GM-42223.

W-Pos102

THE OSMOTIC SENSITIVITY OF NETROPSIN ANALOG BINDING TO DNA. ((N.Yu. Sidorova and D.C. Rau))

NIDDK, NIH, Bethesda, MD 20892 (Spon. by A.N. Schechter)

Direct force measurements have shown the dominating role of water in the interactions between macromolecules. We have now begun measuring the dependence of the binding to DNA of the antibiotic netropsin (Nt) and several of its analogs on water activity in order to correlate changes in binding strength and water release in dilute solution with the condensed phase force measurements. Netropsin itself binds in the minor groove of DNA and shows a strong preference for A-T base pairs. Binding isotherms of two much less tightly bound Nt analogs can be obtained from changes in the circular dichroism of the ligands with binding to calf thymus DNA. For a variety of chemically distinct osmolytes (betaine, sucrose, sorbitol, methanol, and triethylene glycol), binding energies decrease linearly with solution osmotic pressure. The complex appears to bind an extra 30 - 40 water molecules (relatively insensitive to osmolyte species) over the separate components, perhaps due to the extended conformation adopted by the ligand on binding. This unexpected result was further probed by modulating the bulk water entropy with the anion species. In agreement with the osmolyte results, strong water structure formers as fluoride increase peptide binding (it's entropically easier to bind extra water from the bulk) compared with structure breakers as perchlorate. Work is in progress on the linkage between changes in bound water and the differences in binding strength of Nt itself to specific DNA sequences.

W-Pos103

SEQUENCE SELECTIVE BINDING OF ACTINOMCYIN D TO DUPLEX AND SINGLE-STRAND DNA

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The thermodynamic properties associated with the interaction of actinomycin D with DNA duplexes containing both classical -XGCY- and non classical -T(G)$_n$T- binding sites are examined. The influences of flanking base sequences on the binding parameters for the -XGCY- sequences are compared. These flanking base studies revealed that of the two classical actinomycin D intercalation sites examined (both containing -GC-steps), the -TGCA- sequence binds actinomycin D with a higher affinity than the -CGCA- sequence, yet binding to both of these sequences is quite high ($K_{int} = 10^6$ M^{-1}). The non classical -TGGGT- containing sequence was found to have comparable binding properties with the classical -XGCY- intercalation sites; however, the -TGGT- sequence exhibited a considerable drop in binding affinity, as well as marked changes in binding energetics. The binding of actinomycin D is demonstrated to be highly influenced by the sequence both at the intercalation site and by neighboring bases which flank the intercalation site.

In addition, actinomycin D was found to interact with single-strand DNA and this interaction was demonstrated to be highly sequence specific, with binding affinities ranging from 10^4 to 10^7 M^{-1} (strand). Our studies reveal single base changes within the non self-complementary 11-mers can result in drastic changes in binding affinities for these actinomycin D-single-strand DNA interactions.

W-Pos104

CIRCULARIZATION OF SMALL DNAs IN THE PRESENCE OF ETHIDIUM ((James B. Clendenning and J. Michael Schurr)) Department of Chemistry, BG-10, University of Washington, Seattle WA 98195

A rigorous theory is developed for the ratios of topoisomers produced upon ligation of an equilibrium population of non-covalently closed circles in the presence of ethidium. Assuming an unwinding angle $\phi = 26°$ for intercalated ethidium, optimum values of the intrinsic binding constant ($K_E = 7.16 \times 10^4$ M^{-1}), the intrinsic twist ($l_0 = 23.746$ turns), and twist energy parameter ($E_t = 5250$) are obtained by fitting this theory to the data of Shore and Baldwin (1983) for a 247 bp DNA. A very good fit is achieved with these optimum values, but a poor fit results when the parameters estimated by Shore and Baldwin are employed in the same theory. Three assumptions employed in the analysis of Shore and Baldwin are found to be invalid. If the present substantially larger E_t-value is representative of their short DNAs, then the E_t vs. N data of Shore and Baldwin now conform to the shape predicted by Shimada and Yamakawa (1985), and one may conclude that all of their DNAs exist in a state with substantially stiffer than normal torsional rigidity.

Shore, D., and Baldwin, R.L.(1983) *J. Mol. Biol.* **170**, 983-1007.
Shimada, J.H., and Yamakawa, H. (1985) *J. Mol. Biol.* **184**, 319-329.

W-Pos105

LONG DNA STRAND CONSTITUTES A SEPARATE PHASE IN INTERACTION WITH TRACE AMOUNT OF SEQUENCE-NONSPECIFIC LIGANDS. ((Zhijian Huang, Stephen Yue and Richard P. Haugland)) Molecular Probes, Inc., 4849 Pitchford Avenue, Eugene, OR 97402.

Binding of ligand to DNA is a crucial event which leads to regulate gene transformation, mutation and expression. However, quantitation of the binding by the mass-action law could be very complicate when sequence heterogeneity, multiple binding modes, ligand-ligand interactions and DNA strand distortion by ligand are considered. In fact, a long DNA strand can be treated as a separate phase which is associated with dilute amount of ligand in a partition fashion. Under low ligand binding densities, DNA titrations against constant amount of sequence-nonspecific ligands such as fluorescent DNA-binding dyes resulted in hyperbolic isotherms. This observation strongly supports the DNA partition model and can be utilized as to determine the dyes' total DNA affinities. The early hydrolysis of the double-stranded DNA (dsDNA) phase by deoxyribonuclease I (DNase I) was also investigated. As revealed by the fluorescence changes of the dyes labeled on calf thymus DNA upon DNase I action, the enzyme repeatedly and randomly embarks on the nicked but unbroken dsDNA with a certain partition affinity of $9.6 \cdot 10^6$, and cuts the phosphodiester bonds at a catalysis-unit turnover rate of 60 per second weighed by the available substrate fraction. Such substrate-dependence accounts for the exponential auto-retardation kinetics which is well observed in DNase I catalysis. The DNase I nicking mechanism can be generalized as a model of enzymatic turnover of a rigid interface on which an enzyme repeatedly attacks but cannot freely diffuse to catch another substrate bond. Moreover the studies on DNase I kinetics will help interpret or design the experiments of DNA-footprinting and DNA nick translation (for nucleotide labeling) where DNase I is utilized as a tool.

W-Pos106

A THEORETICAL MODEL FOR THE INFLUENCE OF ION EXCHANGE REACTIONS OF PROTEINS ON DNA BINDING. ((Douglas F. Stickle, Nadine L. McCann and Michael G. Fried)) Department of Biological Chemistry, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033.

The observed binding (association) constant (K_{obs}) for DNA-protein interactions measured *in-vitro* is strongly dependent on the concentration of salt in the solution. This dependence is often used to estimate the number of ionic contacts between protein and DNA. The approximately-constant slope of the relationship (F) of $\log(K_{obs})$ vs. $\log([salt])$, observed at high [salt], is predicted when the displacement of ions from the DNA upon protein binding is the predominant ion exchange reaction (Record *et al.*, 1978, *Q. Rev. Biophys.* 11:103-178). Deviations of F from linearity are often observed at low [salt]; in some cases the slope reverses sign. We have examined the predicted influence on F of a specific formulation for the ion exchange reactions of the protein upon DNA binding. The formulation is derived from a mass balance of ions bound to both protein and DNA when some portion of the protein is transferred from the fixed ion concentration in bulk solution to the high [cation] and low [anion] environment that exists in the immediate vicinity of the DNA. Given the probable difference between the affinity of DNA for cations (a high affinity) and the affinity of the protein for cations and anions (a relatively low affinity), the net displacement of ions upon binding from the system comprised of DNA plus protein is dependent on the bulk salt concentration. The predictions of the model are consistent with the deviations of F from linearity that are observed experimentally for a number of DNA-protein binding systems. (Supported by NSF DMB-91-96154)

PHYSICAL CHEMISTRY OF PROTEINS

W-Pos107

MOLECULAR DYNAMICS AND OXYGEN-17 NMR STUDIES OF AMINO ACID HYDRATION AND ACTIVITY IN AQUEOUS SOLUTIONS AS A FUNCTION OF pH. ((I.C. Baianu*, T-C. Wei* and T.F. Kumosinski‡)) *University of Illinois at Urbana, AFC-NMR Facility, Department of Food Science, 580 Bevier Hall, 905 S. Goodwin Avenue, Urbana, IL 61801; ‡Eastern Regional Research Center, USDA, Philadelphia, PA 19118.

Amino acid hydration and activity in aqueous solutions were investigated by ^{17}O NMR measurements as a function of both pH and concentration. Similar behavior was observed also for proline solutions. Charge-charge and dipole-dipole interaction models were employed to fit the ^{17}O NMR data in conjunction with nonlinear regression computer analysis. At concentrations above 1M a thermodynamic linkage model was employed to analyze the aggregation of amino acid molecules. In certain cases, amino acid tetramer formation was found by the thermodynamic linkage analysis of our data. Among the amino acids studied were glycine, aspartic acid, arginine and lysine. Marked differences were observed between the concentration dependences of ^{17}O NMR relaxation rates of glycine at its pK_a and at neutral pH. Molecular dynamics of water in amino acid solutions suggests very fast dynamics and exchange of water between hydration sites and bulk water. These results are consistent with previous reports of protein activity measurements[1-3].

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W-Pos108

GLASS FORMATION IS NECESSARY BUT NOT SUFFICIENT FOR STABILIZATION OF PROTEINS DURING FREEZE-DRYING. ((M.Z. Zhang, S.J. Prestrelski, T. Arakawa, J.F. Carpenter*)) Amgen Inc., Thousand Oaks, CA 91320, CryoLife, Marietta, GA 30067*

To test the hypothesis that glass formation is sufficient for stabilization of proteins during lyophilization, we examined the effect of dextran, an amorphous excipient with a high glass transition temperature, on the structure and activity of two labile enzymes, lactic dehydrogenase and phosphofructokinase, during freeze-thawing and freeze-drying. The dextran used was pre-dialyzed to remove any low molecular weight components. At high concentrations (10-20% w/v) dextran was effective at stabilizing these enzymes against freeze-thawing. At all concentrations tested, dextran did not confer stability during freeze-drying. In fact, dextran solutions destabilized LDH during freeze-drying and were observed to induce aggregation. Infrared studies of dried enzymes lyophilized with dextran showed extensive unfolding. Addition of mono- and disaccharides to dextran mixtures enhanced stability during freeze-drying. These dextran results demonstrate conclusively that glass formation alone is insufficient to stabilize proteins. The enhanced stability observed upon adding saccharides to dextran mixtures indicates that other interactions, presumable hydrogen bonding, are also necessary. The lack of stabilization with dextran alone is likely due to its bulkiness which inhibits interaction with proteins.

W-Pos109

CALORIMETRIC STUDIES OF ELASTASE: THERMAL DENATURATION AND INTERACTIONS WITH LIGANDS. C.S. Randall, S.N. Neff, A.J. Perri and T.D. Sokoloski, Dept. of Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals, King of Prussia, PA. 19406

The stability of porcine pancreatic elastase to heat denaturation has been investigated using differential scanning calorimetry (DSC). In the range of pH 5 to 8, elastase undergoes complex, irreversible unfolding accompanied by aggregation. The temperature of denaturation (Td) can be markedly affected by ligands, e.g., divalent cations. In the presence of calcium ions, the unfolding endotherm is sharpened, suggestive of increased cooperativity, along with a shift in the Td to higher temperature, consistent with increased conformational stability. By contrast, addition of copper ions decreased the Td compared to that of elastase alone, indicating a less stable conformation. By means of differential titration calorimetry (DTC), it is possible to calculate the thermodynamic parameters related to the binding of such ligands, and to postulate a basis for the observed stabilizing or destabilizing effects on the protein.

W-Pos110

THERMODYNAMIC AND STRUCTURAL ASSIGNMENTS OF THE UNFOLDING TRANSITIONS OF DNAK ((D. Montgomery*, R. Jordan+ R. McMacken+, and E. Freire *)) *Biocalorimetry Center and Dept. of Biology, Johns Hopkins University, Baltimore, MD 21218 and +Dept. of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

The thermal unfolding of the *E. coli* 70kD heat shock protein, DnaK, exhibits three well defined transitions. As characterized by high sensitivity differential scanning calorimetry these transitions are centered at 45.2 C^o(K1), 58.0 C^o(K2), and 73.3 C^o (K3). Calorimetric scans as a function of pH can be fit by a four-state model which includes a ΔH, Tm, and ΔCp for each transition. A scan of the isolated N-terminal 44kD proteolytic fragment shows a major transition at 47.8 C^o(N1) and a minor transition at 78.5 C^o(N2). A scan of the C-terminal 23kD proteolytic fragment can be described by three transitions of roughly equal ΔH with Tm's of 50.3 C^o(C1a), 58.2 C^o(C1b), and 70.6 C^o(C2). The low temperature transition of intact DnaK (K1) can be assigned to the N-terminal on the basis of the similarity of the Tm and ΔH of (N1) of the isolated N-terminal fragment. This assignment is supported by the similarity of the Tm (41C^o) obtained from studies of the intrinsic fluorescence as a function of temperature. DnaK contains only one tryptophan which is located at residue 102 in the N-terminal. In addition, the Tm of (K1) increases by 8.7 C^o in the presence of excess ADP which is known to bind to the N-terminal. The middle transition of the intact protein (K2) can be assigned to transitions (C1a) and (C1b) of the C-terminal fragment on the basis of similar Tm's and ΔH's. In the intact protein (C1a) and (C1b) form a single cooperative unfolding unit. In addition, a peptide which is known to bind to the C-terminal affects (K2) by increasing the Tm by 3C^o. Presumably the high temperature transition (K3) contains contributions from both (N2) and (C2), however, the exact structural elements have not yet been identified. (Supported by NIH grants GM37911 (E.F.), RR04328 (E.F.), and G.M.36526 (R.M.))

W-Pos111

INTERACTION OF CALMODULIN WITH A PHOSPHORYLASE KINASE REGULATORY PEPTIDE. ((D. Juminaga, S.A. Albaugh and R.F. Steiner)) Department of Chemistry, University of Maryland Baltimore County, Baltimore, MD 21228

The interaction of Phk13, a regulatory peptide of the γ-subunit of phosphorylase kinase, and calmodulin has been studied by several physical and enzymic techniques. For the purpose of fluorescence studies modified forms of Phk13 have been synthesized in which nitrotyrosine and/or tryptophan are placed at one or both of positions 4 and 21. Static fluorescence measurements indicate that, in the presence of Ca^{2}-ligated calmodulin, the tryptophan groups are in a non-polar, partially solvent-shielded microenvironment. Gel exclusion chromatography, in aqueous buffer or 3M urea, indicates that the 1:1 complex does not self-associate. Fluorescence titration indicates that, at high peptide:calmodulin ratios, calmodulin can bind more than one peptide. Circular dichroism measurements show that no net increase in α-helicity occurs upon binding of peptide. Fluorescence radiationless energy transfer between Trp-4 and NO_2-Tyr-21 suggests that the peptide is highly extended in the complex. Complex formation does not protect the bond between residues 77 and 78 from proteolysis by trypsin. However studies with tryptic and thrombic fragments of calmodulin indicate that the connecting helix is involved in the interaction.

W-Pos112

WATER AS AN INHIBITING LIGAND IN YEAST HEXOKINASE ACTIVITY. ((P. Rand, N.L. Fuller, P. Butko and P. Nicholls)) Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1.

We have used osmotic stress to measure water's role in yeast hexokinase (HK) activity. HK has a cleft whose closure is induced by glucose binding and aided by reducing the activity of water outside it. Osmotic pressure reduces both the glucose binding constant K_d and the Michaelis constant K_m for glucose. The effects are similar (although we are testing whether the binding may be more sensitive) and the pooled equilibrium and kinetic data show that 65±10 water molecules are removed as glucose binds. Water behaves as an inhibiting ligand. The consistency of the kinetic and equilibrium data shows that the hydration/dehydration reactions are present during catalysis and the number of waters suggests a large contribution by solvation to the energetics of the conformational change in catalysis.

Most of this water likely comes from the cleft. Above MW2000 the osmotic effect is independent of molecular weight and methylation of the polyethyleneglycol used to fix the osmotic pressure . For MW 600 PEG, equivalent equilibrium and kinetic data give 11±6 water molecules showing that this osmolyte has access to much of the aqueous compartment that excludes the higher molecular weights. We are using even smaller osmolytes in an attempt to probe energetically significant but smaller aqueous spaces around the protein.

W-Pos113

STUDY OF HELIX CAPPING IN CYTOCHROME b_5 BY NMR SPECTROSCOPY AND SITE-DIRECTED MUTAGENESIS. ((E.A. Baldauf and J.T.J. Lecomte)) Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

While the α-helix is a frequently occurring motif in protein secondary structure, the factors governing helix formation are poorly understood. Amino acid residues whose side chains can form hydrogen bonds with the C=O or N-H moieties of the backbone are often found a the N- and C-terminal boundaries of helices [1]. Presta and Rose hypothesized that the ability to form side chain:main chain hydrogen bonding may constitute a signal for adopting helical structure [2]. We are addressing this question by using cytochrome b_5 as a model system. Residues 80 to 88 form helix VI of cytochrome b_5. The δ-nitrogen of His 80 in the N-cap position acts as an acceptor for the backbone N-H hydrogen of Asp 82 both in the holoprotein and in the apoprotein [3]. To probe the importance of this H-bond in the stabilization of helix VI, we have replaced His 80 by Ala and recorded two-dimensional NMR spectra of the holoprotein and the apoprotein. Comparison to the wild-type data shows that the replacement does not affect the C-terminal portion of the helix. NMR studies of a second mutant (Pro81Ala) will also be presented.

Supported by NIH grant DK-43101

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W-Pos114

ENERGETICS OF SITE SPECIFIC ANTIBODY-CYTOCHROME *C* INTERACTION. ((C. S. RAMAN, B. T. NALL and M. J. ALLEN)) Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78284-7760.

We have used high sensitivity titration microcalorimetry to evaluate the thermodynamics of monoclonal antibody (*mAb*) binding to horse heart cytochrome *c* (*cytc*). *mAb*2B5 binds near the exposed heme crevice and *mAb*5F8 interacts with the opposite side of the molecule. Both antibodies have high affinity for the native protein (K_a = 10^9 - 10^{10} M^{-1}). The enthalpic (ΔH^o) and entropic (ΔS^o) contribution to the binding free energy of both antibodies are large and negative. The *mAb*2B5-*cytc* association exhibits a temperature dependent ΔH^o (ΔC_p = -520 cal mol^{-1} K^{-1}), while that of *mAb*5F8-*cytc* does not (ΔC_p ≈ 0). Further, unlike the *mAb*5F8-*cytc* interaction, binding of *mAb*2B5 to *cytc* involves proton uptake. These results will be discussed in light of the forces governing antibody-protein recognition.

W-Pos115

TEMPERATURE-DEPENDENT HYDRATION FORCES MEASURED BETWEEN COLLAGEN TRIPLE HELICES. ((S.Leikin, D.C.Rau, and V.A.Parsegian)) PSL/DCRT and LBM/NIDDK, NIH, Bethesda, MD 20892.

Forces directly measured between lipid membranes or between linear macromolecules have revealed features quite different from those used in traditional theories of molecular recognition, assembly, and folding. As molecules are brought together, they repel exponentially with a work of approach that appears to involve removal of water organized around the structures of hydrophilic groups on the apposing surfaces. These "hydration forces" have been seen in materials as different as lipid bilayers, DNA double helices, and stiff polysaccharides. Though hydration is generally regarded as essential to protein function and structure, the lack of direct force measurements has allowed the neglect of "hydration forces" in protein association and folding.

We now report their appearance between soluble proteins, specifically between triple helices in native and reconstituted fibers of type I collagen. Measured force-distance curves closely resemble those in spontaneously assembled DNA fibers. An observed decrease in repulsion with increasing temperature implies that there is an *increase* in entropy as molecules are pushed together. Forces vary exponentially with a decay constant that reflects the structure of collagen triple helix. We see these results as an essential step to connect measured forces with protein-protein recognition.

W-Pos116

THERMODYNAMIC AND CONFORMATIONAL ANALYSIS OF COMPLEXES OF THE REPRESSOR OF BIOTIN BIOSYNTHESIS WITH SMALL LIGANDS

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The repressor of biotin biosynthesis, BirA, is an allosteric site specific DNA binding protein. A unique feature of this protein is that it catalyzes synthesis of the molecule, bio-5'-AMP, that is the allosteric effector for DNA binding. In an effort to elucidate the mechanism of coupling of allosteric effector and DNA binding in this system we have investigated energetic and structural features of the complexes of BirA with the small molecules biotin, ATP and bio-5'-AMP.

Results obtained from a combination of fluorescence titrations and kinetic measurements indicate that BirA binds relatively weakly (K_D in the μM range) to ATP and biotin and that the equilibrium dissociation constant for the BirA-bio-5'-AMP interaction is in the subnanomolar range of concentration. Partial proteolytic digestion of BirA and its complexes with the three ligands has been used to probe conformational transitions induced upon ligand binding. The responses of the aporepressor and repressor bound to biotin and ATP to this probe are similar. A significantly different response is observed in the cleavage of the BirA-bio-5'-AMP complex.These results indicate a correlation between tight binding of the allosteric effector bio-5'-AMP and a structural change in BirA. This structural change may be required for specific binding of the protein to DNA.

W-Pos117

RULES FOR MACROMOLECULAR RECOGNITION BY THROMBIN

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The interaction of thrombin with its natural substrate fibrinogen and the potent natural inhibitor hirudin is mediated by two distinct structural domains of the enzyme: the fibrinogen-recognition-site (FRS) and the catalytic pocket (CP). Macromolecular recognition by thrombin hinges on macromolecular "bridge-binding" to these two separate domains. Bridge-binding is very finely tuned by specific ion binding interactions with the CP and FRS. Thrombin exists in two conformations, fast and slow, that differ by one order of magnitude or more in the kinetic processes involving diffusion to and from the CP. The slow→fast transition is allosterically triggered by Na^+ binding in the region of negative electrostatic field surrounding the CP. This binding reaction also induces a major change in the intrinsic fluorescence of Trp residues and molecular ellipticity. Bridge-binding to the slow and fast conformations of thrombin occurs with widely different affinities. Na^+ binding facilitates bridge-binding and hence, by linkage principles, it follows that the slow→fast transition is a major component of macromolecular recognition by thrombin. Binding to the FRS is controlled in a competitive fashion by Cl^-. The control of macromolecular recognition by Na^+ and Cl^- is of physiological relevance. Our findings suggest a possible molecular mechanism for recognition by thrombin which is consistent with recent crystallographic results.

W-Pos118

CHLORIDE BINDING AND DONNAN SWELLING

((*Gerald Elliott **Stuart Hodson and *Justyn Regini))

*The Open University, ORU, Oxford, OX1 5HR and **Vision Sciences, UWCC, Cardiff, CF1 3XF, UK.

In corneal stroma, the cation exchange capacity of the connective tissue creates a Donnan-osmotic swelling pressure and a Donnan electrical potential. These generate passive swelling. The exchange capacity has two contributions: (1) the charged carboxylic and sulfonic acid groups of glycosaminoglycans and (2) a charge resulting from the transient binding of free chloride ions to a ligand. Chloride binding shows first order kinetics and provides most of the stromal cation exchange capacity under physiological conditions. Chloride binding has the consequence of stabilising Donnan swelling on exposure of the cornea to solutions of varying tonicity, and it explains the well documented negative temperature coefficient of stromal swelling. It is suggested that a similar phenomenon explains the temperature-dependent disappearance of the myosin layer lines in the X-ray diffraction pattern of relaxed rabbit muscle, and may also be relevant to cold-cataract in eye lens.

W-Pos119

EQUILIBRIUM BINDING OF SUCROSE OCTASULFATE WITH BASIC FIBROBLAST GROWTH FACTOR. ((T. Arakawa, J. Wen & J. Philo)) Amgen Inc., Amgen Center, Thousand Oaks, CA 91320

Fibroblast growth factors (FGFs) strongly bind to heparin and are thereby stabilized against deactivation and proteolytic cleavage. Sucrose octasulfate (SOS), which has a chemical structure resembling the repeating unit of heparin, has also been shown to enhance stability of basic FGF against thermal denaturation and to induce a small conformational change. We have examined SOS binding to bFGF using equilibrium dialysis. The difference in SOS concentration across the dialysis membrane was measured using a precision density meter, since the density of SOS differs greatly from that of water. With care, this densimetric technique can measure binding with a precision of ±0.1 mole/mole using only 2 mg/ml of protein. These results show that the binding saturates at 2 moles of SOS per mole of bFGF as SOS concentration increases to 4 mM or higher.

The effect of SOS on the thermal stability of bFGF was examined using denaturation at a constant heating rate, by both turbidity and DSC. Since the thermal denaturation is irreversible, the temperature where aggregation abruptly increases was taken to indicate the onset of denaturation. This temperature increased ~12° as [SOS] increased from 0.01 to 4 mM and remained constant above 4 mM, consistent with our binding data if the binding is specific to the native state.

W-Pos120

THERMAL STABILITY OF THE SINGLE-CHAIN ANTIBODY SCA 4-4-20/212

((Joachim D. Müller*, Edward W. Voss Jr. & G. Ulrich Nienhaus)) Department of Physics, Biophysics & Microbiology, University of Illinois at Urbana-Champaign, IL 61801, *Technische Universität München, Germany.

The anti-fluorescyl single-chain antibody SCA 4-4-20/212 of the $(NH_2)V_L$-linker-$V_H(COOH)$ design exibits a reversible cold denaturation. The antibody is based on the high affinity murine monoclonal antibody Mab 4-4-20, which has been extensively characterized in terms of structural, kinetic and spectroscopic properties. The native antibody binds its hapten fluorescein, whereas the denatured form has no affinity to fluorescein. The difference in absorption between the bound and unbound fluorescein serves to probe the folding state of the antibody. T-jump experiments at a variety of temperatures have been performed in glycerol/water mixtures. The denaturation process after the T-jump is observed using time-resolved absorption measurments. Analysis of the T-jump experiments with a singular value decomposition (SVD) algorithm clearly shows a two-state process implying that the cold denaturation is a highly cooperative process. Supported by NIH.

W-Pos121

CHARACTERISATION OF BOVINE ARTICULAR CARTILAGE BIGLYCAN AND BOVINE SKIN DECORIN. ((J. LIU*, L. Rosenberg#, T. Laue*))* Department of Biochemistry, University of New Hampshire, Durham, NH 03824 and # Montefiore Medical Center, Bronx, NY 10467.

Biglycan and decorin are small, leucine rich proteoglycans, present in a variety of extracellular matrices. Little is known regarding their self-association properties. We have used sedimentation analysis to examine the structure and self-association of bovine articular biglycan and bovine skin decorin in a variety of buffers. The monomer molecular weights, obtained in buffer containing 4 M GdnHCl, 50 mM Tris, 5 mM EDTA at pH 7.5, were 86,625 Da for intact biglycan, 44,095 Da for its core protein and 22,159 Da for GAG chain. Under associative condition without Zn^{++} (0.15 M NaCl, 50 mM Tris, 5 mM EDTA, at pH 7.5), the GAG chain remains monomeric, however both biglycan and its core protein appear to form polydisperse oligomers. Dimer is apparently a major species in biglycan self-association. When 5 mM Zn^{++} was introduced, both biglycan and its core protein form larger polydisperse aggregates. Results have also been obtained from bovine skin decorin. In 4 M GdnHCl buffer at pH 7.5, intact decorin exhibits a Mr of 73,750 Da. Under associating condition without Zn^{++}, decorin appears to form dimers. However, adding Zn^{++} yields larger polydisperse aggregates. These results show that both biglycan and decorin undergo polydisperse self-associations in associative conditions, probably mediated by interactions between their core proteins. Supported by NSF DIR 9002027.

W-Pos122

PHYSICAL STUDIES OF THE CLOTTING REACTION OF LIMULUS COAGULOGEN. ((T. Moody and T.M. Laue)) Dept. of Biochemistry, UNH, Durham NH 03824.

The clotting reaction of coagulogen provides a gel forming system that is readily amenable to physical study. Turbidimetric data obtained during trypsin initiated clotting reactions of coagulogen show that the turbidity at 326 nm changes more slowly than the wavelength dependence of the light scattering. On average, the final value of the wavelength dependence rapidly approaches approximately −2. A comparison of pelleting data with spectral data indicates that, early in the reaction, the change in turbidity lags slightly behind the change in the amount of pelletable material. Judging from experiments conducted at 15°C, it would appear that if a critical concentration exists at that temperature, it is close to 0 mg/ml. The pelleted and supernatant parts of aliquots taken at various times during clotting reactions provided useful material for SDS-PAGE experiments. The results of these experiments indicate that coagulogen, and at least one proteolytic intermediate between coagulogen and coagulin, do not participate in gel formation. SDS-PAGE of reduced and unreduced mature coagulin gel suggests that disulfide bonds may stabilize the structure of the gel. Analytical ultracentrifugation experiments should provide further insight into the structure of the mature coagulin gel. The materials currently under investigation in this work include the coagulin gel dissolved in pH 3 chloroacetic acid with and without βME, and the coagulin gel dissolved in pH 8 6M guanidine HCl with and without βME. Supported by NSF DIR 9002027 and NSF DIR 8914571.

W-Pos123

USING SOLUTION EFFECTS TO PROBE THE MECHANISM(S) OF HETEROTROPIC REGULATION IN ASPARTATE TRANSCARBAMYLASE ((Vince J. LiCata and Norma M. Allewell)) Dept. of Biochemistry, University of Minnesota, St. Paul, MN 55108

E. coli aspartate transcarbamylase (ATCase) catalyzes the condensation of aspartate and carbamyl phosphate to produce carbamyl aspartate and inorganic phosphate. In the presence of saturating amounts of carbamyl phosphate, ATCase can be treated as a single substrate enzyme exhibiting both positive homotropic cooperativity and substrate inhibition for aspartate. In addition, ATCase is allosterically regulated by several heterotropic effectors including ATP (positive regulation) and CTP (negative regulation).

We have studied the dependency of the enzyme kinetics of ATCase on [NaCl], temperature, and [ethanol] in the presence and absence of allosteric effectors. The goal of these studies is to characterize the types of bonding interactions involved in heterotropic cooperativity in ATCase by perturbing these heterotropic interactions with changes in solution conditions.

NaCl and ethanol produce inhibitory effects on both the apparent Km and the apparent Vmax for ATCase vs. [aspartate]. The relative [NaCl] and [ethanol] dependencies differ in the presence of ATP vs. CTP, suggesting that ATP and CTP utilize differing non-covalent bonding networks in their "communication" with the active site.

Increasing temperature increases the apparent Vmax (as expected) and increases the apparent Km. Again, the dependence differs in the presence of ATP vs. CTP. If Km = Kd for aspartate, this indicates that aspartate binding is enthalpy driven in the presence of both effectors, and that differential enthalpy-entropy compensation accounts for the net increase (ATP) or decrease (CTP) in free energy of substrate binding.

W-Pos124

ROTATIONAL DIFFUSION OF MYOGLOBIN AND HEMOGLOBIN. ((James Hofrichter, Colleen M. Jones, Anjum Ansari, Eric R. Henry, Olivier Schaad and William A. Eaton)) Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, Maryland 20892

Time-resolved polarized absorption measurements have been used to study the kinetics of the decay of the optical anisotropy produced by partial photodissociation of the CO complexes of sperm whale myoglobin and human hemoglobin. For myoglobin, the rotational correlation time scales linearly with the ratio of solvent viscosity to temperature for glycerol concentrations ranging from 0 to 79% by weight and temperatures ranging from -5° C to 35° C. The rotational correlation time is 12 ± 1 ns at 20°C in aqueous buffer. The corresponding value for hemoglobin is measured to be 36 ± 2 ns. The rotational diffusion coefficients obtained from these results are consistent with those previously reported from dielectric relaxation studies, but are significantly smaller than those obtained using a number of other techniques, including fluorescence depolarization, electron paramagnetic resonance, and γ-γ correlation spectroscopy. To rationalize these values, approximately 10 water molecules/nm^2 of accessible surface area must be included in the hydrodynamic particle. For myoglobin the solvent volume is approximately equal to that of the protein itself. Representations of the hydrodynamic particle obtained by 'shrink-wrapping' with ellipsoids of revolution and by molecular dynamics calculations suggest that the results are consistent with the shapes of these molecules and their optical properties.

W-Pos125

A Scaling Perspective on the Influence of Quantum Energy Flow In Protein Reaction Dynamics

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We present an analysis of quantum energy flow and localization in large molecules from the point of view of a scaling approach. We couple this with the result of a derivation of the rate of electron transfer in large biological molecules such as proteins and the dependence of this rate on vibrational energy flow. The scaling approach is based on earlier scaling theories of Anderson localization in disordered metals. The picture provides a simple general framework for describing both energy flow and the localization of eigenstates. This framework can be applied to molecules with random local transitions between states. We find that when vibational localization occurs the rate of electron transfer is slowed down considerably. We discuss limitations of the model, generalization to other types of reaction, and possible relevence in particular instances.

W-Pos126

BROWNIAN DYNAMICS STUDY OF THE INFLUENCES OF ELECTROSTATIC INTERACTION AND DIFFUSION ON PROTEIN-PROTEIN ASSOCIATION KINETICS. ((Huan-Xiang Zhou)) Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, MD 20892.

We present a unified model for protein-protein association processes (e.g., protein oligomerization, enzyme catalysis, electron and energy transfer) that are under the influences of electrostatic interaction and diffusion. The proteins are modeled as spheres that have point charges embedded in them and undergo translational and rotational Brownian motions. Before association can occur the two spheres have to be aligned properly to form a reaction complex. The reaction complex can either go on to form the product or it can dissociate into the separate reactants. The electrostatic interaction influences every step except the one that brings the reaction complex into the product. The interaction potential is governed by the Debye-Huckel equation outside the two spheres and the Poisson equation inside the spheres. It is obtained by solving an integral equation using basis set expansion. The equilibrium constant for the reaction complex is simply an integral involving the interaction potential. The time-dependent association rate coefficient is obtained through Brownian dynamics simulations. Illustrative calculations demonstrate the subtle way that the protein charge distribution controls the equilibrium constant and the association rate.

W-Pos127

THE EXCLUDED VOLUME EFFECT EXAMINED WITH KINETIC, SELF-AVOIDING, OFF-LATTICE RANDOM WALK POLYPEPTIDES.

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The excluded volume effect represents the fact that real molecules cannot occupy any portion of space that is occupied by another portion of the same molecule at the same time. The effect of excluded volume alone on protein conformations has been difficult to assess theoretically. The statistical parameters of random walk polypeptides are commonly presented *without* explicit account of the excluded volume effect. In addition, few random walk Monte Carlo simulations have been performed off-lattice. Hence a self-avoiding, off-lattice random walk polypeptide simulation is of great utility in assessing protein conformations and comparing them to the "random coil" state. Due to the inherent computational problems involved in generating unbiased random walks which are also self-avoiding, a kinetic or growing walk model is presented. The generation of these kinetic, self-avoiding, off-lattice random walk polypeptides should better represent possible conformations of real random coil polypeptides. Large amounts of Monte Carlo (MC) polyalanine simulations are presented. These random walk polyalanine structures were generated ranging from N=5 to 1000. The radii of gyration, end to end distances, and the distributions of these simulations are reported and compared with values from molecular dynamics, crystallographic and random walk theory. While these parameters show a swelling due to excluded volume, the structures are more compact than reported in previous work using lattices. The number of self-avoidance instances is shown to increase linearly with polymer length N. In addition the limiting characteristic ratio for these polyalanine simulations matches that based on the Flory rotational isomeric state theory model (3, this study, 2.97 (Brant, Miller and Flory 1967 *J. Mol. Biol.* 23:47-65)). However Flory reported the limiting characteristic ratio of polyalanine to be 9 based on a correction for dipole-dipole interactions. This report shows random coil polyalanine is a more compact structure and the dipole-dipole correction of Flory is not consistent with the definition of a random coil polypeptide structure.

W-Pos128

AN ANALYTICAL METHOD FOR NONEXPONENTIAL KINETICS.

((A. Xie, B. Banko, H. Frauenfelder, G. U. Nienhaus, R. Philipp, and R. D. Young)) Department of Physics, University of Illinois, 1110 W. Green Street, Urbana, IL 61801.

Nonexponential kinetics, observed in biological processes of proteins, can be envisioned as composed of many exponential processes. At low temperatures, the variation in the reaction rate within a protein ensemble arises from conformational heterogeneity. In order to elucidate the thermodynamic properties and the structure-function relationship of a protein, we have developed a method (i) to resolve the rate dependencies on temperature of various protein conformations, (ii) to reveal the correlation between activation enthalpy H and prefactor A, and (iii) to deduce the population density g(H,A). To correlate the rates of any specific conformation at different temperatures, we assume that the rates of any two conformations will not cross each other in the concerned temperature range. We have employed this method to investigate the kinetics of geminate ligand recombination to sperm whale myoglobin. The Arrhenius plot of the resulting rate coefficient exhibits three distinct temperature regions: quantum tunneling, Arrhenius, and conformational relaxation. In the Arrhenius region where $k = A(T/T_0)\exp(-H/RT)$, the prefactor A is sensitive to the protein conformation and varies by a factor of more than 10. Within the distributions of A and H, a large prefactor A is coupled to a small activation enthalpy H with a nonlinear correlation. This method also allows the quantitative analysis of conformational relaxation and quantum-mechanical tunneling. (Supported by NIH).

W-Pos129

"COMPLETING THE KNOT"-ANOTHER NEAT DISCOVERY IN EVOLUTION.

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"Knots","matrices" and "surfaces" are the substructures of most proteins (J.Mol. Liquids,42,113(1989)). Thermodynamic stability is primarily due to knots; matrices rarely have maximum stability. Knot-formation is extremely cooperative. A single missing or altered residue usually prevents formation. This is the basis a device of great utility we call "completing the knot".Because it makes the stabilizing free-energy from knot formation available for specific processes, it greatly increases the effective free-energy changes in the latter. One such application is in protein associations. In "leucine zippers" the inter-protein knot is completed by combining groups from two proteins. Many repressor proteins and the HIV-1 protease consist of two identical proteins each of which contributes a single hydrophobic group to complete the knot of the other. In some of these this is supplemented by a "strap-hinge" formed by combining short peptide segments into a two-chain anti-parallel β-sheet knot. An effector which supplies a missing knot residue has major thermodynamic and specificity advantages. The thermodynamic advantage can be used to increase sensitivity as in regulation of enzymes by effectors which prevent formation of one of the two knots; e.g., calcium-ion in α-lactalbumin. Multi-knot proteins with an uncompleted knot are "molten globules". The coordination of expansion-contraction fluctuations of two functional domains, one knot per domain, to act mechanically like our opposed thumbs is called the "pairing principle" It is a major feature of enzymic mechanisms and may be important in antigen-antibody combination and abzyme function. The major factor in contraction as in thermodynamic stability is the arrangement of peptide dipoles. Comparisons of B-factor plots for different kinds of atoms can give rare information about these arrays. Thus the B-factor data for Fab-antidigoxin (Constantine et al,.Proteins:Struc. Func. Gene.,in press) show little change in the mean B for backbone nitrogens in the variable regions on binding digoxin but a large increase in standard deviation.

W-Pos130

DETECTION OF HETEROGENEITY IN SELF-ASSOCIATING SYSTEMS BY EQUILIBRIUM SEDIMENTATION.

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A procedure has been developed for detecting heterogeneity in self-associating systems that combines the merits of equilibrium sedimentation, the power of computers and the discrimination of signal detection theory. In typical experiments with self-associating systems this approach can detect the presence of less than 2% (by weight) of "incompetent monomer". The procedure is based on a nonlinear least square fitting program, NONLIN, used extensively for sedimentation equilibrium studies during past two decades. First we showed that the parameters (apparent molecular weights, association constants, etc.) estimated by NONLIN were asymptotically Gaussian, based on good agreement of the confidence regions of the estimates evaluated by Monte Carlo runs with those calculated according to the Cramer-Rao lower bound. The method of "linear hypothesis testing" then was employed to estimate the dependence of the observed association constants on initial loading concentration and/or speed (both previously indicated to be measures of heterogeneity in self-associating systems). Computer simulation studies with this new approach are underway for various association schemes and types of heterogeneity. The dependence of the sensitivity of this approach on sedimentation speed, loading concentration and association constant will be reported. This method appears useful both for theoretical studies and for practical analyses of self-association systems by sedimentation equilibrium.
(Supported by NSF grant #DIR-8612159)

W-Pos131

Calculation of the dipole moment of Heme Proteins: Use of Protein Data Base.

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We have been using the protein data base to calculate the dipole moment of small proteins . The calculated dipole moments are in general in agreement with the measured. We are now extending the same method to larger proteins such as normal and abnormal sickle cell hemoglobins. Hemoglobin is a protein having a molecular weight of 64000 and consists of two α-chains and 2 β-chains. Because of the nature of our method of calculation of the dipole moment, we are unable to calculate the moment of the whole hemoglobin molecule at once. In order to circumvent the difficulties, we calculate the dipole moments of subunits separately and then sum them up vectorially. The calculated dipole moemnt 501D is in excellent agreement with the measured moment 495D. In addition to the normal hemoglobin A, we calculated the dipole moment of sickle cell hemoglobin S. Experiments indicate that the dipole moment of HbS is considerably smaller than that of HbA. The calculated value turned out to be 297D while the measured value is 286D.

W-VCR3

LIPIDAT: A DATABASE OF LIPID PHASE TRANSITION TYPES, TEMPERATURES AND ENTHALPY CHANGES: AN UPDATE.

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LIPIDAT was initiated approximately four years ago with a view to providing a single, centralized source of all literature data on the mesomorphic and polymorphic transition temperatures and enthalpy changes for synthetic and biologically-derived complex polar lipids. The compilation includes all data for lipids in the dry and in the partially and fully hydrated states. Also included is the effect on these thermodynamic values of pH, and of salt and metal ion concentration and other additives such as proteins, drugs, etc. The experimental methods and conditions used in making the measurements are reported. Bibliographic information includes complete literature referencing and list of authors. As of this writing, the database is current through June, 1990 and contains in excess of 10,000 records. Each record has 28 information fields. The second phase of the database project, namely the compilation of all data regarding the miscibility properties of lipids is now underway. Here, data are being presented graphically in the form of evaluated isobaric and isothermal phase diagrams. To date we have collected approximately 1,300 such diagrams. This database is called LIPIDAG. The electronic version of LIPIDAT will be released in late 1992 and will be demonstrated at the Meeting. A hard copy version of LIPIDAT is in preparation at CRC Press Inc.

W-Pos132

STRUCTURAL DETERMINATION AND PACKING ANALYSIS OF A CHOLESTERYL CAPRATE/CHOLESTERYL LAURATE SOLID SOLUTION.

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The X-ray crystal structure analysis of a cholesteryl ester solid solution, cholesteryl deconoate/cholesteryl laurate in a molar ratio 0.56/0.43 is described. The unit cell is monoclinic with a=12.969, b=9.048, c=31.137Å and β=91.12° and the space group $P2_1$ with Z=4 (two molecules per asymmetric unit). The cell constants closely represent an average value of crystal parameters for the two components (hence, nearly corresponding to Vegard's law). Although the overall monolayer I lamellar packing is superficially similar to the earlier studied cholesteryl undeconoate/laurate solid solution, there appears to be a partitioned distribution of acyl chains, i.e. a microfractionation corresponding to the observed nonideal phase behavior. This structural arrangement is contrasted with the fractional atomic occupancy in an average laurate structure found earlier for the more ideal undeconoate/laurate solid solution. The final weighted R factor for 4578 reflections is 0.138. Difficulties in refinement were experienced in both the isoprenoid chain of one molecule and the acyl terminal chain of the other molecule. The molecular conformations of the molecules are however consistent with previous determinations for the pure components. Increased molecular volume differences in such solid solutions thus lead to a displacemnt of the molecules from one another in an effort to retain important interfacial contacts. Research supported in part by the Helen Woodward Rivas Memorial Trust.

W-Pos133

CALORIMETRIC STUDIES OF MIXED-CHAIN PHOSPHATIDYL-ETHANOLAMINES.

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The lamellar gel to liquid-crystalline phase transitions of aqueous dispersions of four homologous series of mixed-chain phosphatidylethanolamines (PE) and one homologous series of identical-chain PE have been studied by high-resolution differential scanning calorimetry (DSC). Within each homologous series, all members of PE share a common value of ΔC. Here, the term ΔC is defined as the effective chain-length difference, in C-C bond lengths, between the sn-1 and sn-2 acyl chains. The ΔC values of the five series of PE under study are 1.5, 2.5, 3.5, 4.5 and 5.5, respectively. In general, a crystalline (or subgel) to liquid-crystalline phase transition is observed in the first DSC heating scan for all PE dispersions; however, this transition is replaced by the gel to liquid-crystalline phase transition in all subsequent DSC heating scans. The values of T_m and ΔH corresponding to the gel to liquid-crystalline phase transition increase with increasing chain lengths within each series of PE. Moreover, the T_m values of various PE from each homologous series of lipids with a constant value of ΔC are observed to be a linear function of $1/(n_1 + n_2 - 2.1)$, or 1/N, where n_1 and n_2 are the number of carbon atoms in the saturated sn-1 and sn-2 acyl chains, respectively. Based on these linear functions, a general expression of $T_m = 146.2 + 2.73\Delta C - 2388.3\ (1/N) - 143.3\ (\Delta C/N)$ can be derived. This general expression may thus be used to predict the T_m values for all PE with ΔC values in the range of 1.5 to 5.5. Support by NIH grant GM-17452.

W-Pos134

SURFACE BEHAVIOR OF GALACTOSYLCERAMIDES WITH HOMOGENEOUS ACYL CHAINS: EFFECT OF ACYL STRUCTURAL ALTERATIONS.

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Galactosylceramides (GalCer) with homogeneous acyl chains containing none, one, or two double bonds have been synthesized and characterized at the argon/buffered saline interface using a Langmuir film balance. Surface pressure and surface potential were monitored as a function of molecular area at various constant temperatures between 10 and 30°C. In this temperature range, GalCer containing palmitoyl, stearoyl, or lignoceroyl acyl chains form condensed films. Introduction of a single *cis* double bond at the omega-9 position dramatically alters GalCer's behavior if the acyl chain length is between 18 and 24 carbons. With such species, two-dimensional phase transitions of a liquid-expanded (LE)-to-liquid-condensed (LC) nature are observed. The surface pressure and molecular area at which the transitions occur are dependent on temperature and acyl chain length. Introduction of two *cis* double bonds into long chains (≥ 18 carbons) or shortening of saturated chains to ten carbons in GalCer's acyl residue results in liquid-expanded films at all temperatures in the 10 to 30°C range. The results provide an understanding of how GalCer's acyl chain structure regulates its physico-chemical behavior in specialized membranes like myelin where concentrations of this sphingolipid are high. (Supported by USPHS Grant GM-45928 and the Hormel Foundation)

W-Pos135

^{13}C MAS NMR STUDY OF THE INFLUENCE OF A HOMOPOLYMERIC PEPTIDE ON SYNTHETIC LUNG PHOSPHOLIPID.

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Lung surfactant (LS) consists primarily of phospholipids and three hydrophobic proteins. We have used two different ^{13}C magic angle spinning NMR techniques (proton-decoupled MAS with and without cross-polarization) to study an LS model system consisting of polyphenylalanine (average MW = 2,000-5,000) and 1,2-dipalmitoylphosphatidylcholine (DPPC) vesicles. Pure DPPC in the gel phase gave high resolution ^{13}C CP/MAS spectra with resonances for the carbonyl carbon (172.5 ppm), the choline methyl (54.2 ppm) and methylenes (59.8, 66.2 ppm), glycerol C-2 (70.9 ppm), glycerol C-1 and C-3 (59.8 ppm), acyl $(CH_2)_n$ (32.7 ppm), and acyl CH_3 (14.1 ppm). Upon heating through the main phase transition, the carbonyl, all choline carbons and the acyl methyl peaks disappeared in the CP/MAS spectrum, but were clearly present in the ^{13}C MAS spectrum obtained without CP (with the carbonyl peak shifted to 173.6 ppm, the acyl CH_2 shifted to 30.5 ppm). Addition of polyphenylalanine to DPPC (9:1 DPPC:Poly-Phe, w/w) yielded gel-phase CP/MAS spectra with missing carbonyl and glycerol resonances. Upon heating, the ^{13}C MAS spectrum (without CP) showed a broad carbonyl peak (at 173.9 ppm) and a weak resonance at 64.2 ppm (glycerol C-1 and C-3). These data are consistent with the idea that this polypeptide interacts with DPPC mainly in the region of the lipid headgroup.

W-Pos136

STUDIES OF ACYL CHAIN PACKING AND DYNAMICS IN MIXED CHAIN-INTERDIGITATED PHOSPHATIDYLCHOLINE BILAYERS

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The thermotropic phase behavior of unlabeled and ^2H and ^{13}C=O labeled samples of mixed chain phosphatidylcholines, in which the length of one acyl chain was approximately twice that of the other, was studied by DSC, ^2H-NMR and FTIR spectroscopy. The ^2H-NMR order parameter profiles of the long and short chains of these lipids are consistent with a liquid-crystalline bilayer in which there is inter-monolayer contact between the ends of the long chains across the center of the bilayer. In the gel phase the acyl chains adopt a highly ordered packing in which there are long-chain/long-chain and short-chain/long contacts but short-chain/short-chain contacts. Together these data and our DSC results are consistent with the formation of a bilayer in which there is some form of chain interdigitation in both the gel and liquid-crystalline phases. Our results also suggest that there may be two kinetic pathways by which the liquid-crystalline phase freezes to form the interdigitated gel phase. (Supported by operating and major equipment grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)

W-Pos137

MESOMORPHIC AND THERMOTROPIC PROPERTIES OF A 12:0/12:0 PC - 22:0/12:0 PC MIXTURE IN EXCESS WATER

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22:0/12:0 PC is an asymmetry lipid with a pronounced difference in the chain length of the fatty acids at the *sn*-1 and *sn*-2 positions of the glycerol backbone. In excess water at low temperatures this lipid adopts a lamellar gel phase with chains which are untilted but of the mixed interdigitated type (L_{β}^{m},). Heating through the chain order-disorder transition gives rise to the partially interdigitated lamellar liquid crystal ($L_{\alpha}P$,) phase. We were interested in determining how 12:0/12:0 PC, a lipid with a chain length likely to match the chain length difference found in 22:0/12:0 PC, might interact with the latter. To this end, a 22:0/12:0 PC - 12:0/12:0 PC mixture (1:4 mole ratio) was examined by using x-ray diffraction in the heating direction from -7 °C to 25 °C for purposes of phase identification and structure characterization. The tentative phase sequence for the mixture is as follows: ripple - lamellar X (L_X, Hatta et al., Biophys. J. 59:310a,1991) - lamellar gel - lamellar liquid crystal. A more complete structure and thermodynamic characterization of this interesting lipid mixture is in progress.

Supported by NIH DK36849.

W-Pos138

LIPIDAT DATABASE: PHOSPHATIDYLETHANOLAMINE DATA SUBSET ANALYSIS

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LIPIDAT is a computerized database providing access to the wealth of information available in the literature concerning synthetic and biologically derived polar lipid mesomorphic and polymorphic phase behavior. Here we present an analysis of the data subset refering to phosphatidylethanolamines (PE). This class of phospholipids is found in biological membranes and is known to form a variety of mesophases, both lamellar and non-lamellar. The PE subset represents about 13% of all LIPIDAT records. It includes data collected over a 38-year period and consists of 1504 records obtained from 201 articles in 33 different journals. An analysis of the data in the subset has allowed us to identify trends in synthetic PE phase behavior following changes in lipid chain length, chain unsaturation (number, isomeric type and position of double bonds), chain asymmetry and branching, type of chain-glycerol linkage (ether, ester), hydration level, pH, salt concentration, stereochemical purity, etc. Information on the phase behavior of biologically derived PEs is also included. This, somewhat limited analysis provides us with the ability to predict certain features of PE phase behavior and has highlighted deficits in our knowledge of this important lipid class.

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W-Pos139

Equilibrium and Non-Equilibrium Phase Behavior of the Monomyristolein / Water System: An Investigation of the Cubic Phase Undercooling Mechanism

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A temperature-composition phase diagram of monomyristolein in water was constructed using x-ray diffraction. Low- and wide-angle diffraction patterns were collected from samples of fixed hydration as a function of temperature in the heating direction on x-ray sensitive film and/or image plates. The phases identified in the system include a lamellar crystalline phase, L_C, the lamellar liquid crystalline phase, L_{α}, the fluid isotropic phase, FI, and two, most likely inverted, cubic phases, Pn3m (Q^{224}) and Ia3d (Q^{230}). The L_{α} phase exists as a pure phase up to 80 °C with a limiting hydration of approximately 32 % (w/w) water between 0 and 30 °C. In the range of 35 to 40 °C and above 30 % (w/w) water, the L_{α} and Pn3m phases coexist. The hydration boundary of the Pn3m phase lies above 54 % (w/w) water at 45 °C and is near 40 % (w/w) water at 90 °C. Between 28 and 38 % (w/w) water and above 40 °C the Ia3d phase is found.

Cubic phase undercooling was examined by adjusting the temperature of samples in the Ia3d and Pn3m phases to a value where the L_{α} phase represents equilibrium behavior. Cooling-induced structure and phase changes were monitored continuously over a 30 min period by recording low-angle diffraction from the sample using a streak camera. The cubic-to-L_{α} transition was rapid in samples with an overall composition below the hydration boundary of the L_{α} phase. The transition slowed progressively with increasing hydration. The lattice parameter of the undercooled cubic phase is larger than is found when the phase is at equilibrium. [Supported by NIH DK36849 and The Dow Cooperative Research Program, The Dow Chemical Company]

W-Pos140

NEUTRAL LIPID-WATER INTERACTIONS.

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The temperature-composition phase diagram for the monoolein/water system is well established (see for example Hyde et al. 1984. *Z. Krystall.*, 168:1-17). The liquid crystal phases encountered include the lamellar liquid crystalline (L_{α}), the inverted hexagonal (H_{II}) and two cubic phases, Ia3d (Q^{230}) and Pn3m (Q^{224}). We have investigated carefully the hydration properties of the Ia3d phase by using x-ray diffraction. To this end, samples were prepared to a fixed hydration level gravimetrically and by equilibrating the lipid with aqueous polymer solutions of known concentration. Further, the thermal expensivity of a fully hydrated monoolein/alkane mixture in the H_{II} phase has been determined. These data combined provide a measure of the curvature elastic energy function for the cubic phase in hydrated monoolein as follows:

$$E = 2k_c [\langle H^2 \rangle - 2 H_o \langle H \rangle + H_o^2] + k_g \langle K \rangle$$

where k_c is the rigidity curvature constant (= 1.3×10^{-12} erg Å^2), k_g is the Gaussian curvature constant (= 1.5×10^{-13} erg Å^2), H_o is the spontaneous curvature (= 0.0224Å^{-1}), $\langle H^2 \rangle$ is the second moment of the mean curvature, $\langle H \rangle$ is the mean curvature and $\langle K \rangle$ is the Gaussian curvature.

Supported by NIH grant DK36849.

W-Pos141

GEOMETRIC STUDY OF LIPID MONOLAYER ORGANISATIONS IN THE CONDENSED STATE

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Lipids at the air-water interface form a reversible monolayer film in the condensed state when the lateral pressure is sufficient. We model the crystalline structure of these monolayers with rigid cylinders whose orientations depend on the available surface. At the scales of the trough, the domains, and the molecules we evaluate the incidence of this model on the local surface density which we relate to the mean density. We find that the density of the film varies by at least 10% of the real molecular density. This has an incidense on the quantitative predictions of the diverse physical properties of these films. Good agreement is obtained between our theoretical prediction and X-ray diffraction measurements of lipid monolayers on water. Our model is also consistent with the topography of the individual domains imaged by the atomic force microscope.

W-Pos142

CHARACTERIZATION OF DIACYLGLYCEROL CONFORMATIONS IN DETERGENT MICELLES AND BILAYERS.

((Chun Zhou, Karen Lewis, and Mary F. Roberts)) Boston College, Chestnut Hill, MA 02167.

Diacylglycerols have unique roles as intermediates in lipid metabolism and as second messengers. These weakly polar species do not form bilayers by themselves but tend to phase separate. While their solubility in bilayer vesicles has been examined to some extent, there is little information on their behavior in detergent micelles. Understanding their partitioning in the latter is crucial for interpreting kinetics of enzymes which use DAG as a substrate (e.g., DAG kinase) or which generate DAG as a product (e.g., phospholipase C). The solubilization of these lipids (including diC_6-, diC_8-diC_{10}- and diC_{14}-DAG) in a variety of detergent matrices (β-octyl glucoside, Triton X-100, deoxycholate, tetradecyltrimethylammonium bromide, $^2H_{26}$-dioctanoyl-PC) as well as bilayer aggregates has been examined by NMR spectroscopy and interpreted in terms of particle types and detergent/DAG interactions. The DAG chain packing as reflected in the nonequivalence of the α-CH_2 groups is a key parameter in these studies. The nonequivalence of these groups is a function of the detergent matrix as well as the DAG chain length. Limiting particle sizes have been determined by QLS and these structures examined for intermolecular detergent / DAG interactions. Surface accessibility and chain dynamics of the DAG species in different particle geometries has also been examined. The information on DAG solubilization and molecular interactions is used to optimize assay conditions for DAG kinase from *E. coli.* as well as to interpret phospholipase C activity toward a variety of phosphatidylcholines in different aggregates. For the latter, DAG removal from the enzyme appears to be the rate-determining step in catalysis.

W-Pos143

PLASMA MEMBRANE LIPID COMPOSITION AND STRUCTURE: EFFECTS OF LIVER FATTY ACID BINDING PROTEIN EXPRESSION. ((J.K. Woodford, J.R. Jefferson, W.G. Wood, T. Hubbell and F. Schroeder)) University of Cincinnati, Cincinnati, OH 45267. (Spon. by W.J. Ball)

Liver fatty acid binding protein, L-FABP, binds fatty acids and sterols *in vitro*, however, L-FABP *in vivo* function is not certain. The plasma membrane lipid composition, distribution, and physical structure of intact L-cell fibroblasts transfected with cDNA encoding L-FABP were determined. L-FABP expression altered plasma membrane phospholipids by decreasing both phosphatidylethanolamine and esterified oleic acid content (by 13% and 20%, respectively); and increasing sphingomyelin content by 20%. The fluorescent sterol dehydroergosterol was used to examine sterol distribution in the transfected cell plasma membrane. The sterol/phospholipid ratio was decreased to 0.58 in the inner leaflet due to sterol depletion. Concomitantly, the halftimes of intermembrane sterol transfer from both the rapidly and slowly exchangeable lateral sterol domains were increased from 25 to 45 min. and from 177 to 237 min., respectively. The fluidity of the plasma membrane was measured with the fluorescent molecule diphenylhexatriene by multifrequency phase and modulation fluorometry. Diphenylhexatriene anisotropy decreased significantly by 0.013 and 0.046 ($p<0.01$) in the bulk plasma membrane and the plasma membrane outer leaflet, respectively. The lifetime distributional width also displayed a decrease, of 0.102 ($p<0.005$) and 0.298 ($p<0.05$) nsec., within the above two membranes. Thus, both the bulk plasma membrane and the plasma membrane outer leaflet lipids were fluidized in transfected cells. These alterations of plasma membrane structure and composition are consistent with a role for L-FABP in regulating intracellular sterol and fatty acid distribution and thereby membrane lipid domain structure. [Funded in part by USPHS, GM31651]

W-Pos144

FATTY ACID-PHOSPHATIDYLCHOLINE (FA-PC) MISCIBILITY IN LIQUID-EXPANDED MONOLAYERS. ((J. M. Smaby, J. M. Muderhwa and H. L. Brockman)) The Hormel Institute, University of Minnesota, Austin, MN 55912.

Classical analysis of surface pressure-area (π-A) isotherms for monolayers which exist only in the liquid-expanded state strongly suggests, but does not prove, that FA and PC are miscible up to at least 0.67 mol fraction of FA. Miscibility behavior at 1:1 FA:PC is important to assess because FA becomes accessible to lipases in a critical manner as FA mole fraction exceeds that of PC [J.M. Muderhwa and H.L. Brockman, J. Biol. Chem., *in press*]. To assess miscibility, π-A isotherms were determined for FA-PC mixtures in which one of the components showed a liquid-expanded to liquid-condensed phase transition. For each set of mixtures analysis of phase transition data according to the 2-dimensional phase rule indicated miscibility at equimolar FA and PC in the liquid-expanded state. Measurements of FA accessibility to lipases in these films showed the critical behavior about 1:1 FA:PC. Thus, the critical change in substrate accessibility to lipases does not occur as a result of lateral phase separation in the lipid-water interface. [Supported by NIH Grants HL17371, HL49180 and by the Hormel Foundation].

W-Pos145

CRYSTALLINE AND LIQUID CRYSTALLINE CHOLESTERYL ESTERS (CE): ^{13}C MAGIC ANGLE SPINNING NMR STUDIES ((James A. Hamilton and Wen Guo)) Biophysics Department, Boston University School of Medicine, 80 East Concord St., Boston MA 02118.

CE are a major lipid constituent of plasma lipoproteins and atherosclerotic lesions. Crystalline and liquid crystalline phases of several CE [oleate (C18:1, ω-9), eruate (22:1, ω-9), hexanoate (C6:0), decanoate (C10:0), undecanoate (C11:0), myristate (C14:0), and stearate (C18:0)] have been studied by natural abundance ^{13}C magic angle spinning (MAS) NMR at 75 MHz (7.05 T). Solid state spectra obtained with MAS, high power proton decoupling, and cross polarization (CP) were highly resolved for crystalline CE. Acyl chain carbons had narrower lines than protonated steroid ring carbons, reflecting differential motions in the crystal. Esters which crystallize into the monolayer type II structure, in which all molecules are equivalent, gave rise to a single resonance for each carbon; esters of the monolayer type I and bilayer structures, in which there are two nonequivalent molecules in the unit cell, had two resonances (equal intensity and linewidth) for several carbons, such as the carbonyl and the steroid ring C5 and C6. Spectra for liquid crystalline phases did not show inequivalency of signals for the same carbon and were not enhanced by CP. These changes are a result of increased molecular motions in the liquid crystals, which average the nonequivalent environments and reduce dipolar interactions. Liquid crystalline phases of cholesteryl oleate and myristate were distinguished by the broader C=0, C5 and C6 signals for the cholesteric compared with the smectic phase. ^{13}C MAS NMR is a powerful approach for examining structure and motions of crystalline and liquid-crystalline CE.

SPECTROSCOPIC STUDIES (OTHER)

W-Pos146

SIMULTANEOUS ACQUISITION AND GLOBAL ANALYSIS OF MULTIPLE SPECTROSCOPIC PARAMETERS TO MONITOR THE THERMAL UNFOLDING OF PROTEINS. ((G. Ramsay and M.R. Eftink)) Univ. of Mississippi, University, MS 38677

An accepted means to test the validity of the "two-state" model for protein unfolding is to experimentally observe the transition by two or more methods and to compare the resulting thermodynamic parameters. Unfortunately, such a comparison can be questionable if identical solution conditions (pH, concentration, temperature, etc) are not employed. Our approach to this problem has been to modify a commercial spectropolarimeter to enable the simultaneous measurement of CD, transmission, and fluorescence emission. Such measurements can be made at several excitation wavelengths on a single sample during a thermal scan. This reduces the problem of sample variation and slight differences in temperature measurements, which occur if two or more separate instruments are used. Changes in the tryptophan environment, measured by its aromatic CD, and/or steady-state fluorescence, and changes in the secondary structure, measured in the far UV CD, are observed. Temperature is controlled by a thermoelectric cell holder, with measurement of temperature and feedback control via a thermistor located inside the cuvette. The resulting set of melting profiles are first analyzed individually, to check that they are consistent with one another and that they are consistent with a two-state model. Then the group of data sets is analyzed globally in terms of $\Delta H°$, $\Delta S°$, and ΔC_p of unfolding (and locally in terms of the profiles' individual baseline slopes and intercepts) via non-linear least squares. The standard deviations of the individual data points are used as weighting factors during the analysis. Such a global analysis allows us to more accurately determine thermodynamic parameters and to test the validity of selected unfolding models. Examples of this approach will be presented for the proteins nuclease A and ribonuclease T_1 and selected mutants. This research was supported by NSF grant DMB 91-06877.

W-Pos147

RESONANCE RAMAN STUDIES OF BETA-SUBSTITUTED METALLO TETRAPHENYLPORPHYRINS AND THEIR CATION RADICALS

((Sharon A. Sibilia[1], Roman Czernuszewicz[2] and Thomas G. Spiro[1]))
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[2]Department of Chemistry, University of Houston, Houston, TX

Metalloporphyrins are important redox agents in biological systems. Substituents to the aromatic ring of these chromophores are known to perturb the two closely spaced highest occupied molecular orbital energy levels. We have investigated the effect of substitution by a series of electron-withdrawing to electron-donating groups on one beta-pyrrole position of copper(II) 5,10,15,20-tetraphenylporphyrin and vanadium(IV)-oxo 5,10,15,20-tetraphenylporphyrin and their cation radicals. We present the Soret-enhanced resonance Raman spectra of the neutral and cationic species of these porphyrins which indicate the symmetry of the highest occupied molecular orbital via shifts in the vibrations identified as marker bands.

W-Pos148

INFRARED STUDIES ON THE INTERACTION OF CARBON MONOXIDE WITH NICKEL IN HYDROGENASE. ((K. A. Bagley, C. Van Garderen, S. P. J. Albracht, and W. H. Woodruff)) INC-14, Los Alamos Nat'l. Lab., Los Alamos, NM 87545 and The E.C. Slater Institute for Biochemical Research, University of Amsterdam, The Netherlands.

The enzyme hydrogenase catalyzes the reversible oxidation of H_2 to H^+. We have used infrared spectroscopy to study the binding of carbon monoxide, an inhibitor to the enzyme's activity, to the nickel containing hydrogenase from *Chromatium vinosum*. We present infrared spectra attributable to an EPR silent Ni(II)-CO species in the enzyme. In these IR spectra we detect an IR band at 2060 cm^{-1} arising from the ν(CO) of a Ni(II)-CO species that is photolabile. Additionally, we detect bands at 2082 cm^{-1}, 2069 cm^{-1}, and 1929 cm^{-1} which shift in frequency upon CO photolysis but which do not arise from ν(CO) based on the fact that their frequency is unaffected by exposure of the enzyme to ^{13}CO. The nature of these non-CO bands will be discussed with regard to the possibility that they arise from one or more of the following: ν(S-H)'s from metal coordinated sulfhydryl species, ν(M-H)'s from metal hydrides, or vibrational frequencies associated with coordinated dihydrogen.

W-Pos149

CONFORMATIONAL CHANGES IN TRANSFERRIN FOLLOWING IRON BINDING AS DETECTED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY. ((J.J. Lorio, F.R. Dellacroce, Y.C. Ho, J.A. Watkins, and J. Glass)) Hematology/Oncology Section, Center of Excellence for Cancer Research, Treatment, and Education, LSUMC-Shreveport, LA.

Transferrin (Tf) delivers iron (Fe) to cells via an endocytic process whereby Fe dissociation from Tf is promoted by binding of Tf to its receptor (R_{Tf}). In order to study structural changes in solution, the Fourier transform infrared spectrum in the 1000 to 2000 cm^{-1} region was investigated and analyzed using Fourier self-deconvolution (FSD), second derivative (SD), and Gaussian-Lorentzian curve fitting methods. In the amide I region (dominant C-O peptide stretch) of the vibrational spectrum, significant changes in the α-helix, β-sheet, β-bend, and non-ordered coil bands of the spectrum are apparent. Preliminary analysis suggest larger changes in the secondary structure for the molecule in solution than observed in the crystal structure. The amide II region shows large changes in the global dynamics as well as polypeptide backbone and side chain residue coupled vibrations upon iron binding. In agreement with Raman spectral studies, we have observed transitions in the amide III region which can be tentatively assigned to the Fe-ligand vibrations in the 1225-1325 cm^{-1} region. The proposed Fe-ligand transitions show broad bandwidth doublets at 1283 and 1261 cm^{-1} in the raw spectrum and quadruplets or pentuplets upon FSD or SD analysis. The results indicate large changes in dynamic coupling of the entire molecule as well as the vibrations coupled to the Fe binding sites. The resolution of vibrational modes for the Fe-ligand structures provides an avenue for investigating the dynamic conformers of Tf and their interconversions resulting in the negative cooperativity between the N and C lobes of the molecule.

W-Pos150

VIBRATIONAL SPECTROSCOPY OF KERATIN FIBERS.

((C. Pande* and B. Lerner)) **Clairol Research Laboratories, Stamford, CT. and Nicolet Instruments, Madison, Wi.*

Human hair is composed of keratin proteins which are characterized by their high disulfide content. We are developing spectroscopic techniques to probe the structure of keratin fibers, *in situ*. We have previously shown that tryptophan in hair can be conveniently monitored using the fluorescence technique[1]. FTIR microspectrometry has been used to measure cysteic acid, an oxidation product of the disulfide bonds, and to obtain the amide vibrations due to the crystalline phase, using the polarization technique[2]. Using FT-Raman spectroscopy, we now show that rich vibrational spectra can be obtained from keratin fibers. These spectra show a strong S-S vibrational band at ~510 cm^{-1}. The large band-width (ca. 40 cm^{-1}) indicates heterogeniety of the disulfide environment. Since the disulfide bonds are believed to be intimately associated with the integrity of keratin fibers, this technique provides a convenient means to determine the role of these crosslinks during chemical degradation, or weathering, of these fibers. The characteristic tyrosine doublet at 850/ 830 cm^{-1}, and the 1000 cm^{-1} band due to phenylalanine, are also clearly discernible in these spectra. Other spectral details, and comparisons with FTIR data, will also be presented.

1) Pande, C. *Biophys. J.* 59, 40a, 1991.; 2) Pande, C. and Hull, J. *Biophys. J.* 61, A479, 1992.

W-Pos151

SEPARATION BY FACTOR ANALYSIS OF THE SPECTRA OF THE AGGREGATED SPECIES OF AMPHOTERICIN B IN AQUEOUS SOLUTIONS

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The usefulness of amphotericin B (AmB) as an antibiotic to treat mycotic infections is limited by its toxicity due the formation of aggregates. To evaluate the abundance of each AmB species in aqueous solutions, the absorption spectra were obtained from a fixed amount of AmB dissolved in several aqueous solutions of propanol and lauryl sucrose (LS). The spectra of AmB obtained in these two media are complex due to the absorption of several species that absorb in a limited spectral range. A method based on factor analysis is used to separate the spectra of the individual species present in the experimental spectra. With this method five species of AmB were identified in each of the two media: two monomers and three aggregates. Two of the aggregates are toxic. The abundance of each species varies with the concentration of the amphiphillic molecules. The correlation between spectroscopy/data-treatment and activity/toxicity results will be made in order to asses the usefulness of the method.

W-Pos152

SYNTHESIS AND PRELIMINARY CHARACTERIZATION OF PHOTOLABILE CAGED COMPOUNDS FOR TIME-RESOLVED, PULSED-LASER PHOTOACOUSTICS. ((Jeanne Rudzki Small and Ernest C. McGoran)) Eastern Washington University, Department of Chemistry & Biochemistry, Mail Stop 74, Cheney, WA 99004-2499 (jsmall@ewuvms.bitnet).

Photolabile caged compounds are biologically inert molecules which, after absorbing a photon, decompose to release a biologically active species, as well as the former caging group. Caged compounds have been used in the field of "light-flash physiology" for many years [e.g., A.M. Gurney and H.A. Lester, *Physiol. Revs.* 67, 583-617 (1987)] and may hold promise for the technique of time-resolved, pulsed-laser photoacoustics. The photoacoustic technique relies on a flash of laser light to trigger a photoinitiated reaction which may involve both heat release and conformational changes of molecules. The photoacoustic measurements are performed on the ns and μs timescales. We are synthesizing and characterizing a number of photolabile caged compounds, including forms of caged acetate, caged proton, caged phosphate, and caged hydroxide. Our preliminary data will include summaries of synthetic methods, product solubilities and photolysis quantum yields, and photoacoustic measurements. (Supported by NIH grant GM-41415.)

W-Pos153

TIME-RESOLVED OPTICAL SPECTROSCOPIC STUDIES OF DYNAMICS OF TRIPLET STATES OF METALLOPORPHYRINS

((Doug H. Kreszowski and G.T. Babcock)) Department of Chemistry, Michigan State University, MI 48824.

Charge separation in photosynthetic reaction centers involves generation of a photochemically active excited state chlorophyll and subsequent electron transfer to produce anion and cation product species. To gain insight into the detailed mechanisms by which excited porphyrin-based macrocycles perform their functions, knowledge of conformation within the electronic structure of the relevant excited state is needed. Pump/probe time-resolved Raman techniques were previously applied to characterize Jahn-Teller distortion effects in the lowest lying triplet excited state of Zn(II) tetraphenylporphyrin (Walters, V.A.,Depaula, J.C.,Babcock, G.T., Leroi, G.E., JACS JACS 111,8300). Transient electronic states of free base and metalloporphyrins are presented and the dynamics of these states are compared with ZnTPP. D_{4h} point group porphyrins (ZnTPP and ZnOEP) observe large scale vibrational shifts consistent with lowering of point group symmetry and are attributed to the Jahn-Teller effect. Free base porphyrins with D_{2h} symmetry observe lesser vibrational shifts which are attributed to changes in electron occupancy of porphyrin molecular orbitals.

W-Pos154

CONFORMATIONAL SUBSTATES IN BLUE COPPER PROTEINS. ((D. Ehrenstein, M. Engelhard†, M. Filiaci, H. Frauenfelder, G.U. Nienhaus, and B. Scharf†)) Physics Dept., Univ. of Illinois, Urbana, IL 61801 and †Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm 201, 4600 Dortmund, FRG.

Azurin and halocyanin are small, blue copper proteins involved in bacterial electron transfer which form photodissociable complexes with nitric oxide (NO) at low temperatures. We have exploited this property to test the generality of protein dynamics principles learned from photodissociation experiments on heme protein systems. We studied the temperature dependence of the ligand binding equilibria and the kinetics of the association reaction after photodissociation over a wide range of temperature (80 – 280 K) and time ($10^{-6} - 10^{2}$ s). We found an exponential, bimolecular rebinding process at high temperatures and a non-exponential, geminate process at lower temperatures, analogous to the heme protein data. We model the low-temperature rebinding using a Gaussian distribution of enthalpy barriers based on our successful model for heme protein rebinding. We conclude that the understanding of dynamics and conformational substates developed through heme protein experiments is also applicable to proteins with different active sites and secondary structures. (Supported by NSF.)

MECHANISMS OF GENE REGULATION AND TRANSCRIPTION

W-Pos155

LOCAL MELTING OF DNA BASE PAIRS BY DNA POLYMERASE. ((D.P. Millar, R.A. Hochstrasser, T.E. Carver and L.C. Sowers*)) Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037 and *Division of Pediatrics, City of Hope National Medical Center, Duarte, CA 91010.

Formation of a mismatched base pair by DNA polymerase stalls further elongation of the primer strand and leads to proofreading by a 3'-5' exonuclease. Mismatch recognition by DNA polymerase may involve preferential local melting of mismatched base pairs. We have examined local melting of 3' terminal base pairs by the Klenow fragment of Pol I using a novel fluorescence decay method. Oligonucleotide primer/templates were synthesized with the fluorescent nucleotide analog 2-aminopurine (AP) located at the primer 3' terminus. The fluorescence decay of AP was highly sensitive to base pairing interactions and the fraction of paired AP bases could be determined under a variety of conditions. The stabilities of AP·X base pairs in solution and bound to Klenow fragment were compared, where X = T, C, G and A. The polymerase amplified differences in base pair stability in this series and extensively melted the least stable pairs. The differences in base pair stability were also reflected in the local mobility of AP within the polymerase, as detected by fluorescence anisotropy decay. Local rotation of AP was very rapid in the highly disrupted base pairs. Two exonuclease-deficient mutant Klenow fragments were compared (D424A and D355A, E357A). These proteins did not behave identically, which may reflect different partitioning of DNA into the separate polymerase and exonuclease domains.

Supported by NIH Grant GM44060.

W-Pos156

EVIDENCE FOR TWO OPEN COMPLEXES (RP_{o1}, RP_{o2}) AT THE λP_R PROMOTER *IN VITRO*: STRUCTURAL CHARACTERIZATION OF RP_{o1} & RP_{o2}. W.-C. Suh, S. Leirmo, and M. T. Record, Jr. Department of Biochemistry and Chemistry, University of Wisconsin, Madison, WI. 53706.

In vitro structural studies on the steps of the process of DNA strand opening at the λP_R promoter, using potassium permanganate, DNase I, and dimethyl sulfate footprinting, demonstrate the existence of the intermediate open complex RP_{o1} between the closed complex RP_{c2} and the transcriptionally-competent open complex RP_{o2}. Strand opening in RP_{o1} (at most 12 base pairs) is less complete at the start point of transcription (+1, +2) than in RP_{o2} (-14 base pairs). The conversion of RP_{o1} to RP_{o2} is strongly dependent on the presence of Mg^{2+}, indicating that uptake of Mg^{2+} is required for the conformational transition of RP_{o1} to form RP_{o2}. Kinetic studies on the λP_R promoter provide further evidence for the existence of at least two mechanistically-significant forms of open complexes. According to the mechanism (1) deduced from kinetic studies, RP_{o1} is converted to RP_{o2} upon uptake of approximately 3 Mg^{2+}, presumably at specific sites on RNA polymerase.

$$R + P \rightleftharpoons RP_{c1} \rightleftharpoons RP_{c2} \rightleftharpoons RP_{o1} \overset{3Mg^{2+}}{\rightleftharpoons} RP_{o2} \qquad (1)$$

W-Pos157

NON-SYMMETRIC CONTACTS BETWEEN LAC REPRESSOR AND OPERATOR REVEALED BY ^{19}F-NMR ((F. Rastinejad, P. Artz, and P. Lu)) Depts. of Chemistry, and Biochemistry & Biophyics, University of Pennsylvania, Philadelphia, PA 19104

The *E. coli lac* operator DNA contains two sequence repeats related by a pseudo-dyad. Deviations from symmetry occur at two pairs of symmetry related sites (+15/+7, +13/+9) and at the center (+11). We used ^{19}F-NMR to examine if the operator specifies distinct half-site interactions with the *lac* headpiece. To observe protein interactions simultaneously at operator half-sites using ^{19}F-NMR, three pairs of symmetric thymines (at +6/+16, +8/+14, and +1/+21) were replaced pair-wise by 5-fluorodeoxyuridines (5-FdU). Two repressor headpiece polypeptides (residues 1-56, and 1-64) were employed to remove steric constraints of protein-protein contacts. Spectral changes associated with headpiece binding to left half-site DNA differ from those caused by binding to equivalent sequences on the right side, suggesting non-equivalent protein-DNA interactions. Three mutant *lac* operator sequences with improved symmetry, bearing 5-FdU substitutions, were used to identify the relative importance of the three asymmetric positions in the operator DNA. Our results indicate that key interactions with the left side +9 (G•C) are altered at the symmetrically related right side +13 (A•T) accounting for the non-equivalent *lac* repressor-operator interactions as far as 3 bp away. ^{19}F-{^{1}H}-heteronuclear Overhauser spectroscopy (HOESY) is used to identify intermolecular contacts.

W-Pos158

INTERACTION OF MISMATCHED DNA WITH KLENOW FRAGMENT. ((T.E. Carver, R.A. Hochstrasser and D.P. Millar)) Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037 (Spon. by P. Eis)

Klenow fragment of DNA Pol I contains a 3' to 5' exonuclease activity that enhances the fidelity of DNA synthesis by excising mismatched bases from the nascent primer strand. This activity resides in a separate domain of the protein, roughly 30 Å from the polymerase active site. In order for proofreading to occur, a mechanism must exist that recognizes mispaired bases and preferentially removes them from DNA. This mechanism could involve selective partitioning of mismatched primer strands into the exonuclease domain of the protein following melting of several terminal base pairs.

Using time-resolved fluorescence spectroscopy of dansyl-labeled DNA, we have examined directly the binding of DNA to the different domains of Klenow fragment. We measured the effects of divalent cations, single and multiple mismatches, and different mutations in the protein upon the extent of partitioning of DNA into the exonuclease domain. Several important results were obtained. First, single 3' terminal mismatches promote the partitioning of DNA into the exonuclease domain. Multiple consecutive mismatches have a dramatic effect, causing most of the DNA to bind the exonuclease domain of the protein. The presence or absence of divalent cations also influences binding of DNA to the exonuclease domain, but binding is not abolished in their absence. These effects will be discussed for the D424A mutant and the D355A, E357A mutant of Klenow fragment.

Supported by NIH Grant GM44060.

W-Pos159
RELATIVE TRANSACTIVATION EFFICIENCIES OF RAR AND RXR CHIMERIC RECEPTORS CONTAINING THE HORMONE BINDING DOMAIN OF THE VITAMIN D RECEPTOR

((S.M. Pemrick, L.J. Sturzenbecker, Cl. Kratzeisen, P. Arbuzua, M.S. Marks*, J.A. Medin*, K. Ozato*, A.A. Levin, W. Hunziker+, and J.F. Grippo)) Dept. Invest. Toxicol., Nutley, NJ and Pharma Research+, Basel, Switzerland, Hoffmann-La Roche Inc. and Lab. of Cell Biol., Nat'l Cancer Inst., NIH, Bethesda, MD*.

The Vitamin D receptor (VDR) and the chimeras, RAR/VDR and RXR/VDR bound [^{3}H]1,25 dihydroxy Vitamin D3 (D3) with high affinity. In CV-1 cells, RAR/VDR mediated transcriptional activity of a RARE (β-RARE3)-thymidine kinase(tk)-luciferase reporter gene but not that of a RXRE (ApoAI)-tk-luciferase reporter gene, after treatment with D3. Unliganded, RAR or RAR/VDR, inhibited transcriptional activity of the RARE mediated by liganded RAR/VDR or liganded endogenous RAR receptors, respectively. RXR/VDR was ineffective, compared to RXR, in mediating transcriptional activity of the RXRE reporter gene. However, RXR/VDR could inhibit RAR/VDR mediated transcriptional activity from the βRARE. Two additional receptors, both containing the DNA-binding domain of RAR and the ligand binding domain (LBD) of the estrogen receptor (ER) failed to mediate transcriptional activity of the RARE, although both ER chimeras were capable of binding [^{3}H] estradiol. Co-precipitation assays with RXR and gel retardation assays showed that both RAR and RAR/VDR but not the RAR/ER chimeras formed heterodimers with RXR. These results indicate that formation of a functional RAR or RXR chimeric receptor requires a LBD capable of interacting appropriately with RXR.

PROTEIN SYNTHESIS

W-Pos160
A FLUORESCENCE STUDY OF THE INTERACTION OF WHEAT GERM INITIATION FACTOR 4B WITH OLIGORIBONUCLEOTIDE ANALOGUES OF mRNA

((Sha Ma, M.Luisa Balasta, and Dixie J. Goss)) Chemistry Department, Hunter College of CUNY, New York, NY 10021.

The binding of oligoribonucleotide analogues of the 5'terminus of mRNA to wheat germ protein synthesis initiation factor eIF-4B was measured by direct fluorescence techniques. An analysis of the equilibrium association constants (Keq) indicates that the eIF-4B binds neither to m'GTP cap structure nor to AUG. eIF-4B is insensitive to the hairpin structures within the oligoribonuclectide. The binding site size is 14 bases. The binding of oligoribonucleotide to eIF-4B as a function of pH and ionic strength is also described. The pH-dependent binding showed an increase in binding with increasing pH in contrast to the sharp pH optimum observed for cap binding protein eIF-4E. Iodine quenching showed that the tryptophan residues are on the surface of the eIF-4B protein. Grant support: NSF, AHA, PSC-CUNY Faculty Award (D.J.G).

W-Pos161
FLUORESCENCE SPECTROSCOPIC STUDIES OF THE INTERACTIONS OF WHEAT GERM EUKARYOTIC INITIATION FACTORS eIF-4F, eIF-4B, AND eIF-4A WITH RNA.

((M. L. Balasta, D. E. Friedland, M. Sha, and D. J. Goss)) Chemistry Department, Hunter College of CUNY, New York, NY 10021.

The study of the interactions of wheat germ protein synthesis initiation factors with RNA is important in gaining a better understanding of the process of translation. The binding of eIF-4F, eIF-4B and eIF-4A to oligoribonucleotides was investigated using direct fluorescence titration techniques. Some of these interactions are known to be ATP-dependent and to cause ATP hydrolysis associated with the helicase activity of these factors. The results indicate that the presence of ATP enhances the formation of the eIF-4F:eIF-4A binary complex, but not that of the eIF-4F:eIF-4B complex nor of the protein:RNA complexes. Protein:protein:RNA ternary complex formation was also studied in the presence of ATP in order to elucidate mechanisms of binding of the initiation factors to RNA. Grant support: NSF, AHA, PSC-CUNY Faculty Award (D.J.G.), and Eugene Lang Student Fellowship (D.E.F.).

W-Pos162
CHARACTERIZATION OF PROTEIN SYNTHESIS IN ISOLATED MITOCHONDRIA FROM *ARTEMIA* EMBRYOS: EVIDENCE FOR REGULATION BY EXTRAMITOCHONDRIAL pH

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Optimal conditions were developed for measuring rates of protein synthesis in isolated mitochondria from encysted *Artemia franciscana* embryos in order to 1) identify the required chemical constituents, and 2) assess the influence of extramitochondrial pH on protein synthesis. Isolation procedures resulted in intact, highly coupled mitochondria (RCR = 5.8 ± 0.6; n = 15). Chemical requirements for optimal protein synthesis, measured as incorporation of ^{3}H-leucine, included an oxidizable carbon source (10 mM succinate), exogenously supplied adenine nucleotides (guanine nucleotides had no effect), phosphate (10 mM), K^+ (125 mM), Mg^{2+} (10 mM), amino acids (0.3 mM for each), 60 uM ^{3}H-leucine, sucrose or trehalose (500 mM), EGTA (1 mM), and 1 mg/ml BSA. Incorporation was linear for 60 min at 25°C. Fluorography of translated products revealed 8 major proteins. Previous research has shown that anoxia-induced acidification of intracellular pH (pH_i) results in suppression of protein biosynthesis, as judged by cytochrome c oxidase (COX) synthesis. Since the COX holoenzyme consists of both mitochondrial and nuclear encoded subunits, suppression of COX synthesis could be due to an arrest of either nuclear or mitochondrial subunit expression, or both. In the present study, mitochondrial protein synthesis was acutely sensitive to external pH, with 86% inhibition observed by lowering pH from 7.5 to 6.8 (the observed pH_i under anoxia). Thus acidification of pH_i may contribute to the suppression of both cytoplasmic and mitochondrial protein synthesis during quiescence in *Artemia*. [Supported by NSF grant DCB-9018579]

W-Pos163

THE DISTINGUISHING CHARACTERISTICS BETWEEN A- AND B-DNA HELICES. ((Jeffrey L. Nauss[1] and Wilma K. Olson[2]) [1] Walter Reed Army Institute of Research, Department of Gastroenterology, Washington, DC, 20307 and [2] Department of Chemistry, Rutgers - The State University of New Jersey, Piscataway, NJ 08855

To better understand the distinctions between A- and B-type polynucleotide helices at the base-base level, a series of regular repeating polynucleotide models were formed. The model structures were built with systematic variations of six of twelve local rotational and translational parameters. The resulting model helices were classified as either A, A*, B, B*, or AB (intermediate) helices based upon their helical parameters. The selection criteria for A- and B-type helical parameters were determined from representative examples of X-ray crystal structures. Variations in the local rotational parameters twist and roll and in the translational parameter slide were shown to have the greatest affect on the helical parameters and, as a consequence, the helical type.

W-Pos164

CORRELATION OF PSEUDOROTATION, PUCKER AMPLITUDE AND THE N-GLYCOSIDIC TORSION IN 2'-DEOXYNUCLEOSIDES. **((H.A. Gabb and S.C. Harvey)) Department of Biochemistry, University of Alabama at Birmingham, Schools of Medicine and Dentistry, Birmingham, AL 35294-0005.**

A comprehensive molecular modeling study of the four commonly occurring 2'-deoxynucleosides (dN) was carried out to determine whether pseudorotation phase angle (P), the N-glycosidic torsion (χ) and pucker amplitude (υ_m) are energetically coupled. To this end, the AMBER all-atom force field (Weiner *et al., J. Comp. Chem.* 7:230, 1986) was rigorously parameterized for ribose and 2'-deoxyribose to best fit existing data with both energy minimization (EM) and molecular dynamics (MD). Twenty 300-K, 1-ns *in vacuo* MD simulations were carried out for each dN to sample thermodynamically accessible regions of conformational space. This data was then used to construct free energy (potential of mean force) surfaces of $E(P, \chi)$. Fluctuations in υ_m were also examined. Adiabatic mapping was used to generate potential energy surfaces of $E(P, \chi)$ at various values of υ_m. We also used two newer methods to examine the major conformational transition in dN's. Specifically, we used the Ulitsky and Elber algorithm (*J. Chem. Phys.* 92:1510, 1990) and the CONTRA MD algorithm developed in this laboratory to determine the preferred pathway, both statically and dynamically, for the C2'-endo/*anti* to C3'-endo/*syn* transition. Our results suggest that P and χ are not energetically coupled. However, P and υ_m are dependent to a degree previously unrecognized.

W-Pos165

THE BASES ARE INCLINED FROM PERPENDICULAR FOR NATURAL B-FORM DNA IN SOLUTION. ((Ping-Jung Chou and W. Curtis Johnson, Jr.)) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305

A sophisticated algorithm is developed to analyze flow linear dichroism data on nucleic acids for individual base inclinations. Measured absorption and linear dichroism data for synthetic AT and GC polymers, and natural DNAs are analyzed. For the first time, specific base inclinations are derived for all four bases individually. For B-form DNA in dilute aqueous solution the inclinations from perpendicular are 16, 25, 18 and 25 degrees for d(A), d(T), d(G) and d(C), respectively. Our results indicate that the bases in synthetic and natural DNAs are not perpendicular to the helix axis, not even in the B-form.

W-Pos166

UV AND IR FLOW LINEAR DICHROISM DEMONSTRATE THAT THE BASES ARE SIGNIFICANTLY INCLINED IN POLYNUCLEOTIDES. ((Hunseung Kang and W. Curtis Johnson, Jr.)) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305 USA

Vacuum ultraviolet (UV) and infrared (IR) flow linear dichroism measurements have been performed on synthetic polynucleotides and natural DNA to probe their conformation in aqueous solution. UV linear dichroism demonstrates that the bases in poly[d(AC)]·poly[d(GT)] and poly[d(AG)]·poly[d(CT)] in the B-form have a sizable inclination. Inclination is increased in the A-form, as expected. In all cases the pyrimidines are more inclined than the purines. These angles of inclination estimated for A- and B-form are larger than those reported for fiber diffraction data. Similar information about base inclination has been obtained by using IR flow linear dichroism measurements. IR flow linear dichroism has also been used to extract structural information about phosphate backbone geometry on these polynucleotides.

W-Pos167

INFRARED CIRCULAR DICHROISM OF POLYNUCLEOTIDES. ((Ting Xiang, Miriam Gulotta, Max Diem, and Dixie J. Goss)) Chemistry Department, Hunter College of CUNY, New York, NY 10021.

Infrared Circular Dichroism (VCD) has been observed for poly(rC)·poly(rG), poly(rC), poly(rG). Using DECO (Degenerate Extended Coupled Oscillator) model, we have simulated the experimental spectrum of single stranded poly(rC). Poly(rG) can be simulated with the assumption that the spectrum results from the combination of single stranded and tetra stranded oligoribonucleotides. New calculational models, NECO (Nondegenerate Extended Coupled Oscillator) and PSS (Pseudo-single stranded ECNO) have been explored to deal with poly(rC)·poly(rG) which show contributions from nondegenerate transitions. The comparison of the simulation with these three models will be presented. We have also measured the VCD for poly(rA), poly(rU)·poly(rA), and poly(rU). The poly(rU) did not show any secondary structure at the experimental conditions. We can interpret the IR and VCD signal for poly(rA) and poly(rU)·poly(rA) in terms of the DECO model and modified oscillator geometry. Grant support: NSF, AHA, PSC-CUNY Faculty Award (D.J.G.), and NIH (M.D.).

W-Pos168

VIBRATIONAL CIRCULAR DICHROISM OF NUCLEIC ACIDS I. A-, B-, & Z-FORM SPECTRA IN THE PO_2^- STRETCHING REGION. II. POLY(DI-DC)•POLY(DI-DC) ((Lijiang Wang & Timothy A. Keiderling)) Department of Chemistry, University of Illinois at Chicago, Box 4348, Chicago, IL 60680 USA

VCD of several natural and model DNAs (single & double stranded, oligomers & polymers) in the B-form, poly(dG-dC)•poly(dG-dC) in the Z-form, and RNA in the A-form in H_2O were measured in the PO_2^- stretching region. Changes of the VCD spectra in the PO_2^- stretching vibration are consistent with the base region spectral differences for these three conformational types. The Z-form spectrum is characterized by an inversion of the VCD with respect to the B-form spectrum which is consistent with the change in helical sense for the nucleic acid backbone. A-RNAs, being right-handed, show same sense of couplet VCD spectra as do B-DNAs. This spectral change can be successfully modelled using dipole coupling calculations. It is, therefore, both empirically and theoretically verified that VCD is a reliable indicator of the helical structure of nucleic acids. As an application, VCD spectra of Poly(dI-dC)•poly(dI-dC) in low salt conditions were measured in both base and PO_2^- stretching regions. The positive couplet bandshapes are consistent with those of A- and B- form DNAs, which indicates that poly(dI-dC) is definitely in a right-handed helical conformation. Thermal denaturation of this duplex was studied by comparing loss of base stacking interaction with loss of phosphate coupling as monitored by the VCD intensity. A cooperative collapse of both signals occurs at about 65°C. The conformational properties of poly(dI-dC)•poly(dI-dC) at high salt concentration have also been studied via VCD spectra.

W-Pos169

The Effect of Sequence-Directed Bending on the Global Equilibrium Configurations of DNA

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A newly developed finite element formalism [1] is used to obtain the three-dimensional equilibrium configurations and associated elastic free energies under various stressed states of a long (~ 6400 bp) intrinsically straight DNA containing a few short (~ 200 bp) highly bent segments. The DNA is modeled as an isotropic elastic rod with large intrinsic curvature within the bent stretches. We examine how this sequence-directed bending affects the global equilibrium configurations of the closed polymer chain when it is bent and twisted. Our numerical results show that both the magnitude of the intrinsic curvature and the positions of the curved segments have significant effects on the topology of the highly stressed DNA. The intrinsically curved segments can preferentially orient the knotting and supercoiling of the DNA.

[1] Yang, Y., Tobias , I. & Olson, W. K., "Finite Element Analysis of DNA Supercoiling," (1992) *J. Chem. Phys.*, in press.

W-Pos170

DNA TWISTING ESTIMATED BY THE ELECTROPHORETIC MOBILITY AT DIFFERENT TEMPERATURES.

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To analyze the temperature dependence of DNA twisting and curvature, a series of 21-base oligonucleotides containing An:Tn blocks was synthesized: A5-X5-A6-X5, where X5 are C5, G5, T5, CTCTC, GAGAG, TATAT and TCTCT. These 21-bp precursors were ligated and the electrophoretic mobility of their multimers in polyacrylamide gel was measured over the temperature range from 5° to 55°C. The temperature dependence of R-factor characterizing anomalous mobility of DNA is found to be extremely sequence dependent. For X5= GAGAG, G5 and C5, the R-factor is maximal at T= 5°C; for CTCTC and TCTCT max(R) corresponds to 15-20°C, and for TATAT and T5 the maximum is reached at 35° and 55°C respectively. Thus, our data refute the common belief that DNA curvature monotonically decreases with an increase in temperature. We interprete the non-monotonic R(T) dependence by a transition in the DNA shape from a right-handed superhelix to the planar loop (maximum R-factor), and then to a left-handed superhelix. In its turn, this macroscopic transition is a consequence of the decrease in the local DNA twisting upon heating. Based on the above interpretation, we estimate the DNA twisting angle Ω in seven dimeric steps: $\Omega(AG)+\Omega(GA)<\Omega(AC)+\Omega(CA)<\Omega(AT)+\Omega(TA)<2\Omega(AA)$. The electrophoresis data are compared with Monte Carlo simulations of DNA bending and twisting. The results are important for developing quantitative theory of the polyacrylamide gel electrophoresis of DNA.

W-Pos171

NMR INVESTIGATION OF THE STRUCTURE OF DNA OLIGONUCLEOTIDES CONTAINING A_nT_n TRACTS.

((R. Michalczyk and I. M. Russu)), Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459-0175

Previous work from this and other laboratories has shown that A_nT_n tracts possess unique properties, such as DNA bending, specific patterns of hydroxyl radical cleavage and anomalously long base-pair lifetimes. In order to understand the structural basis of these properties we have undertaken an NMR investigation of the following self-complementary DNA oligomers containing A_nT_n tracts of various length: 5'-d(CGCGAATTCGCG)-3', 5'-d(CGCAAATTTGCG)-3' and 5'-d(CGCAAAATTTTGCG)-3'. Phase-sensitive NOESY spectra in H_2O were collected for each oligonucleotide as a function of mixing time in the range from 30 to 150 ms at 5°C. The buffer conditions were chosen such that the exchange of imino and amino protons with water protons did not contribute significantly to the relaxation of these protons. Build-up curves of the NOESY cross-peaks between imino and amino protons indicate the existence of a significant conformational variability along the DNA helix. Unique conformational features are observed, for example, between the A·T base-pair at the 5'-end of the tract and the base-pair situated at the 3'-end of the flanking sequence. Differences in structure between the tract and the flanking sequence were also observed. The results for the three oligomers studied will be compared and the relationship between them and various structural models for A_nT_n tracts will be discussed. (Supported by NSF)

W-Pos172

PURINE C8-HYDROGEN EXCHANGE KINETICS IN DNA DODECAMERS CONTAINING A_nT_n TRACTS.

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Proton nuclear magnetic resonance (NMR) spectroscopy is being used to measure the deuterium exchange rates of C8-protons in individual purines of the following three DNA dodecamers: 5'-d(CGCGAATTCGCG)-3', 5'-d(CGCAAATTTGCG)-3', and 5'-d(CGAAAATTTTCG)-3'. The dodecamers contain central A_nT_n tracts where n=2, 3, and 4. C8-proton resonances are assigned to individual purines using NOESY experiments in the same conditions as those used for the exchange measurements (i.e., 0.01 M phosphate buffer containing 0.6 M NaCl and 0.002 M EDTA in D_2O at pH 7.0 and at 40°C). The results indicate that the rates of C8-proton exchange in Adenines are three- to eight-fold lower than that in 5'-adenosine monophosphate. This retardation effect is found to be dependent on the exact location of the Adenine in the A_nT_n tract. The relationship between these results and the structural properties of A_nT_n tracts will be discussed (supported by the Hughes Program in the Life Sciences, Wesleyan University).

W-Pos173

RESTRICTION ENZYMES HAVE ALTERED CLEAVAGE RATES AT SITES NEAR CERTAIN SEQUENCES.

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Under appropriate environmental conditions, e.g. supercoiled DNA, 50 -100 uM cobalt hexamine, the DNA sequence $(CG)_4$ forms non-B or Z-type structures. Restriction enzymes, e.g. BglI, EcoRV, HaeIII, MboI, NotI, PstI, TaqI, cutting at sites up to circa 50 base pairs from inserted $(CG)_4$ segments show enhanced cleavage rates (in the presence of cobalt hexamine) relative to cleavage rates for DNA fragments not containing the $(CG)_4$. We have previously shown that the sequence TCTTG is a hot spot for the binding of the carcinogen N-acetoxy-N-acetyl-2-aminofluorene. Restriction enzymes cleaving near an inserted TCTTG segment have decreased reaction rates. Gel retardation binding studies and exonuclease footprinting studies suggest that the observed alterations in restriction enzyme reaction rates arise from changes in the binding of the restriction enzymes to DNA when either $(CG)_4$ or TCTTG segments are present. These studies suggest that certain sequences can modulate DNA functions. (This work supported by PHS MBRS GM 08205-07 (SAW) and NSF DMB-8996232 (RDS).)

W-Pos174

A COMPARISON OF DYNAMICS OF TWO EPR ACTIVE PROBES ON DUPLEX DNA

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DNA dynamics have been studied by EPR spectroscopy using spin-labeled analogues of thymidine [1,2]. The two spin-labeled thymidines differ in the length of the tether between the base and the spin label: T* has a single acetylenic group as the tether [1], while T** has a diacetylenic tether [2]. The EPR spectra of T* labeled DNA duplexes are quite sensitive to length suggesting that there is little independent motion of the spin-label relative to the DNA [3]. However, it is possible that the T* label may restrict the internal motion of the DNA. EPR spectra of the self-complementary dodecamers 5'-d[CGCGAA TT*CGCG] and 5'-d[CGCGAATT**CGCG] were obtained for the duplexes both in buffer and bound to DEAE-Sephadex. We present a detailed, self-consistent analysis of the dynamics of these two probes. Studies of the duplexes bound to DEAE-Sephadex show that the T** probe undergoes nearly unimpeded rotation about a single axis with a characteristic rotation time of 1.0 nsec, while the T* probe is as reported [3] nearly immobile relative to the DNA. The EPR spectra of both spin-labeled DNA duplexes in solution are quantitatively accounted for by the same overall tumbling motion expected for a 12 bp duplex. There is no evidence of additional large amplitude local base motion in the T** labeled duplex, where the possibility of the probe interfering with base motion is small. The self-consistent analysis of the EPR spectra suggests that the T* label does not interfer with local base motion.

1. Spaltenstein, A., Robinson, B.H., & Hopkins, P.B. (1989a) *Biochemistry* **28**, 9484-95.
2. Kirchner, J.J., Hustedt, E.J., Robinson, B.H.,& Hopkins, P.B. (1990) *Tet. Lett.* **31**, 593-6.
3. Hustedt, E.J., Spaltenstein, A., Kirchner, J.J., Hopkins, P.B. & Robinson, B.H. (1993) *Biochemistry*, in press.

W-Pos175

USE OF ^{15}N NMR TO PROBE H-BONDING IN A SPECIFICALLY LABELED O^6-METHYL G•T BASE PAIR

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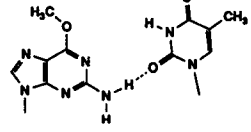
O^6-Methylguanine is known to be a mutagenic lesion in DNA and preferentially incorporates thymine. However, its hydrogen-bonding properties have not yet been clearly defined. We have synthesized two ^{15}N-labeled analogs of a self-complementary DNA fragment that forms a duplex containing two O^6MeG•T mispairs, {d[CGTGAATTC(O^6Me)GCG]}$_2$. In one fragment, the label is at the N1 (imino) and in the other, at the N2 (amino) of the O6-methylguanine residue. The ^{15}N chemical shift of each of these molecules was monitored as a function of temperature. We find that the N2 (amino) chemical shift is sensitive to the helix-coil transition and displays a sigmoidal curve. Thermodynamic values derived from this local monitor agree well with those obtained by more global optical melting experiments. In contrast, the N1 (imino) chemical shift is not sensitive to the helix-coil transition and displays only a linear downfield drift with increasing temperature. These results indicate that this O^6MeG•T mispair is directly H-bonded only at the O^6MeG N2, and not at the N1.



W-Pos176

T-T BASE MISMATCHES ENHANCE DRUG BINDING AT THE BRANCH SITE IN THREE AND FOUR-ARM DNA JUNCTIONS

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Branched DNA molecules have unique structural and physical properties, including the capacity to bind intercalative drugs tightly. We have investigated the effect of base mismatches--non Watson-Crick pairing between bases--on the binding properties of three and four arm DNA junctions, constructed from oligonucleotide strands. T-T mismatches destabilize duplex DNA, and all the DNA junctions we have examined. The patterns of destabilization differ however; T-T mismatches at the branch of four arm junctions cause a greater reduction in enthalpy than in three arm junctions. Changes in conformation of the three arm species can be readily detected from gel mobility experiments. Junctions containing T-T mismatches at the branch bind methidium and other intercalators with higher affinity than junctions lacking mismatches.

W-Pos177

DETERMINING THE STRUCTURE OF A G:U WOBBLE BASE PAIR IN AN RNA HELIX BY NMR

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The goal of this study is to determine the structure of a G:U base pair in an RNA helix. We showed previously that purine-purine stacking is the dominant interaction in the G:U pair in Helix I of 5S rRNA. Three guanines are involved in this stacking and their structure is entirely consistent with that predicted for an A-form helix.

In order to develop a more detailed structural picture of the G:U wobble base pair, a centrosymmetric 8 base pair helix was synthesized chemically and purified. The G:U is still has the same neighbors, but an extra G:C base pair has been added for further stability.

A series of two-dimensional NMR experiments has been completed and assignments for both protons and phosphorus will be presented. NOEs between the imino protons indicate that the smaller molecule's purine stacking is identical to that found in Helix I. Both phosphorus chemical shifts and coupling constants will be used to define the RNA's backbone torsional angles.

This work was supported by NIH GM41651 to P. B. Moore.

W-Pos178

THE INFLUENCE OF SEQUENCE ON THE THERMODYNAMICS OF DNA MELTING AND LIGAND BINDING PROPERTIES FOR A FAMILY OF OCTAMERIC DUPLEXES.

((Danuta Szwajkajzer and Kenneth Breslauer)) Department of Chemistry, Rutgers University, Piscataway, NJ 08855.

Specially designed and synthesized oligonucleotides provide a unique opportunity to evaluate the influence of sequence on DNA properties. In this study, we have used a combination of spectroscopic and calorimetric techniques to characterize the influence of sequence on the melting thermodynamics and the ligand binding properties for a family of octameric DNA duplexes. In this family of duplexes, the sequence has been varied systematically, while the base composition has been held constant at 50% AT and 50% GC. We used netropsin, an A-T-specific, minor groove binding compound, as the DNA binding ligand. Our spectroscopic and calorimetric measurements reveal sequence-dependent differences in the thermodynamic parameters for melting of the drug-free DNA duplexes and for netropsin binding to these duplexes. These differences will be discussed in terms of potential molecular interpretations. This work was supported by GM 23509 and GM 34469.

W-Pos179

STRUCTURAL BASIS FOR ENHANCED AFFINITY OF RNA HAIRPIN LOOP-LOOP INTERACTIONS

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Replication of plasmid Col E1 is regulated by the action of two complementary RNAs, termed RNA I and RNA II, and a protein, ROM. Binding of the two RNAs, which leads to inhibition of plasmid replication, begins with the interaction of complementary loop regions in the two RNAs. Individual RNA hairpin loops, of numerous sequences and sizes, form similar complexes and bind ROM. Inverting the wild-type loop sequence 5'-to-3' results in a complex which is 1000-fold more stable than wild-type and has markedly slower association and dissociation rates.[1]

We have used UV melting and stopped-flow kinetic methods to screen mutants to determine the sequence motif necessary for tight binding and slow kinetics. Whereas mutations in the center of the loops result in complex stabilities which can be explained in terms of Watson-Crick pairing, mutations at the 5' or 3' ends of the loops result in stability and kinetic constants which cannot be explained by nearest-neighbor interactions alone. Model RNA systems indicate that coaxial stacking of the helices formed in the complex enhances binding affinity, but it does not fully explain the observed increase in stability. One- and two-dimensional NMR experiments on both wild-type and inverted loop sequences elucidate principal structural determinants of variations in physical properties, particularly in the stem-loop junction regions.

[1]Eguchi, Y. and Tomizawa, J. (1991) J. Mol. Biol., 220, 831-842.

W-Pos180

THE THERMODYNAMICS AND TEMPERATURE DEPENDENT CONFORMATIONAL CHANGES OF SYNTHETIC 45 BASE-PAIR PHASED $d(A)_5$ TRACKS.

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Using synthetic 45-bp long DNAs of known sequences, we have studied: (i) the unusual structure that the phased A-tracts have at temperatures below about 37°C; (ii) the thermodynamics of the loss of that structure well below the duplex melting temperature; (iii) and the conformational changes that occur with temperature. We show by circular dichroism that there is a low temperature structural transition in a 45-mer duplex with 4 segments of phased $d(A)_5$ tracks separated by 5 segments of 5 randomized G•C pairs, but none in the corresponding random sequence isomeric 45-mer duplex. Differential scanning calorimetry measurements show that the enthalpy of the structural transition is 3.5 kcal/mole-AT pair or 4.4 kcal/mole-AA step, consistent with the idea of one H-bond breakage per AT pair or AA step. The integrated structural enthalpy change is only about 16% of the global duplex-to-single-strands melting enthalpy change and is relatively broad (about 30°C spread). Electric birefringence decay shows that from 5-40°C the phased duplex is bent with a projection length less than a straight rod, and that the bend straightens over the same temperature range where the CD spectra changes and the excess heat capacity is observed. We believe that these kinds of sequence dependent structures which "melt" at physiological temperatures may be relevant to DNA topology and function.

W-Pos181

THE INTERACTION OF CIS-DDP WITH SYNTHETIC DNA.

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The antitumor action of cis-DDP on DNA is thought to occur by inhibiting DNA replication. The mechanism of this phenomena is possibly caused by intrastrand chelation of two guanines separated by a third nucleotide, which in turn inhibits replication processes. We have investigated the interaction of cis-DDP with synthetic DNA (three different 20 b.p double stranded oligos). Previous data, based on the analysis of the helix-coil transition, indicated, a sequence dependent binding effect of cis-DDP to these oligos. New data, with the titration of cis-DDP demonstrate a preferential inhibition of annealing and melting. The 20-mer with repeating G-C showed the greatest amount of inhibition of the helix-coil transition, followed by G-A, then closely by G-T as compared to the negative control. The effects were such that the T_m, velocity of annealing and denaturation significantly changed differentially between the three different sequences, upon the increasing addition of cis-DDP. Data from C.D and U.V spectrophotometric tecniques further corroborate these trends. Based on C.D data, the effect of cis-DDP on the B to Z transition of these oligos was found to also be preferential, with the G-C oligo being inhibited the most, followed by G-A, then closely by G-T, under high salt induction of the Z-structure. PAGE under non-denaturing conditions indicate that the double stranded G-C sequence had more platinum bound, than both the G-A and G-T sequence. The amount of cis-DDP bound to these double stranded oligos was further analyzed with atomic absorption spectroscopy using a platinum window and shows that the G-C sequence had twice the amount of cis-DDP bound to it when compared to the the G-A and G-T sequences, thus supporting our proposed mechanism and hypothesis. The proposed mechanism of intrastrand chelation will be futher analyzed with the use of H^1 NMR using a GN-300.

(Supported by MBRS 8167-14)

W-Pos182

DISSOCIATION OF ACTINOMYCIN D FROM DNA OLIGOMERS WITH HAIRPIN MOTIFS.

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The SDS-induced dissociation of 7-amino-actinomycin D (7-AM-ACTD) from some oligonucleotides has been investigated by fluorescence. It is found that the dissociation kinetitcs of this drug from the self-complementary dodecamer d(ATGCATATGCAT) cannot be adequately described by a single rate process but requires a double-exponential fit, with the fast component contributing about 20% at 20°C. Two rate constants are also found for d(ATGCAT-T-ATGCAT) and d(ATGCAT-A-ATGCAT) except now the contributions from the fast components increase to about 75%. These results are interpreted in terms of the drug coming off from both hairpin and dimeric duplexes, with the faster rate constants corresponding to those from the hairpins. Studies with d(ATGCAT-TTT-ATGCAT) and d(ATGCAT-AAA-ATGCAT), which are found to be exclusively in the hairpin form at 20°C, revealed that 7-AM-ACTD binds strongly to these oligomers and dissociates single exponentially with a slow rate process. These results indicate that although ACTD binds to hairpins as strongly as to the dimeric duplexes, the rates of dissociation depend intimately on the structural features near the loop regions.

W-Pos183

BINDING OF *TRIS* (PHENANTHROLINE)RUTHENIUM(II) TO DNA.

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The interaction of Δ and Λ $[Ru(o\text{-}phen)_3]^{+2}$ with calf thymus DNA has been characterized using a variety of physical techniques. The mode of binding of these compounds to DNA is a matter of controversy. Employing equilibrium dialysis, both isomers of $[Ru(o\text{-}phen)_3]^{+2}$ were found to bind, but weakly, to calf thymus DNA, with binding constants of 4.9 (±0.3) x 10^4M^{-1} and 2.8 (±0.2) x 10^4M^{-1} determined for the Δ and Λ isomers, respectively, at 20° C in 5mM Tris HCl(pH 7.1), 10mM NaCl. The quantity ($\delta \log K/\delta \log [Na^+]$) was found to be 1.37 and 1.24 for the Δ and Λ isomers respectively, showing that the binding is primarily electrostatic in nature. Equilibrium dialysis experiments designed to assess the base and helical specificity of the isomers revealed that Δ Ru has weak specificity for GC sites while Λ weakly favors AT regions. Neither isomer binds preferentially to Z DNA but both favor poly(dGm^{5}dC) rather than poly(dGdC) regardless of conformation (B or Z DNA). Viscosity experiments show that binding of the Λ isomer does not alter the relative viscosity of DNA to any appreciable extent, while binding of the Δ isomer *decreases* the relative viscosity of DNA. Thermal difference spectra clearly show that the enthalpy of the Ru-DNA interaction for both Δ and Λ Ru is a small positive value. Analyses of steady state fluorescence quenching data using Singular Value Decomposition revealed that binding is a two state process. Collectively these studies show that each isomer has only *one* bound form with the Λ isomer outside or groove bound and the Δ isomer partially inserted so as to produce a kinked DNA structure.

W-Pos184

$[Co(III)(NH_3)_5(OH_2)]^{+3}$ INDUCED CONFORMATIONAL TRANSITIONS IN DNA OLIGOMERS.

((David M. Calderone, Edgardo J. Mantilla, Daniel H. Huchital, W. Rorer Murphy, Jr. & Richard D. Sheardy)), Department of Chemistry, Seton Hall University, South Orange, NJ. 07079

It is well-known that $[Co(NH_3)_6]^{+3}$ induces the B to Z transition of DNA oligomers and polymers with alternating CG sequence motifs and an unusual B-like conformation in a DNA oligomer containing $(dC\text{-}dG)_4$ [Winkle et al, *J. Biomol. Struct. Dyn.*, in press]. Being interested in the effects of derivatives of cobalt (III) on the conformational properties of DNA, we report here a preliminary comparison of $[Co(NH_3)_6]^{+3}$ and $[Co(NH_3)_5(OH_2)]^{+3}$ induced conformational transitions for some DNA oligomers. What is particularly noteworthy about the results is that the nature of the effect of high concentrations (ca. 100 - 200 uM) of $[Co(NH_3)_5(OH_2)]^{+3}$ on $(d5meC\text{-}dG)_4$ is dependent upon how the sample was prepared. For example, if the cobalt complex is added to the oligomer, immediately heated and allowed to slowly cool, the UV spectrum is hyperchromic at all wavelenths relative to an untreated DNA sample. In addition, the CD spectrum is characteristic of B-type DNA. On the other hand, if this cobalt complex is added to a previously heat-treated DNA oligomer, the resultant UV spectrum is essentially unchanged from that of an untreated sample. Furthermore, the melting profiles of the two cobalt treated samples are quite different from each other and from that of a normal B-type DNA oligomer. The results are discussed in terms of the interaction specificities of the cobalt complexes with DNA oligomers. Supported by NSF DMB-8996232 (RDS).

W-Pos185

STRUCTURAL FEATURES OF THE RNA STEM-LOOP OF THE EQUINE INFECTIOUS ANEMIA VIRUS TAR ELEMENT.

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The equine infectious anemia virus (EIAV) has recently become the focus of considerable attention because it has several features in common with human immunodeficiency virus type-1 (HIV-1). Each virus encodes a protein (Tat) that mediates an increase in gene transcription via an interaction with a specific RNA stem-loop, referred to as the trans-activation response element, or TAR. A knowledge of the structural basis for the Tat-TAR interaction would directly contribute towards a better understanding of both HIV-1 and EIAV.

A 25 nucleotide RNA with the sequence of the equine infectious anemia virus TAR element was prepared by *in vitro* transcription with T7 RNA polymerase. The RNA structure was analyzed using multi-dimensional homonuclear and heteronuclear NMR methods, and was found to consist of an A-helical stem capped by two non-Watson-Crick U-G base pairs and a compact four nucleotide loop. Near the 5' end of the molecule, the stem contains a single bulged cytosine. A 3-dimensional homonuclear NOESY-TOCSY spectrum was found to be particularly useful in increasing the number of observable NOEs. A general method for distinguishing RNA stem-loops from palindromic dimers will be described, and was used to confirm that the RNA has a stem-loop structure at the conditions of the NMR experiments.

W-Pos186

DNA JUNCTIONS, ANTIJUNCTIONS, MESOJUNCTIONS AND KNOTS.

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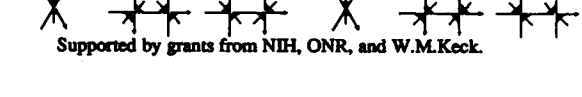
By equating a half-turn of double helical DNA with a node, it is possible to devise a diagram that directs the synthesis of knots from single-stranded DNA. It is often feasible to condense isolated half-turns into larger structures, such as full turns of duplex, or branched junctions. We have built trefoil and amphichiral figure-8 knots from a motif containing two separate turns of DNA connected by four oligo-dT linkers; minimal linker size is 6 (figure-8) or 7 (trefoil) nucleotides. We display branched junctions with 3-arms (3_3) and 4-arms (4_4) flanking polygons below: Each contains a half-turn of DNA per double helical segment; arrows are 3' ends, parallel lines are base pairs, and helix axes are shown perpendicular to base pairs. The 'antijunction' arrangement shown as 4_0 appears in the diagram for a 9_{49} knot. In contrast to 3_3 and 4_4, its helix axes are circumferential, not radial. Switching only some helices from radial to circumferential produces 'mesojunctions.' Two helices must switch at once, to avoid long linkers. 3_1, $^14_2$, and $^24_2$ are all mesojunctions for 3- and 4-strand complexes. We have built all the structures below, in molecules containing 1.5 helical turns per domain. Antijunctions and mesojunctions do not form as readily as junctions; care must be taken to destabilize alternative structures. We have characterized their melting and electrophoretic behavior, and have performed Fe(II)EDTA2- autofootprinting.



Supported by grants from NIH, ONR, and W.M.Keck.

W-Pos187

Dynamics of Spontaneous DNA Branch Migration

Igor G. Panyutin and Peggy Hsieh

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DNA branch migration, a process whereby two homologous DNA duplexes exchange strands, is an essential component of genetic recombination. In the simplest model, the elementary step of branch migration is movement of the Holliday junction, which is the branch point between two duplexes, one base pair in either direction with equal probabilities. Therefore, spontaneous DNA branch migration according to this model proceeds as a random walk process. An important issue in genetic recombination is whether spontaneous DNA branch migration is capable of traversing sequence heterology such as mismatches, insertions and deletions. We use a model four-strand system to examine the effect of mispaired or unpaired bases on branch migration. Our results demonstrate that a single base mismatch, insertion or deletion is sufficient to pose a substantial barrier to spontaneous branch migration. In the presence of magnesium ions, branch migration through such sequence heterologies is almost completely blocked. Using the same DNA substrates we are studying the rate of spontaneous DNA branch migration as a function of temperature, ionic strength and type of metal ions present in solution.

W-Pos188

STRUCTURE TRANSITIONS OF TELOMERIC DNA PROBED BY RAMAN SPECTROSCOPY ((Takashi Miura and George J. Thomas, Jr.)) School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri 64110.

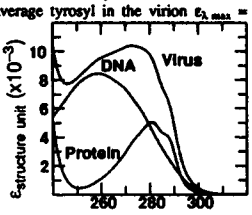
Telomeres are the termini of eukaryotic chromosomes, characterized by tandem-repeated, guanine-rich sequences in the DNA 5' → 3' strand and cytosine-rich sequences in the complementary strand. In addition to conventional Watson-Crick base pairing, telomeric DNA may form antiparallel-foldback and parallel-extended quadruplex structures stabilized by intrastrand or interstrand associations. To elucidate the mechanisms of inter-quadruplex and duplex-quadruplex conversions, we have investigated the structure of $d(T_4G_4)_4$, a telomeric DNA model, over wide ranges of solution temperature and ionic strength by Raman spectroscopy. In the presence of Na^+, $d(T_4G_4)_4$ can form either an antiparallel-foldback quadruplex (with both C2'-endo/syn and C2'-endo/anti dG conformers) or a parallel-extended quadruplex (with only C2'-endo/anti dG). The latter structure, not detected in previous structural studies of model telomeric DNA, is favored by high Na^+ concentration and exhibits greater thermostability than the antiparallel form. In both the parallel and antiparallel quadruplexes, the conformation of the phosphodiester backbone is similar to that of the canonical B form of DNA, but with important characteristic differences, which shall be discussed in relation to Watson-Crick DNA. [Supported by NIH Grant AI18758.]

W-Pos189

HYPERCHROMICITY OF DNA BASES AND OF TYROSINES IN Pf1 VIRUS. ((L.G. Kostrikis[1,2], D.J. Liu[1,3], and L.A. Day[1])) [1]The Public Health Research Institute, New York, NY 10016, [2]Dept of Chemistry, New York University, New York, NY 10003, and [3]Dept of Biochemistry, N.Y.U. School of Medicine, New York, NY 10016.

The bacterial virus Pf1 consists of a single-stranded circular DNA molecule of 7349 bases held by a surrounding protein coat, the entire structure being close to 2000 nm long and 7 nm in diameter; the coat is primarily subunits of 46 amino acids, two being tyrosines. We report that uv spectra of highly purified samples disrupted in (0.2 M NaOH, 1% SDS, 8 M urea) are fit almost exactly with spectra for purified DNA and protein in this solvent (data every 0.1 nm from 250 nm to 360 nm). The fits yield nucleotide/subunit molar ratios of 1.00 ± 0.01 based on extinction coefficients for the separate components. The spectrum of the intact virus, corrected for light scattering, has $\varepsilon_{\lambda\,max}$ = 10,400 and ε_{259nm} = 9,450 per structure unit (one protein subunit plus one nucleotide). Subtraction of protein absorbance at 259 nm yields 8400 ± 200, which we take as $\varepsilon(P)_{259nm}$ for the average nucleotide in the virion. The DNA in Pf1 is thus hyperchromic relative to base-stacked DNA. The spectrum for protein, generated as the difference between spectra for virus and DNA, looks like a pure tyrosyl spectrum, and its numerically generated derivative spectrum has features characteristic of tyrosyl model compounds. For the average tyrosyl in the virion $\varepsilon_{\lambda\,max}$ = 2500 in the near uv, higher than for most tyrosines.

The CD of the virus has the shape of pure α-helical reference CD, with both $[\theta_{208nm}]$ and $[\theta_{222nm}]$ more negative than -42,000 units, larger than for most proteins. There are no obvious base-stacking contributions to the CD from 245 nm to 300 nm. This and the high $\varepsilon(P)$ lead us to argue that the bases do not stack in Pf1. The combined new data support a previously proposed model for the DNA helix in Pf1 with phosphates inside and bases outside.



W-Pos190

THE STRUCTURE OF THE CATALYTIC DOMAIN OF HUMAN HEPATITIS DELTA VIRUS GENOMIC RNA - A 600 MHZ PROTON NMR STUDY

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We have synthesized 10 mg of a 74-mer RNA which was derived from the catalytic domain of hepatitis delta virus genomic RNA (HDV RNA). This 74-mer lost the self-cleavage activity because of a single base mutation (G → C) at the cleavage site. One- and two-dimensional proton NMR spectroscopy at 600 MHz has been applied to study the conformation of this fragment at temperatures between 2 °C and 77 °C, with and without added Mg^{++}. Deconvolution of the NMR spectrum obtained at 32°C indicates that the 74-mer RNA molecules may exist in multiple conformations in equilibrium. The major conformer contains two A-U base-pairs and 13±1 G-C base-pairs. It appears to contain no standard G-U base-pair. NMR Melting study suggests that there are at least two stems. From one- and two-dimensional NOE data we identify one of the stem to be a tetra-loop (Tinoco et al, Biochemistry 30, 3280). Based on this study and other biochemical evidence, models of the secondary structure are discussed.

W-Pos191

PLASMID FUSION: A POSSIBLE CAUSE FOR REDUCED LEVELS OF EXPRESSION ((Katsuhide Mabuchi)) Dept. of Muscle Research, Boston Biomedical Research Institute, 20 Staniford Street, Boston, Ma. 02114

Expression of the troponin C (TnC) gene carried on the plasmid pKP1500 in several cell lines of E coli KP3998 was so unstable that by the time the culture was brought to a larger volume the majority of bacteria had ceased to express TnC. This was accompanied by a reduction in the amount of plasmids and changes in their gel-electrophoretical pattern. In order to investigate the significance of the different gel-electrophoretic patterns, 3 types of plasmid bands, differing in their migration velocity --fast, intermediate and slow were isolated using low melting agarose gel electrophoresis. Electron microscopy showed that all three types contained circular double-stranded DNA but there were inverse correlation between size and velocity. Digestion of these differently sized plasmids by EcoR 1 created the same gel electrophoretic pattern corresponding to a pKP1500 vector and a TnC insert, thus it appears that the slower migrating, larger plasmids are fused ones. In general, characteristics of plasmids in the re-transformants of the three types were similar to their respective ones used for the re-transformation. However, one colony among the re-transformants of the slow-migrating plasmid expressed the largest amount of TnC and its plasmid pattern was not of the slow migrating but of fast migrating plasmids. These observations suggest that there is a mechanism that prevents fusion of plasmids and that failure of this mechanism leads to the reduced level of TnC expression.

W-Pos192

THREE DIMENSIONAL MATCHING AND DOCKING OF PROTEIN MOLECULES.

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Recently we have developed and adapted a computer-vision based technique to the problem of matching of three dimensional structures of macromolecules. The method enables searching the structural database, looking for geometrical, substructural motifs in proteins. Since we look at atoms as points in space, we are able to detect "real" three dimensional substructures, completely disregarding the contiguity of the amino acids in the chain. We are thus able to detect substructural motifs in the interior of proteins, or on their surfaces. The former have direct relevance to the protein folding problem; the latter are directly implicated in the problem of biomolecular recognition. We have further extended our approach, and adapted it to the problem of matching of complementary surfaces, that is, the docking of a ligand onto a receptor surface. Our matching technique is very efficient. We have already applied our method to both types of problems. Several "real" 3-D similarities in the interior, and separately, on the surfaces of proteins have already been detected.

W-Pos193

STRUCTURAL ENERGETICS OF PEPTIDE RECOGNITION: ANGIOTENSIN II / ANTIBODY BINDING. ((Kenneth P. Murphy, Dong Xie, K. Christopher Garcia, L. Mario Amzel and Ernesto Freire)) Department of Biology and Biocalorimetry Center, The Johns Hopkins University and Department of Biophysics and Biophysical Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21218

The prediction of the strength of association of peptide hormones or other ligands with their protein receptors is fundamental in the fields of protein engineering and rational drug design. To form a tight complex between a flexible peptide and its receptor, the large loss of configurational entropy must be overcome. Recently, the crystallographic structure of the complex between Angiotensin II and the Fab fragment of a high affinity monoclonal antibody has been solved. We have carried out a quantitative prediction of the binding of this complex utilizing the x-ray coordinates and the set of fundamental thermodynamic parameters (ΔH, ΔS, ΔC_p) that define the energetics of each of the interactions. The validity of this analysis has been tested by performing an experimental dissection of the binding energetics using high sensitivity calorimetric measurements. The calculated and experimentally determined thermodynamic quantities are in close agreement. The loss of configurational entropy is more than compensated by the entropy gain from the solvent release. Binding is favored by polar interactions (hydrogen bonding) but is partially offset by the enthalpically unfavorable burial of apolar surfaces. These effects add up to a net binding free energy of about -11 kcal mol^{-1} at 30°C, pH 7. The problem of binding flexible hormones thus is solved in much the same way as the folding of a polypeptide chain into a globular protein. (Supported by National Institutes of Health RR04328, NS24520 and GM37911(E.F.) and GM44692 (L.M.A.). KPM and KCG were also supported by a NSF postdoctoral fellowship (DIR8721059))

W-VCR4

MOLECULAR SHAPE COMPARISON OF ELASTASE INHIBITORS: QUANTITATIVE SURFACE SEARCHING OF THE TURKEY OVOMUCOID INHIBITOR WITH A TRIPEPTIDE INHIBITOR, ((Brian B. Masek and James B. Matthew)) Medicinal Chemistry Dept., ICI Pharmaceuticals Group, A business unit of ICI Americas Inc., Wilmington, Delaware 19897-2500

Molecular shape comparison (MSC) seeks to find the spatial properties common to two or more molecules. A new and powerful analytical method for comparing molecular shapes by optimizing the overlap of molecular "Skins" has been developed. This method provides a quantitative measure of the shape similarity by maximizing the intersection volume of molecular Skins; a molecular surface of finite thickness. We report shape matching of a small tripeptide inhibitor of elastase class proteins with the 56 residue turkey ovomucoid inhibitor (TOMI). Skin based comparisions using either the binding loop of TOMI alone (residues 15-20) or the entire TOMI protein successfully found the alignment expected from comparison of their crystallographic complexes (i.e. HLE/TOMI complex and PPE/tripeptide complex). To match a large elastase inhibitor such as TOMI with a small inhibitor or drug we found that it is important to use a skin match rather than a volume match since the skin of the small inhibitor is matched only to the exposed skin of the larger molecule. In the MSC of the tripeptide with the entire TOMI protein, random or blind searching for skin matchs involved optimization of the shape match from 172 starting positions. The tripeptide center of mass was placed at points randomly selected from a set of 500 points on the TOMI van der waals surface (within 10 Å of the Leu 18 on the TOMI binding loop (1 point/Å^2)). Its orientation was randomized before optimization was initiated. The best match, 86.4 Å^3, was found three times and corresponds to the experimental alignment. The next best match was 78.1 Å^3 giving a discrimination factor in this case of 10%.

W-Pos194

The Dynamics of Simulated Molecular Recognition

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Molecular dynamics simulations of a complex between aglycovancomycin and various dipeptides have been performed using thermodynamic cycle perturbation methods to predict their thermodynamic behavior. The results are compared to experimental measurements which provide the free energy, enthalpy, and entropy of complex formation. The simulations correctly predict that the binding affinity for Ac-D-ala-gly (AcDAG) is 0.8 kcal/mole less than for Ac-D-ala-D-ala (AcDADA), and that Ac-D-ala-L-ala will not bind. However, the binding of AcDAG has a 0.7 kcal/mole enthalpic advantage over that of AcDADA, in agreement with measurements. This indicates that the enthalpy advantage for AcDAG binding is more than compensated by an entropic cost of 1.5 kcal/mole. Although it is likely that much of this entropic cost involves solvent interactions with the complex, the simulation indicates that the AcDAG complex is more compact and that low frequency vibrational modes are attenuated. Thus, some of the entropic cost may be due to reduced configurational entropy.

Supported by HL47469, the Minnesota Heart Association, and the L. P. Markey Charitable Trust.

W-Pos195

THE ROLE OF HYDROPATHY IN COMPUTER SIMULATED, SEQUENCE-SELECTIVE ADRIAMYCIN INTERCALATION INTO DNA DOUBLE-STRANDED HEXANUCLEOTIDES.((Frank A. Fornari, David A. Gewirtz, Gwen B. Bauer, Donald J. Abraham and Glen E. Kellogg)) Departments of Pharmacology/Toxicology and Medicine and Department of Medicinal Chemistry , Medical College of Virginia, Richmond, VA 23298

The anthracycline antibiotic, adriamycin, inhibits DNA biosynthesis and transcription as a result of intercalation into DNA, a process in which the planar aromatic rings of the drug insert between adjacent base pairs. Employing the program HINT (Hydropathic Interactions), an empirical hydropathic field-like 3D function was calculated and used to study computer-simulated, sequence-selective adriamycin binding in the following hexanucleotides: d(CGATCG), d(CGTACG), d(CGCGCG), d(TGATCA), d(TACGTA) and d(TATATA). Regarding the two regularly alternating hexanucleotides, d(CGCGCG) and d(TATATA), preliminary analysis of the empirically calculated binding constants generated by HINT revealed adriamycin binds preferentially to d(TATATA), which corresponds with previous experimental data (Graves & Krugh 1983). In addition to hydrophobic interactions, the HINT model, based on experimental solvent partitioning phenomena, inherently includes electrostatic, steric, entropic and solvation effects. The hydrophobic interaction is thought to be very important for drug binding and activity and the use of the HINT model, in these studies, may extend the current understanding of adriamycin/DNA interactions.

W-Pos196

COMPUTER SIMULATED HYDROPATHIC ANALYSIS OF CHLORAMBUCIL ADENINE ADDUCT FORMATION IN THE MINOR GROOVE.((Gwen B. Bauer, Peng Wang, Lawrence F. Povirk, Donald J. Abraham and Glen E. Kellogg)) Departments of Pharmacology/Toxicology and Medicine and Department of Medicinal Chemistry, Medical College of Virginia, Richmond, VA 23298

Previous work demonstrates the predominant mutations induced by the aromatic nitrogen mustards melphalan (l-phenylalanine) and chlorambucil in the shuttle vector pZ189 are AT->TA transversions, suggesting that alkylations of adenine are important premutagenic lesions. The nonaromatic analogues mechlorethamine and phoshoramide mustard are much less effective than melphalan and chlorambucil in producing thermolabile adenine adducts. Thus, we propose that the aromatic nitrogen mustards may have greater affinity for the minor groove resulting in adenine N-3 monofuctional alkylations. The DNA-drug complex associated with N-3 alkylations was modeled using the program Sybyl and minimized with the Tripos force field. Extensive conformational analysis was performed using the gridsearch option in Sybyl with final minimization of every conformer. Employing the program HINT (Hydropathic Interactions), based on experimental solvent partitioning phenomena, we found several minimized structures which gave the lowest energy and least distortion of DNA in addition to possessing favorable hydrophobic interactions between the phenyl ring of chlorambucil and the faces of the minor groove. These interactions may account for the high level of adenine N-3 alkylations by aromatic nitrogen mustards.

W-Pos197

SITE SELECTIVITY OF DAUNOMYCIN.

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Daunomycin is an antitumor drug that has been used for the treatment of leukemia. The binding properties of the drug were re-examined in the presence of oligonucleotides, sixteen bases long, that were designed to contain published binding sites for the drug. The sequences contained a six base-pair core flanked on the 5′ and 3′ sides with tracts of A's. Four major sequences were examined in the six base-pair core, CGTACG, TAGCTG, TCATCC and $(TA)_3$ and compared with calf thymus DNA. Fluorometry, absorption and stopped flow techniques were used to examine the binding. Scatchard-type graphs were used to estimate the total binding affinity, and the number of sites per oligonucleotide. In all cases the affinity for the drug was enhanced relative to DNA. Association constants ranged from 1.0×10^6 to 3.0×10^7. Stopped flow data was used to measure dissociation rates, and support the total number of sites.

W-Pos198

Ca^{2+} IONOPHORETIC ACTIVITIES OF PEPTIDE HORMONES AND DRUGS.

((V.S. Ananthanarayanan)) McMaster Univ., Hamilton, Ont., Canada L8N 3Z5.

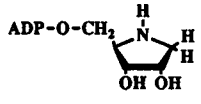
With a view to understanding the possible role of extracellular Ca^{2+} in hormone and drug action, we have examined the ability of these molecules to interact with Ca^{2+} in the lipid milieu. Binding studies in membrane mimetic solvents (using spectral changes) showed that a variety of peptide hormones (eg. bombesin, glucagon, LHRH, substance P) and drugs (eg. verapamil, diltiazem, salbutamol, lidoflazine, cyclosporin) bind Ca^{2+} stoichiometrically. All of them translocated Ca^{2+} across the lipid bilayer in unilamellar dimyristoylphosphatidylcholine vesicles containing arsenazo III inside and suspended in a neutral pH buffer. Leakage of vesicular contents induced by these agents was relatively small at low concentrations (<50 μM) as tested with fluorescent dyes. Interestingly, segments of peptide hormones that are important for signal transduction were much better Ca^{2+} binders and/or transporters than the other segment(s). These data suggest that the Ca^{2+}-bound forms of hormones and drugs may be their bioactive conformations recognized by the membrane-bound receptor, as proposed in a recent hypothesis on signal transduction (V.S.A. Biochem. Cell Biol. 69, 93-95, 1991). Supported by Med. Res. Council of Canada.

W-Pos199

CONFORMATIONAL ANALYSIS OF POLY(ADP-RIBOSE) GLYCOHYDROLASE INHIBITORS.

((Annise M. Younes, Rita Lodaya, James P. Slama and Leonore A. Findsen)) Department of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, 2801 W. Bancroft, Toledo, OH 43606.

Poly (ADP-Ribose) Glycohydrolase is important in the repair of DNA in cells. Adenosine diphosphate hydroxypyrrolidinediol (ADP-HPD), shown below, is a potent and specific non-competitive inhibitor of the protein. Evidence indicates that the pyrrolidine part of the molecule is important in the activity since ADP-ribose is inactive as an inhibitor of the protein. To identify the cause of this difference in activities, the conformational analysis of the pyrrolidine analogs, ribose, and the traditional transition state analogs, lactam and lactone, are examined. These studies, in conjunction with experimental binding studies of the molecules, have provided great insight into the mechanism of inhibition of ADP-HPD.



LIPID–PROTEIN INTERACTIONS II

W-Pos200

CONFORMATIONAL ANALYSIS OF A PEPTIDE MODEL OF THE HUMAN APOLIPOPROTEINS

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The apolipoproteins are responsible for lipid transport, lipoprotein particle structure and the activation of enzymes in lipid metabolism. These proteins share common primary sequence repeats which exist in tandem throughout most of these sequences. A peptide model consisting of a dimer of a consensus of these tandem repeats, $(PLAEELRARLRAQLEELRERLG)_2\text{-}NH_2$, has been synthesized and purified using cation and reverse phase HPLC. Column chromatography, calorimetry, electron microscopy, and circular dichroism spectroscopy (CD) were used to characterize the peptide and complexes formed with dimyristoyl-phosphatidylcholine (DMPC). The results are contrasted with similar studies carried out with apolipoprotein A-I (apoA-I). The peptide has a solution structure of 87% helix, which increases to 97% in the presence of lipid, Trifluoroethanol, and octyl β-glucopyranoside. In contrast to apoA-I, no cooperative unfolding is observed in the peptide as determined by CD as a function of temperature. The peptide forms nacent HDL disc-like structures with DMPC that have an average diameter of 160±25Å with a mass stoichiometry of 5.6 lipid : peptide, similar to those formed by apoA-I which have a diameter of 136±25Å and a mass stoichiometry of 2.6 lipid : protein. The size and conformational details demonstrate that 1 peptide molecule is equivalent to 1/3 of an apoA-I molecule with respect to interaction with lipid. This supports the concept of 6 helical binding segments in apoA-I. Scanning calorimetry of peptide / DMPC complexes exhibited order disorder lipid transitions at 24.5° (ΔH=6.3 Kcal/mole) intermediate between that for uncomplexed lipid (23°) and apolipoprotein A-I/DMPC (27.0°, ΔH=6.4 Kcal/mole). Particle disruption occurs at a significantly higher temperature (90°) in the peptide complexes compared to those formed with apoA-I (78°).

W-Pos201

PULMONARY SURFACTANT PROTEIN SP-C AND PHOSPHOLIPIDS IN SPREAD MONOLAYERS AT THE AIR/WATER INTERFACE.

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The interaction of SP-C with dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphospatidylglycerol (DPPG) has been studied. The presence of < 27 wt% SP-C in binary protein/phospholipid monolayers gave a positive deviation from the ideal behavior of the mean area/molecule with the magnitude being higher in SP-C/DPPG films. SP-C alone did not sustain surface pressure (π) higher than 32-34 mN/m. At low protein concentrations (~3 wt%) SP-C/DPPC and SP-C/DPPG monolayers had collapse pressures at 70 mN/m and 65 mN/m, respectively. At initial SP-C concentration ≥ 4 wt%, a kink point at π~ 50 mN/m appeared in the isotherms implying possible exclusion of material from the monolayers. Analysis of the mean molecular area as a function of the monolayer composition and π suggested that SP-C, associated with some phospholipid, was squeezed out from the monolayers at π of 50 mN/m, indicating a potential role for SP-C in the process of refinement of the monolayer at the alveolar/air interface. (Supported by MRC Canada.)

W-Pos202

STRUCTURE OF LOW DENSITY LIPOPROTEIN IN VITREOUS ICE

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We have completed preliminary analysis of the structure of low density lipoprotein (LDL) in ice at 34 Å resolution. An image set of 480 LDL particles was used in this analysis. Initial reference images were selected at random. An automated, iterative, single particle averaging procedure consisting of translational and rotational alignment in real and Fourier space was used to select image sets representing different views of the LDL particle. Correspondence analysis using cluster validity, fuzzy and hierarchical classification was employed to demonstrate the uniqueness of the averaged images. Studies using two dimensional factor maps and image reconstitution showed that the first four eigenvectors, comprising 46.51% of the variance between image sets, contained the relevant features for image alignment. Images that failed to segregate using these factors were excluded. Images in a final set were used as alternate references in the automated procedure and required to yield other images from the set as a criterion for inclusion. Five independent sets containing 4-12 images were obtained. The image processing program SPIDER was used for all manipulations, alignments, and analysis. The independent views indicate that LDL is a semi-spherical, 200-220 Å diameter particle, with an area of low density (lipid) surrounded by a ring (in projection) of high density believed to represent apolipoprotein B-100. This ring is seen to be composed of four or five (depending on view) regions of high density material that may represent protein super-domains linked by areas of somewhat lower density.

W-Pos203

MODULATION OF ACETYLCHOLINE RECEPTOR STRUCTURE AND FUNCTION BY LIPIDS.

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Fluorescence quenching and Fourier transform infrared (FTIR) techniques have been used to probe lipid-protein interactions in reconstituted membranes containing purified nicotinic acetylcholine receptor from *Torpedo californica*. FTIR analysis of the protein reveals that negatively charged lipids and cholesterol increase the alpha helix and beta sheet content of the receptor. Analysis of the lipid reveals that pKa values of phosphatidic acid molecules in contact with the protein are increased by 2 pH units from 6.5 to 8.5. The functional activity of the receptor, as measured by carbamylcholine-stimulated ion influx at different pH values, correlates directly with the amount of dianionic phosphatidic acid, suggesting a direct link between lipid charge, lipid-protein interactions, and protein function. Fluorescence quenching of pyrene-labeled receptor by spin labeled lipids has been used to obtain a more detailed picture of the local protein structure at the lipid-protein interface. The sensitivity of the quenching profile to activating ligands is consistent with an allosteric linkages among the extracellular binding sites, the lipid-protein interface, and the ion channel. (Supported by NIH Grant NS13050 to MGM).

W-Pos204

^{19}F-NMR INVESTIGATION OF MEMBRANE-BINDING DOMAIN OF D-LACTATE DEHYDROGENASE OF *E. COLI*

((Z.-Y. Sun, E. A. Pratt, H.-T. N. Truong, D. C. Sutherland, C. E. Kulig, R. J. Homer, and C. Ho)) Dept. of Bio. Sci., Carnegie-Mellon Univ., Pittsburgh, PA 15213

^{19}F-NMR spectroscopy and site-specific Trp substitution have been used to probe the lipid-binding domain of the membrane-associated D-lactate dehydrogenase (D-LDH) of *E. coli*. It has been proposed that D-LDH has the typical two-domain structure of the cytoplasmic dehydrogenases, with the addition of a membrane-binding domain between the catalytic and cofactor-binding domains [Peersen, Pratt, Truong, Ho, & Rule, Biochemistry 29, 3256 (1990)]. Forty mutants containing an additional Trp in place of a hydrophobic residue have been constructed in our laboratory. ^{19}F-NMR spectra of the 5F-Trp labeled active D-LDH mutants, in the presence of lysolecithin and 8-doxyl palmitic acid, have been acquired. The 5F-Trps in the lipid-binding region can be identified when the ^{19}F resonances are broadened by the lipid-bound paramagnetic nitroxide. In addition to mutations of amino acid residues at positions 243, 309, 340, and 361 which have been found in the membrane-binding region by previous studies, we have produced new mutations at Y228, F251, F270, Y280, Y321, and F369. The ^{19}F-NMR results suggest that amino acid residues at positions 228, 280, and 369 are in the membrane-binding region, while amino acid residues at positions 251 and 270 are not. It is proposed that the membrane-binding domain of D-LDH is located in the region between amino acid residues 228 and 369, and is formed from separated segments rather than being a continuous strand. [This work is supported by a research grant from the NIH (GM-26874).]

W-Pos205

EFFECTS OF MEMBRANE THICKNESS ON THE DYNAMICS OF RECONSTITUTED SARCOPLASMIC RETICULUM (SR) Ca-ATPASE.

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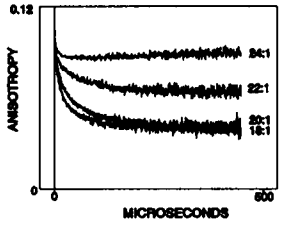
It has been previously shown that the activity of the reconstituted SR Ca^{2+}-ATPase is sensitive to the phospholipid fatty acid chain length. The phospholipid supporting the highest activity is di(18:1$^{\Delta 9}$)PC. Monounsaturated phosphatidylcholines (PC) with longer or shorter fatty acid chains support lower activities. To evaluate the effects of membrane thickness on the molecular dynamics of the Ca^{2+}-ATPase, we have used time-resolved phosphorescence anisotropy (TPA). In the TPA experiments, we used Ca^{2+}-ATPase reconstituted in exogenous PCs with monounsaturated chains 14 to 24 carbons long. Our results show that chain lengths longer or shorter than the optimal 18 result in a significantly decreased mobility of the Ca^{2+}-ATPase, indicated by higher residual anisotropy (r_∞) and suggesting protein aggregation. This effect can be attributed to a mismatch between the chain length and the thickness of the hydrophobic surface of the enzyme. In contrast to the large effects on protein mobility, these changes in chain length had little or no effect on hydrocarbon chain mobility as detected by 5-SASL. This is consistent with our proposal that protein dynamics and protein-protein interactions are of primary importance to the Ca^{2+}-ATPase mechanism.



W-Pos206

INTERACTIONS BETWEEN THE MYELIN BASIC PROTEIN AND EGG PHOSPHATIDIC ACID STUDIED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY.

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Myelin basic protein (MBP) is an extrinsic protein which binds to acidic lipids initially by electrostatic interactions. It has also several short hydrophobic segments which are believed to intercalate into the lipid bilayer. Fourier transform infrared spectroscopy has been used to investigate the binding of MBP with the natural lipid Egg-phosphatidic acid (Egg-PA) at pH 7. Our results reveal that the strong binding of MBP to Egg-PA induces the ionization of the second proton of the phosphate group. The analysis of the phospholipid carbonyl stretching band shows that in the liquid-crystalline phase, the protein prevents the penetration of water into the interfacial region of the lipid bilayer. The band due to the CH_2 symmetric streching mode of the acyl chains indicates that the protein induces a decrease of the temperature of the gel to liquid-crystalline phase transition from 19 to 5°C and perturbs the lipid acyl chain order in both phases. Finally, investigation of the secondary structure of the protein using the amide I band reveals that the content of α-helix in the protein increases when it is bound to Egg-PA. The domains of structured conformation in the protein have also been located by combining different methods of secondary structure prediction.

W-Pos207

MECHANISTIC STUDIES OF THE HYPOLIPIDEMIC ANSAMYCINS GP 43371 AND CGS 24565.

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The ansamycins (CGP 43371 and CGS 24565) are monopivaloyl oxazole derivatives of rifamycin which lack the antibacterial activity of their parent. They have been shown to have marked hypolipidemic properties in animal studies. The results of biochemical studies indicate that these agents are not operating via modification of hepatic cholesterol synthesis, but by another mechanism(s). To help discover this mechanism, studies were undertaken to examine the molecular interactions of these ansamycins with isolated and purified lipoproteins plasma proteins, isolated liver plasma membranes, and synthetic lipid vesicles. Our results indicate that these compounds have a very high and preferential affinity for the lipoprotein particles themselves, that the maximum carrying capacity (but not affinity) of the lipoproteins increases with increasing particle mass, and that these compounds are directly intercalating into the particles. In contrast, the ansamycins were found to interact only minimally with serum proteins, or the liver plasma membrane. Together, our results suggest that the ansamycins may be acting, at least in part, by intercalating into the lipoprotein particles and modifying their physical-chemical properties.

W-Pos208

SPIN-LABEL STUDIES INTO THE THE EFFECTS OF ETHANOL ON LIPID-PROTEIN INTERACTIONS IN RECONSTITUTED ACETYLCHOLINE RECEPTOR VESICLES

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The nicotinic acetylcholine receptor from *Torpedo* has been used as a model for general anesthesic action; it's agonist affinity is increased by ethanol. Using stearic acid spin-labeled at the 6^{th}, 9^{th}, 12^{th}, and 14^{th} positions, we have probed the lipid-protein interface of the nicotinic acetylcholine receptor from *Torpedo* reconstituted into dioleoylphosphatidylcholine vesicles and examined the effects of ethanol. The fraction of the motionally restricted component was found to be independent of the position of the nitroxide reporter group on the acyl chain. The rotational correlation times for motionally restricted stearic acid was between 10 - 20 ns, varying with both temperature and nitroxide position. For all 4 spin-labels, the addition of 0.5 to 3 M ethanol consistently increased the motion of bulk lipid, but did not alter the rotational mobility nor the fraction of motionally restricted stearic acid. This suggests that ethanol does not exert its effects on the acetylcholine receptor via alterations in lipid-protein interactions. (Supported by the National Institute on Alcohol Abuse and Alcoholism AA 07040 (KWM) and the Foundation for Anesthesia Education Research (DER)).

W-Pos209

TRANSLATIONAL DIFFUSION OF BOVINE PROTHROMBIN FRAGMENT 1 WEAKLY BOUND TO SUPPORTED PLANAR MEMBRANES

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Previous work has shown that bovine prothrombin fragment 1 (BF1) binds to supported planar membranes composed of phosphatidylcholine and phosphatidylserine in a Ca^{2+}-specific manner and that the average membrane residency time of BF1 is ≈ seconds (Pearce et al., 1992, *Biochem. 31*, 5983). Here, fluorescence pattern photobleaching recovery with epi-illumination has been used to show that saturating concentrations of BF1 and Ca^{2+} reduce the diffusion coefficients of nitrobenzoxadiazol-conjugated phosphatidylserine (NBD-PS) and nitrobenzoxadiazol-conjugated phosphatidylcholine (NBD-PC) by factors of approximately four and two, respectively. In addition, fluorescence pattern photobleaching recovery with evanescent illumination has been used to measure the diffusion coefficient of the weakly bound BF1. The results show that the BF1 diffusion coefficient is approximately 10-fold slower than that of the fluorescent lipids. This work is one of the first measurements of the translational diffusion coefficient of a protein that is weakly bound to a phospholipid membrane surface. The measured values of the NBD-PC, NBD-PS and BF1 diffusion coefficients are interpreted with several models for lipid and protein diffusion in or on two-dimensional fluid bilayers. This work was supported by NSF grant DMB-9024028.

W-Pos210

CALORIMETRIC AND SPECTROSCOPIC STUDIES OF THE INTERACTION OF *MANDUCA SEXTA* APOLIPOPHORIN III WITH ZWITTERIONIC, ANIONIC AND NONIONIC LIPIDS.

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The interactions of *Manduca sexta* Apolipophorin III (apoLp-III) with a variety of zwitterionic and anionic phospholipids and with two nonionic glycolipids was investigated by DSC and ^{31}P-NMR. MGDG, PE and PA (pH 7.2) exhibit the weakest interaction with apoLp-III. The main phase transition of these lipids is little affected by the binding of apoLp-III. DGDG, PC, PS and PA (pH 8.8) exhibit somewhat stronger interactions with apoLp-III. ApoLp-III induces the formation of lipid domains which melt less cooperatively and at higher temperatures than do the pure lipids, whilst having little effect on the melting enthalpy. ApoLp-III severely disrupts their bilayer structure of PG and PA (pH>9.3) resulting in marked reductions in the cooperativity and enthalpy of the main phase transition of the lipids. Our results indicate that the binding of apoLp-III to lipid bilayers is mediated primarily through polar and/or ionic interactions at the lipid bilayer surface. Our results also suggest that the interaction of apoLp-III with lipid bilayers promotes the hydration of their surfaces, a property which is consistent with the proposed *in vivo* functions of this protein. (Supported by the Medical Research Council of Canada, the Alberta Heritage Foundation for Medical Research, and a U.S. National Institutes of Health grant.)

W-Pos211

THE ROLE OF DRUG-LIPID INTERACTIONS IN THE BIOLOGICAL ACTIVITY OF MODULATORS OF MULTI-DRUG RESISTANCE (MDR)

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A wide range of compounds have now been shown to circumvent acquired drug resistance in cultured cells. Thus far, little or no structure-activity relationship has been found among these compounds, although their proposed mechanism of action is through modulation of function of a ~170 KD membrane glycoprotein known as p-glycoprotein (pgp). While it has been suggested that the mechanism of this inhibition is a direct interaction of the modulators with pgp, we show here that such a model seriously neglects the effects many of these compounds have on lipid physical properties. We have measured the interactions between 10 structurally diverse modulators of MDR and a variety of lipids by three techniques. We have examined the inhibitory effects of the modulators on the membrane binding of a fluorescent dye recognized by MDR, and have shown a good correlation of the measured K_i values with the effectiveness of these compounds *in vivo*. We have determined the effects of the modulators on detergent micellization, and have shown substantial changes on the cmc of the detergents in the presence of the drugs. Finally, we have examined the changes in model membrane "fluidity" induced by the modulators. The results, taken together, indicate that both direct and indirect interactions of MDR modulators with pgp should be considered in understanding the mechanism by which these compounds reverse multi-drug resistance.

W-Pos212

SURFACE HYDROPHOBICITY AND MEMBRANE INTERACTIONS OF *Bacillus thuringiensis* TOXIN cryIC.

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Interactions between the Bacillus thuringiensis delta-endotoxin cryIC and phospholipid vesicles were studied by means of fluorescence spectroscopy. Ability of cryIC to dissipate membrane potential was measured with a potential-sensitive dye $diSC_2(5)$. Surface hydrophobicity of the cryIC molecule was assessed by binding of a polarity-sensitive probe bis-ANS. It was found that (i) cryIC dissipates membrane potential by creating ion-permeable channels in lipid bilayers; (ii) the activity of cryIC is enhanced at low pH, which is parallelled by increasing hydrophobicity of the surface of the toxin molecule, and hence its increasing propensity to interact with the core of the lipid bilayer; (iii) the increased activity at low pH can be further enhanced by the presence of acidic phospholipids in the membrane, which indicates some involvement of electrostatic interactions in binding of cryIC to the membrane.

W-Pos213

INVESTIGATION OF SECONDARY AND TERTIARY STRUCTURAL CHANGES OF CYTOCHROME C IN COMPLEXES WITH ANIONIC LIPIDS USING AMIDE HYDROGEN EXCHANGE MEASUREMENTS : AN FTIR STUDY.

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The structure of cytochrome c bound to anionic lipid membranes (dioleoyl-,dimyristoyl-, dipalmitoyl- phospatidylglycerol, bovine heart cardiolipin) has been investigated by Fourier transform infrared spectroscopy. Only small changes in secondary structure were observed upon binding at temperatures below that of denaturation of the protein, and these were not coupled to the thermotropic phase transitions of the lipids. The denaturation temperature of the protein decreased by ca. 25°-30° upon binding. Large changes in the amide proton exchange characteristics, as monitored by the spectral shifts in the amide I band of the protein in D_2O, were observed on binding cytochome c to the lipid membranes. For the slowly exchanging population, the amide deuteration rates were nearly independent of temperature, whereas those of the bound protein increased by up to two orders of magnitude over the temperature range from 10°C to 40°C. A structural transition in the bound protein was detected as a discontinuous step in Arrhenius plots of the deuterium exchange rates which occurred at a temperature in the region of 22° to 29°C, depending on the lipid, far below that of denaturation. The temperature of this transition was determined by the physical state of the lipid, being 7° lower for the lipids in the fluid state than for those in the gel state, and occurred at an intermediate temperature, being controlled by the lipid chain-melting transition at 27°-28°C for complexes with dimyristoyl phosphatidylglycerol. These results provide evidence for a coupling of the tertiary structure of the membrane-bound protein with the physical state of the membrane lipids.

W-Pos214

pH DEPENDENT STABILITY AND MEMBRANE INTERACTION OF THE PORE FORMING DOMAIN OF COLICIN A ((A. Muga, J.M. Gonzalez-Manas, J.H. Lakey, F. Pattus, W.K. Surewicz)) Inst. Biol. Sci., NRC, Ottawa, Canada K1A OR6 and EMBL, Heidelberg, Germany.

Thermal stability of the pore-forming domain of colicin A was studied by high-sensitivity differential scanning calorimetry and circular dichroism spectroscopy. In the pH range between 8 and 5, the thermal denaturation of the protein in solution occurs at 66-69 deg C and is characterized by the calorimetric enthalpy of approximately 90 kcal/M. At pH below 5, there is a rapid pH-dependent destabilization of the pore-forming domain resulting in the lowering of the midpoint denaturation temperature and the decrease in the calorimetric enthalpy of denaturation. Circular dichroism spectra in the near and far ultraviolet show that the thermotropic transition is associated with collapse of the native tertiary structure of the pore-forming domain, while a large proportion of the helical secondary structure remains preserved. Association of the pore-forming domain with phospholipid vesicles of dioleoylphosphatidylglycerol results in total disappearance of the calorimetric transition, even at pH values as high as 7. Since lipid-binding also induces collapse of the near ultraviolet circular dichroism spectrum, these data indicate that interaction with the membrane facilitates a conformational change within the pore- forming domain to a looser (denaturated-like) state. These findings are consistent with the view that a flexible "molten globule" state is an intermediate on the pathway to membrane insertion of colicin A.

W-Pos215

INVESTIGATION OF THE CONFORMATIONAL DYNAMICS OF LEUCINE ENKEPHALIN AT A PHOSPHATIDYLCHOLINE INTERFACE BY SOLID STATE NMR AND MOLECULAR DYNAMICS. ((James P. Schwonek and Charles R. Sanders, II)) Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106. (Spon. by Thomas Gerken).

The low affinity binding of leucine enkephalin to phosphatidylcholine (PC) bilayers is a phenomenon which is probably relevant to the mode by which this opioid pentapeptide interacts with its receptors *in vivo*. Approximately 20 ^{13}C chemical shift anisotropies (CSA) and ^{13}C-^{1}H dipolar coupling constants from surface-associated leucine enkephalin were measured using unlabeled peptide and magnetically oriented model membranes composed of mixtures of dimyristoyl phosphatidylcholine and dihexanoyl phosphatidylcholine (Sanders and Schwonek, Biochemistry, *Biochem.* 31, 8898-8905 (1992)). Molecular dynamics (MD) simulations were run using the Dreiding force field with added energy/force terms modeling peptide-surface interactions in terms of electrostatic and hydrophobic peptide interactions with a dielectric gradient (derived from the work of Ram et. al., *Biophys. J.* (in press)). A program was written (SANDMODS) to back calculate CSAs and dipolar couplings from the ensembles of interconverting structures produced during the MD runs. In this manner we tested the simulations for their ability to reproduce the experimental data.

W-Pos216

THE MICELLE HYPOTHESIS OF ALPHA-CRYSTALLIN AGGREGATION: FORMATION OF PROTEOLIPOSOMES ((J.F. Koretz and L.W. Radlick)) Center for Biophysics and Dept. of Biology, Rensselaer Polytech. Inst., Troy, NY 12180-3590

α-crystallin, the major protein component of the mammalian eye lens, has been suggested to aggregate into micellar structures (Augusteyn and Koretz, 1987), based on primary sequence analysis, heterogeneity of native populations, and other factors. It has been shown more recently (Radlick and Koretz, 1992) that α-crystallin behavior under increased hydrostatic pressure and in its effect on aqueous surface tension are both consistent with the micelle hypothesis. Further to test this model, attempts were made to prepare proteoliposomes of α-crystallin and lecithin using four different standard methods and two different protein concentrations, one well above and one well below the critical micelle concentration. Under all conditions, proteoliposomes were formed, as validated by FPLC, sucrose gradient centrifugation, and other methods. These results indicate that the hydrophobic region of α-crystallin is unbroken by polar or charged groups, and the ease with which the proteoliposomes formed suggest that the α-crystallin aggregate is highly dynamic. It has been shown that α-crystallin is present in non-lenticular tissues, where it is believed to serve as a molecular chaperone and/or heat shock protein; this class of proteins is believed to function in part due to the accessibility of hydrophobic surfaces with which partially folded structures can interact. The present results are consistent with this possible function and mode of action.

Supported by grant EY02195.

W-Pos217

X-RAY SCATTERING WITH MOMENTUM TRANSFER IN THE PLANE OF THE MEMBRANE: SIZE AND DISTRIBUTION OF MEMBRANE PROTEINS.
((H.W. Huang, K. He, S. J. Ludtke, and Y. Wu)) Rice Univ., Houston, TX 77251-1892.

We demonstrate a technique for measuring x-ray (or neutron) scattering with the momentum transfer confined in the plane of the membrane, for the purpose of studying lateral organization of proteins and peptides in membrane. Unlike freeze-fracture electron microscopy or atomic force microscopy which requires the membrane to be frozen or fixed, in-plane x-ray scattering can be performed with the membrane maintained in the liquid crystalline state. As an example, the controversial question of whether gramicidin forms aggregates in the membrane was investigated. We used dilauroyl phosphatidylcholine (DLPC) bilayers containing gramicidin in the molar ratio of 10:1. Very clear scattering curves reflecting gramicidin channel-channel correlation were obtained, even for the sample containing no heavy atoms. Thallium ions bound to gramicidin channels merely increase the magnitude of the scattering curve. Analysis of the data shows that the channels were randomly distributed in the membrane, similar to a computer simulation of freely moving disks in a plane. We suggest that oriented proteins may provide substantial x-ray contrast against the lipid background without requiring heavy-atom labelling. This should open up many possible new experiments.

W-Pos218

MOLECULAR DYNAMICS SIMULATION OF A LIPID BILAYER-PEPTIDE SYSTEM.((K. V. Damodaran and Kenneth M. Merz Jr.)) 152 Davey Laboratory, Department of chemistry, The Pennsylvania State University, University Park, PA 16802.

Molecular dynamics simulations of a tripeptide Ala-Phe-Ala -O -tert -butyl interacting with a dimyristoylphosphatidylcholine (DMPC) bilayer have been performed. The head group hydration and the peptide-bilayer interactions have been examined with the help of head group-water pair distribution functions, molecular order parameters and by visual examination of the peptide-bilayer interaction sites. Comparison of the theoretical results to the experimental results of Jacobs and White (1) have also been made. The dynamics of the water, peptide and the lipid bilayer have been investigated using the velocity autocorrelation functions and mean square displacements. It has been observed that the peptide is mostly confined to the head group water interface region with only marginal penetration of the central residue into the bilayer as was observed experimentally.

1. Jacobs, R. E. and White, S. H. 1989. The Nature of the Hydrophobic Binding of Small Peptides at the Bilayer Interface: Implications for the Insertion of Transbilayer Helices. *Biochemistry* 28:3421-3437.

W-Pos219

PHYSICAL STUDIES OF THE INTERACTION OF FATTY ACIDS WITH SERUM BASIC PROTEIN III.
((Mahnaz Motevalli, Peter Kwiterovich, and Robert Steiner))
Johns Hopkins School of Medicine, and University of Maryland Baltimore County.

Basic protein III (MW 55,000, pl 8.7) is one of three novel basic proteins (BP) in Normal human serum that stimulates fatty acid incorporation into cellular lipids. BP III was isolated from normal serum using affinity and ion exchange chromatography.
From interaction of BP III with toluidinyl naphthalene sulfonate (TNS), BP III appears to contain one or more binding sites capable of combining with TNS to yield a fluorescent complex. The addition of sodium oleate (0.8 mM) or sodium palmitate (0.3 mM) to BP III results in a significant enhancement of tryptophan fluorescence (15% and 20%), indicating that both unsaturated and saturated fatty acids influence the micro-environment of one or more of BP III tryptophan. Further evidence for the interaction of fatty acids was obtained by addition of palmitate with a complex of BP III and TNS, resulting in a 20% reduction in and a blue shift of the TNS fluorescence. These data indicates that palmitate competitively displaces TNS from one or more of its non-polar binding sites on BP III. The effect of fatty acids on the structure of BP III may be important in understanding the role of BP III in fatty acid and lipid metabolism.

W-Pos220

NONBILAYER-FORMING LIPIDS INFLUENCE RHODOPSIN PHOTOCHEMICAL FUNCTION. ((Nicholas J. Gibson, Robin L. Thurmond, and Michael F. Brown)) Department of Chemistry, University of Arizona, Tucson, Arizona 85721.

Here we have tested the proposal that nonbilayer-forming lipids govern visual **signal transduction** by shifting the MI-MII conformational equilibrium of rhodopsin.[1,2] The influences of the membrane lipid headgroup and acyl chain composition were investigated using **flash photolysis** techniques. Bovine rhodopsin was combined with DOPC and varying amounts of DOPE to form membrane recombinants in which only the identity of the lipid headgroups was varied. The ratio of MII / MI produced by an actinic flash in recombinants with DOPC/DOPE was studied as a function of pH and increased with the amount of DOPE. At pH 7 a native-like headgroup ratio (1:1) was not sufficient to produce a native-like MII / MI ratio, but the DOPC/DOPE (1:3) sample yielded very close to full activity. Thus it is possible to reconstitute the signalling event in vision using non-native lipids. Moreover, when native-like headgroup ratios are used we find that docosahexaenoyl (22:6ω3) phospholipids are essential for optimal rhodopsin activity. The results are discussed in terms of the bulk **material properties** associated with the lipid/water interfaces including the area elastic stress and the curvature stress. [1]N.J. Gibson and M.F. Brown (1990) *Biochem. Biophys. Res. Commun.* 169:1028. [2]N.J. Gibson and M.F. Brown (1991) *Biochem. Biophys. Res. Commun.* 176:915. Supported by NIH Grant EY03754.

W-Pos221

BINDING OF FATTY ACIDS (FA) TO THE INTERPHOTORECEPTOR-RETINOID BINDING PROTEIN (IRBP). ((John Boylan*, Barbara Wiggert#, Gerald Chader#, Roberta Reed+ and James A. Hamilton*)) *Biophysics Department, Boston Univ. School of Medicine, 80 E. Concord St., Boston MA 02118, #National Eye Institute, NIH, Bethesda MD 20892, +Mary Imogene Bassett Hospital, 1 Atwell Rd., Cooperstown NY 13326

IRBP (145 Kd) is the major soluble protein in the interphotoreceptor matrix, the space between the pigment epithelial cells and the photoreceptors of the retina. In addition to binding retinoids, this glycoprotein binds unesterified FA noncovalently [Bazan et al., J. Biol. Chem. 260, 13677 (1985)]. We have studied the binding of two long chain FA, oleic (OA) and palmitic (PA), to IRBP by ^{13}C NMR spectroscopy and a competitive equilibrium binding assay. Equilibrium binding results showed two binding sites with affinities comparable to the first two sites of bovine serum albumin. The ^{13}C NMR spectra of a 2:1 mole ratio of ^{13}C-carboxyl enriched PA or OA to IRBP, showed two FA carboxyl signals of about equal intensity at 181.1 ppm and 182.2 ppm. At higher mole ratios, 1-2 additional carboxyl signals were seen between the two peaks representing the high affinity sites. The NMR spectra thus reveal several structurally distinct binding sites for FA on IRBP in which FA anions are stabilized by interactions with basic amino acids. When complexes of PA with IRBP (6:1 mole ratio) were added to FA-free phospholipid vesicles (1g phospholipid/g protein), ^{13}C spectra showed that 1-2 moles of PA were bound per mole of IRBP and the remainder to the vesicles. Thus, IRBP contains both high and low affinity binding sites for FA and can donate to, or accept FA from, membranes.

W-Pos222

LIPID-PROTEIN INTERACTIONS: THE ROLE OF BILAYER FREE VOLUME AND POLYUNSATURATION IN MODULATING RHODOPSIN ACTIVATION.

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Metarhodopsin II (meta II), the rhodopsin photointermediate which activates G_t, exists in metastable equilibrium with its precursor, meta I. Here, we extend our recent studies of the relationship between bilayer acyl chain packing free volume and K_{eq} for the meta I↔meta II equilibrium with measurements made on rhodopsin containing, large unilamellar vesicles composed of PC's with higher levels of polyunsaturation in the *sn-2* position (PDPC), and a dipolyunsaturated PC (DAPC). Analysis of the depolarization dynamics of the fluorescent membrane probe DPH yields a single parameter, f_v, which characterizes the lipid acyl chain packing free volume. K_{eq} was found to be linearly correlated with f_v for each lipid studied. The steepness of the K_{eq} - f_v correlation line indicates the ease with which meta II can form in any given bilayer. The slope of the correlation lines increased markedly with higher levels of polyunsaturation in the acyl chain at the *sn-2* position, and was further increased by the substitution of a second polyunsaturated acyl chain at the *sn-1* position. Acyl chain polyunsaturation, which leads to increased packing free volume, imparts unique properties to the lipid bilayer relative to the modulation of integral membrane protein conformational equilibrium. (Supported by NIH grant EY00548).

W-Pos223

^{2}H-NMR STUDY ON THE ORDER AND DYNAMICS OF A FATTY ACID COVALENTLY COUPLED TO GRAMICIDIN IN ORIENTED BILAYERS.

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We have used acylated analogs of gramicidin as a model to study the interaction between a covalently coupled fatty acid and the hydrophobic part of a membrane spanning protein in a bilayer environment. The acylchain was covalently coupled to the C-terminal ethanolamine group of gramicidin which is located near the membrane interface, mimicking a situation as found in acylated proteins. Either perdeuterated palmitic acid, or palmitic acid deuterated at only C2, C3, C5-C6, C7-C8, C9, or C13 was coupled to gramicidin and examined by ^{2}H-NMR in oriented bilayers. In this way, quadrupolar splittings of deuterons at specific carbons were assigned. The quadrupolar splittings and T_1 values were compared to those of free palmitic acid in oriented bilayers, with and without gramicidin. The results indicate that the covalently coupled fatty acid is highly immobilized near the carboxyl-terminus because double quadrupolar splittings and very low T_1 values (4 ms) are found for the -CD_2- deuterons at carbon atoms C2 and C3. Control experiments with free fatty acid show single quadrupolar splittings and higher T_1 values (13 ms) for this segment of the fatty acid. In contrast, the methyl-end (C10-C16) of the covalently coupled fatty acid has quadrupolar splittings and T_1 values very similar to that found for free fatty acids. This indicates that the order and dynamics of the fatty acid near the methyl-end is dominated by the surrounding lipids and is not significantly affected by the neighboring peptide.

W-Pos224

THE MOLECULAR SPRING MODEL FOR DOCOSAHEXAENOIC ACID FUNCTION IN BIOLOGICAL MEMBRANES. ((L.L. Holte and E.A. Dratz)) Dept. of Chem. & Biochem., Montana State Univ., Bozeman, MT 59717

No generally accepted model for the function of polyunsaturated fatty acids in biological membranes has emerged to date. We have proposed that docosahexaenoic acid, 22:6ω3, may aid in protein conformational changes required for the function of rhodopsin in rod outer segment disk membranes (Proc. 3rd Int. Cong. on Essential Fatty Acids and Eicosanoids, Adelaide, Aust., 1992, *J. Am. Oil Chem. Soc.*, in press). We propose that 22:6 exists in a compact, coiled conformation that thins the membrane. Upon light excitation, rhodopsin is thought to open up and expand in planar area. The 22:6 may facilitate the opening of rhodopsin, especially at the metarhodopsin II (MII) intermediate, by reducing lipid planar area and thickening the bilayer. A key prediction of the molecular spring model is that after rhodopsin is excited by light the lipid bilayer becomes thicker.

Rhodopsin was reconstituted in membranes containing $16{:}0_{ds}$ 22:6 PC/PE/PS (3:3:1). Absorbance measurements were taken on a thin film of the solid membranes to characterize rhodopsin intermediates formed after bleaching and wideline deuterium NMR spectra were measured over the same time scale. Sample temperature was maintained at -13.5°C to better stabilize MII and other products close to MII after bleaching. The average ^{2}H NMR order parameter, $\langle S_{CD} \rangle$, was calculated from the first moment of the deuterium spectrum. $\langle S_{CD} \rangle$ is proportional to the length of a hydrocarbon chain. A series of measurements after the sample was bleached showed that $\langle S_{CD} \rangle$ increased 6% relative to pre-bleaching. This increase in average order implies that the fatty acid chains become more extended and is consistent with an increased bilayer thickness when rhodopsin is bleached, as predicted by the molecular spring model.

W-Pos225

DETERMINATION OF COLICIN E1 MEMBRANE-BOUND TOPOGRAPHY BY DEPTH-DEPENDENT FLUORESCENCE QUENCHING ((L.R. Palmer* and A.R. Merrill)). Guelph-Waterloo Center for Graduate Work in Chemistry, Dept. of Chem. & Biochem., Univ. of Guelph, Guelph, Ont., N1G 2W1.

Previously, conformational changes of the colicin E1 channel peptide associated with the insertion-competent state as determined by fluorescence methods were reported (Merrill *et al.*, Biophys. J. 59, 458a, 1991). Also, part of the structure of the colicin E1 channel in the membrane, including the helical membrane anchor domain and the voltage-sensitive region has recently been determined (Merrill and Cramer, Biochemistry 29, 8529-8534, 1990). In the present study, a number of single tryptophan-containing peptides of colicin E1 were prepared to facilitate the use of the tryptophan as a probe for the topography of the COOH-terminal channel peptide in the membrane-bound state. The membrane-associated form of the channel peptide was studied by binding protein to POPC/POPG LUV's (60:40, mol:mol; 0.1 μm dia. vesicles prepared by the extrusion technique) at low pH (pH < 4). The uniformity of the size distribution of both the liposomes and proteoliposomes was assessed using the quasi-elastic light scattering technique. Depth-dependent fluorescence quenching (Parallax Method) involving nitroxide-labeled phospholipids was used to determine the membrane location of a number of tryptophan residues for the vesicle-bound peptide. The three naturally occurring Trp residues in the colicin channel peptide, W-424, W-460 and W-495, were found to reside at membrane depths (from the C-2 carbon of the fatty acyl chains) of 5.9 ± 0.8 Å, 2.4 ± 1.0 Å, and 6.7 ± 0.7 Å, respectively. Additionally, the membrane depths of Trp residues in the single Trp channel peptides, W-507, W-443, W-431, and W-413 were 3.2 ± 0.9 Å, 6.4 ± 1.0 Å, 8.4 ± 1.4 Å, and 6.6 ± 0.9 Å, respectively. In light of these results, a model for the membrane-associated structure of the colicin E1 channel peptide is proposed [supported by the Medical Research Council of Canada, ARM].

W-Pos226

DISTRIBUTION ANALYSIS OF MEMBRANE PENETRATION BY DEPTH DEPENDENT FLUORESCENCE QUENCHING

(Alexey S. Ladokhin) Biology Department and McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, MD 21218.

A new approach is presented to evaluate depth dependent quenching (by brominated lipids, spin labels or other contact quenchers) of the fluorescence of membrane bound probes and integral proteins. Fluorescence intensities (F) should be measured as a function of vertical distance from the bilayer center to the quencher (h) and normalized to the concentration of quenchers. At least 3 measurements with the quenchers of known and distinctly different depth are required as well as a control measurement with all nonquenching lipid (F_0). These data are fitted to the following: $F_0/F(h) = \exp\{G(S,\sigma,h-h_m)\}$, where G is the Gaussian distribution. Position of the maximum (h_m) represents the most probable depth of the probe; dispersion (σ) will depend on 1) size of probe and quencher and 2) thermal fluctuations of both; area (S) is a function of quenching efficiency and concentration of quenchers. The latter parameter could be normalized to the S of another system to yield relative exposure of particular chromophore to lipid phase. For instance exposure of tryptophanyls in integral proteins could be quantitatively determined with respect to the model compound (tryptophan octyl ester). A number of published data were reevaluated with the proposed method. (Supported by NSF Biological Research Centers Award, DIR-8721059 and W.M. Keck Foundation Award.)

W-Pos227

FLUORESCENCE LIFETIME HETEROGENEITY AS A PROBE OF PROTEIN-LIPID INTERACTIONS: USE IN DETECTING MODIFICATIONS RESULTING FROM CHRONIC ETHANOL INTOXICATION.

((C. Ho, M. B. Kelly and C. D. Stubbs)) Department of Pathology and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107.

The membrane protein-lipid interface of liver microsomes prepared from animals subjected to chronic ethanol ingestion was probed using diphenylhexatriene (DPH) attached to phosphatidylcholine (DPH-PC). It was found appropriate to describe the fluorescence decay as a range of decay rates, approximated by fitting the data to a bimodal lifetime distribution. Thus, the DPH-PC experienced excited state environmental heterogeneity, a property of the protein-lipid interface, since for vesicles of extracted lipids the decay was homogeneous. The degree of environmental heterogeneity, assessed from the width of the distribution at half-maximum, was found to markedly increase as a result of the chronic ethanol treatment, while the lifetime center decreased. These effects are presumably due to the lipid compositional changes known to occur as a result of ethanol ingestion. Upon addition of ethanol, the width of the distribution increased, indicating increased environmental heterogeneity. However, these effects were diminished in microsomes from the ethanol-fed animals (membrane tolerance), indicative of an adaptation to the presence of ethanol. Thus membrane tolerance appears to be manifest at the protein-lipid interface, an effect that could be responsible for functional effects of ethanol ingestion. Supported by US Public Health Grant AA08022.

W-Pos228

FLUORESCENCE DYNAMICS OF RECOMBINANT HUMAN STEROL CARRIER PROTEIN-2: INTERACTION WITH STEROLS.

((S.M. Colles, J.K. Woodford, S.C. Myers-Payne, D. Moncecchi, L.R. McLean, J.T. Billheimer, G. Nemecz, and F. Schroeder)) University of Cincinnati, College of Pharmacy, Cincinnati, OH 45267. (Spon. by F. Schroeder)

Sterol carrier protein-2 affects several lipid and steroid synthesis reactions in vitro. SCP-2 may enhance these reactions through acting as an aqueous sterol carrier. We present for the first time data on human liver SCP-2 sterol interactions. Recombinant human liver SCP-2 bound the fluorescent cholesterol analog, dehydroergosterol, with a $K_D = 2.2 \pm 0.6$ μM. Lifetime analysis indicated dehydroergosterol was sequestered in a non-polar environment. SCP-2 contains a single tryptophan and no tyrosine residues. Analysis of the SCP-2 tryptophan emission spectra indicated the absence of energy transfer, in contrast to earlier findings with SCP-2 isolated from rat liver (Schroeder et al. J. Biol. Chem., 255:151, 1990) where changes in dehydroergosterol emission were interpreted as indicating possible energy transfer. Structural analysis of SCP-2 using circular dichroism suggested conformational changes within SCP-2 upon cholesterol binding. Acrylamide quench studies of tryptophan emission, in which the K_w decreased from 4.19 to 2.22 μM upon sterol binding, also provided agreement with these conclusions. Conformational changes were further substantiated through SCP-2 tryptophan lifetime analysis. In addition, recombinant SCP-2 enhanced intermembrane dehydroergosterol transport in the same manner as native rat liver SCP-2. Thus recombinant human liver SCP-2 appears to be an excellent source of protein for studying and characterizing human SCP-2. (Supported in part by USPHS, DK41402)

W-Pos229

ANALYSIS OF MEMBRANE PROTEIN SELFASSOCIATION IN LIPID SYSTEMS BY FLUORESCENCE PARTICLE COUNTING. APPLICATION TO THE DIHYDROPYRIDINE RECEPTOR.

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Fluorescence Particle Counting (FPC) is first employed to identify conditions for which purified Dihydropyridine Receptor (DHP-R) remains singly distributed in detergent micelles, in lipid vesicles, and in lipid monolayers generated from the lipid vesicles. In monolayers, the DHP-R showed selfassociation starting from monomeric distribution. The average molecularity (m(t)) of associates was followed by FPC in time as well as the lateral diffusion constant (D_{lat} (m,π)) for particular values of m and of the surface pressure (π). By studying m(t) in dependence of DHP-R concentration (c), π, $D_{lat}(\pi)$, and salt concentration (c_s) an empirical expression for the association rate (k_a) and for m(t) is derived, which fits the experimental m(t) relations. Theoretical justification for these expressions is obtained from general collision theories. DHP-R association is irreversible, its rate is not diffusion limited but a large number of collisions is required to overcome an interaction energy barrier of about 6 to 11 kT, depending on m and c_s but not on π. The increase in association rate with increasing m is related to van der Waals attraction, while the increase in rate with increasing c_s relates to decreasing electrostatic repulsion. The energy barrier is governed, however, by a third force, most likely hydration forces, not dependent on the variables studied.

W-Pos230

PERCOREGULATION OF LIPOLYSIS AND IMPLICATIONS FOR LIPID - MEDIATED SIGNAL TRANSDUCTION.

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In mixed monolayers of prelabelled 13,16-docosadienoic acid ($^{18}O,^{18}O$-DA) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), both the extent and mechanism of lipase-catalyzed ^{18}O exchange exhibit characteristics of a critical transition at 0.5-0.6 mole fraction (MF) of DA [Biochemistry 31, 141 and 149 (1992)]. These results are consistent with reactant percolation being a regulator of lipase activity in monolayers (J. Biol. Chem., in press). To determine if this critical behavior is relevant to bilayer membranes, unilamellar vesicles of $^{18}O,^{18}O$-DA and POPC were used. In the range of 0 to 0.625 MF of DA, the ^{18}O exchange occurred in a lipid-composition, enzyme-concentration and time-dependent manner. The reaction proceeded to completion with long incubations or high enzyme levels. Both a critical increase of activity and shift in mechanism were observed. Kinetic analysis using a double exponential decay model suggests coexistence of two equal pools of DA representing, presumably, DA on the inside and outside of the bilayer leaflets. Overall, the data suggest that the percoregulation model for lipolysis is also relevant to substrate-containing bilayer membranes and may play an important role in lipid-mediated signal transduction. [Support: NIH Grants HL17371, HL49180 and Hormel Foundation]

W-Pos231

CHOLESTEROL AND PHOSPHOLIPID UNSATURATION MODULATE PROTEIN KINASE C ACTIVITY.

((S.J. Slater, C. Ho, F.J. Taddeo, M.B. Kelly, and C.D. Stubbs)) Department of Pathology and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107.

Protein Kinase C (PKC) is a Ca^{2+}, Mg^{2+}, anionic phospholipid (e.g. PS), and *sn*-1,2-diacylglycerol dependent enzyme that is important in signal transduction. It is hypothesized that the conformational change and/or insertion that the enzyme undergoes on binding to the cell membrane may be influenced by bilayer structural parameters, such as lipid order ("fluidity"). To investigate this possibility, the effects of cholesterol, and altering the PC unsaturation were determined in vesicles comprised of PS and PC (1:4 molar). The fluorescence anisotropy of diphenylhexatriene was used to assess lipid order. It was found that cholesterol, which profoundly increases lipid order, inhibited PKC in a concentration dependent manner, while increasing PC unsaturation, which decreases lipid order, activated the enzyme. A plot of the values for activities and lipid order from these two experiments revealed a simple relationship between activity and lipid order. Further, the two data sets formed a continuous curve, which at a value of lipid order corresponding to ~four double bonds/PC reached an optimal value, after which activity declined. This suggested that there may be an optimal "lipid order" or related parameter(s) for optimal insertion and therefore of expression of PKC activity.

W-Pos232

THE SPECIFIC VOLUME OF WATER IN REVERSE MICELLAR SOLUTIONS CONTAINING PROTEIN. ((P.C. Kahn, W. Urbach, and M. Waks Rutgers Univ., New Brunswick, NJ 08903, USA (PCK). Laboratoire des Systemes Moleculaires Organises, Univ. Rene Descartes, UFR Biomedicale and URA1458 CNRS, Paris, France (WU and MW).

Using small angle neutron scattering, Rahaman & Hatton (J. Phys. Chem. 95:1799-1811, 1991) recently determined the water core radii of reverse micellar solutions in which some of the micelles contained a molecule of chymotrypsin and some did not. We have analyzed their data, which, we find, cannot be explained unless the water in the micelles has a volume which differs, sometimes greatly, from normal.

In micelles prepared by shaking isooctane solutions of AOT, the detergent, with excess aqueous buffer containing the protein, the water collapses to specific volumes smaller than normal. When micelles are prepared by injecting small amounts of aqueous buffer containing protein into AOT in isooctane, the resulting micelles are not saturated with water, a single phase micellar system results, and the water is "stretched" to volumes exceeding that of bulk solvent.

W-Pos233

THE SPECIFIC VOLUME OF WATER IN REVERSE MICELLAR SOLUTIONS CONTAINING AOT, WATER AND ISOOCTANE. ((B. Etchells Basham and P.C. Kahn)) Department of Biochemistry & Microbiology, Rutgers Univ., New Brunswick, NJ 08903,

The specific volume of water in reverse micelles of sodium 2-bis (ethylhexyl) sulfosuccinate (AOT) in isooctane has been determined from high precision solution density measurements. The AOT concentration was held constant at 105 mM, the water concentration being allowed to vary over the entire range within which the micelles are stable at 25 C. At low $W_0 = [H_2O]/[AOT]$ the water collapses to a specific volume of approximately 0.5 ml/g. The specific volume rises rapidly with W_0 to a plateau at which its volume is 0.95 ml/g, about 5% less than bulk water. At no water concentration does it attain the normal value. The sharp volume collapse is due apparently to disruption of the hydrogen bonding lattice which maintains the low packing density of this solvent.

W-Pos234

ELECTRON PARAMAGNETIC RESONANCE STUDIES OF THE EFFECTS OF METHOXYACETIC ACID ON ERYTHROCYTE MEMBRANES. ((Chafia Hegase de Trad,[a] Jinbo Lee,[b], and D. Allan Butterfield[b,c])), [a]Department of Physics, American University of Beruit, 850 Third Avenue, New York, 10022, [b]Department of Chemistry and [c]Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506-0055.

Methoxyacetic acid (MAA) is a potent, teratogenic toxic chemical formed as a metabolite from methoxyethanol, a compound used as an anticing agent in jet fuels and other applications. MAA has a long biological half-life (20 h); therefore, repeated daily exposures to MAA is dangerous because the chemical accumulates in the organism. The interaction of methoxyacetic acid with human erythrocyte membranes was investigated using electron paramagnetic resonance spin labeling techniques. Cytoskeletal proteins were selectively spin labeled with MAL-6 (2,2,6,6-tetramethyl-4-maleimidopiperidin-1-oxyl). The MAA decreased the motion of the spin label consistent with the idea that this agent increased cytoskeletal protein-protein interactions. In separate studies, MAA had no effect on the physical state of the lipid bilayer. Cell-surface carbohydrates were selectively and covalently spin labeled with the amine spin label Tempamine (2,2,6,6-tetramethyl-4-aminopiperidin-1-oxyl) by reductive amination. Information on the rotational rate of spin-labeled galactose and sialic acid residues was obtained from EPR spectra by calculating apparent correlation times. MAA appeared to cause a change in motion of cell-surface carbohydrates, with galactose residues on Band 3 affected the most. These results are discussed in terms of the toxicity of MAA. Supported in part by grants from NSF (EHR-9108764) and NIH (AG-10836) (D.A.B.).

W-Pos235

^{31}P NMR AND X-RAY DIFFRACTION STUDIES ON THE PHASE BEHAVIOUR OF N-BIOTINYL PHOSPHATIDYLETHANOLAMINES ((Musti J. Swamy, U. Würz* and D. Marsh))
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The structures of the phases formed in excess buffer (at pH 7.4) by a homologous series of saturated diacyl N-biotinyl phosphatidylethanolamines with chainlengths of C(12:0) to C(20:0) were investigated using ^{31}P NMR spectroscopy and low angle x-ray diffraction. In 1 M NaCl, all lipids display ^{31}P NMR spectra characteristic of a lamellar gel phase at low temperature. In the fluid phase, the lipids of C(12:0) and C(14:0) chainlengths display isotropic ^{31}P NMR spectra, corresponding to structures with high surface curvature, whereas those with C(18:0) and C(20:0) chainlengths display sharp axial powder patterns characteristic of a lamellar (L_α) phase. The lipid of intermediate C(16:0) chainlength displays a complex temperature dependence in the fluid phase. With increasing temperature the spectra convert from an axial powder pattern of unusually low chemical shift anisotropy to one characteristic of an L_α phase. The low angle x-ray diffraction patterns of the lipids in 1 M NaCl have lamellar repeat spacings in the gel phase which increase linearly with chain length and are consistently lower than those in the fluid phase [for chainlengths C(16:0) to C(20:0)]. The gradient in long spacings in the gel phase is approximately half that expected for a gel phase with untilted, all-*trans* chains, indicating that the lipid chains are interdigitated in the gel phase (L'_β). In the absence of salt, all the lipids are in a micellar phase at higher temperatures and those with chainlengths from C(16:0) to C(20:0) adopt a lamellar gel phase at low temperature, as deduced from ^{31}P NMR spectroscopy. On prolonged incubation at low temperature the C(14:0) lipid also forms a gel phase.

W-Pos236

FORMATION OF MEMBRANE DOMAINS INDUCED BY THE G AND M PROTEINS OF VESICULAR STOMATITIS VIRUS.
((P. Luan and M. Glaser)) Department of Biochemistry, University of Illinois, Urbana, IL 61801.

Vesicular stomatitis virus (VSV) buds from specialized regions or domains on the plasma membrane of infected cells. These domains are composed mainly of viral proteins and they are enriched in certain lipid species. The properties of the two envelope-associated proteins of VSV, the glycoprotein (G) and the matrix protein (M), were investigated in reconstituted lipid vesicles in order to understand the mechanism of virus budding and domain formation. Fluorescence digital imaging microscopy was used to determine if the proteins could alter the lateral distribution of the phospholipids in the vesicles. Large domains enriched in phosphatidic acid (PA) were formed when either G or M protein was reconstituted into the vesicles. Analysis of the images showed that M protein could further enrich the PA domains caused by G protein in the membranes. M protein also had the ability to induce the formation of domains enriched in phosphatidylserine (PS). When both G and M proteins were incorporated into vesicles at the same time, sphingomyelin was sequestered into the domains in addition. Thus, G and M protein together could induce the formation of large domains enriched in PA, PS, sphingomyelin and the viral proteins. Phosphatidylcholine (PC) was excluded from the domains. The polypeptide ionophore gramicidin was chosen to represent the distribution of an integral membrane protein when domains were induced in the vesicles by the two viral proteins. Gramicidin showed no lipid specificity and was preferentially excluded from the domains and became associated with the PC enriched areas in the vesicles. These observations suggest that the two envelope associated proteins of VSV may be important in the formation of domains during the process of virus budding from the plasma membrane of the host cells.

W-Pos237

DIACYLGLYCEROL IN PHOSPHATIDYLCHOLINE BILAYERS: A DISSECTION OF ITS INTERFACIAL STRUCTURE USING SOLID STATE NMR, DIACYLGLYCEROL ANALOGS, AND COMPUTATIONAL METHODS. ((Charles R. Sanders, II and James P. Schwonek)) Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106.

Previous high resolution NMR results (Hamilton et. al., JBC 266, 1177-1186 (1991)) have suggested that saturated diacylglycerol maintains an averaged conformation in phosphatidylcholine (PC) bilayers which is very similar to the averaged conformation of L_α PC and phosphatidylethanolamine. In order to obtain a more complete understanding of its interfacial structure and dynamics sn-1,2-dimyristoyl glycerol (DMDAG), sn-1,2-dimyristoyl-3-fluoro propanediol (DMFPD), and dimyristoyl ethylene glycol (DMEG) were synthesized in acyl perdeuterated $(d_{27})_2$ and carbonyl $(^{13}C{=}O)_2$ labeled forms. These compounds were dispersed at very low concentration (ca. 1:35, relative to PC) in magnetically orientable dimyristoyl PC bilayers (Sanders and Schwonek, *Biochem.* 31, 8898-8905 (1992)) and solid state ^{13}C and ^{2}H NMR spectra were acquired. In conjunction with molecular dynamics simulations, the spectra were evaluated in both qualitative and quantitative terms with respect to positioning of the lipids in the bilayer, their order profiles, acyl chain inequivalencies, and conformational dynamics.

W-Pos238

CONFORMATIONAL STUDY OF MEMBRANE BOUND SULFOQUINOVOSYL DIACYLGLYCEROL USING NMR-MOLECULAR MODELING METHODS.((Kathleen P. Howard and James H. Prestegard)) Department of Chemistry, Yale University, New Haven, CT 06511

SQDG (sulfoquinovosyl diacylglycerol) is a glycolipid which occurs widely in higher plants, algae and photosynthetic microorganisms. Aside from their natural function, SQDGs have recently attracted attention because of their inhibition of HIV-1 in cultured human lymphoblastoid T-cell lines. As with other glycolipids, their natural site of action is likely to be at a membrane surface. Hence, conformational properties in a membrane-like environment are of interest. Here we present a study of the membrane bound conformation of SQDG isolated from extracts of algae grown on ^{13}C enriched media. The study is conducted using methods which rely on the observation of dipolar interactions between neighboring spin 1/2 nuclei in ^{13}C labeled molecules. Dipolar couplings are measured in a model membrane system consisting of phospholipid bilayers which orient in an applied magnetic field. Molecular modeling using a version of AMBER modified to include a membrane interaction energy term is used to study the energetics of membrane surface interactions and aid in analysis of the experimental observations.

W-Pos239

RECOVERY OF PASSIVE HUMAN NEUTROPHILS AFTER SMALL DEFORMATIONS. ((R. M. Hochmuth, H. P. Ting-Beall, D. Needham, and R. Tran-Son-Tay)) Department of Mechanical Engineering and Materials Science. Duke University. Durham, North Carolina 27708-0300.

Passive neutrophils undergoing small deformations are studied by aspirating them into a glass pipet with a diameter that is only slightly smaller than the diameters of the spherically shaped cells. After being held in the pipet for at least seven seconds, the cells are rapidly expelled and allowed to recover their undeformed, spherical shapes. The recovery takes about 15 seconds. An analysis of the recovery process that treats the cells as simple Newtonian liquid drops with a constant cortical tension gives a value of $3.3x10^{-5}$ cm/s for the ratio of the cortical tension to cytoplasmic viscosity. This value is about twice as large as a previously published value obtained with the same model from studies of large deformations of neutrophils. This discrepancy indicates that the passive neutrophil does not behave like a simple Newtonian liquid drop in that small deformations are inherently different from large deformations. An extrapolated value for the cytoplasmic viscosity at zero deformation is about 600 poise when a value for the cortical tension of 0.0024 dyn/cm is assumed. More complex models consisting either of two or more fluids or multiple shells must be developed. The validity of a power law model for describing the mechanical behavior of passive neutrophils under deformations will be discussed. The complex structure inside the neutrophil is shown in scanning electron micrographs of osmotically burst cells and cells whose membrane has been dissolved away.

This work was supported by NIH Grant No. HL 23728

W-Pos240

EFFECT OF LIPID COMPOSITION ON HYDROCARBON CHAIN ORDER AND PHASE PREFERENCE IN *ACHOLEPLASMA LAIDLAWII* MEMBRANES: A ^{2}H-NMR STUDY. ((M.A. Monck[1], M. Bloom[2], R.N.A.H. Lewis[3], R.N. McElhaney[3]* and P.R. Cullis[1])) Depts. Biochemistry[1] and Physics[2], Univ. British Columbia, Vancouver V6T 1Z3 and [3]Dept. Biochemistry, Univ. Alberta, Edmonton, Canada T6G 2H7.

^{2}H-NMR has been applied to *A. laidlawii* B membranes containing roughly equal amounts of biosynthetically incorporated perdeuterated palmitic acid and a nondeuterated fatty acid of varying length or degree of unsaturation and various levels of cholesterol. At 37°C, the chain-average order decreases as the degree of unsaturation of the second fatty acyl group increases or as its chain length decreases, as expected. The presence of cholesterol increases the average orientational order of the hydrocarbon chains with all fatty acid compositions and at all temperatures studied. We also find that at the same absolute temperature the phosphatidylglycerol and monoglucosyldiacylglycerol components of the total membrane lipids possess a higher degree of hydrocarbon chain orientational order than do the diglucosyldiacylglycerol or glucolipid X components of similar fatty acid composition. This effect appears to reflect primarily the relatively higher gel to liquid-crystalline phase transition temperature of the former compounds as compared to the latter. In all cases the lipids of intact membranes remain in the lamellar phase over the temperature range studied, although nonlamellar phases can be formed by aqueous dispersions of the total membrane lipids at high temperatures. However, this organism does not coherently regulate the bilayer/nonbilayer phase transition temperature of the total membrane lipid dispersions, in contrast to the closely related A strain. We also find evidence for two populations of membrane-associated cholesterol in *A. laidlawii* membranes, one population residing in the lipid bilayer and the other in a different environment. Interestingly, the isolated glycerylphosphoryldiglucosyldiacylglycerol actually forms a micellar phase at 37°C. (Supported by the Medical Research Council of Canada.)

W-Pos241

THERMODYNAMIC BASIS FOR THE MOLECULAR INTERACTIONS OF LACIDIPINE WITH MEMBRANES.((Leo G. Herbette, Pamela E. Mason, Giovanni Gaviraghi*, R. Preston Mason and Mark Trumbore.))Departments of Radiology, Medicine, Biochemistry, Biomolecular Structure Analysis Center, University of Connecticut Health Center, Farmington, CT 06032; Glaxo Research Laboratories, Verona, Italy*

Lacidipine is a long lasting, 1,4 dihydropyridine (DHP) calcium channel antagonist. It is unique amongst the DHP antagonists in that it exhibits both a high membrane partition coefficient ($K_{P[mem]}$ = approximately 136,000 in the absence of cholesterol in the membrane) and a slow "waskout" rate (k_{wo} = approximately 60 minutes) from membranes. These two parameters may work to keep Lacidipine within the therapeutic "window" for activity longer than most other drugs in this class. Increasing cholesterol, results in a decrease in $K_{P[mem]}$ and a faster k_{wo}. These results are consistent with changes in the time averaged location of Lacidipine as the amount of cholesterol in the membrane is increased. Further analysis by two dimensional differential scanning calorimetry is providing a thermodynamic description of the ΔG for partitioning by decomposing it into its entropic and enthalpic components. Thus, this model at the membrane molecular level may, in part, explain the long duration of action of Lacidipine by a unique mechanism involving the environment of the target membrane bound protein receptor. Supported by a grant from Glaxo Research, Verona, Italy.

W-Pos242

X-RAY DIFFRACTION ANALYSIS OF ARTERIAL SMOOTH MUSCLE PLASMA MEMBRANE AS A FUNCTION OF DIETARY ATHEROSCLEROSIS.
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Small angle x-ray diffraction was used to examine oriented arterial smooth muscle (ASM) cell plasma membrane from atherosclerotic New Zealand rabbits. These studies demonstrated progressive changes in the structure of ASM plasma membrane as a function of time on a cholesterol augmented diet, including an increase in hydrocarbon core electron density and width. Membrane structure changes were directly related to the membrane unesterified cholesterol:phospholipid (FC:PL) mole ratio. Under conditions of severe atherosclerosis, in which the membrane FC:PL mole ratio increased two-fold to $\geq$0.9:1, two separate lamellar phases were observed at 37°C in the oriented membranes: a typical membrane lipid bilayer phase (56Å) and a cholesterol monohydrate phase (34Å). In samples with lower membrane FC:PL mole ratios (0.6-0.7:1), the cholesterol monohydrate phase was observed only at lower temperatures (<15°C) and was fully reversible upon warming the sample to 37°C. These data provide evidence for changes in the structure and lipid organization of ASM plasma membrane as a result of dietary atherosclerosis which may, in turn, account for changes in the function of certain membrane proteins. Supported by research grants from Pfizer Laboratories (RPM), AHA S.E. PA predoctoral fellowship (MC) and HL-30496 (TNT).

W-Pos243

X-RAY DIFFRACTION BASED STRUCTURAL STUDIES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR MEMBRANES ISOLATED FROM *T. CALIFORNICA* AND *T. NOBLIANA*. ((Howard S. Young, Victor Skita, Mark Trumbore, Rondi Butler, and Leo G. Herbette)) Biomolecular Structure Analysis Center , Univ. of Conn. Health Ctr., Farmington, CT 06030

The nicotinic acetylcholine receptor (AChR) is a neurochemically-gated cation-selective channel found in the neuromuscular junction of vertebrates and the electrogenic organs of marine elasmobranchs and teleosts. This receptor is a large molecular weight (~295kDa), oligomeric ($\alpha\beta\alpha\gamma\delta$ hetero-pentamer), integral membrane protein whose three-dimensional shape has been determined by electron microscopy and low-resolution x-ray diffraction. In the absence of a crystal structure, an x-ray diffraction based structure analysis of the native AChR membrane is currently being performed in our laboratory. The AChR-enriched membranes from *T. californica* and *T. nobliana* have been purified to near homogeneity (≥65% and ≥80% AChR, respectively). Fully oriented AChR membrane multilayers yield reproducible lamellar Bragg diffraction with a unit cell dimension of approximately 500Å for the double-membrane unit cell and a resolution of approximately 10Å. Due to the large unit cell size and the weak nature of the membrane diffraction from this system, the low angle diffraction data has been collected at a synchrotron and correlated with the higher angle diffraction data collected in our laboratory. The diffraction data appears to be Bragg diffraction modulated by continuous diffraction due to severe lattice disorder in these samples. The AChRs are organized in a hexagonal lattice in the plane of the membrane resulting in Bragg sampling at low angle. If d_{hk} is the spacing of the lattice planes (h,k), then the (1,0), (1,1), and (2,0) reflections are observed with a lattice constant of approximately 75Å. At higher angle, the in-plane structure gives rise to a diffuse ring at 4.6Å characteristic of liquid crystalline acyl-chain packing, and a 10Å diffuse ring characteristic of α-helix packing. Structure determination from this diffraction data is currently in progress. [Supported by Miles Pharmaceuticals Division, Miles Inc., a Univ. of Conn. Health Ctr. HCRAC Grant, and NIH 2P01HL18608]

W-Pos244

THE ORIGIN OF THE ANOMALOUS AMIDE I VIBRATIONAL FREQUENCY OF PURPLE MEMBRANE. ((John F. Hunt, Thomas N. Earnest‡, Olaf Bousché‡, Donald M. Engelman, and Kenneth J. Rothschild‡)) Yale University and (‡) Boston University. (Spon. by B. Kobe.)

Purple membrane (PM) exhibits an anomalously high amide I absorption frequency, at approximately 1668cm^{-1} in polarized FTIR difference spectra. Purple membrane contains trimers of the integral membrane protein bacteriorhodopsin arranged in a two-dimensional hexagonal lattice. We have produced monomeric BR by reconstituting the protein at a high lipid-to-protein ratio in DMPC. Monomeric BR exhibits a normal amide I frequency, at approximately 1659cm^{-1} in polarized FTIR difference spectra. The protein is photochemically active in this form, and its photocycle is very similar to that in PM as assayed by low temperature FTIR difference spectroscopy. Since this technique is exquisitely sensitive to structural perturbations, we conclude that the secondary and tertiary structure of BR are the same in the monomeric state and in PM, which implies that the anomalous amide I frequency of PM must be attributable to the quaternary structure of the protein. We hypothesize that the frequency shift is caused by transition dipole moment coupling between the peptide bond oscillators in the different α-helices. Model calculations support this hypothesis, indicating that an increase in the observed amide I frequency is expected in an extended parallel or anti-parallel array of α-helices compared to an isolated α-helix with an identical conformation.

W-Pos245

SARCOLEMMAL PROTEIN CHANGES IN CANINE SUBACUTE MYOCARDIAL INFARCTION.

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Previous studies have shown degradation of cardiac structural proteins and disruption of the sarcolemma as a result of acute myocardial infarction. However, there is no evidence to date on changes in sarcolemmal membrane proteins induced by experimental subacute myocardial infarction. We studied subepicardial layers overlying myocardial infarct 4 days following ligation of the left anterior descending coronary artery in 12 dog hearts. We first demonstrated that this layer provides the anatomic-electrophysiologic substrate for reentrant arrhythmias using activation mapping techniques and histologic correlations. The makeup of membrane proteins was studied using SDS polyacrylamide gel electrophoresis, peptide mapping, and laser densitometry. Sarcolemmal membrane proteins were isolated by ultracentrifugation through a sucrose gradient. We found that a sarcolemmal polypeptide (MW 126,000; n=12) in the normal tissues has a different mobility than the corresponding protein (MW 124,000; n=12) of the ischemic tissues although their peptide analysis appeared similar, suggesting that the protein undergoes a post-translational modification. In addition , two proteins (MW 75,000; n=12 and MW 88,000; n=12) were present in greater amount in the ischemic than in the control tissues suggesting either acceleration in protein synthesis or slow down of degradation turnover. These results demonstrate that specific changes occur in membrane proteins subjected to ischemic insults which might be responsible for membrane alterations following ischemia and may contribute to the abnormal electrophysiologic properties and arrhythmia seen in vivo at this stage.

W-Pos246

INTERACTION OF CHOLESTEROL WITH SYNTHETIC SPHINGOMYELINS IN MONOLAYERS: VARIATION IN THE POSITION OF A *CIS* DOUBLE BOND IN THE *N*-ACYL CHAIN.

((R. Bittman, C.P.R. Kasireddy, A-L. Östmann and J. P. Slotte))
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We demonstrated recently that sphingomyelin (SPM) interacts with cholesterol (Ch) by van der Waals rather than by specific hydrogen bonding interactions [*Biochemistry* **1991**, *30*, 7759 and 10746]. We synthesized a series of C(16:1)-SPMs in order to examine how the position of a single *cis* double bond in the *N*-acyl chain influences the Ch-SPM interaction. Force-area isotherms of monolayers at 20° gave the following mean molecular areas at 20 mN/m: Δ3, 39Å^2; Δ4, 38Å^2; Δ9, 69Å^2; Δ11, 48Å^2; Δ13, 45Å^2. All of the SPMs are in the liquid-crystalline phase at room temperature and all showed a collapse pressure of ~50 mN/m. In mixed Ch/SPM monolayers, the order of condensation of molecular packing was Δ9> Δ11> Δ4> Δ13> Δ3. Thus, the strongest interaction with Ch occurs when the double bond is positioned in the middle region of the *N*-acyl chain of SPM. The rate of enzyme-catalyzed oxidation of Ch indicated that Ch and Δ3-SPM are laterally segregated in monolayers. However, a break in the oxidation rate vs. Ch/SPM molar ratio plot was found at 2:1 Ch/SPM stoichiometry with Δ9, Δ11, Δ13 (as in egg and brain SPM: *Biochemistry* **1992**, *31*, 5472), and at 1:1 with C(16:1 Δ4)-SPM.

Supported by NIH Grant HL-16660 and by the Sigrid Juselius Foundation and Academy of Finland.

PHYSICAL PROPERTIES OF BILAYERS II

W-Pos247

STUDIES OF THE FRACTAL DIMENSION OF LIPID VESICLES.

((L. Huang, Y. X. Chen, M. Ge, B. D. Zion and M.F. Blackwell)) Department of Chemistry, Lawrence University, Appleton, WI 54912.

We report the results of theoretical and experimental investigations which suggest that lipid vesicles have a fractal structure on small time and distance scales. Molecular motion in lipid monolayers was studied by Monte Carlo calculations of random walks on square percolation lattices. After t random walk steps, the mean square displacement behaves as $\langle R^2(t) \rangle \propto t^{2/2+\theta}$ on time and distance scales for which the lattice has a self-similar (fractal) structure. The exponent θ is related to the fractal dimension of the medium. If the medium is homogeneous, θ=0; otherwise, θ>0. The solid membrane model, in which only one lipid performs a random walk and the rest are spatially fixed, gives us θ ~ 0.85 for 100 steps at the percolation threshhold p=pc. The fluid membrane model, in which all lipids are performing simultaneous random walks, gives values of θ that depend on both p and t, suggesting that fluid lipid monolayers have a fractal structure on small time and distance scales. We have also carried out experimental investigations of the fractal dimensionality of soybean phosphatidylcholine vesicles and ethanol. Quinone diffusion coefficients in PC vesicles, estimated by the probe fluorescence quenching technique, were found to depend upon the fluorescence lifetime (τ) of the probe. We measured θ from the slopes of plots of log10D vs. log10 τ and found that, in PC vesicles, θ ~ 0.7, whereas in ethanol, a homogeneous environment, θ~0. Pyrene monomer and excimer fluorescence kinetics studied in PC liposomes and in ethanol were analyzed assuming that the excimer formation rate coefficient is of the form $k \sim t^{-h}$. In ethanol the we found that h~0, corresponding to classical kinetics, whereas in PC liposomes h>0,suggesting once again that PC vesicles display a fractal geometry on the measurement time/distance scale. (Supported by ACS-PRF 24266-B4, NSF-ILI USE-9152434, and HHMI 71191-529201.)

W-Pos248

LIPID-CHOLESTEROL INTERACTIONS IN THE $P_{\beta'}$ PHASE: APPLICATION OF A STATISTICAL MECHANICAL MODEL . H.L. Scott and W. Scott McCullough, Department of Physics, Oklahoma State University, Stillwater, Ok. 74078.

We describe a statistical mechanical model for lipid-cholesterol mixtures in the $P_{\beta'}$ (ripple) phase of lipid bilayers. The model is a simple extension of an earlier model for the ripple phase in pure lipid bilayers. The extension consists of adding a degree of freedom to allow for the occupation of underlying lattice sites by cholesterol molecules, and adding a lipid-cholesterol interaction term to the model Hamiltonian. The latter term was constructed after numerical calculations of lipid-cholesterol energies were calculated for several different packing juxtapositions of the two molecules. The extended model does not contain any arbitrary parameters, and therefore the model serves as a test of the original model. Properties of the model were calculated using the Monte Carlo method. Results are displayed as snapshots of the ripple configurations at different cholesterol concentrations. The spacing of the ripples increases with increasing cholesterol concentration and the rate of increase compares very well with experimental data. The success of this model strongly supports the conclusion drawn earlier that frustration arising from anisotropic packing interactions is responsible for the ripple phase in lipid bilayers, and for the selective partitioning of cholesterol in the regions between the ripples.

W-Pos249

MONTE CARLO SIMULATION OF LIPID MIXTURES: FINDING PHASE SEPARATION. ((J. Huang and G. W. Feigenson)) Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Recently, we developed a treatment of the mixing of phosphatidylserine (PS) and phosphatidylcholine (PC) in a fluid bilayer to enable comparison of experimental results with a model that accounts for interactions that cause nonideal mixing. We now find that reasonable values for the nonideal interaction energy ΔE_m should result in lateral separation of fluid bilayer phases that differ in PS/PC ratio. Monte Carlo simulations of PS/PC mixing, using Kawasaki relaxation, are performed for various values of ΔE_m. Using Kirkwood's coupling parameter method, the excess free energy of the mixing is calculated. Plotting the total free energy of mixing against composition reveals whether phase separation occurs at each given value of ΔE_m. In this way, the Monte Carlo simulation corresponding to the maximum possible deviation from ideality for a one-phase mixture can be visualized.

W-Pos250

IR STUDIES OF THE STRUCTURE AND PHASE BEHAVIOR OF MIXED MONOLAYERS COMPOSED OF [d_{62}]-DPPC AND DOPG. A MODEL FOR PULMONARY SURFACTANT. ((A.D. Williams, R.A. Dluhy and F.R. Rana)) Department of Chemistry, University of Georgia, Athens, GA 30602

Combined IR and ^{31}P NMR studies will be presented that are designed to evaluate the postulated "squeezing out" mechanism of pulmonary surfactant physiology. The dynamic phase behavior of lipid monolayers at the air-water interface was measured for pure films and binary mixtures of [d_{62}]-DPPC and DOPG at 88:12 mol ratios, respectively. The structure of the monolayers was probed using ATR-IR spectroscopy. Langmuir-Blodgett techniques were used to transfer the monolayers to Ge ATR crystals at molecular areas representative of the different regions of the surface pressure-molecular area (π-A) isotherm. Wavenumber shifts in the C-H and C-D stretching regions (located between 3000-2800 and 2300-2000 cm^{-1}, respectively) were used to monitor the relative hydrocarbon chain conformation in the two lipid components. In addition, the IR dichrosim of the band intensities may be used to estimate the molecular orientation of each component. The exact fractional composition of each component in the binary mixture as a function of surface pressure may be obtained from correlating the IR and ^{31}P NMR intensities of calibration standards.

W-Pos251

SOLUBILIZATION STEPS OF DARK-ADAPTED PURPLE MEMBRANE BY TX-100: A SPECTROSCOPIC STUDY

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Ultraviolet-visible spectroscopy is used to follow the solubilization of the dark-adapted purple membrane (PM) of *Halobacterium Halobium* by Triton X-100. Evolutions of turbidity at 700nm and absorbance at 560 nm of purple membrane fragments and maximal absorption wavelength and intensity of bacteriorhodopsin were recorded during continuous addition of detergent for different [PM]s. Each experiment was monitored during 12 hours in order to allow the equilibration of the detergent partitioning between purple membrane and aqueous medium. All solubilization profiles exhibit several break points corresponding to transitions between the stages of the solubilization process. The analysis of the dependence of [TX100] on [PM] allowed the determination of the detergent to protein + lipid ratios in mixed aggregates at the corresponding break points. Interestingly, the evolutions of the turbidity of purple membrane and of the spectral characteristics of BR during the solubilization process do not follow the same pattern, indicating that the microenvironment of the solubilized BR in mixed micelles is still modified by increasing detergent concentration beyond PM micellization point. This technique is a convenient and powerful tool in the quantitative study of biomembrane to micelle transition.

W-Pos252

COMBINED IR SPECTROSCOPY AND SCANNING PROBE MICROSCOPY OF LANGMUIR-BLODGETT AND SELF-ASSEMBLED BIOMEMBRANE MONOLAYERS. ((S.M. Stephens[1], B.W. Gregory[1], R.A. Dluhy[1] and L.A. Bottomley[2])) [1]Department of Chemistry, University of Georgia, Athens, GA 30602, [2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332.

Current research in this laboratory involves studies of the structure of well-ordered biomembrane monomolecular films, prepared by either Langmuir-Blodgett (L-B) or self-assembled (SA) methods. We are presently using a combined spectroscopic/imaging strategy which uses IR spectroscopy to obtain compositional and conformational information and scanning tunneling/atomic force microscopies (STM/AFM) to acquire complementary spatial distribution and morphological information about these biomembrane models. This combined approach provides the kind of detailed information that is unobtainable by either of these methods alone. We have studied SA monolayers of alkanethiols (particularly $C_{18}H_{37}SH$) on Au substrates; we have also studied L-B monolayers of phospholipids (*e.g.* DPPC and DPPG) and lipid-peptide mixtures transferred to Ge and alkanethiol-covered Au substrates. Our initial investigations have focused on mixtures of PC or PG with the peptide alamethicin, which is known to form voltage-gated ion channels upon incorporation into lipid bilayers. Structural information obtained from our integrated studies will be presented.

W-Pos253

N-ACYLPHOSPHATIDYLETHANOLAMINES: EFFECT OF THE CHAIN LENGH ON THE ORIENTATION OF THE N-ACYL CHAIN

((Jean-Erik Blochet and Michel Pézolet)) CERSIM, Département de Chimie, Université Laval, Québec, CANADA

N-acylphosphatidylethanolamines (NAPE) have been isolated for the first time from wheat flour where they represent about 50% of the polar lipids. Later, they have been found in numerous tissues involved in degenerating processes (deshydrated endosperm of seeds, erythrocyte membranes, BHK cells) or cell injury (Ischemia). It has been shown that NAPEs stabilize liposomes. In order to determine the conformation and orientation of the N-acyl chain, NAPE with deuterated N-acyl chains have been synthesized and studied by transmission and ATR infrared spectroscopy. The results show that in the case of the N-C16 NAPE, the N-acyl chain has the same orientation as the two acyl chains, while for the N-C6 NAPE, the N-acyl chain is randomly oriented. These results demonstrate unambiguously that for the N-C16 NAPE, the N-acyl chain is completely embedded into the hydrophobic core of the bilayer while for N-C6 NAPE, the N-acyl chain remains in the polar environnement of the lipid head group.

W-Pos254

TREHALOSE PRESERVES MEMBRANES AND PROTEINS IN INTACT CELLS. ((S.B. Leslie[1], E. Israeli[2], and J.H. Crowe[1])) [1] Dept. of Zoology, University of California, Davis CA 95616 and [2]Institute of Biological Research, PO Box 19, Ness-Zione, Israel.

Trehalose has previously been shown to preserve the structure and function of liposomes, isolated biological membranes, and isolated proteins during dehydration (Crowe *et al.*, 1988. *BBA*). In the present work we investigate the ability of trehalose to stabilize membranes and proteins in intact *Escherichia coli* and *Bacillus thuringiensis*. Both species show a substantial increase in survival when dried in the presence of trehalose compared to cells dried without trehalose. Fourier transform infrared spectroscopy (FTIR) was used to investigate the effect of trehalose on the membranes of each organism. *E. coli* dried with trehalose show a gel to liquid phase transition (T_m) 35°C lower than those dried without trehalose. Trehalose had a similar effect on *B. thuringiensis*, lowering the T_m from 45°C in cells without trehalose to 8°C. In both organisms the T_m of the cells dried with trehalose was near that of the hydrated cells. Use of an FTIR microscope made it possible to investigate protein within cells by observing the amide I and amide II bands. While values obtained are an average for all the proteins in the cell, they indicate overall protein condition. In both organisms the amide II band shifts from 1545 to 1530 wavenumbers when the cells are dried without trehalose. When dried with trehalose there is no shift of the amide II band, indicating the proteins remain in their hydrated conformations. (Supported by NSF grant DCB89-18822).

W-Pos255

OBSERVATION OF MERIDIONAL X-RAY SCATTER FROM A DIPALMITOYL-PHOSPHATIDYL CHOLINE MONOLAYER AT THE AIR-LIQUID INTERFACE.

((V. Skita[1], H.S. Young[1], R.A. Butler[1], D.W. Chester[1] and R.F. Fischetti[2])) [1]Univ. Ct. Health Ctr, Farmington, CT 06030 and [2]Biostructures Inst., Phil., PA 19104.

Pulmonary surfactant is a complex mixture of phospholipids, neutral lipids, glycolipids, and protein at the air-liquid interface of the alveoli. It is composed primarily of phospholipid of which dipalmitoylphosphatidyl choline (DPPC) is the largest constituent. The surfactant monolayer significantly reduces the surface tension at the air-liquid interface at functional residual capacity (FRC), thus reducing the amount of work required to inflate the lungs. Structural information as to the conformation and interaction of the various components of the surfactant monolayer may lead to the development of better replacement compounds, as well as improve our basic understanding of how phospholipids and proteins behave at an air-liquid interface. We report the observation of meridional $(0,0,s_z)$ scatter from a DPPC monolayer spread at a Langmuir surface (z axis $\perp$ to the liquid surface). An optical flat was oriented in the experimental hutch to deflect a monochromatic (11.3 keV) x-ray beam and provide control of the grazing angle, ω, of the incident beam with the Langmuir surface. The x-rays were focused vertically (280μm) at the center of a KSV mini-trough and horizontally (670μm) at the detector (373mm from trough center). Real time observation of the specular reflection of the incident x-ray beam (ω = 1.4 mrad) from the air liquid interface was used to verify and optimize our alignment. At surface pressures ranging from 20 - 40 mN/m we observed three broad maxima along the meridian corresponding to the structure factor modulus squared of a single monolayer. The observed intensity is qualitatively very similar to the scattering observed from a hexadecyltrichlorosilane monolayer covalently bound to a solid substrate. In addition, we observed that with increasing x-ray dosage both the intensity of the scattered x-rays and the monolayer surface area (at constant Π) decreased, presumably due to radiation damage. The authors would like to thank Mr. Greg Wills of KSV Instruments (Riverside, CT) for the use of their mini-trough. [Supported by NIH HL45284, NIH RR01633, and the ALA National Res. Award]

W-Pos256

AQUEOUS PROPERTIES OF POLYMER LIPID DERIVATIVES: LIGHT SCATTERING OF PEG-PE.

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Incorporation of polymer lipid derivatives such as polyethyleneglycol-distearoylphosphatidylethanolamine (PEG-PE) in liposomes reduces both their interaction with cells and their uptake in vivo which has been attributed to formation of a steric barrier on the liposome surface (Woodle and Lasic, 1992, BBA 1113, 171-199). These lipids are also interesting in that they form clear solutions in water at up to relatively high concentrations (>10 mg/ml) with the ability to solubilize egg phosphatidylcholine when present at greater than about 40 mol% as with single chain PEG surfactants ($C_{12}E_8$). Properties of PEG-PE in pure water and 0.9% saline were studied by both static and dynamic light scattering. The results show behavior expected for nonionic surfactants with these ionic materials: a cloud point like phenomena observed with ^{5000}PEG-PE increases with temperature but decreases with increasing ionic strength. The shorter ^{2000}PEG-PE doesn't exhibit it in the concentration and temperature ranges examined. Contrary to expectations, substitution of distearoylamine for DSPE as the hydrophobic lipid portion forms a neutral derivative which doesn't exhibit the cloud point like phenomena but is ionic strength independent. Other differences in the micelles formed with the latter lipid analog were also found and will be described. The results will be compared with those from single chain surfactants ($C_{12}E_8$ and $C_{16}E_8$) and discussed with respect to biological relevance.

W-Pos257

BILAYER STRUCTURE AND LATERAL ORGANIZATION OF A LONG-CHAIN DIACETYLENIC PHOSPHATIDYLCHOLINE.

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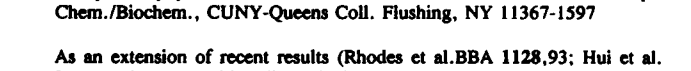
As an extension of recent results (Rhodes et al.BBA 1128,93; Hui et al. Langm., in press) with a diacetylenic phosphatidylcholine (DAPC), bilayers of 1,2-bis(pentacosa-4,6-diynoyl)-sn-glycero-3-phosphocholine (1) were



(1)

investigated using x-ray diffraction and electron diffraction. Monolayers of this lipid form domains at $\pi > 14$ dyn/cm. Electron diffraction data indicated that the chain spacing is 4.25 Å and that the chains are tilted at angle of ~35° relative to the bilayer plane. X-ray data agreed and showed that the lamellar repeat was 70 Å at high humidity and ≤60 Å at low humidity. The bilayer electron density profiles indicated a bilayer structure with no interdigitation. High angle reflections indicate that the principal acyl chain repeat is preserved as a function of hydration but some rearrangement occurs for other reflections. The ~10Å reflection corresponding to the headgroup spacing previously observed with DAPC bilayers was not observed. The results are interpreted in terms of a packing model and possible limitations or constraints to the polymerization process. Support: NSF (CTS-8904938, DGR), Glaxo (DGR), NIH (HL-16660, RB and GM-28120, SH)

W-Pos258

FREEZING AND MELTING OF ICE IN LAMELLAR LIPID-WATER DISPERSIONS

((J. T. Gleeson, M. E. Wall, Shyamsunder Erramilli & S.M. Gruner)) Physics Dept., Princeton University, Princeton, NJ, USA

Small- and wide-angle x-ray diffraction, as well as dielectric response measurements, were used study the formation and melting of ice in lamellar dispersions of phospholipids in water. For dielaidoylphosphatidylethanolamine, we find: (i) At hydrations greater than 15%, ice crystals form on cooling at $\approx -12°C$, and on heating, melt abruptly at $\approx 0°C$, (ii) At hydrations between 15% and 9%, ice crystals do not form unless cooled to as low as $-30°C$; on heating, the ice line intensity decreases continuously with temperature, disappearing altogether at $\approx -8°C$. (iii) At hydrations less than 7%, ice is not observed down to at least $-34°C$. Ice formation is always accompanied by an abrupt decrease in the unit cell spacing of the lamellar lattice, a, to a value below that observed in samples sufficiently dehydrated so that ice does not form. This suggests that water must be expelled from the lattice to freeze, and that interlamellar ice does not occur. It has been suggested that sufficiently cold water which does not freeze must become glassy. Frequency-dependent dielectric susceptibility measurements are employed to investigate this possibility.

Work supported by the Office of Naval Research, U.S. Department of Energy and the National Institutes of Health.

W-Pos259

X-RAY LINEWIDTHS FROM DPPC LIPID BILAYERS AND INTENSITY CORRECTION FACTORS FROM UNDULATION THEORY.

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Our measurements show that the h=1 and h=2 low angle peaks of fully hydrated $L_{\beta'}$ phase of DPPC, measured with instrumental resolution of 0.011°, are very narrow, with half widths at half maximum scattering angle 2θ (HWHM) of about 0.018° for an intrinsic HWHM (IHWHM) of 0.014°. At the same resolution, the h=1 peak of the L_α phase is even narrower, being resolution limited, but the h=2 peak is broader (IHWHM = 0.02°), the h=3 peak is very broad and small, and no higher orders are observable. In contrast, we observe that the higher order peaks of the gel phase broaden only gradually after applying the correction for sample size. Using the undulation-fluctuation theory of Caille' and DeGennes our calculations exhibit a strong decrease in peak intensity with increasing order h accompanied by a much smaller increase in width. We conclude that the absence of h>3 peaks for the L_α phase is due to undulations and that the intensity of even the h=2 peak is strongly corrupted for the purpose of obtaining single bilayer structure factors.

W-Pos260

THE STRUCTURE OF PIG STRATUM CORNEUM. A WIDE AND SMALL ANGLE X-RAY STUDY.

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The outermost layer of the skin, the stratum corneum consists of corneocytes embedded in lipid lamellar regions. The lipids mainly consist of ceramides, free fatty acids, cholesterol and frequently triglycerides. Since the stratum corneum is the main diffusional barrier for substances through the skin, an understanding of the lipid arrangement is of predominant importance.

In this study the structure of pig stratum corneum has been studied as function of hydration and temperature using X-ray diffraction. The lipid structure has also been studied after recrystallisation of the lipids from several temperatures.

The combination of all the data led us conclude that the lipids are arranged in at least two lamellar structures with repeat distances of 6 and 9 nm. The lamellar structure became disordered between 60 and 66°C. Upon hydration no swelling of the bilayers was observed. The lateral packing of the lipids is partly hexagonal and partly liquid. After recrystallisation from 120°C only one lamellar structure was observed with a repeat distance of 13.0 nm.

W-Pos261

PHASE BEHAVIOR OF MEMBRANE LIPIDS CONTAINING POLYENOIC ACYL CHAINS.

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The low temperature thermal behavior of di-18:2 phosphatidylcholine and di-18:3 phosphatidylcholine is shown to be characterized by similar broad low-enthalpy transitions to those previously reported for polyenoic samples of phosphatidylcholines (Keough and Kariel (1987) Biochim. Biophys. Acta, 902, 11-18). Real-time X-ray diffraction measurements indicate that these transitions correspond to transitions between the gel (L_{β}) and liquid crystal-line (L_{α}) phases of the lipids. The gel phase of these lipids is, however, much more loosely packed than the corresponding phases of membrane lipids containing monoenoic or fully-saturated acyl chains. The low enthalpy and reduced co-operativity of the L_{β}→L_{α} phase transitions of the polyenoic lipids is attributed to the reduced contribution of van der Waals interactions between their acyl chains in the gel-state of these lipids rather than an inherent inability of polyenoic chains to organize into regular arrays.

W-Pos262

TIME-RESOLVED PRESSURE-JUMP STUDIES ON MEMBRANE LIPIDS BY SYNCHROTRON X-RAY DIFFRACTION.

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The pressure induced structural phase transitions of membrane lipids were investigated by time-resolved X-ray diffraction at the synchrotron beamline X9A at NSLS. Fast pressure jumps (7 ms) of lipid-water suspensions were achieved within a high-pressure X-ray specimen cell capable of withstanding pressures up to 4 kbar. Simultaneously X-ray diffraction patterns were recorded with 9 ms time-resolution. We studied the transition kinetics of some phosphatidylethanolamine (PE) lipids, and the glycerolipid monoelaidin as a function of p-jump amplitude at various temperatures. The PE-lipids undergo transformations between lamellar and inverted hexagonal phases. We observed that the transition kinetics and dynamics depended on the magnitude of the jump applied. When using jump amplitudes > 1 kbar, the phase changes occur within the time resolution of our system. Transition rates from 100 ms to several seconds were observed for smaller jump amplitudes. The lamellar lattice responds much more quickly (< 30 ms) to pressure perturbation than the hexagonal lattice, which changes size with a several hundred ms time constant. This can be attributed to the different ways water moves into and from these lattices during the transformation. The analysis of the data also shows the transition proceeds via a complex power law type of conversion.

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W-Pos263

STUDYING THE MECHANISM OF L_{α}/H_{II} PHASE TRANSITIONS WITH TEMPERATURE-JUMP CRYO TRANSMISSION ELECTRON MICROSCOPY (TJC-TEM)

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We have built apparatus to apply 1-ms temperature jumps to cryo TEM specimens about 9 ms before vitrification. We used it to study the mechanism of the L_{α}/H_{II} transition in LUV of DOPE-Me T-jumped from 25°C to T > T_H. In DOPE-Me, large oligolamellar structures with many ILAs [1] and small domains of H_{II} phase are present 9 ms after heating 10 - 25° above T_H. The extent of conversion may be limited by the small area of membrane-membrane apposition compared to MLV. We observed no inverted micellar intermediates (IMIs) [1] or stalks [2], which have been proposed as intermediates. It is conceivable that such structures are too labile to survive vitrification (ca. 1 ms cooling time). We observe some evidence for the line defects postulated to arise from either IMIs or stalks [1,2]. ILAs are expected to form slowly if they form from IMIs (ca. 1 s [1] vs. < 10 ms observed). A stalk-based mechanism [2] is more consistent with our results. The transition progresses significantly within several ms in LUV, faster than transition time constants for MLV (0.1 s) determined via X-ray diffraction with similar superheating [3,4]. Transport & L_{α} phase defect density may affect the transition kinetics differently in MLV vs. LUV [3].

[1] Siegel, Biophys. J. 49:1155 & 1171, [1986]; [2] Siegel, abstract at this meeting; [3] Tate et al., Biochemistry 31:1081 [1992]; [4] Laggner et al., J. Appl. Cryst. 24:836 [1991].

W-Pos264

INTERACTION OF ANTIFREEZE GLYCOPROTEINS WITH LIPOSOMES

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Antifreeze glycoproteins (AFGP) found in blood of polar fish at concentrations as high as 35 g/L are known to prevent ice crystal growth and depress the freezing temperature of the blood. The AFGPs are polymers of Ala-Alal-Thr with a disaccharide residue bound to Thr. The sugars are coplanar, located on one side of the protein backbone which is believed to have a flexible-rod conformation. Recently, Rubinsky *et al.* (1992. *Am. J. Physiol.*) provided evidence that AFGPs block ion fluxes across membranes during cooling, an effect that they ascribed to interactions with ion channels. We investigated the interaction of AFGPs with 100 nm liposomes (DEPC, which has a T_m of 12°C), in which carboxyfluorescein was trapped. As these liposomes are cooled through T_m they leak about 60% of their contents. Addition of as little as 1 mg/ml AFGP prevents up to 90% of this leakage. It is necessary to add the AFGP above T_m to achieve this effect. AFGPs denatured by heating to 80°C continue to prevent ice crystal growth but lose the capacity to inhibit leakage from the liposomes. Non-glycosylated antifreeze proteins show no protection against liposome leakage as well. We conclude that AFGPs stabilize liposomes during thermotropic phase transitions and may have similar effects on the lipid components of native membranes. (Supported by NSF DCB89-18822).

W-Pos265

INTERACTION OF HYDROPHOBIC IONS WITH BIOLOGICAL MEMBRANES.

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Adsorption of tetraphenylborate ($TPhB^-$) to biological membranes from photosynthetic bacteria (characterized by a lipid to protein ratio of 0.8+/-0.2 $\mu mol\ mg_P^{-1}$) was measured by means of equilibrium microdialysis under different conditions of pH and ionic strengths. Binding is fully characterized by the Langmuir-Boltzmann-Grahame model in which surface charge effects at the membrane-water interface are considered. The data indicate that $TPhB^-$-membrane interactions are pH- and ionic strength-unaffected provided that surface charge densities of (-0.5+/-0.3) and of (-1.3+/-0.3)$x10^{-3} e/Å^2$ are assumed for pH values ranging from 5.5 to 6.5 and from 7.5 to 8.5, respectively. Moreover, when binding parameters on biological and lipid membranes are compared, it is concluded that in the presence of membrane proteins, $TPhB^-$ saturation is unaffected while its partition coefficient in the fluid lipid bilayer is five fold decreased.

W-Pos266

INTER-LIPID HYDROGEN BONDING IN LIPID BILAYERS.

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Compounds that can potentially compete for direct inter-lipid hydrogen bonding between head group dipoles, indirect inter-lipid hydrogen bonding via associated water molecules (the "hydration layer"), and hydrophobic interactions were used to investigate lipid bilayer stability. To accomplish this the effect of ethanol, ethylamine, cholesterol and of increased fatty acyl chain unsaturation on the desorption rate of (7-nitrobenzo-2-oxa-1,3-diazole-4-yl)aminohexanoate labeled phospholipids from bilayers were measured. Use of these lipids conferred a fast and easily measurable rate as well as reducing the magnitude of the normally dominant hydrophobic interactions. Ethanol and ethylamine accelerated the rate of desorption of labeled PC, while labeled PE and PS were less effected. This showed that direct inter-lipid hydrogen bonds involving PE and PS are too strong for competition, compared with indirect hydrogen bonding in the hydration layer, that occurs between PC molecules. Interestingly, cholesterol had virtually no effect on labeled PC, PE, or PS desorption rates, indicating that neither structurally important hydrogen bonding, nor hydrophobic interactions are much effected by this bilayer component. Increasing unsaturation was found to increase the desorption rate of the labeled PC. Both ethanol and ethylamine potentiated this effect, which may be due to the increased head group spacing weakening the extended hydrogen bonded network of water molecules in the hydration layer.

W-Pos267
DETERGENT STABILITY OF LIPOSOMES FORMED WITH 1,2-BIS(TRICOSA-10,12-DIYNOYL)-SN-GLYCERO-3-PHOSPHOCHOLINE ($DC_{8,9}PC$) AND DINONANOYLPHOSPHATIDYLCHOLINE (DNPC). ((E.L. Chang, M. Markowitz, and A. Singh)) Center for Bio/Molecular Science and Engineering, Code 6900, Naval Research Laboratory, Washington, D.C. 20375-5320.

The addition of short chain "spacer lipids", such as DNPC, to $DC_{8,9}PC$ allows extensive polymerization of the mixed-lipid liposomes. The spacer lipids also permit incorporation of membrane proteins into pre-polymerized liposomes, thereby avoiding UV irradiation damage to the proteins. However, it is not known whether these liposomes, formed with a high content of short-chain lipids, still possess semi-permeable membranes. We report here that such liposomes are intact. In addition, we find the mixed lipid vesicles to possess a very high degree of stability against detergent lysis. For instance, a 1:1 mixture of unpolymerized DNPC/ $DC_{8,9}PC$ requires over 4 mM of Triton X-100 to induce leakage of carboxyfluorescein from the vesicles. This is approximately 16 times the nominal cmc of Triton. For comparison, leakage of vesicles composed of egg PC or dipalmitoylPC, at the same lipid concentration, can occur with Triton X-100 concentrations under 0.05mM. The effect of polymerization and detergent/lipid ratios on leakage will be discussed.

W-Pos268
INTERNAL ELECTROSTATIC POTENTIALS IN BILAYERS. MEASURING AND CONTROLLING DIPOLE POTENTIALS IN LIPID VESICLES. ((J. Craig Franklin and David S. Cafiso)) The Department of Chemistry and Biophysics Program at the University of Virginia, Charlottesville, VA 22901.

The membrane binding and translocation of positive and negative hydrophobic ion spin labels was measured in unilamellar vesicle systems formed from phosphatidylcholine. Cationic and anion probes exhibit dramatically different binding and translocation rates in these systems that can be understood in terms of a point dipole model described previously (see Flewelling and Hubbell. 1986. *Biophysical J.* 49:541-552). Phloretin, a molecule that reduces the magnitude of the dipole potential, increases the translocation rate of hydrophobic cations, while decreasing the rate for anions. In addition, phloretin decreases the free energy of binding of the cation, while increasing the free energy of binding for the anion. The incorporation of 6-ketocholestanol, produces differential changes in the binding and translocation rates of hydrophobic ions, but in an opposite direction to those produced by phloretin. This is consistent with the view that 6-ketocholestanol increases the magnitude of the membrane dipole potential. A quantitative analysis of the binding and translocation rate changes produced by ketochloestanol or phloretin is well accounted for by a point dipole model that includes a dipole layer due to phloretin or 6-ketocholestanol in the membrane-solution interface. This approach allows dipole potentials to be estimated in membrane vesicle systems, and permits predictable, quatitative changes in the magnitude of the internal electrostatic field in membrane vesicles.

(This research was supported by NIH grant GM 35215)

W-Pos269
LONG LIVED REVERSIBLE PORES IN GIANT LIPOSOMES: A NOVEL METHOD FOR DETERMINATION OF PORE SIZE AND PORE LINE TENSION. ((Doncho V. Zhelev* and David Needham)) Department of Mechanical Engineering and Materials Science, Duke University, Durham N.C. 27706. * Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria.

Using a new experimental technique for studying the electropermeabilization of lipid membranes, giant liposomes (from 25 to 56 μm in diameter) were subjected to single, square, electric pulses (duration 150 μs and electric field strength from 63 to 126 kV/m). The liposomes were held by a micropipet and small membrane tensions were created by controlling the pipet suction pressure. By adjusting the membrane tension, pores were kept open, and pore lifetimes varied from tenths of a second to several seconds. The pore size was determined from the volumetric flow in the pore region and the measured pressure differences across the bilayer. It was clear from the experiments that only one pore remained opened after the pulse. The estimated pore radii were on the order of one micrometer. The pores were in a quasi-equilibrium state and when they closed they did so spontaneously in milliseconds. The isotropic membrane tension was determined for the same measurements and from determinations of both pore size and dynamic membrane tension the pore line tension was found. The line tension of the pore region was determined for two lipid compositions, SOPC and SOPC:CHOL (1:1), and the obtained values for single bilayers were 0.92×10^{-11}N and 3.05×10^{-11}N respectively. The presented analysis and experimental data support the notion that non-attached lipid bilayer membranes with zero tension will always tend to form a closed vesicle surface. (NIH Grant GM 40162)

W-Pos270
FORCE CHARACTERIZATION OF DPPC AND DOPC: A DIFFERENT LOOK AT THE HYDRATION FORCE. ((J.M. Collins)) Physics Department, Marquette University, Milwaukee, WI 53233.

Previous studies of the interactive forces between lipid bilayers have modeled the hydration force as an exponentially decaying pressure whose force strength and decay length were determined by the slope and intercept of the measured force decay for the lipid bilayers. There was a theoretical justification for this approach given in 1976 by Marcelja and Radic (Chem. Phys. Let. 42, 1976) which has been questioned by many investigators over the intervening years. An alternative procedure is to model the electrodynamic forces along with the thermodynamic fluctuation forces and subtract the resultant force from the measured force decays. This has been done for DPPC and DOPC in fully hydrated suspensions at room temperature using independent determinations of the electrodynamic and thermomechanical fluctuation force parameters.

W-Pos271
EFFECTS OF CHAIN UNSATURATION ON EQUATION OF STATE FOR MONOLAYER AT AIR-WATER INTERFACE ((Si-shen Feng and Robert C. MacDonald)) Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2153 Sheridan Road, Evanston, IL 60208. (Spon. by I. M. Klotz)

We present a state equation, formulated from a theoretical model for molecular structure and intra- and intermolecular interactions, of lipid monolayers in the liquid state at air-water interface with the goal of studying the effects of the number and position of the double bonds in the unsaturated hydrocarbon chain. The formulation begins with a description of the partition function for the lipid monolayer system. The total Hamiltonian is assumed to include three primary parts, 1) the position distribution of both the lipid and the water molecules on the surface, 2) the chain conformation and 3) a supplementary part, which includes all other intra- and intermolecular interactions within and between the lipid and the water molecules. The formulation of the translational partition includes the effects of water molecules as a component of the surface phase. The description for the partition due to the chain conformations given by Berde et al. (*Biochemistry*, 19, 4279-4293, 1980) in their study of surface layer phase transitions is utilized to formulate the effects of head group structure and the effects of the number and location of double bonds in the unsaturated chain. The supplementary part is assumed to be independent of the unsaturation of lipid molecules with specified chain length and head group size and therefore, can be determined by fitting the theory to experimental data for a lipid monolayer with a specified unsaturation in the chains. Various curves of the surface pressure vs. surface area per molecule are produced from the theory to study the effects of the number and location of double bonds in the chain. The results will be compared with experimental measurement.

W-Pos272
EQUILIBRIUM PHOSPHOLIPID MONOMER CONCENTRATION AND THERMODYNAMICS OF BILAYER ASSEMBLY ((J. T. Buboltz and G. W. Feigenson)) Section of Biochemistry, Mol. and Cell Biology, Cornell University, Ithaca New York 14853

In aqueous suspension, bilayer phase phospholipid will come to equilibrium with monomeric phospholipid. This equilibrium monomer concentration (EMC) is an extremely valuable thermodynamic parameter, as discussed by Gershfeld [(1989) Biochemistry 28]. When accurately determined, the EMC provides a direct measure of the free energy of bilayer assembly; as a function of temperature, it can dissect assembly free energy into enthalpic and entropic contributions. Even non-ideal mixing behavior of membrane components should be amenable to EMC analysis. Typical phospholipid EMCs appear to be in the nanomolar regime, and so their measurement presents an experimental challenge. We have characterized several important phenomena that must be taken into account in order to conduct meaningful EMC studies. A method for phospholipid EMC measurement, developed in light of these discoveries, is presented here. Employing this method, we report the determination of the EMC for ^{14}C-labelled dilauroyl-PC, between 4°C and 40°C. The thermodynamic implications for phospholipid bilayer assembly are discussed.

W-Pos273

SELF-ASSEMBLED MONOLAYERS AS SUBSTRATES FOR PLANAR MODEL MEMBRANES. (Anne L. Plant, Michael Tarlov) National Institute of Standards and Technology, Gaithersburg, MD. 20899

Alkanethiols, which spontaneously form stable monolayers on gold films, can be used as a substrate for planar phospholipid model membranes. A strong attraction between the thiol moieties and gold results in the formation of a stable hydrophobic monolayer. We are studying the interaction of phospholipids with these hydrophobic monolayers as an easy and reproducible way to create stable planar single bilayer membranes. Electrochemical data indicate that the addition of POPC vesicles to a hexanethiol monolayer on gold results in a decreased capacitance compared to the monolayer, and an estimated bilayer distance of 28 Å. The electrochemical activity of $K_3Fe(CN)_6$ at the membrane-coated electrode is greatly attenuated, and is consistent with the presence of a significant diffusional barrier. The addition of micromolar concentrations of melittin results in enhanced electroactivity of $K_3Fe(CN)_6$, consistent with the formation of pores by mellitin in the model membrane. This model system is applicable to studies of interfacial phenomona and membrane protein activity.

W-Pos274

Theoretical studies of model biosurface properties. *K. Hristova and D. Needham, Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27708*

To enhance our understanding of cell surface properties and to characterize a new Stealth drug delivery system we study model systems of lipid bilayers with grafted polymers. For single bilayers we show that the grafted polymer is expected to decrease the tensile strength and hence reduce the mechanical stability of the bilayer. The influence of the polymer on membrane elastic properties (area expansibility and bending) is modeled in terms of scaling theory and the dependence of K_A, k_c and $\bar{k}_c$ on the grafting concentration and polymer molecular weight is derived. For two bilayers in close proximity, we show that the grafted chains increase interbilayer repulsion even at low concentration (the so-called "mushroom" regime). For small compressive pressures ($\ln P \propto 8 - 10\ dyn/cm^2$), this repulsion is due to an entropy decrease; upon further compression, the spread mushrooms begin to overlap, giving rise to an additional osmotic force and increasing the repulsion to a new level ($\ln P \propto 15\ dyn/cm^2$). Finally, the "soft" liquid membranes are found to influence the confinement energy of the polymer. The additional energy, required to "trap" the polymer between two thermally undulating membranes is calculated. This energy depends on the bending stiffness of the membrane, which in turn is affected by the presence of the polymer.

W-Pos275

DYNAMIC STUDIES OF TETHER FORMATION FROM LECITHIN VESICLES ((Robert M. Raphael and Richard E. Waugh)) Dept. of Biophysics, Univ. of Rochester Medical Center, Rochester, NY 14642

Formation of thin membrane "microtubes" from bilayer membrane vesicles provides a powerful physical approach for investigating the elastic and dynamic properties of lipid bilayers. Theoretical analysis predicts that after a step change in membrane tension, a rapid exponential increase in microtube length to a new equilibrium value should be observed, and that the time constant for tube growth should be determined in large part by a mechanically driven "slip" between the leaflets of the bilayer and the accompanying interlayer drag (Evans et al. *Biophys. J.* 59:498a,1991). Observations confirm the predicted short term exponential response, but instead of approaching equilibrium, the microtube continues to grow, following either a linear or slow exponential time course. Different mechanisms are likely to account for a linear as opposed to an exponential time course: A linear time course would be consistent with an exchange of material between the constituent monolayers of the bilayer (flip-flop), and an exponential time course would correspond to residual, long lived inter-leaflet tractions in the vesicle surface. Technical refinements in the experimental approach have been introduced to investigate the dynamic aspects of membrane behavior over extended times, making it possible not only to distinguish between alternative mechanisms, but also to provide a quantitative measure of the rate of interleaflet exchange or the relaxation of interlayer stress. (Supported by PHS under grant no. RO1-HL31524.)

W-Pos276

Electrophoresis of plant cells and protoplasts

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The electrophoretic mobility of *Catharanthus roseus* cells and protoplasts was measured by microelectrophoresis in order to compare the surface charge density of the cell wall with that of the protoplast membrane. The origin of surface charge groups was studied and the effect of different negatively and positively charged groups was evaluated.

C.roseus cells had net negative ζ-potential of -29.5±4mV (in 10 mM phosphate buffer, pH 5.5). The surface charge density was calculated to be -34.52 mC/m^2. With the addition of 0.5 mM EDTA, the surface charge density increased to -48.23 mC/m^2. This corresponds to a density of one negative charge per 33.14 A^2. The average ζ-potential of *C. roseus* protoplasts measured in10 mM phosphate buffer, 0.35M sucrose, pH 5.5, was -24.89±2.8 mV, with a surface charge density of -19.51 mC/m^2. This corresponds to one negative charge per 510.76 A^2. The presence of 1mM EDTA did not alter significantly the surface charge density.

The origin of surface charge groups was determined by treatin *C.roseus* cells and protoplasts with glutarardehyde, alkaline phosphatase and morpholine-N,N'-dicyclohexylcarboxamidine for determination of amino, phosphate and carboxylic groups respectively.

W-Pos277

Pressure Induced Fusion (PIF) Liposomes: A Solventless Method to Make Liposomes of High Captured Volume.

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Multilamellar liposomes made from certain saturated phospholipids are known to interdigitate when inducers such as ethanol are added or pressure is increased beyond some critical point. We found that in order for interdigitation to occur in highly curved small liposomes there must first be a change in vesicle geometry. We have previously demonstrated (with ethanol as the inducer) [Boni et al. (1991) Biophys. Journal 59: 503a; Ahl et al. (1992) Biophys. Journal 61: 1401a] that this shape change results in liposome-liposome fusion to form extended bilayer "sheets". Macroscopically a sample of these intermediary sheets appear as a viscous gel. Light microscopy revealed that these sheets spontaneously form much larger liposomes when the temperature is raised above the main phase transition of the lipid. We called these systems interdigitation-fusion vesicles (IFVs). Here we show that small liposomes comprised of DPPC and DSPC, following an increase in hydrostatic pressure (1 to 7 kbar), form similar viscous gels that also give rise to large liposomes of high captured volume upon heating. POPC SUVs did not undergo any changes in morphology when subjected to the same high pressures.

W-Pos278

ROLE OF VITRIFICATION IN STABILIZATION OF DRY LIPOSOMES. ((J.H. Crowe and L.M. Crowe)) Biophysics Group, UC Davis

Freeze-dried liposomes can be preserved with trehalose or sucrose (reviewed in Crowe *et al.* 1988. *BBA*). We have shown previously that the sugars obviate fusion and phase transitions, which we ascribed to interactions with the polar headgroups of the phospholipids. Recently, it has been suggested that vitrification alone may be sufficient to achieve stabilization (Levine and Slade. 1992. *Biopharm*). Thus, we have investigated this possibility, with these results: (1) Maintenance of frozen liposomes (9 egg PC: 1 PS) in a vitrified state is necessary. If such liposomes are incubated at temperatures above T_g for trehalose (-32°C), they are destabilized. (2) Dextran (T_g= -10°C) is ineffective in preserving dry liposomes. If vitrification were sufficient, it should be more effective than trehalose, since it vitrifies more readily. (3) Kinetic analysis showed that dextran actually *inhibits* protection by trehalose, by noncompetitive inhibition. (4) At very high concentrations, dextran inhibits fusion during drying, but it has no effect on phase transitions. By contrast, trehalose reduces T_m in dry lipids by about 80°C. (5) Eu^{3+} competitively inhibits stabilization by trehalose, suggesting that it competes with trehalose for the same binding site. We conclude that vitrification appears to be necessary, but it is not in itself sufficient. The evidence is consistent with direct, specific interactions between the sugars and dry phospholipids. (Supported by NSF grant DCB89-18822).

W-Pos279

Incorporation of Lipids Which Do Not Interdigitate Into Interdigitation-Fusion Vesicles (IFV's)

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Interdigitation-fusion (IF) is a new method for producing lipid vesicles with high internal volumes [Ahl et al. (1992) Biophys. J. 61: 243a]. Small unilamellar vesicles (SUV's) formed from saturated acyl chain phospholipids such as DPPC spontaneously fuse together in the presence of 3 M ethanol to form interdigitated membrane sheets [Boni et al. (1991) Biophys. J. 59: 503a]. Interdigitation-fusion vesicles (IFV's) are formed from the sheets when the temperature is raised above the T_m of the lipid. SUV's composed of unsaturated lipids such as egg PC which do not undergo bilayer interdigitation do not undergo ethanol-induced interdigitation-fusion. Furthermore, the addition of unsaturated lipids or cholesterol to the DPPC SUV's severely inhibits the ethanol-induced fusion. In order to circumvent these problems, we have developed a modification to the basic IF process which allows incorporation of significant amounts of non-interdigitating lipids such as cholesterol into IFV's. Additional SUV's containing the non-interdigitating lipid were added to the ethanol-induced interdigitated membrane sheets. The lipid from these SUV's was incorporated into the IFV's when the temperature was raised above the T_m of the lipid in the sheets. We have used this IF modification to prepare relatively high internal volume DPPC IFV's (10 - 15 ul/uM P_i) which contain significant mole fractions of cholesterol (0.15). IFV's may be useful as drug carriers since they allow for a high drug to lipid ratio.

W-Pos280

INTERACTION OF THE PEPTIDE FRAGMENT 828-848 OF THE CARBOXY-TERMINAL REGION OF THE HIV ENVELOPE GLYCOPROTEIN WITH PLANAR LIPID BILAYERS.

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We investigated the membrane effects of the carboxy-terminal region of the HIV transmembrane protein (gp41), which has been implicated in T cell death. This protein segment forms a positively charged amphipathic α–helix and is located in the intra-viral or cytoplasmic compartment. The structure of the corresponding peptide fragment is similar to the structure of known cytolytic peptides. We have studied the interaction of the isolated, synthetic amphipathic helix of gp41 with planar phospholipid bilayer membranes using voltage clamp, potentiodynamic, and stability measurements. The peptide binds strongly to planar bilayers of negatively charged phosphatidylserine. More than 60% of the membrane lipids are bound to the peptide. The interaction results in decreased membrane stability, decreased membrane surface tension, and increased membrane conductance. In the presence of micromolar concentrations of the peptide the formation of voltage-dependent pores with cationic selectivity and a modal conductance of 4 nS was observed. The membrane-perturbing activity of the peptide suggests that the interaction of the carboxy-terminal fragment of HIV-1 envelope protein with the membrane of infected T cells could disrupt the membrane barrier function, leading to sodium and calcium flux into cells, osmotic swelling, and T cell death.

W-Pos281

CH_2 WAGGING MODES AS QUANTITATIVE IR PROBES OF ACYL CHAIN CONFORMATIONAL ORDER IN PHOSPHOLIPID MEMBRANES.

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Two IR experiments involving CH_2 wagging vibrations have been developed to quantitatively probe conformational order in alkanes and in phospholipid acyl chains. The first experiment exploits a series of localized methylene wagging modes in the 1320-1370 cm^{-1} region of liquid alkanes and L_α and H_{II} phospholipid phases which are characteristic of particular two- and three-bond conformational states as follows: gg state, 1353 cm^{-1}; end-gauche state, 1340 cm^{-1}; and the sum of gtg' (kink) + gtg states, 1368 cm^{-1}. We have used the measured intensities of these bands in liquid alkanes along with the Rotational Isomeric State model to develop extinction coefficients from which the concentration of these particular conformers in phospholipids has been deduced. The second experiment exploits the coupled wagging progression bands in the range 1180-1380 cm^{-1} in relatively ordered phospholipid phases. A simple model has been developed which allows an estimate of conformational order in the liquid-ordered phase of cholesterol/DPPC and in CaATPase reconstituted with a variety of PC's.

W-Pos282

QUANTITATIVE IR EVALUATION OF ACYL CHAIN CONFORMATIONAL ORDER AND THERMOTROPIC PROPERTIES IN C14, C15, AND C16-ENRICHED A. LAIDLAWII B.

((D.J. Moore and R. Mendelsohn)) Dept. of Chemistry, Newark College, Rutgers University, Newark, NJ 07102.

Recent advances in our understanding of the quantitative relationships between CH_2 wagging bands and hydrocarbon chain conformational states have permitted the evaluation of the physical state of A. laidlawii B membranes in vivo. The thermotropic behavior of the membranes of the live organism has been compared to that of the isolated membrane and the extracted membrane lipids. Whereas gross differences are apparent between the extracted membrane lipid and the live cells, no such differences are apparent between the isolated membranes and the membranes of the living organism. The persistence of CH_2 wagging progression bands at the growth temperature in the live A. laidlawii B and in the isolated membrane suggests that a substantial degree of order remains in the membrane acyl chains under physiologically relevant conditions. The CH_2 wagging progression bands are not evident in the extracted membrane lipids at the growth temperature.

GRAMICIDIN CHANNELS

W-Pos283

HIGH RESOLUTION PROTEIN STRUCTURE DETERMINATION BY SOLID STATE NMR

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Oriented samples of the monovalent cation selective channel formed by a dimer of gramicidin A in hydrated lipid bilayers are used to obtain orientational constraints. These constraints yield structural detail rarely achieved for proteins. Samples have been labeled with ^{13}C, ^{15}N and ^{2}H for a variety of studies involving the observation of chemical shifts, dipolar couplings and quadrupolar couplings. The Φ and Ψ backbone torsion angles have been solved from solid state NMR data alone. This structure has been refined by determining the ω torsion angle which describes the non-planarity of the peptide linkages. It is thought that distortions of the peptide plane occur as the backbone carbonyl oxygens solvate cations in their passage through the channel. The orientation of the four indole rings of the gramicidin monomer have been determined with respect to the bilayer normal. The orientations are very similar and the N-H groups are all directed toward the bilayer surface rather than the bilayer center. Consequently, these groups could be hydrogen bonded to the water of the aqueous interface or to the lipid backbone carbonyl oxygens. These interactions appear to be responsible for the stability of the single stranded gramicidin helix in a lipid bilayer versus the double stranded conformations that exist in organic solvents. Furthermore, the dipole moments of the indole rings are predominately aligned with the channel axis and oriented in such a way as to reduce the potential energy barrier at the bilayer center.

W-Pos284

THREE-DIMENSIONAL STRUCTURE OF GRAMICIDIN IN SDS MICELLES.

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CD measurements indicate that the conformation of gramicidin incorporated into SDS micelles is quite similar to the conformation of gramicidin incorporated into lipid bilayers. Deuterated SDS micelles therefore provide an excellent model membrane environment for the determination of gramicidin structures by NMR. Three dimensional structures have been determined for gramicidin A, [Phe-1] gramicidin A, and [Phe-1] gramicidin C. For each peptide, 100 structures were generated from NOESY data using distance geometry and simulated annealing. An average structure was then calculated from the 10 best structures. Superposition of the 3 average structures indicated near identical backbone conformations and similar Trp sidechain orientations. Electrostatic modeling was used to show qualitative differences in the electrostatic fields of the peptides.

W-Pos285

SYNTHESIS AND PRELIMINARY CHARACTERISATION OF 6-AMINO-HEXANOYL GRAMICIDIN.

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Since the basic architecture of the gramicidin channel is well-established, the molecule is an attractive target for ion channel engineering and design. We have begun to explore modifications to the chemical structure of gramicidin which might impart novel properties to gramicidin channels and report here the synthesis and preliminary characterisation of 6-amino-hexanoyl-gramicidin (6-AH-gram), *i.e.* gramicidin with a positively-charged C-terminal extension. We expected that this derivative might show 'burst-like' single channel openings due to transient occlusion of the channel mouth by the extension during the lifetime of a conducting gramicidin dimer. Furthermore, if the positive charge on the extension could sense the transmembrane potential, then single-channel voltage-gating might be seen - in a manner conceptually analogous to the 'ball and chain' model of sodium channel inactivation. The synthesis was accomplished in a simple two-step procedure: the esterification of gramicidin with FMOC-6-amino-hexanoic acid followed by removal of the FMOC protecting group. Mass spectrometric analysis confirmed the identity of the product. 6-AH-gram was found to have conformational behaviour similar to the parent compound; CD analysis indicated that typical 'pore' forms were adopted in organic solvents and 'channel' forms in phospholipid vesicles. Preliminary characterisation of 6-AH-gram single-channel behaviour in planar lipid bilayers confirmed the expectation of 'burst-like' openings with conductances similar to native gramicidin channels. Possible effects of voltage on this activity are currently being investigated. (Supported by grant #DMB88-16981 from the U.S. N.S.F. & a postdoctoral fellowship from the Canadian MRC (to G.A.W.))

W-Pos286

NONCONDUCTING STATE OF GRAMICIDIN.

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One major unknown concerning the mechanism of the gramicidin (GA) channel is what is its closed state. It has been suggested that (a) it is an intertwined dimer, the crystal form of GA grown from organic solvents; (b) it is monomers in beta-helical form and they remain in the monolayers; or (c) it is monomers (in an unspecified form) on the membrane surface, because they are unstable in the bilayers. We will describe a technique of x-ray scattering with the momentum transfer in the plane of the membrane that can distinguish between these three cases. To create a nonconducting state of gramicidin, we use BOC-gramicidin (BOC-GA), a synthetic analogue in which the formyl group of the natural GA is replaced by a BOC group. BOC-GA has exhibited channel activities four orders of magnitude less than natural GA.

W-Pos287

CHARACTERIZATION OF [L-ALA^1-D-ALA^2] GRAMICIDIN A: STABILIZING EFFECT OF THE GLY^2 --> D-ALA SUBSTITUTION.

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Valine 1 gramicidin A (Val^1 gA) forms right-handed $\beta^{6.3}$ helical transmembrane channels by the dimerization of two monomers via H-bond formation of the formyl-NH ends. Additions, deletions and substitutions at this head-to-head interface affect both the conductance and stability of the channel, and in some cases the structure. Substitution of L-Val^1 by L-Ala decreases the mean single-channel duration in diphytanoylphosphatidylcholine/n-decane bilayers from 990 to 150 ms, and increases the single-channel conductance from 14.4 to 16.7 pS (1 M NaCl, 200 mV). The additional replacement of Gly^2 with D-Ala increases the mean channel duration to 2100 ms and increases the conductance to 18.7 pS. Hybrid channel experiments show that Val^1 gA and [L-Ala^1-D-Ala^2]gA have the same backbone structure. Circular dichroism spectroscopy also indicates that this analogue adopts a $\beta^{6.3}$ "channel" conformation in dimyristoyl-phosphatidylcholine vesicles and in sodium dodecyl sulfate (SDS) micelles. Two-dimensional NMR techniques, DQCOSY, NOESY and TOCSY, have been used to investigate the structure of this stabilized analogue in d_{25} SDS micelles. The "fingerprint" region of the spectra confirms both the alanine substitutions and the right-handed $\beta^{6.3}$-helical motif. Distance geometry and simulated annealing are being used to refine a molecular model of both the backbone and sidechains of [L-Ala^1-D-Ala^2]gA.

W-Pos288

DYNAMICS OF THE GRAMICIDIN A CHANNEL: MOLECULAR REORIENTATION RATES AND BACKBONE LIBRATIONS.

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Gramicidin A is a 15 residue polypeptide which forms a monovalent cation selective channel as an end-to-end helical dimer spanning a lipid bilayer. The dynamics of the gramicidin A channel in oriented bilayers of DMPC have been studied by solid-state NMR. By simulating ^{2}H line shapes in spectra of $Ala_{3,5}$-d4 obtained with various quadrupole echo delay times the rate of global molecular reorientation about the helical axis has been found to be in the μsec timescale. The correlation time for rotation of the channel is strongly dependent on temperature near the gel to liquid crystal phase transition. Measurement of the global correlation time has allowed this parameter to be held constant in interpretations of T_1 relaxation times for backbone amide ^{15}N and alpha ^{13}C. Field dependent T_1 experiments on ^{15}N demonstrate consistent rates of peptide plane libration at sites throughout the channel on the order of 10^{-8} seconds with amplitudes of 12-17 degrees. Field dependent T_1 experiments on alpha ^{13}C sites demonstrate significant motion of the C_α-H bond at rates comparable to rates for peptide plane libration.

W-Pos289

DURATION AND CONDUCTANCE OF GRAMICIDIN A CHANNELS WITH NON-DIPOLAR POSITION #1 SUBSTITUTIONS

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Dipolar position #1 substitutions in [Val^1]gramicidin A have profound effects on the single-channel conductance (g) and average duration (τ) of the channels that are formed by the sequence-substituted analogues (Russell et al. *Biophys. J.* 49:673, 1986; Koeppe et al. *Biochemistry* 29:512, 1990). The consequences of non-dipolar substitutions are not as well understood. We have therefore examined how alterations in side chain geometry among non-dipolar aliphatic and aromatic side chains affect g and τ in 1.0 M NaCl. Alterations in side chain bulk can alter τ by about 10-fold (between [Gly^1]gA, $\tau \simeq 60$ ms, and [PhenylGly^1]gA, $\tau \simeq 800$ ms), and g by about 3-fold (between [Gly^1]gA, $g \simeq 5.5$ pS, and [Norleu^1]gA, $g \simeq 15$ pS). With respect to the alterations in τ, the side chains can be classified in four groups (listed in order of increasing τ): Gly; straight-chain aliphatic (Ala - Norleu); γ-branched aliphatic and Phe; and β-branched aliphatic and PhenylGly. With respect to alterations in g, the classification is somewhat different (again listed in order of increasing g): Gly; PhenylGly; γ-branched aliphatic and Phe; β-branched aliphatic; and straight-chain aliphatic. These results show that channel function is surprisingly sensitive to alterations in the position #1 side chain's geometry, which can alter side chain-backbone interactions (and thus the "stiffness" of the peptide backbone) as well as side chain-side chain interactions across the dimer join, where Val^1 of one monomer has been shown to be in close contact with Ala^5 of the other monomer (Killian et al. *Biochemistry* in press).

W-Pos290

FORMAMIDINIUM BINDS STRONGLY TO AND STRONGLY STABILIZES GRAMICIDIN A CHANNELS.

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Methylammonium and formamidinium exhibit self block, suggesting multiple occupancy in the gramicidin A channel. Formamidinium has a relatively high binding affinity, the dissociation constant (K_1) being 22 mM from the Eadie-Hofstee plot. The rate limiting step for the ion is translocation judging from supralinear I-V relations. 1M formamidinium solutions yield exceptionally long single channel lifetimes (20-fold longer than methylammonium which yields lifetimes similar to those found with alkali metal cations) which significantly decreases with increasing voltage and whose temperature and concentration dependences were steep at lower voltages (≤100mV). At very low salt concentrations (0.01M, 100mV), there was no difference in average lifetime from those in other ionic solutions. All the above results suggest that single formamidinium binding stabilizes the gramicidin A channel effectively. The exceptional behavior of the ion compared to other ions in stabilizing the dimer was also observed in N-acetyl gramicidin A but disappeared in the gramicidin M dimer. We conclude that formamidinium can effectively stabilize the gramicidin A dimer at a binding site near the mouth probably by affecting the orientation or H-bonds of Trp side chains near the C-terminus.

W-Pos291
EFFECTS OF AQUEOUS AND LIPID ENVIRONMENT ON COLLECTIVE MOTIONS AND STRUCTURE OF THE GRAMICIDIN ION CHANNEL: A POSSIBLE BASIS FOR THE HANDEDNESS OF THE CHANNEL? ((S.-W. Chiu and E. Jakobsson)) Biotechnology Center, National Center for Supercomputing Applications, and Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801. (spon. by H. J. Stapleton)

In time-correlation fluctuation analysis of molecular dynamics simulations, we find that there are collective damped periodic motions of the backbone. We are now exploring whether these periodicities depend on interactions with channel water and whether they are substantially modified by inserting the channel into a lipid environment. In computations on PC bilayers, we have found the existence of a large electric field in the headgroup region. This field apparently corresponds to the dipole potential measured experimentally. We are exploring whether this field may be important in orienting the gramicidin tryptophan rings. Simple energetic calculations suggest that interactions between this field and the tryptophans may account for the fact that the bilayer favors the head-to-head helix over the double-helical form of the channel. The interactions may also be strong enough to determine the handedness of the channel. We are testing these hypotheses by molecular dynamics simulations. Support by NIH.

W-Pos292
LIPID CHAIN LENGTH DEPENDENCE OF RECIPROCAL GRAMICIDIN-LIPID INTERACTIONS
((Roger E. Koeppe II & Denise Greathouse)), Dept. of Chem. and Biochem., Univ. Arkansas, Fayetteville, Arkansas USA 72701.

A membrane or micelle environment appears required for linear gramicidins to adopt the $\beta^{6.3}$ channel conformation, as opposed to the double-helical conformation observed in organic solvents. Lipid-incorporated gramicidin A (gA) can modulate the structure of surrounding lipids, inducing micelle-to-bilayer (Killian et al. *1983* Biochim. Biophys. Acta 728:141) or bilayer-to-H_{II} (van Echteld et al. *1981* Biochim. Biophys. Acta 648:287) transitions. We have previously used circular dichroism to show that gA adopts the $\beta^{6.3}$ channel conformation if $n \geq 12$ in di-(C_n)-PC, but not if $n < 8$ (Biophys. J. 61: A526). For $n = 8$ or 10, the results are intermediate, depending on both sample preparation methods, as well as 'solvent history'. Upon addition of gA to di-(C_9)-PC, the lipids remain micellar (25-50 nm), based on ^{1}H-NMR linewidths and electron microscopy, and the gA remains non-channel-like, based upon CD spectra. In constrast, upon incorporation into di-(C_9)-PC as the $\beta^{6.3}$ channel, electron microscopy reveals that gA induces both an increase in micelle size, as well as the formation of vesicles. ^{1}H-NMR linewidths and ^{31}P anisotropies support these findings. The results support previous suggestions that the lipid-modulating activities of gA correlate with the $\beta^{6.3}$-helical channel conformation.

W-Pos293
MODULATION OF GRAMICIDIN CHANNEL FUNCTION BY THE HOST BILAYER: EFFECT OF LYSOLIPIDS. ((Jens A. Lundbæk and Olaf S. Andersen)) Cornell Univ. Medical College, New York, NY 10021.

Lysophospholipids and other amphipatic molecules modulate the function of a large number of membrane proteins including ion channels and pumps. Given that different classes of membrane proteins are affected by structurally diverse classes of amphipatics, it is likely that these alterations in function result from alterations in the physical properties of the host bilayer. In order to examine this possibility we have studied how lysolipids affect gramicidin channels. The average conductance and lifetime of gramicidin channels were examined as lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol and lysophosphatidylserine (0.5-4 μM) were added to the electrolyte bathing the membrane. The effect on the channel formation constant was determined from the increase in number of conducting channels as the lysolipids were added. All lysolipids decrease the single-channel conductance. The uncharged LPC and LPE are the most effective. All lysolipids increase the channel duration and the channel formation constant, whereas the energetic cost of channel formation is decreased. These latter effects are proportional to the size of the polar head group and are not affected by the charge. The surface tension of phospholipid monolayers is not detectably changed by lysolipids in equivalent concentrations. The results are consistent with notion that lysolipids alter membrane stiffness.

W-Pos294
PROPERTIES OF GRAMICIDIN A CHANNELS INCORPORATED IN ERYTHROCYTE MEMBRANE
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The channel former gramicidin A incorporated into the erythrocyte membrane increases the membrane cation permeability. It is considered as model of the integral membrane channels. The ionpermeability properties of the gramicidin A channel have been monitored by measuring the exchange diffusion of radioactive tracer cations.

Channel inactivation, a time dependent decrease of the high ionpermeability has been observed in cholesterol containing erythrocyte membranes. The rate of channel inactivation strongly depends on the phospholipid to cholesterol molar ratio of the membrane and no inactivation can be observed on cholesterol depleted erythrocyte membrane. The channel inactivation is suggested to be the result of an interaction between gramicidin and cholesterol in a stoichiometry of 1:5. When cholesterol in the membrane is substituted by 7-dehydrocholesterol, which has a second double bound in the B ring of the molecule, no sign of inactivation has been found indicating the importance of the B ring of the sterol molecule in the inactivation process.

W-Pos295
HOW POLARIZABILITY AND HELIX FLEXIBILITY AFFECT ION-WATER CORRELATIONS IN A GRAMICIDINLIKE CHANNEL. ((Karen A. Duca and Peter C. Jordan, Department of Chemistry, Brandeis University, Waltham, MA 02254))

Previous work (Jordan, Biophys. J., 59, 320a [1991]) has indicated that interaction between a gramicidinlike channel (a configurationally constrained polyglycine analog) and sodium is distinctly different than that between the channel and potassium or cesium. With sodium present, electrostriction was so pronounced that water-water distances were sharply reduced. An extra water was present in the single file and the waters were more orientationally ordered. These phenomena were attributed to sodium's markedly larger ability to polarize water. To test this hypothesis, we have carried out calculations focusing on two features of the original model: the explicit inclusion of polarizability and the nature of the constraint which maintains helical integrity. We find that the qualitative picture is not altered if the helix is modeled more realistically and allowed greater flexibility. In contrast, we find that the explicit introduction of polarizability is critical; with non-polarizable water, there is no electrostriction. We confirm earlier observations: 1) water-water correlations are notably dependent upon a cation's size and its location in the channel; 2) cesium and potassium interact with gramicidin in basically quite similar fashions while sodium differs notably from its larger relatives.

W-Pos296
$T\ell^+$ ION VELOCITY DISTRIBUTIONS IN GRAMICIDIN CHANNELS OBSERVED WITH LASER DOPPLER VELOCIMETRY. ((F. Macias and Michael E. Starzak)) Department of Chemistry, State University of New York, P.O. Box 6000, Binghamton, NY 13902-6000

Velocity distributions for $T\ell^+$ ions permeating through gramicidin channels in bilayer membranes are observed with laser Doppler velocimetry using a He-Ne laser. The technique permits detailed observation of ion kinetics within the gramicidin channels. The Doppler frequency and corresponding velocity distributions at each transmembrane potential have a single maximum produced by a steady diffusional velocity of the ions in the channels. These velocities range from .0375 m/sec to .914 m/sec for transmembrane potentials from 10 mV to 150 mV, respectively. Distribution half-widths suggest the ions can fluctuate about this steady velocity as they interact with the gramicidin channel. (Sponsored by the Office of Naval Research.)

W-Pos297

EXTENDED DIPOLAR CHAIN MODEL FOR ION CHANNELS: ELECTROSTRICTION EFFECTS AND THE TRANSLOCATION ENERGY BARRIER. ((Miguel Sancho*, Michael B. Partenskii and Peter C. Jordan)) Department of Chemistry, Brandeis University, Waltham, MA 02254; *Departamento de Fisica Aplicada III, Universidad Complutense de Madrid, E-28040 Madrid, Spain.

The dipolar chain model introduced to describe the aqueous pore of single-file transmembrane ion channels (TMIC) indicates that the susceptibility of water in unoccupied channels is high while the presence of a monovalent ion reduces water's orientational susceptibility to nearly zero (Partenskii, Cai & Jordan, Chem. Phys., 153, 125 [1991]). The associated energy barrier for translocation is high, calling into question the electrostatic rationalization of channel function. Static ion-pore interaction is insufficient to lower the energy barrier to physiologically reasonable values (Partenskii and Jordan, Quart. Rev. Biophys., in press). Here, we modify the analysis to account for non-electrostatic ion-water and water-water Lennard-Jones interactions. We focus on a model system with gramicidinlike dimensions and find that electrostriction has only a limited influence on the total energy barrier for permeation. However, there is a clear ion-dependent modulating effect on translocation that corresponds to Eisenman selectivity sequence I (low site field strength), in complete accord with experiment. The computed ratios of translocation rates are in reasonable agreement with those found experimentally.

W-Pos298

INFLUENCE OF A CHANNEL FORMING PEPTIDE ON ION ENERGY BARRIERS VIEWED IN A CONTINUOUS THREE-DIELECTRIC MODEL FOR ION CHANNELS. ((Michael B. Partenskii and Peter C. Jordan)) Department of Chemistry, Brandeis University, Waltham, MA 02254.

A three dielectric model of transmembrane ion channels of finite length is presented, with ϵ_1 the permittivity of interchannel water ($r < R_1$), ϵ_2 that of the highly polarizable region of the channel forming peptide ($R_1 < r < R_2$) and ϵ_3 that of the remainder of the system, the membrane and the nonpolar regions of the peptide ($r > R_2$). Setting $\epsilon_w = \infty$ for bulk water and using the method of images the electrostatic problem is exactly soluble. Comparison with numerical solutions to the Poisson-Boltzmann equation with $\epsilon_w = 80$ demonstrates that the model is accurate, especially for ionic strengths ≥ 0.5M. The analysis is applied to a system with gramicidinlike geometry. Choosing $\epsilon_1 \sim 4$-5, to account for the strongly nonlinear dielectric behavior of water in the ionic field (Partenskii and Jordan, J. Phys. Chem., 96, 3906 [1992]), we varied ϵ_2 to assess the possible influence of peptide. It appears difficult to reproduce the results of our previous semimicroscopic study of the influence of the carbonyls on the ionic energy barrier within a continuum approach for reasonable values of ϵ_2. The discrepancies arise because ϵ_2 is actually nonlocal and because the model's fixed geometry can not account for significant local reorientation of polar groups near the ion.

SARCOPLASMIC RETICULUM I

W-Pos299

BLOCK OF THE RYANODINE-MODIFIED SR Ca^{2+}-RELEASE CHANNEL. ((A. R. G. Lindsay, A. Tinker and A. J. Williams.)) National Heart and Lung Institute, University of London, LONDON, SW3 6LY, UK.

We have previously reported that the large, impermeant, cations tetrabutyl ammonium (TBA), tetrapentyl ammonium and the local anaesthetic QX314 interact with the cytosolic vestibule of the conduction pathway of the purified sheep cardiac sarcoplasmic reticulum (SR) ryanodine receptor-channel. The interaction of each of these cations results in the production of a unique reduced conductance state, the occurrence of which is both voltage- and concentration-dependent (Tinker et al., 1992, Biophys. J., 61: 1122-1132). Following modification with ryanodine, which causes the receptor-channel to enter a reduced conductance state with long open dwell time, these cations block the receptor-channel to a level which is indistinguishable from the closed state. The voltage-dependence of TBA's interaction both before and following modification was determined by Boltzmann analysis and by determining k_{on} and k_{off}. In control, the two methods yield an equivalent gating charge of 1.66 and 1.81 respectively. Following modification these values are 1.74 and 1.61. TBA concentration dependence has been examined in control and after modification; K_ds are 45 and 165 μM respectively. We suggest that the interaction of ryanodine with the receptor-channel involves an alteration to the cytosolic vestibule which is reflected in the differing affinity of the TBA site. We have also examined the interaction of several polycations which are known to inhibit Ca^{2+}-release from isolated SR vesicles. Gentamycin, neomycin, streptomycin, spermine and spermidine block the ryanodine-modified receptor-channel. Unlike the large tetraalky ammonium cations, these polycations block to a reduced conductance state.

This work was supported by the British Heart Foundation and the MRC.

W-Pos300

CHARGED LOCAL ANAESTHETICS BLOCK THE SHEEP CARDIAC RYANODINE RECEPTOR-CHANNEL. ((A.Tinker and A.J. Williams.)) National Heart & Lung Institute, University of London, London SW3 6LY, UK.

We have examined the effect of QX314, QX222 and Procaine on K^+ conduction in the ryanodine receptor-channel purified from sheep cardiac sarcoplasmic reticulum. QX222 and Procaine act as classical voltage-dependent blockers from the cytoplasmic face of the channel. At $\leq$ 500 Hz filtering this is manifest as a smooth reduction in single-channel current amplitude at positive holding potentials. Quantitative analysis gives an effective valence of approximately 0.9 for both ions and $K_d(0)$s of 9.2 and 15.8 mM for QX222 and Procaine respectively. Analysis of the concentration-dependence suggests that QX222 is binding to a single site with a K_m of 491 μM at 60 mV holding potential. Investigation of the excess open channel current noise at higher frequencies of filtering by amplitude distribution analysis reveals that both the voltage-dependence and concentration-dependence occur due to changes in K_{on}. The addition of QX314 to the cytosolic face of the channel is different and leads to the production of a substate with an amplitude ½ of control. Quantitative analysis reveals that the effect is highly voltage-dependent with a valence of approximately 1.5 accounted for by nearly equal changes in K_{on} and K_{off}. Analysis of concentration-dependence reveals positive co-operativity due to changes in K_{on} with at least two QX314s binding to the conduction pathway. A paradoxical increase in K_{off} at high positive holding potentials and with increasing concentration at 80 mV suggests the existence of a further QX314-dependent reaction. The substate block is interpreted as a form of partial occlusion in the vestibule of the conduction pathway reducing current by electrostatic means.

Supported by the MRC and BHF.

W-Pos301

HYDROGEN PEROXIDE MODIFIES THE GATING OF THE SHEEP CARDIAC SARCOPLASMIC RETICULUM CALCIUM-RELEASE CHANNEL ((A. Boraso & A. J. Williams)) National Heart & Lung Institute, University of London, LONDON SW3 6LY, UK

Some pathophysiologic conditions cause an overproduction of reactive oxygen species (ROS) to such an extent that intracellular defence mechanisms may be unable to limit the damage. The ROS singlet oxygen has been applied to the cardiac sarcoplasmic reticulum (SR) Ca^{2+}-release channel and shown to cause an initial increase in open probability (Po), followed by irreversible loss of channel function (Holmberg et al. Cardioscience (1991), 2: 19-25). The effect of the ROS hydrogen peroxide has been investigated on the channel in this study. Vesicles of heavy SR from sheep cardiac muscle were incorporated into planar phospholipid bilayers and Ca^{2+} used as the permeant ion. Free $[Ca^{2+}]$ on the cytosolic side of the channel was initially 10μM and was 40-60mM on the luminal side. At 10μM cytosolic $[Ca^{2+}]$ hydrogen peroxide (1-10mM) increased the open probability of the sheep cardiac SR Ca^{2+}-release channel, but no loss of channel function was observed. In a second series of experiments, the cytosolic $[Ca^{2+}]$ was lowered from 10μM to 0.5nM. The Po of the Ca^{2+}-release channel dropped to zero and, under these conditions, hydrogen peroxide (3-5mM) produced openings within 3-5mins. After washing out hydrogen peroxide, raising the $[Ca^{2+}]$ to 10μM or lowering it to nM or pM levels no longer modified the Po of the channel. No change in channel amplitude at 0mV was seen. The ryanodine binding site appeared to be unaffected by hydrogen peroxide (3mM) since ryanodine (1-2μM) still characteristically locked the channel into a subconductance open state. These results suggest that hydrogen peroxide induces Ca^{2+}-independent openings of the sheep cardiac SR Ca^{2+}-release channel.

W-Pos302

ACTIVATION OF THE SHEEP CARDIAC CA^{2+}-RELEASE CHANNEL BY CARDIAC GLYCOSIDES : STUDIES ON THE STRUCTURAL REQUIREMENTS FOR ACTIVITY. ((S.J.McGarry and A.J. Williams)), National Heart and Lung Institute, University of London, LONDON, SW3 6LY, UK.

The effects of cardiac glycosides on sheep cardiac and skeletal sarcoplasmic reticulum (SR) Ca^{2+}-release channels have been studied using rapid $^{45}Ca^{2+}$ efflux from SR vesicles and at the single-channel level. Therapeutic concentrations of digoxin (1nM) increased rapid $^{45}Ca^{2+}$ efflux at 0.1μM extravesicular Ca^{2+} from 144 $\pm$ 20 to 258 $\pm$ 24 nmoles $^{45}Ca^{2+}$/mg protein/sec. At an activating $[Ca^{2+}]$ of 0.1μM, 1nM digoxin increased single-channel Po from 0.012 $\pm$ 0.003 to 0.110 $\pm$ 0.029 when added to the cytosolic face of the channel; at 10μM Ca^{2+}, 1nM digoxin increased Po from 0.048 $\pm$ 0.012 to 0.281 $\pm$ 0.065. The increase in Po was achieved mainly by increasing the frequency of channel opening. However, higher concentrations of digoxin increased Po by increasing the duration of open events as well, giving an Ec50 of approximately 0.91nM for digoxin. Ouabain was less potent than digoxin. At 10μM Ca^{2+} 10nM ouabain increased Po from 0.040 $\pm$ 0.011 to 0.232 $\pm$ 0.061. There was no effect of cardiac glycosides on skeletal muscle Ca^{2+}-release channels. Prednisolone, β-estradiol, chlormadinone and spironolactone failed to activate the cardiac SR Ca^{2+}-release channel or affect the digoxin-activation of the channel. Similarly, digoxigenin and ouabagenin, and digitoxose and rhamnose, at up to 100μM failed to increase single-channel Po. The genins also failed to affect the digoxin or ouabain-induced increases in Po. In conclusion, both sugar moieties and a genin or steroid nucleus are required for binding to the glycoside site on the cardiac channel and the composition, and possibly the number, of the sugar residues are determinants of activity.

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