M-Pos41

APPLICATIONS OF MAGNETICALLY COUPLED RELAXATION SPECTROSCOPY TO UNDERSTANDING MAGNETIC RELAXATION IN TISSUES AND PROTEIN SYSTEMS. Robert G. Bryant, Biophysics Department, University of Rochester, Rochester, NY 14642.

Coupled magnetic relaxation is well known and forms the basis for solution of macromolecular structures in solution phase experiments. However, the full practical and theoretical implications of this coupling have been overlooked in attempts to understand the behavior of water at interfaces. New experiments that exploit this magnetic relaxation coupling permit direct characterization of the proton spectrum of the solid components while observing the narrow liquid components. The analysis and extension of this experiment provides the fundamental basis for a quantitative understanding of the magnetic field dependence of proton relaxation in heterogeneous systems. The consequences have a profound impact on the interpretation of magnetic relaxation in other liquid systems including the controversial case of protein solutions.

M-Pos42

STUDIES OF BIOLOGICALLY ACTIVE PROTEIN FRAGMENT CONFORMATIONS USING 2D NMR METHODS J.E. Furstenau*, J.A. Starkey*, H.E. Hamm+, P.A. Hargrave#, and E.A. Dratz*, *MT State Univ., +Univ. Ill Med Center, #Univ. Fl (sponsored by A. Jesaitis).

Biological control and/or adhesion systems often depend on interactions between proteins that cannot be crystallized and/or that tumble too slowly to allow structural studies by solution NMR. In an number of interesting cases, synthetic peptide fragments of one protein cause the full biological activity in the other member of the interacting pair.

We are studying solution conformations (using primarily ROESY and TOCSY) and receptor bound conformations (using primarily transferred NOESY) of several systems. Laminin peptide 11, which binds to the laminin receptor on endothelial cells and inhibits metastatic tumor invasion and retinal rod GTP-binding protein (transducin) Ac-311-329 and 340-350 segments, which bind to light-activated rhodopsin and hold it in metarhodopsin II (the form that binds to and activates the full GTP-binding protein). (Support: Tobacco Res. Council and MT Center for Excellence in Biotechnology.)

M-Pos43

^{31}P NMR METABOLIC IMAGES OF THE ARM. T.R. Brown, S.J. Nelson, J.S. Taylor, J. Murphy-Boesch, D.B. Vigneron, Dept. of NMR and Medical Spectroscopy, Fox Chase Cancer Center, Phila., PA

Measuring changes in metabolism within specific muscle groups requires precise localization and correlation with anatomical features. Chemical shift imaging (CSI) produces spectra from well defined voxels which can be mapped directly onto corresponding proton images. To analyze this CSI data one must first quantify individual spectra and estimate metabolite concentrations. We have developed automatic quantification procedures and applied them to extract images of the spatial distribution of phosphorous metabolites in the arm. A single turn 125 mm diameter surface coil was placed around the arm and 2D-CSI datasets collected with spatial resolution as low as 5 mm. Localization perpendicular to the plane of the coil was approximately 80 mm. Metabolic images of P_i, PCr, γ-ATP, α-ATP and β-ATP showed excellent correlation with the anatomy obtained from proton images. Data acquisition times as short as 1' were used to follow changes in the ^{31}P metabolites during exercise and recovery. Images of the metabolites showed changes in PCr, P_i and pH were localized to specific muscle groups: the flexor digitorum superficialis, the flexor digitorum profundis and the extensores carpi radialis.

M-Pos44

NMR VISIBILITY OF SODIUM IN RAT MUSCLE

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The NMR visibility of the Na-23 nucleus has been debated for many years. Theoretically, it is possible that only 40% of the signal is observable.

We have used the ratio method of Thulborn and Ackerman [1] to quantitate total Na levels in vivo in rat leg (n=6) and in excised rat gastrocnemius muscle (n=4). At the end of each NMR experiment, samples were extracted for measurement of Na by flame photometry. Comparison of the two methods allows determination of NMR visibility of Na-23. We have found more than 80% of the Na to be visible in both sample types.

Na-23 T_2 relaxation times in intact rat leg are biexponential with values of 4.4ms (40%) and 37ms (60%). The T_1 value is 51ms. These values compare to NaCl in aqueous solution for which $T_1=T_2=55$ms.

[1] K.R. Thulborn and J.J.H. Ackerman. Journal of Magnetic Resonance, 55: 357-371, (1983).

This work was supported by the Medical Research Council of Canada (MT-625).

M-Pos45

^{19}F NMR ANALYSIS OF TRIFLUORETHANOL METABOLITES IN RAT URINE

Barry S. Selinsky and Daniel M. Rusnyiak, Department of Chemistry, Villanova University, Villanova, PA 19085

Trifluoroethanol (TFE) is a common industrial solvent and a known metabolite of the inhalation anesthetic fluoroxene (2,2,2-trifluorcethyl vinyl ether). The urinary metabolites of rats dosed with 0.21 g/kg (i.p.) trifluoroethanol were monitored using ^{19}F NMR spectroscopy. The predominant early metabolite observed in urine was the glucuronide conjugate of TFE, with concentrations approaching 30 mM in urine collected eight hours after TFE injection. The oxidation products of TFE, trifluoroacetaldehyde and trifluoroacetate, are also observed in rat urine following TFE treatment. A fourth metabolite, yet to be identified, is also found; its excretion parallels the excretion of trifluoroacetaldehyde. This study demonstrates the utility of ^{19}F NMR to examine the metabolism of fluorinated xenobiotics.

M-Pos46

TRNOE STUDY OF THE CONFORMATION OF MGATP BOUND TO NUCLEOTIDYL AND PHOSPHORYL TRANSFER ENZYMES S. B. Landy, K. B. Lipkowitz, B.D. Nageswara Rao IUPUI, Indpls, In., 46205, and P. Plateau, Ecole Polytechnique, France.

The conformations of MgATP bound to a nucleotidyl transfer enzyme, methionyl tRNA syntetase, and a phophoryl transfer enzyme, pyuvate kinase, were studied by ^{1}H NMR transferred NOE (TRNOE) measurements. Selective inversion of chosen resonances was accomplished with a DANTE sequence NOE measurements were made at 8 delay times ranging from 20 ms to 500 ms. A full complement of ten NOE build-up curves, for each enzyme complex, was analyzed by using the complete relaxation-matrix method modified to include exchange between bound and free substrate. Molecular mechanics computations were used to examine the energetic implications of the NOE-determined structures. The final structures obtained for MgATP bound to the two enzymes were very similar to each other, with a 3' - *endo* sugar pucker and an *anti* conformation with a glycosidic torsional angle (O'_4 - C'_1- N_9- C_8) of $39^o \pm 4^o$. (supported in part by NSF DMB 8608185 and NIH GM 43966)

M-Pos47

NMR AND FLUORESCENCE OF 5-FLUORO-TRP-MELITTIN. Arthur J. Weaver†, Marvin D. Kemple‡, and Franklyn G. Prendergast§, †Howard Hughes Med. Inst., UT Southwestern, Dallas, TX 75235, ‡Dept. of Physics, IUPUI, Indianapolis, IN 46205, §Dept. of Biochem. and Mol. Biol., Mayo Foundation, Rochester, MN 55905.

An analog of the cytolytic peptide melittin was synthesized with 5-fluoro-tryptophan at position-19 and ^{13}Cα-glycine at position-12. Column-chromatography experiments indicate that this peptide forms a NaCl-induced tetramer. NMR chemical shift measurements of ^{13}Cα-gly-12 were consistent with tetramer formation. The ^{19}F-NMR signal from trp-19 registered a chemical shift change of 0.9 ppm upon formation of the putative tetramer in aqueous solution. Chemical shift changes of the ^{19}F resonance induced by D_2O are considered to be indicative of solvent exposure of the residue in question. For the monomer a solvent-induced shift identical to that of free 5-fluoro-trp was observed while for the tetramer no shift was seen implying complete solvent exposure of trp-19 in the former and little exposure in the latter. Properties of this peptide under various solvent conditions as monitored by NMR and fluorescence will be described. Supported in part by NI486K0521 from ONR.

M-Pos48

THE BEHAVIOR OF HALOTHANE IN RABBIT TISSUES AS MEASURED BY 19F SPIN-SPIN RELAXATION TIMES.

Mitsue Miyazaki, Eugene Y. Shen, and Alice M. Wyrwicz. Department of Radiology, University of Illinois at Chicago, Chicago, Illinois 60680.

The mechanism(s) of general anesthetic action remains unknown. To gain further understanding of their behavior in tissues, 19F NMR spin-spin relaxation times(T2) of a fluorinated anesthetic, halothane, were measured using a CPMG pulse sequence. The T2 values for halothane in various rabbit tissues given below, were measured at ambient temperature(~18 °C) on a Bruker WP-200 NMR spectrometer. Halothane in the brain cerebrum and cerebellum gives similar 19F T2 values of 3-4 msec. There is no distinction in this value between the white and the grey matter. This short T2 relaxation time is unique for the brain tissue and indicates a considerably decreased mobility for halothane. The other tissues have much longer T2 relaxation times: muscle, T2=391 msec; adipose, T2=471 msec; infraorbital glands, T2=334 msec. At higher inspired cocentratios of halothane an additional longer T2(182 msec.) component is observed predominantly in the white matter of the brain. This suggests additional binding site(s) with higher mobility for halothane in the white matter.

M-Pos49

^{13}C CP/MAS SOLID STATE NMR SPECTROSCOPY AND X-RAY STUDIES OF THE CARBOXYLATE COORDINATION OF PARAMAGNETIC SHIFT REAGENTS

E.A. Adams, S.L. Helgerson, and E.A. Dratz, Chemistry Department, Montana State University, Bozeman, MT 59717.

We have carried out model experiments to investigate the use of paramagnetic shift reagents in solid state NMR to identify side chains involved in calcium-binding membrane proteins. Paramagnetic (Eu^{+3}, Sm^{+3}, Pr^{+3}) and diamagnetic (La^{+3}, Ca^{+2}) ions were complexed with ethylenediamine tetraacetic acid (EDTA). Eu^{+3} and Pr^{+3} EDTA produced broad, weak carboxylate peaks shifted greater than 50 ppm. Sm^{+3} shifts the carboxylate resonances of EDTA downfield by 12 ppm with only moderate broadening. Sm^{+3} also splits (5 ppm) the carboxylate resonances of EDTA into two equal peaks. X-ray diffraction shows that two pairs of carboxylate groups in Sm:EDTA are coordinated with different bonding geometries. The main La:EDTA carboxylate peak, is three carboxylate groups, and a downfield shoulder equal to a longer C-O distance. (ONR K0278, NIH EY06913.)

M-Pos50

^1H NMR SPECTROSCOPY OF A SYNTHETIC 23-MER FRAGMENT OF HUMAN RELAXIN.

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The human relaxin molecule, a peptide hormone with 53 residues, is involved in the dilation of the mammalian birth canal. The mechanism by which relaxin participates in this process is unclear, in part, due to a lack of structural information. Relaxin appears to form aggregates in solution, making NMR analysis of its native conformation difficult. Because fragments of relaxin show less tendency to aggregate, their solution structures may be more amenable to NMR analysis. Based on relaxin's disulfide homology to insulin, we have selected and synthesized a 23-mer fragment of the human sequence which runs from residue 7 to 17 of chain A and from residue 7 to 18 of chain B, including both the one intra-A-chain and one inter-chain disulfide bonds of the intact hormone.One dimensional ^1H NMR spectroscopy of the 23-mer indicated that its single histidine titrates with an abnormally low pK_a of 5.6. From two-dimensional spectroscopy, we have assigned nearly all of the ^1H resonances of the the residues sequence specifically. In order to understand the basis of the depressed pK_a of HisA12, we have used a series of low resolution absolute value mode COSYs to determine the pH profile of most all side chain proton resonances.

M-Pos51

SOLUTION STRUCTURES OF PEPTIDES CONTAINING THE RECEPTOR BINDING SEQUENCE ARG-GLY-ASP.

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Two-dimensional proton NMR studies of a synthetic 14-residue peptide (YAVTGRGD-SPASSC), based on residues 80 to 92 of fibronectin, were carried out at 600 MHz, 27°, 150 mM NaCl, and pH 5.9. Sequence-specific assignments of resonances were made by double quantum filtered COSY, TOCSY, and NOESY spectra with jump-return solvent suppression. The results are consistent with a type II β-turn at the GRGD position, preceded and followed by long β-strands which approach each other, as shown by remote NOEs from Hα of Ala-2 and Val-3 to HN of Ala-11, and by several slowed NH exchange rates. However, a fully formed antiparallel β-sheet is not detected. This secondary structure differs in register from that of the simpler pentapeptide (GRGDS) which consists primarily of a type II β-turn in the RGDS position (Cachau et al., these proceedings). Because of such differences, it would be most appropriate to study the conformations of receptor-bound RGD ligands.

M-Pos52

MULTIPLE CONFORMATIONS OF WILD TYPE AND MUTANT STAPHYLOCOCCAL NUCLEASE

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We have investigated multiple conformations of staphylococcal nuclease by studying the His^{121} $^1H^{\delta 2}$ and $^{13}C^{\delta 2}$ NMR signals in nuclease wild type (wt) and in the mutant in which glycine 79 is changed to serine (G79S). In nuclease wt labeled with ^{13}C 26% (ul) histidine, we find two $^1H^{\delta 2}/^{13}C^{\delta 2}$ crosspeaks in the $^1H\{^{13}C\}$ single bond correlation experiment. Both signals are strongly upfield shifted (4.3/116.5 and 4.8/121.0), presumably due to the ring current effect of nearby Tyr^{91}, and are found in an intensity ratio of 10:1. We attribute this heterogeneity at His^{121} $^1H^{\delta 2}$ to the *cis:trans* isomerization about the Lys^{116}-Pro^{117} peptide bond. In the nuclease mutant G79S, this pattern is reversed. Based on their response of the His^{121} $^1H^{\delta 2}$ signals of G79S to inhibitor binding, we assign the conformation about the Lys^{116}-Pro^{117} peptide bond as *cis* in nuclease wt and *trans* in nuclease G79S.

We also have observed two signals for His^{46} in the one-dimensional ^1H NMR spectrum. This heterogeneity is evident for both unligated nuclease and the nuclease·Ca^{++}·inhibitor ternary complex in nuclease wt and mutants. In addition, mutant enzymes showing reversed N/N' ratios for the $^1H^{\varepsilon}$ of His^{121}, exhibit a wt-like distribution of peak intensities for His^{46}. Thus, the heterogeneity we observe at His^{46} does not appear to be due to isomerization about the Lys^{116}-Pro^{117} peptide bond.

M-Pos53

^{1}H-NMR STUDIES OF PSEUDOMONAS PUTIDA FERREDOXIN. Hong Cheng, Klaus Grohmann, William Sweeney. Dept. Chemistry, Hunter College, 695 Park Ave., N.Y., NY 10021

P. putida ferredoxin contains a 3Fe-4S and a 4Fe-4S cluster, and exhibits extensive sequence homology to Azotobacter vinelandii ferredoxin. The proton NMR spectrum exhibits six resolved downfield resonances. All six resonances exhibit T_1 times ≤10 msec, indicating all arise from protons near an iron-sulfur center. Direct labeling studies have shown that the five most downfield resonances (31, 25, 22, 18, and 16 ppm) arise from β-cysteinyl protons. Conflicting reports have assigned the resonance at 16 ppm to a proton near the 3Fe center or near the 4Fe center. Current studies suggest that this resonance arises from a β-proton on a cysteine bound to the 4Fe center but near the 3Fe center. The sixth resonance (10.5 ppm) appears not to arise from a cysteinyl proton. At temperatures ≤15° and ≥30° this resonance splits into two peaks of approximately equal intensity. pH titration studies show that the three most downfield resonances titrate with a pK close to 5.6. The resonances at 18 and 16 ppm titrate with pKs of 4.5 and 7, respectively. Supported by NSF grant DMB-84 16808.

M-Pos54

PROTON-DETECTED NATURAL ABUNDANCE ^{13}C NMR SPECTROSCOPY OF THE ZINC FINGER DNA-BINDING DOMAIN XFIN-31. Arthur G. Palmer, III and Peter E. Wright, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

The increased sensitivity of proton-detected heteronuclear NMR spectroscopy, compared to conventional methods, facilitates the study of heteronuclei at natural abundance in biomolecules. Recently, the zinc finger motif has garnered attention as a structural domain for the specific recognition of DNA sequences. The DNA-binding domain Xfin-31 is a 25-residue peptide, derived from the Xenopus laevis protein Xfin, that binds a single zinc atom and forms a compact globular structure in solution. Proton-detected ^{13}C heterocorrelation and relayed heterocorrelation spectroscopies have been used to assign the ^{13}C spectrum of Xfin-31 and two-dimensional proton-detected measurements of ^{13}C longitudinal and transverse relaxation have been initiated. The results indicate the utility of proton-detected heteronuclear spectroscopy for spectral assignment, conformational and structural analyses, and investigation of relaxation in biomolecules.

M-Pos55

NMR DETECTION OF CREATINE KINASE EXPRESSED IN THE LIVER OF TRANSGENIC MICE: DETERMINATION OF FREE ADP LEVEL.

M.J. Brosnan*, L. Chen*, J. Chen°, T.Van Dyke°, and A.P. Koretsky* Depts of Bio. Sci., Carnegie Melon Univ.* and Univ. of Pittsburgh°, Pittsburgh, PA 15213

ADP is an important regulator of a number of processes, such as gluconeogenesis and oxidative phosphorylation. The equilibrium established by creatine kinase (CK) has been successfully used to determine free ADP in muscle, brain, and heart. Liver lacks CK, therefore, it has been difficult to determine free ADP levels in liver *in vivo*. To remedy this we have expressed the B isozyme of CK in the liver of transgenic mice. A DNA construct containing the liver specific control region from the transthyretin gene and the coding region of the mouse brain CK gene was used to generate three lines of transgenic mice. These mice have CK activity in liver ranging from 80-250 μmoles/g wet wt at 25°C; this compares to <1 μmoles/g wet wt in control liver and 250 μmoles/g wet wt in mouse heart. ^{31}P NMR spectra from transgenic liver contain a peak due to phosphocreatine which is absent from control liver spectra. Using the equilibrium established by CK a free ADP level of 0.059±.004 μmoles/g wet wt was found in transgenic liver. This value was constant over a broad range of CK activity and total liver creatine levels. These mice provide a valuable tool for understanding the role of ADP in hepatic energy metabolism.

M-Pos56

DIVALENT METAL BINDING TO CHEY: EFFECT ON PROTEIN CONFORMATION AND PHOSPHATE BINDING

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§Department of Microbiology and Immunology
University of Illinois at Chicago

The interaction of the bacterial chemotaxis protein, CheY, with ATP and a variety of other related molecules has been studied in the presence and absence of divalent metal ions. These studies have led to the discovery of two conformations of the protein in water. It is shown that, in the metal-bound conformation, CheY will bind to nucleotide phosphates and phosphates in general; but in the metal-free conformation the protein loses its affinity for phosphates. Comparison of double quantum filtered COSY spectra from the metal bound and metal free forms of the protein indicates that two phenylalanines and one threonine are most significantly affected by the conformational change involved. These same residues are also indicated to be close to the phosphate binding site in NMR experiments with spin labeled ATP, suggesting that the loss of metal alters the conformation of the phosphate binding site, thereby reducing binding affinity for phosphates. The possible significance of metal binding on the phosphorylation & dephosphorylation of CheY is discussed.

M-Pos57

^{31}P NMR STUDY OF THE EFFECT OF PB^{2+} ON ATP SYNTHESIS RATE IN BONE CELLS.

Dowd, T.L., Rosen, J.F. and Gupta, R.K. Albert Einstein College of Medicine, New York, N.Y. 10461.

Evidence from various studies suggests perturbations in cellular energetics as the molecular basis for Pb^{2+} toxicity. We have investigated the effects of Pb^{2+} on high energy phosphates and on the unidirectional rate of ATP synthesis in a continuously perfused rat osteoblastic bone cell line (ROS 17/2.8) using ^{31}P NMR and saturation transfer. The mechanism of $ATP\gamma \rightarrow P_i$ saturation transfer was also investigated. The unimolecular rate constant for ATP synthesis in untreated cells was $.25 \pm .04$ sec^{-1}. Upon incubation of the cells with 10 μM Pb^{2+} no saturation transfer was detectable with our signal to noise ratio indicating a reduction in the rate of ATP synthesis. In addition there was a 34% decrease in ATP and a 39% decrease in PCr upon exposure to 10 μM Pb^{2+}. This provides direct evidence of Pb^{2+} induced alterations in cellular energetics and may explain some previously observed functional impairments.

M-Pos58

A UV-RESONANCE RAMAN INVESTIGATION OF TRYPTOPHAN IN AZURIN. J. A. Sweeney, P. A. Harmon and S. A. Asher, Dept. of Chem., Univ. of Pittsburgh, C. M. Hutnik and A. G. Szabo, Div. of Biol. Sciences, Nat'l. Res. Council of Canada, Ottawa,

We investigated the role of Trp in the blue-copper protein azurin from Pseudomonas aeruginosa which serves as a redox carrier in electron transfer. Fluorescence studies of holo and apo azurin indicate interactions between the Trp and the Cu which depends upon the Cu oxidation state. We use Raman Saturation Spectroscopy to monitor repopulation of ground state molecules subsequent to Raman excitation. The reciprocal of TRP ground state repopulation rates in holoazurin and apoazurin qualitatively agree with the fluorescence data. TRP radical cation is not observed. The fast recovery of ground state Trp in holoazurin, indicates that the lifetime of TRP radical must be less than 500 ps. We also examined the Raman cross sections of Trp of Cu(I) and Cu(II), and guanidine hydrochloride denatured azurin. These results reflect the hydrophobic Trp environment in the solvent-inaccessible β-barrel region of azurin and the highly polar aqueous environment of the exposed Trp in the denatured protein.

M-Pos59

FLUORESCENCE LIFETIME DISTRIBUTION OF TRYPTOPHANYL RESIDUE AS FUNCTION OF POLYPEPTIDE CHAIN LENGTH.

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The aim of this report is to correlate the emission lifetime distribution parameters to the polypeptide chain length. In order to exclude effects due to non-covalent interactions, we studied the fluorescence decays of protein fragments, in 6.0 M guanidine, obtained from enzymatic digestion of tuna apomyoglobin,which contains a single indole residue. Three fragments of different length were purified. Lifetime determinations were performed by frequency domain fluorometry. The results show that the tryptophanyl lifetime distribution width of peptides containing more than 14 residues is correlated to the chain length whereas the emission decay of shortest peptides is similar to that observed for N-acetyltryptophanylamide.

M-Pos60

NANOSECOND TRANSIENT ABSORPTION SPECTROSCOPY OF COENZYME B_{12}: QUANTUM YIELDS AND SPECTRAL DYNAMICS. Chen, E., Chemistry Department, Georgetown University, Washington, DC 20057.

For many B_{12} enzyme reactions, cleavage of the cobalt-carbon bond of adenosylcobalamin (Ado-Cbl) leads to the generation of a Co^{2+} ion and a free radical. Photolysis of Ado-Cbl, which also leads to homolytic cleavage, may mimic the structure and dynamics of enzyme induced cleavage, providing a model system for enzymes. Therefore, we have used laser photolysis and nanosecond transient absorption spectroscopy with a double diode array detector, in order to measure the quantum yields and spectral dynamics of cobalamin compounds. The nanosecond quantum yield of photolysis for Ado-Cbl is 0.23 $\pm$ 0.04, higher than previously reported. "Base-off" B_{12}, a more stable, acidified form of Ado-Cbl, has a fivefold lower quantum yield at 0.045 $\pm$ 0.015, showing that quantum yields reflect intrinsic bond strength. The minimal wavelength dependence of the quantum yield for Ado-Cbl, demonstrates at least 50% efficiency in coupling of the corrin ring energy to the cobalt-carbon bond. In addition, these results suggest that geminate recombination may play a significant role in the mechanism of homolytic cleavage.

M-Pos61

STRUCTURES OF INTERMEDIATES OF COENZYME B_{12} CATALYSIS. Sagi, I., Wirt, M., Chen, E., Frisbie, S., and Chance, M., Georgetown Univ., Dept. of Chemistry, Wash.,D.C.,20057.

The structures of the catalytic intermediates of adenosylcobalamin (Ado-Cbl) dependent enzyme systems are not well understood and have been difficult to crystallize. Using EXAFS spectroscopy, we have examined the structure of the Co^{+1}, Co^{+2}, and "base-off" forms of B_{12}. For the Co^{+2} species, the average of the Co-N equatorial distances of the corrin ring are 1.87$\pm$0.01 A, and the Co-nitrogen ($Co-N_d$) distance to the dimethyl-benz-imidazole ligand is 1.99$\pm$0.02 A. The large reduction in the $Co-N_d$ distance relative to Ado-Cbl, where $Co-N_d$ is 2.24 A, is consistent with the cobalt ion moving out of the plane of the corrin nitrogens, and suggests a mechanism for cobalt-carbon bond homolysis. When the cobalt-carbon bond breaks, the $Co-N_d$ bond becomes much stronger and electron density is reoriented away from the breaking bond. We also present the structures of Co^{+1} and base-off B_{12} and discuss their significance to B_{12} catalysis.

M-Pos62

X-RAY EDGE SPECTROSCOPY OF COBALT (I, II, III) B_{12}. Wirt, M., Sagi, I., Chen, E., Frisbie, S., and Chance, M., Dept. of Chemistry, Georgetown Univ., Wash., D.C. 20057

We present the first direct structural studies of the cobalt(I, II, III) and "base-off" forms of B_{12} using x-ray edge spectroscopy. In addition, model cobalt compounds, including cobalt (I, II, III) dimethylglyoxime (DMG) were examined. A surprisingly small 0.5±.2 eV shift to lower energy was observed for each change in oxidation state from Co^{+3} to Co^{+2} to Co^{+1} B_{12}. In general, a one electron reduction of a metal ion shifts the edge to lower energy by 1-5 eV. We attribute these small shifts to a delocalization of electron density off the cobalt atom in the two reduced forms. No edge shift was observed for the Co^{+3} DMG compared to Co^{+2}, indicating that the unpaired electron density may be localized on the axial nitrogen of the pyridine ligand. A 2.0 eV shift to lower energy was observed in the comparison of Co^{+3} DMG to Co^{+1}, indicating full Co^{+1} character for that species. Also, assignments for other cobalt compounds are presented.

M-Pos63

FTIR STUDIES OF B_{12} COMPOUNDS. Taraszka, K., Chen, E., and Chance, M., Dept. of Chemistry, Georgetown Univ., Washington, D.C. 20057

These studies represent the first IR spectra of B_{12} compounds observed in solution and are relevant to B_{12} enzyme dynamics. The major band of B_{12} compounds in D_2O is observed at about 1630 cm^{-1} and may be attributed to the acetamide and propionamide side chains of the corrin ring. This band is observed at 1633 cm^{-1} for vitamin B_{12}, 1629 cm^{-1} for coenzyme B_{12}, 1632 cm^{-1} for dicyanocobinamide and is shifted to a higher frequency, 1651 cm^{-1}, for "base-off" B_{12}. In addition, we have observed a major band originally seen in solid spectra by Hogencamp et al (J. Biol. Chem., 1965, 240, p.3641) at 1574 cm^{-1} for vitamin B_{12}, 1570 cm^{-1} for coenzyme B_{12}, and 1582 cm^{-1} for dicyanocobinamide, while no band was observed for base-off B_{12}. This may be attributed to displacement of Co from the plane of the equatorial nitrogen groups. We also report the spectra of Co^{+2} B_{12} and aquocobalamin and the observation of further spectral modes from 1400-1500 cm^{-1}.

M-Pos64

PURIFICATION OF TRANSCOBALAMIN II Frisbie, S.M., and Chance, M., Dept. of Chemistry, Georgetown University, Washington, D.C.,20057

Transcobalamin II is responsible for transporting vitamin B_{12} in the blood stream. In order to better understand the mechanisms of binding, we have purified transcobalamin II from chicken serum using an affinity matrix. Aminopropylcobalamin was made from the reaction of cyano-cobalamine with 3-chloropropylamine, then immobilized on Sephacryl (Jacobsen and Huennekens, Methods in Enzymology 123, 1986, 28, with minor changes). The aminopropyl-cobalamin-bound Sephacryl was used to extract transcobalamin II from the serum. The interaction of B_{12} and its binding proteins leads to spectroscopic changes similar to those observed in B_{12} dependent enzyme systems. Therefore, binding proteins are also a model system for enzyme catalysis. The isolated protein has been used to study protein-ligand interactions using resonance Raman and other optical spectroscopies.

M-Pos65

CRYOGENIC OPTICAL SPECTROSCOPY OF B_{12} COMPOUNDS. Chen, E. and Chance, M., Dept. of Chemistry, Georgetown Univ., Washington, D.C.,20057

At low-temperature, the vibrational splitting of cobalamin pi-pi* optical transitions becomes pronounced. Previous investigations (Firth et al., Biochem., 1967, 6, p.2178.) have suggested that a vibrational overtone of 1300-1350 cm^{-1} exists for many B_{12} compounds. We have re-investigated these spectra at liquid helium temperatures with a high resolution intensified diode array spectrograph. For vitamin B_{12}, we observe the 1,2 progression of 1300 cm^{-1} from the second lowest energy peak. Further towards the blue is a 1,2 progression of 1800 cm^{-1} relative to the second overtone of the main band.In adenosylcobalamin, different overtones of 900-950 cm^{-1} are seen. These results suggest the presence of metal-ligand charge transfer or d-d transitions in addition to the classical pi-pi* transitions. Since vibrational modes are related to excited states, these results are relevant to dynamics of cobalamin photolysis.

M-Pos66

VIBRATIONAL CIRCULAR DICHROISM, RAMAN, FTIR AND ELECTRONIC CD STUDIES OF AZIDE BINDING TO HEME PROTEINS. S. Asher[a], P. Larkin[a], N. Ragunathan[b], T. Freedman[b], L. Nafie[b], B. Springer[c], S. Sligar[c], R. Noble[d]. [a]Dept. of Chem., Univ. Pittsburgh, [b]Dept. of Chem., Syracuse Univ., [c]Dept of Biochem., Univ. of IL, Urbana, IL, Dept. Med. and Biochem., State Univ. of NY, Buffalo, NY.

We report novel vibrational CD studies of azide binding to heme proteins which when coupled to additional optical studies using more classical techniques ?clarify? the role of the distal heme pocket in defining the ligand binding geometry and affinity. We examine sperm whale Mb and a mutant in which the distal his is replaced by gly, horse Mb, human and carp Hb and proteins reconstituted with modified hemes.

Following the earlier pioneering study by Marcott et. al. (Optical Activity and Chiral Discrimination, Ed. Mason, F., Reidel Publishing, 1979), the work here is the first use of VCD to study ligand binding in heme proteins.

M-Pos67

SPECTROSCOPIC STUDIES OF THE NUCLEOTIDE BINDING SITE OF GTPases AND ATPases. John F. Eccleston, Sally K.A. Woodward, Tazeen F. Kanagasabai and Stephen R. Martin. National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

The 2'(3')-O-methylanthraniloyl (mant) derivatives of nucleotides are valuable probes of the structures and mechanisms of GTPases and ATPases. Derivatives of ribonucleotides exist as an equilibrium mixture of the 2' and 3' isomers but derivatives of 2'-deoxyribonucleotides consist of a single isomer. Mant-GDP binds to elongation factor Tu (EF-Tu) with the same affinity as GDP and its fluorescence is enhanced by 60%. Mant-dGDP binds four-fold weaker than GDP but shows no enhancement of fluorescence. Mant-GDP has relatively intense CD in solution which is increased on binding to EF-Tu whereas mant-dGDP has weak CD whether in solution or bound to EF-Tu. These results are discussed in relation to the known three-dimensional structure of EF-Tu and compared with results for the binding of mant-ADP and mant-dADP to myosin subfragment 1 where both derivatives show enhancements of fluorescence and CD on binding.

M-Pos68

TIME-RESOLVED CIRCULAR DICHROISM STUDIES OF PROTEINS

Sofie C. Bjorling, Cora M. Einterz, James W. Lewis, Robert A. Goldbeck and David S. Kliger; Chemistry Department, University of California, Santa Cruz

A technique to measure circular dichroism (CD) spectra with a nanosecond time resolution has recently been developed. Linear birefringence and linear dichroism artifacts produced by photoselection can distort the CD signal when it is measured during the time scale of molecular reorientation. Extensive theoretical analysis using Jones calculus has been done to find the conditions where these artifacts are eliminated and the analysis has been tested experimentally. The technique has been applied to the study of structural and conformational changes occuring in several proteins (including retinal and heme proteins) after their photolysis. Theoretical and experimental results will be presented.

M-Pos69

FLUORESCENCE DECAY OF S-100a PROTEIN. Chien-Kao Wang[2], Rajam S. Mani[3], Cyril M. Kay[3], and Herbert C. Cheung[2]. [2]University of Alabama at Birmingham, Birmingham, Alabama, and [3]University of Alberta, Edmonton, Alberta, Canada.

The emission and anisotropy decays of the single tyrptophan of bovine brain S-100a protein was studied by picosecond time-resolved spectroscopy. With 295 nm excitation the emission decay was resolved into 3 components. At 20°C and pH 7.2 the decay times were 0.43, 1.42, and 4.05 ns with fractional intensities of 0.31, 0.31, and 0.38, respectively. While Mg^{2+} had a negligible effect on the decay pattern, Ca^{2+} induced a 12% decrease in the shortest decay time and an increase of 55% and 14% in the other two decay times. The fractional intensity of the shortest decay time was reduced to 0.05, whereas the fractional intensities of the other two components were increased to 0.39 and 0.55. Similar Ca^{2+}-induced changes in the decay parameters were observed at lower temperatures. The decay kinetics may be indicative of multiple local conformations. The anisotropy decay will be discussed. (Supported by NIH AR-25193 and MRC Protein Group, Canada.)

M-Pos70

POLARIZED ELECTRONIC SPECTRA OF Z-DNA SINGLE CRYSTALS

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Polarized electronic absorption spectra of the (100) face of single crystals of the Z-form double helical duplex of d(m^5CGUAm^5CG) have been obtained from Kramers-Kronig analysis of reflection data. The *c* crystallographic axis is parallel to the helix axis and shows but weak absorption. The *b* axis is perpendicular to the helix axis and shows a structureless absorption band centered at 270 nm with an oscillator strength of 0.26. These are the first polarized spectra obtained for a polynucleotide with precisely known structure, conformation and orientation. Calculations of the crystal spectra utilizing available transition moment data for the individual chromophores have been carried through using the oriented gas model (no interbase interactions) and, again, employing all base-base interactions (point dipole) in the duplex. The calculated hypochromism of the 270 nm band is much less than the experimental value obtained from the crystal data, and this difference is attributed to chain length effects. The crystal spectra appear to be representative of Z-form double helices of essentially infinite length and not of a collection of twelve base duplexes. No evidence for $n\pi^*$ transitions polarized parallel to the helix axis is found.

M-Pos71

THE DETERMINATION OF THE pKa's OF AMINO ACID GROUPS IN PROTEINS BY RAMAN DIFFERENCE SPECTRSCOPY.

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Sensitive Raman difference spectroscopy can be used to study the behavior of small molecules and/or small molecular groups inside proteins or other large macromolecules (Yue, K. T. et al., *J. Raman Spectrosc.* (1989) 20, 541-545). We have measured the Raman spectra of human transferrin at different pH's, titrating its various histidine residues. For example, the difference spectrum between pH 6 and pH 8 of transferrin is very similar to the difference spectrum of histidine in solution, indicating that differemce spectroscopy can be used to study behavior of individual residue inside proteins. Similar experiments with lactate dehydrogenase, which contains an essential histidine at its active site, will also be presented.

M-Pos72

FLUORESCENCE SPECTROSCOPY OF HUMAN IMMUNODEFICIENCY VIRUS-1 rev

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HIV-1 rev, a viral-encoded protein essential for replication of the virus has been purified. HIV-1 rev contains a single TRP (residue 45) which is located within an ARG-rich region of the protein (10 ARG within residues 35-50). The TRP residue has an emission maximum at 344 nm, characteristic of the amino acid in an aqueous environment. Quenching by acrylamide and iodine was measured and Stern-Volmer constants for each quencher determined. Together with polarization and anisotropy measurements (0.15 and 0.10, respectively) the results indicate that TRP-45 is located on the surface of the protein. Possible importance of the TRP residue and the Arg-rich region in RNA binding will be discussed.

M-Pos73

SPECTROSCOPIC EVIDENCE OF HETEROGENEITY IN THE TRP ENVIRONMENT OF NEUROTOXINS

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The photo-excited triplet state of the three homologous single trp-containing nicotinic acetylcholine receptor neurotoxins, α-bungarotoxin (BgTX), α-cobratoxin (CbTX) and cobrotoxin (CoTX), have been investigated using phosphorescence and optically detected magnetic resonance (ODMR) spectroscopy. Each shows similar phosphorescence from the functionally invariant trp28 with the intensity of BgTX and CbTX quenched relative to that of CoTX. ODMR signals from BgTX and CbTX clearly exhibit multiple trp resonances which change features upon varying excitation wavelength. This is interpreted as heterogeneity in the trp28 environment of the unquenched ensemble detected via ODMR. CoTX shows no excitation-dependence of its ODMR signals. Reduction of the cys29-cys33 disulfide bridge of BgTX (also present in CbTX but absent in CoTX) produces increased trp emission and an ODMR spectrum similar to that of CoTX suggesting the role of disulfide in quenching trp28. Supported by NIH Grant ES-02662.

M-Pos74

FLUORESCENCE STUDIES OF NONENZYMATICALLY GLYCOSYLATED ENZYMES.

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Nonenzymatic glycosylation of the amino groups on enzymes may interfere with their degradation by the ubiquitin mediated pathway of protein degradation and result in the accumulation of modified enzymes in cells. As a preliminary evaluation of this hypothesis, we have investigated the susceptibilities of several enzymes of the glucose metabolism to nonenzymatic glycosylation. When rabbit muscle aldolase is incubated with 3 mM fructose-1,6-diphosphate in Tris buffer, pH 7.5 at 30°C, changes in the absorption spectrum of the enzyme at around 330 nm, representing the formation of glycosylation products, are observed within 20 hours. A fluorescence emission spectrum (excitation at 360 nm), with a peak at 430 nm and a broad shoulder at about 480 nm, is obtained within less than 5 days of incubation. The enzyme is also glycosylated by ribose-5-phosphate and fructose-1-phosphate. Unphosphorylated glucose, fructose, galactose and ribose are, however, ineffective in glycosylating the enzyme even after 37 days of incubation at 30°C. Rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GPDH) and baker's yeast glucose-6-phosphate dehydrogenase are glycosylated by ribose-5-phosphate, producing emission spectra resembling the spectrum of glycosylated aldolase.

M-Pos75

RESONANCE ENERGY TRANSFER (RET) FROM A CYLINDRICAL ASSEMBLY OF DONORS TO A PLANE OF ACCEPTORS - LOCATION OF THE APO-B100 PROTEIN ON THE SURFACE OF THE LDL PARTICLE.

J. Eisinger, P. Bastiaens, A. de Beus, H.M. Lacker, P. Somerharju* and M. Vauhkonen*. Dept. of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029. The rate of RET from a donor cylinder (e.g. a membrane protein) to a planar acceptor assembly (e.g. membrane probes) was evaluated by (1) analytical solutions for uniform and continuous donor (D) and acceptor (A) distributions, and (2) by computation of the RET rates between pairs of discrete D's and A's, for many random distributions of D and A. Results for the two approaches, presented in terms of Förster distance-independent parameters, are in good agreement, with the latter providing a measure of the error expected for unknown D locations. This methodology is illustrated by analyzing the RET from the 37 tryptophan residues of the apo-B100 protein to a series of pyrenyl acceptor probes in the phospholipid monolayer of the human LDL particle and it is concluded that the protein is partially immersed in the particle's phospholipid surface.

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M-Pos76

INTERMOLECULAR INTERACTIONS IN MELANIN - A FLUORESCENCE STUDY

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Melanins are the natural pigments responsible for the color of hair and skin. They are formed *in vivo* by enzymatic oxidation of tyrosine. The main structural units in natural melanins are believed to be 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), but the structure is not well understood.

Melanins have unusual optical properties; the absorption spectra, irrespective of the origin, are featureless, and the absorption increases monotonously from the near infrared to the ultraviolet region. Fluorescence specroscopy, on the other hand, appears to be quite sensitive to the melanin type[1].

Using fluorescence spectroscopy, we show that melanin solutions in dimethyl sulfoxide (DMSO) exhibit strong intermolecular interactions (aggregation). These interactions were not observed in aqueous solutions. We attribute these to ground-state interaction between the polymeric chains, rather than exciplex formation, since both the excitation and emission spectra are affected. For sepia melanin, these interactions result in ca. 90 nm (4000 cm^{-1}) shift in the emission maximum. The likely mechanism of these interactions and their relevance to the optical properties of melanin will be discussed.

[1] C. Pande and T. Schultz, *Biophys. J.* (abst.) 1989, 214a.

M-Pos77

CONSEQUENCES OF THE HYDROGEN BOND STRENGTH IN THE SYSTEM $C{=}NH^+ \cdots {}^-OOC$ ON THE RAMAN EXCITATION PROFILE OF PROTONATED SCHIFF BASE. IMPLICATIONS FOR BACTERIAL AND VISUAL PIGMENTS.

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The retinyl Schiff base (SB. hereafter) was synthesized by condensation of all-trans-retinal and tert-butylamine and is the chromophore commonly encountered in bacterial and visual pigments under a protonated form ($C{=}NH^+$). This SB was then dissolved in chloroform and complexed with organic acids of pK_a ranging from ca.0 to 5 units in water, in order to characterize the consequence of the H^+ charge perturbation on the π-π^* electronic transition of this double bond conjugated system.

The behavior of these compounds was not followed by UV absorption, but preferably by recording their Raman spectra with various excitation wavelengths between 457.9 and 514.5nm, which are coincident with the long-wavelength side of the π-π^* transition maximum of the fully protonated SB (This absorption band is centered at ca.453nm when a strong organic acid is used as a proton source). All the Raman data collected between 1100-1800 cm^{-1} are dominated by the strong emission of the stretching vibration of the C=C moiety and part of an excitation profile can be drawn from this particular mode. Unfortunately, a severe deviation from normal expectations is observed, due to the presence of species such as C=N....HOOC, $C{=}NH^+ \cdots {}^-OOC$ and $C{=}NH^+ \cdots {}^-OOC$....HOOC which are in equilibrium and governed by a multiple-well potential.

In this situation the resonance Raman regime is affected, and early theoretical calculations have proved that the appearance of the spectra is more dependent on the displacement of the potential surface in the resonating excited state than on the population of each individual well. In addition, a qualitative picture of the excited state shape can also be deduced. These findings will also be used to give a possible explanation to the color of the retinyl Shiff base in visual pigments.

M-Pos78

CAROTENOID FLUORESCENCE. W.I. Gruszecki, B. Zelent* and R.M. Leblanc. Centre de recherche en photobiophysique, Université du Québec à Trois-Rivières, Québec, Canada, G9A 5H7.

It has been established that carotenoid pigments form aggregates in the hydrated organic solvents [1]. On the other hand, apart from the existing conviction that these pigments do not fluorest, some investigations on the carotenoid fluorescence have been recently reported [2,3]. We have studied the fluorescence properties of zeaxanthin and violaxanthin aggregates in water-ethanol (9:1, v/v) solvent system. The analysis of the fluorescence emission and the fluorescence excitation spectra of these carotenoid aggregates lead us to the conclusion, contrary to the other reports [2,3], that the observed fluorescence is related to the radiative deactivation of the symmetrically forbidden lowest excited electronic singlet state $^1A_g^*$ [4]. Along the presented data, the biological importance of the carotenoid fluorescence from the $^1A_g^*$ state will be discussed.

1. Hager, A., Planta 91, 38 (1970); 2. Gillbro, T. and Gogdell, R.J., Chem. Phys. Lett. 158, 312 (1989); 3. Bondarev, S.L., Bachilo, S.M., Dvornikov, S.S. and Tikhomirov, S.A., J. Photochem. Photobiol., A:Chemistry 46, 315 (1989); 4. Thrash, R.J., Fang, H.L.-B. and Leboi, G.E., Photochem. Photobiol. 29, 1049 (1979).

M-Pos79

BINDING OF INDOLE DERIVATIVES TO PROTEIN RECEPTORS

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The mechanisms involved in the recognition of specific molecules in ligand-receptor complexes involve a complicated array of interactions. The specific binding of many drugs is based upon aromatic ring systems, presumably due to interactions unique to these residues. Charge transfer interactions, most often involving aromatic components, can provide high affinity and highly specific binding between molecules. The heterocyclic aromatic molecule indole, commonly involved in charge transfer interactions with other molecules, is a component of a number of drugs such as yohimbine and serotonin. We are evaluating the binding affinity and the specificity of charge-transfer (CT) complexes that form between indole-based molecules and protein receptors or with electron acceptors, as a function of covalent modifications on the indole chromophore. These properties are being correlated to the ground and excited state electronic properties of the indole chromophores. Indole derivatives have been shown to bind to the Alpha-2 receptor of human platelets (5-amino indole> indoline> 5-hydroxy indole) as well as to the enzyme tryptophanase. The binding affinities of these derivatives to the protein receptors are not identical, and the properties of these molecules that facilitate interaction with the receptor systems will be evaluated.

M-Pos80

BOMBESIN-LIKE PEPTIDE INTERACTIONS WITH MODEL MEMBRANES. P. Cavatorta[1], G. Farruggia[2], P. Neyroz[2], G. Sartor[2], A.G. Szabo[3], [1]Department of Physics and [2]Institute of Biological Chemistry, University of Parma, 43100 Parma, Italy; [3]Division of Biological Sciences, NRC, Ottawa, Canada K1A 0R6.

Bombesin (BMB), gastrin releasing peptide (GRP) and litorin (LIT) share a widespread spectrum of hormonal activity. They act as growth factors for certain cell lines. In solution they exist as an ensemble of unstructured, flexible conformers. With DMPS vesicles an increase of tryptophan fluorescence and changes in ellipticity show that the peptides bind to the lipids with induction of α-helical structure. The binding constants and amount of induced α-helix varies in the order BMB<LIT<GRP. The pH behaviour and salt effects indicate that the binding is both electrostatic and hydrophobic in nature. On the contrary, DMPC vesicles do not show any detectable interaction with the peptides. Mixed DMPS-DMPC vesicles, however, show a binding constant similar to that of DMPS alone. These results suggest a special requirement for negatively charged lipids in the binding process. This requirement should be important for a membrane assisted hormone-receptor interaction mechanism.

M-Pos81

MYOGLOBIN: EFFECT OF HEME LIGATION ON THE FLUORESCENCE DECAY KINETICS AND EVIDENCE FOR DISCRETE LIFETIME COMPONENTS. K.J. Willis, A.G. Szabo, M. Zuker, J.M. Ridgeway and B. Alpert*. Div. of Biological Sciences, N.R.C., Ottawa, Canada K1A 0R6. *Laboratoire de Biologie Physico-Chimique, Université de Paris VII, 75251, Paris, France.

Myoglobin from sperm whale, purified by HPLC displays picosecond decay kinetics exclusively. Observed lifetimes of 24 ps for Trp^{14} and 122 ps for Trp^{7} (oxy, pH 7) agree with theoretical predictions. The lifetimes are subtly sensitive to changes in the heme ligand and pH. The increase in the fluorescence lifetime observed on O_2 binding (ca. 6 ps for Trp^{14} and 20 ps for Trp^{7}) is mainly due to heme absorption spectra changes, which reduce the rate of energy transfer from trp to heme. This correlates with crystal structure data which indicates that the trp-heme separation and relative orientation are unchanged on ligand binding. Analysis of the fluorescence data as a continuous Gamma distribution of exponential decays resulted in extremely narrow distributions which do not support the existence of multiple, structurally distinct conformational states in Mb.

M-Pos82

ROTAMERS AND EXCITED STATE REACTIONS OF TRYPTOPHAN ZWITTERION. A.G. Szabo, K.J. Willis and D.T. Krajcarski. Division of Biological Sciences, National Research Council, Ottawa, Canada K1A 0R6.

The understanding of the fluorescence properties of tryptophan is vital to its use as an intrinsic probe of protein structure and dynamics. It has been generally accepted that at pH 7, the fluorescence of tryptophan decays with double exponential decay kinetics, and that this behaviour may be attributed to different rotamers of the alanyl side chain. The rationalization of the increased quantum yield in D2O solution has not been completely elucidated. We have reexamined the fluorescence decay behaviour of tryptophan and some of its derivatives in D2O and H2O under a variety of excitation conditions and at different emission wavelengths. The results complement the original data, but new details of the fluorescence decay behaviour, including excited state reaction, have been observed which may require a reinterpretation of the rotamer model. These results will be presented and possible explanations will be discussed.

M-Pos83

RESONANCE RAMAN SPECTRA OF COPPER(II) AND NICKEL(II) OCTAETHYLISOBACTERIOCHLORIN

David F. Bocian and Alexander D. Procyk

Resonance Raman (RR) spectra are reported for Cu(II) and Ni(II) octaethylisobacteriochlorin (OEiBC). The spectra were obtained by using a variety of excitation wavelengths which span the B, Qx, and Qy regions of the absorption spectrum. The RR intensity enhancement patterns are found to differ markedly at the various excitation wavelengths, particularly at different wavelengths within the B band. The majority of the high-frequency skeletal vibrations of the macrocycle are assigned by examining selectively meso-deuterated CuOEiBC and both high- and low-spin NiOEiBC. Normal coordinate calculations were also performed in order to determine the forms of the vibrational eigenvectors. Collectively, these studies lend insight into the structural and electronic properties of the OEiBC macrocycle.

M-Pos84

Detection of ATP, ADP, and Phosphate in solution by Near Infra-Red Spectroscopy

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The direct measurement of ATP, ADP, and phosphate in a buffered solution has been carried out using a temperature controlled, nitrogen purged cell in an LT Industries Quantum 1200 NIR scanning spectrometer. Region scanned was 900 to 1800 nanometers at a resolution of 0.75 nm. Scanning time is 200 milleseconds. 100 scans were used for all concentration ranges from 25 to 1 micromolar. All runs were done at 4°C, in 0.2M Bis-tris pH 7.40 and 0.1M KCl. At pH 8.4, only the phosphate moiety in ATP and ADP were seen, thus all samples looked alike. At pH 6.2, in Bis-tris, ATP and ADP looked alike but were different from phosphate. Spectral analysis and correlation with concentration is done by single-valueddecomposition (SVD) (1,2) and partial-least-squares (PLS) (3) using MATLAB running on an IBM AT. Extensive experience with NIR has been reported in other fields (4,5) but little work has so far been done in biochemistry and biophysics. It is hoped this technique may be of use particularily to workers in transport research.

M-Pos85

INFLUENCE OF DIFFUSION ON LONG-RANGE ENERGY TRANSFER BY FREQUENCY-DOMAIN FLUOROMETRY.

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We used frequency-domain fluorometry to study long-range intermolecular energy transfer in presence and absence of translational diffusion. Frequency-domain data of indole fluorescence (donor) in the absence and presence of dansylamide (acceptor) in propylene glycol (low diffusion) and methanol (high diffusion) were fitted to Forster and/or Gosele et al. models, the latter of which contains diffusive terms. This donor-acceptor system ($R_o \approx 25Å$) is commonly used in intramolecular energy transfer measurements. The Forster model provides acceptive fit in propylene glycol, but fails in methanol. The Gosele et al. model gives reasonable fits and parameters (R_o and D) in both solvents. Analysis of simulated and measured data show usefulness of frequency-domain fluorometry in energy transfer studies. From analysis of simulated data we estimated the limit of resolution of diffusion coefficient ($D > 10^{-7} cm^2/s$). In a separate study, using 2-aminopurine as a donor and 2-aminobenzophenone as an acceptor, we observed systematic deviations from the Forster model at higher concentrations of the acceptor. (From the Center for Fluorescence Spectroscopy, University of Maryland.)

M-Pos86

DIELECTRIC DISPERSIONS OF BACTERIORHODOPSIN IN THE MHz REGION

Idwar Bakarudin. Institute of Basic Sciences, University of Agriculture Malaysia, Bintulu Campus, 97008 Bintulu, Sarawak.

Dielectric measurements around the MHz region in aqueous solution containing bacteriorhodopsin (bR) have shown the existance of β_1-dispersion with five distinct parts. This dispersion and the β-dispersion at low frequencies conform approximately to the Debye relaxation equations. The derived apparent relaxation times showed dependence on physical parameters. The relaxation effect at low frequencies is attributed to reorientation of bR chromopore within the purple membrane (PM) fragments. The mechanism which give rise to the β_1-dispersion may well be due to the Maxwell-Wagner effect, although the first two parts of the β_1-dispersion may also be attributed to counterion relaxation or bR reorientation.

M-Pos87

CONFORMATIONAL CHANGES OF BOVINE BRAIN S100b INDUCED BY TERBIUM AND CALCIUM.

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S100b is an acidic, homodimeric protein which exposes a hydrophobic domain upon binding calcium. Using circular dichroism (CD) and UV difference spectroscopy, terbium was used to study the calcium binding sites of S100b. The addition of Tb^{3+} decreased the ellipticity at 222 nm of S100b (14 μM), and induced a UV difference spectrum, both similar to that observed with calcium. The secondary structure estimations of the Tb^{3+} induced far UV CD spectra of S100b are consistent with an α-helix to coil transition, as observed in the presence of calcium. The UV difference spectral molar extinction values ($\Delta\varepsilon$) at 259.5 nm, characteristic of the blue shifted phenylalanine residues, and at 282 nm, representing the red shifted tyrosine residue, were less for calcium than for terbium at identical concentrations. However, in contrast with Ca^{2+}, higher concentrations of terbium precipitate S100b. These data suggest that Tb^{3+} and Ca^{2+} have similar binding sites with a higher affinity for terbium.
(supported by a grant from The Graduate School, Wayne State University)

M-Pos88

THE METAL SITE STRUCTURE OF CON A IN CRYSTAL AND SOLUTION

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A.J. Kalb (Gilboa), Dept. of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

X-ray Absorption Fine Structure Spectroscopy (XAFS) is employed for an investigation of the comparative structure of Concanavalin A (Con A) in forms of crystal and solution. Con A has two distinctive metal-binding sites. One of these, the transition metal-binding site, has a different structure and temperature dependence in the two forms. The crystal has one less ligand than the solution. The crystal also has an anomalous temperature dependence: The disorder of the site is not reduced as the temperature is decreased, while the metal-ligand bond lengths increase. Normal behavior is observed for the solution form. We suggest that the constraints of the crystal structure cause a compression in the transition metal site; the compression increases with temperature, compensating the normal thermal effect. Our results suggest caution in assuming that crystal and solution forms of proteins are always the same.
[Research supported by NSF grant DMB-8613948]

M-Pos89

Nonaqueous Hydrogen Bonding and Proton Transfer by Aromatic Alcohols: Steady-State and Time-Resolved Fluorescence Studies.

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In water, aromatic alcohols, such as tyrosine and the estrogen 17β-dihydroequilenin (17β-DHE) which are both fluorescent, can become stronger acids in the excited state; proton dissociation may take place on the same time scale as the fluorescence decay of the protonated alcohol. However, the local protein environment of a tyrosine or an estrogen binding site is not completely aqueous. To investigate the possibility of this two-state excited-state reaction occurring in a nonaqueous environment, we have titrated 2-naphthol and phenol in organic solvents with the proton acceptor triethylamine (TEA). Based on shifts in absorption spectra, TEA and the alcohol form a ground-state hydrogen-bonded complex with K_a's on the order of 100 M^{-1}. From shifts in steady-state emission spectra and the characteristics of the fluorescence intensity decay kinetics, we find that any subsequent excited-state proton dissociation depends on solvent polarizability. Furthermore, alcohol not complexed with TEA is dynamically quenched by TEA.
Supported by NIH grants GM39750 and DK39548.

M-Pos90

Tyrosine Fluorescence in Oxytocin: Rotamers and Nearest Neighbor Interactions.

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Oxytocin (OT) is a nonapeptide hormone containing a single aromatic residue, Tyr^2. The $Cys^1 \rightarrow Cys^6$ disulfide bond statically quenches one of the three $C\alpha$-$C\beta$ phenol side chain rotamers [*Biochemistry* 25, 607 (1986)]. We now report that the N-terminal amino group also influences the phenol environment. Steady-state Stern-Volmer fluorescence quenching studies (pH 3) indicate that the apparent quenching constant, k_q, for acrylamide is smaller for tyrosine in OT than for two desamino OT analogs, suggesting steric hindrance by the amine. Furthermore, the apparent k_q of iodide ion is larger for OT than for the analogs, indicating electronic attraction by the protonated N-terminal amine. The disulfide bridge does not, however, significantly affect the efficiency of these fluorescence quenching agents. Initial time-dependent fluorescence quenching studies indicate that each $C\alpha$-$C\beta$ rotamer has a distinct k_q for each quenching agent, supporting the concept, based on their distinct lifetimes (*ibid.*), that each rotamer has a different environment. Supported by NIH grants DK39548, DK10080, and GM39750.

M-Pos91

[Lys(DNS)^8] Vasotocin: A Highly Fluorescent Neurohypophyseal Hormone Analog.

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and J.D. Glass, Medical Department, Brookhaven National Laboratory, Upton, NY

The neurohypophyseal hormones oxytocin (OT; lactation and uterine contraction) and vasopressin (VP; renal water retention) are homologous nonapeptides cyclized by a $Cys^1 \rightarrow Cys^6$ disulfide bond. These hormones bind to their storage/carrier protein neurophysin (NP). To measure binding interactions, we have synthesized a dansyl (DNS) OT analog, [Lys(DNS)^8] vasotocin, by conjugation of the cyclic tocinoic acid (-S-Cys-Tyr-Ile-Gln-Asn-Cys-S-) with the "vaso" tripeptide Pro-Lys(DNS)-$GlyNH_2$. In aqueous solution (100 mM KCl, pH 6.4 to 7), the DNS group of the OT analog displays fluorescence properties characteristic of fully–hydrated DNS models. This OT analog is capable of *tight, reversible* binding to NP, as shown by an increase in fluorescence intensity, a corresponding spectral shift of the emission to higher energies, and an increase in fluorescence anisotropy for the DNS probe on titration with NP; these changes are lost upon addition of an excess of native OT. Supported by NIH grants DK39548 and DK10080.

M-Pos92

Tryptophan-A^{14} and Tryptophan-A^{19} Insulins: Steady-State and Time-Resolved Fluorescence.

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The A and B chains of insulin each contain two tyrosine residues. To study the local environments of the A^{14} and A^{19} tyrosine sites, and to study the fluorescence characteristics of a tryptophan residue in a well-defined system, a tryptophan residue was substituted for either tyrosine-A^{14} or A^{19}. Usually, chemical modification of tyrosine-A^{14} has little effect upon biological activity, but modification of tyrosine-A^{19} generally results in diminished activity; the same behavior is observed for the tryptophan analogues. The fluorescence emission of tryptophan-A^{14} is comparable to that of model compounds, but the emission of tryptophan-A^{19} has its peak 4 nm to higher energy. The fluorescence intensity decay of each tryptophan analogue is complex but emission wavelength independent. While each tryptophan has similar kinetic constants, the *mean* intensity decay times of the A^{14} and A^{19} analogues are 5.7 ns and 3.3 ns, respectively, since each similar kinetic constant has a different fractional intensity. The fluorescence results can be correlated with the local environments of the probe residues. Supported by NIH grants GM39750 and DK12925.

M-Pos93

FLUORESCENCE OF TRYPTOPHAN DIPEPTIDES.

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When comparing dipeptides of the type W-X and X-W, where W = Trp and X = another amino acid, the following were found for W-X : 1. Absorption and emission spectra were blue shifted. 2. Quantum yields were higher for both zwitterionic and anionic forms. 3. pK_{α} values for the amino group were lower. 4. The fluorescence decays were more nearly exponential. The parameters for W-W were like those for X-W, showing that the C-terminal W acts as an energy sink. These results, as well as the decay-associated spectra, suggest predominance of a rotamer of W-X where the indole and $-NH_3^+$ groups are in close proximity. While spectral shifts in proteins are usually interpreted as reflecting degree of W exposure, the present results indicate that electrostatic factors can play a part.

M-Pos94

DISTRIBUTION OF TOMAYMYCIN BONDED TO DNA. Mary D. Barkley, Fahmida N. Chowdhury and Karol Maskos, Dept. of Chemistry, Louisiana State University, Baton Rouge, LA 70803

The fluorescence decay of tomaymycin bonded to calf thymus DNA is biexponential with lifetimes of 3.5 and 6.7 ns at 5°C. Studies with synthetic DNA oligomers and polymers show that the values of the lifetimes and the relative amplitudes of the two exponential components depend on the flanking base sequence of the covalently modified guanine. Tomaymycin and other pyrrolo[1,4]benzodiazepine antibiotics have four possible bonding modes: two stereochemistries of the covalent linkage and two possible orientations of the drug on the DNA helix. The two fluorescence lifetimes of tomaymycin probably represent the two stereochemical linkages. To determine whether the two exponential decays represent particular adducts at preferred bonding sequences or a distribution of adducts, the fluorescence decay data were fitted to discrete and continuous lifetime distributions. Time-resolved fluorescence data from experiments performed at different excitation and emission wavelengths, pH, DNA concentration and chain length, and nucleotide/drug ratio were analyzed simultaneously in global programs. Supported by NIH grant GM35009.

M-Pos95

TRYPTOPHAN EXCITON COUPLING IN THE fd PHAGE Gregory E. Arnold & A. Keith Dunker, Washington State University, Pullman, Washington.

The fd phage coat protein (8P) contains 50 amino acids, and is predominantly helical. The bulk of the fd capsid consists of a tight interlocking network of about 2700 copies of the 8P protein arranged as closely packed helical layers, with their axes approximately parallel to the particle axis. The CD spectrum of the native virus has a highly unusual shape, exhibiting both the 208 nm π-π^* and 222 nm n-π^* transitions typical of a helix, but with the 222 nm trough being significantly deeper than the one at 208 nm. Previous interpretations ascribed this irregularity to optical distortions (absorbtive flattening and/or differential light scattering) arising from the large particulate size of the virus as compared to a protein uniformly disbursed in solution. It is our contention that the anomalous CD intensities arise, not from optical artifacts, but rather from 8P tryptophan absorbtion. We present the results of CD, Raman, UV, and fluorescence studies on the oxidation of the fd virus by NBS. These studies suggest that NBS, in low concentrations, specifically oxidizes the 8P tryptophan without altering the secondary structure of 8P and, concomitantly, eliminates the anomalous spectral shape. A CD difference spectrum (fd spectrum - NBS treated fd spectrum) provides evidence that the unusual spectral shape is, in large part, due to exciton coupling between tryptophans in the 8P capsid network.

M-Pos96

REACTIONS OF EXCITED TRIPLET STATES OF Pd* AND Zn MYOGLOBINS C. M. Phillips, J. M. Vanderkooi & S. Papp, Dept. of Biochem. & Biophys. and Dept. of Chem., Regional Laser & Biotechnology Laboratory, Univ. of Pennsylvania, Philadelphia PA 19104

The triplet state of Zn and Pd derivatives of myoglobin were studied by transient absorption and emission techniques. The transient absorption spectra following laser flash excitation showed a new absorption band at 460 nm and 418 nm for the Zn and Pd derivatives, respectively, with decay parameters comparable to phosphorescence. For Zn-myoglobin, the spectra did not exhibit an isobestic point and the decay kinetics to the ground state depended upon the wavelength of measurement, indicating that there was more than one form of emitting species. For Pd myoglobin, a single exponential decay and an isobestic point on the transient absorption spectra were observed. Possible reason for these different decay phenomena can be related to the different geometries: Zn porphyrin, five coordinated, can undergo out-of-plane distortions (as does deoxy-myoglobin) whereas Pd, which cannot accept a fifth ligand, is likely to form a derivative which resembles oxy myoglobin. The quenching rate constant for oxygen at 20°C was 9.7×10^7 $M^{-1}s^{-1}$ for both derivatives, suggesting no difference in oxygen penetrability. The quenching by anthraquinone sulfonate was examined on a time scale of psec to msec. The data suggest that a fast transfer process occurs, without indication of a formation of a transient cation. This result would be predicted for porphyrins in hydrophobic environments as in the heme pocket. (Supported by NIH Research Grants GM 21487 and 5-P41-RR-01348-08).

M-Pos97

PROTEIN PHOSPHORESCENCE QUENCHING BY DIFFUSION ENHANCED ENERGY TRANSFER J.V. Mersol, A. Gafni, M. Model, D.G. Steel (Intro. by Robert Zand) The University of Michigan Ann Arbor, MI 48109.

Room temperature tryptophan phosphorescence from deoxygenated aqueous protein solutions can be used for detailed study of protein structure and dynamics. In this study the distances of closest approach between phosphorescent tryptophan residues in several proteins and freely diffusing quenchers of various sizes were determined, using resonance energy transfer measurements in the rapid diffusion limit. From the rate of quenching of alkaline phosphatase (AP) phosphorescence by the heme group of myoglobin (Mb) due to dipole-dipole energy transfer, a distance of closest approach of 21 Å between this group and the phosphorescent tryptophan was obtained, yielding a distance of 16 Å from the latter residue to the surface of AP accessible to Mb. In contrast, using freely diffusing small dye molecules as energy acceptors yielded distances of ~2 Å for the depth of the same residue beneath the enzyme surface. This apparent discrepancy is alleviated by assigning the phosphorescence to Trp 109 in AP which is close to the surface of the active site cleft into which the small dyes, but not Mb, can diffuse. The unrealistically small distances of closest approach obtained with the dyes also reflect the fact that mechanisms other than resonance transfer dominate the quenching rate below ~10 Å. These include electron transfer and exchange interactions.

M-Pos98

ANALYSIS OF THE SELF ASSOCIATION OF BILIRUBIN DITAURATE (BDT) AND THE INFLUENCE OF BILE SALTS USING ANALYTICAL ULTRACENTRIFUGATION. [1]Ridgeway, T.M., [2]Carey, M.C., [3]Neubrand, M.W. and [1]Laue, T.M., [1]Dept. Biochemistry, U. New Hampshire, Durham, NH 03824, [2]Harvard Med. School, Boston, MA 02115, [3]Klinikum Großhadern, Munich, Fed. Rep. Germany

Bilirubin ditaurate (BDT, Mr=850) is a good analog to the unstable bilirubin glucuronide conjugates that constitute the major bile pigment of humans. It is of physiological importance to understand the secretion of bilirubin conjugates. Therefore, the aggregation state must be assessed under a variety of conditions, including the effects of various bile salts. The extent of BDT association has been examined as a function of BDT concentration at fixed ionic strength, as a function of ionic strength at fixed BDT concentration, and in the presence of different taurine-conjugated bile salts at non-micellar concentrations. BDT self associates to tetramer, with association facilitated by increased ionic strength. BDT self association and BDT association with bile salt appear to result in spectrally distinguishable complexes. Advantage may be made of the spectral differences to determine the effects of BDT association with bile salts on BDT self association. Supported by NSF BBS 86-15815.

M-Pos99

CONFORMATIONAL ANALYSIS OF β-CASOMORPHIN ANALOGUES BY SPECTROSCOPIC AND BIOACTIVITY MEASUREMENTS

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The solution conformations of a series of Trp[3] substituted β-casomorphin-5 analogs were investigated by spectroscopic and bioactivity (BA) measurements. Sequences were identical with variance only at Pro[2] and Pro[4] positions. CD measurements revealed a direct correlation between certain CD parameters and BA activity. Fluorescence (F) energy transfer experiments established a rank order correlation between the Tyr[1]-Trp[3] intramolecular distance and BA. F(τ) data of all analogs were best fitted to a double exponential decay model. Short-τ values were shown, by global analysis of the decays to be identical for all three analogs studied. The results of our biological and spectroscopic experiments indicate that casomorphins which can form pseudocyclic structures in solution are preferred for binding to μ receptors.

M-Pos100

A CONFORMATIONAL STUDY OF SYNTHETIC ANALOGUES OF THE SIDEROPHORE ENTEROBACTIN USING VIBRATIONAL CIRCULAR DICHROISM

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We have investigated the solution conformations of three tripodal peptides, $R(CH_2NH\text{-}COCHR'NHCOBoc)_3$, with R = Ph or $N(CH_2)_3$ and R′ = CH_3 or $CH_2CH(CH_3)_2$, that serve as precursors in the synthesis of iron binders. Interchain hydrogen bonds between the amino acid carbonyl of one chain and NH of an adjacent chain can form, resulting in a conformer with C_3 symmetry. From the vibrational circular dichroism (VCD) spectra, we were able to determine the handedness and extent of the hydrogen bonding network. The compounds were studied in $CHCl_3$ or C_2Cl_4 solution at a concentration of 5×10^{-4} M. Spectra were recorded in the NH and carbonyl stretching regions. The (+,-) VCD couplet observed at 1676 cm^{-1} arising from the amino acid carbonyl stretches is consistent with a clockwise arrangement of the amino acid carbonyl groups about the C_3 symmetry axis. Interchain hydrogen bonded NH stretches give rise to a negative VCD band at 3350 cm^{-1}, which correlates with the orientation of the amino acid NH bonds.

M-Pos101

IONIC SPECIES IN HEMATOPORPHYRIN IX IDENTIFIED BY FLUORESCENCE SPECTROSCOPY

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The protonation state of the imino nitrogen of hematoporphyrin IX (Hp) in solution in water or in a mixture of SDS in water is evaluated by fluorescence spectroscopy between pH 0.1 and 13. At pH greater than 6 the prodominant species is the free base and at pH lower than 2, the predominant one is the dication. In water solutions the spectra are complicated by aggregation. In SDS solutions, the problem is less severe. The spectrum of the free base and that of the dication are subtrated from the spectra of the species obtained at intermediate pH to obtain the spectrum of the monocation. The relative amount of the dication, monocation and free base along with the aggregation formation of Hp as a function of pH are discussed.

M-Pos102

TIME-RESOLVED FLUORESCENCE USED TO ANALYZE pH-DEPENDENT CHANGES IN THE UNFOLDING KINETICS OF IL-1β. P. HENSLEY+, P. YOUNG+, T. PORTER+, D. PORTER-, K. KASYAN+ AND J. KNUTSON-. + Smith, Kline, and French, King of Prussia, PA., 19406,- LC, NHLBI, NIH, Bethesda, MD, 20892.

Interleukin-1β, an important immunomodulator, is a single Trp, 17kD protein, displaying a pH-dependent fluorescence intensity profile (pKa ~ 6.6). GdmHCl unfolding kinetics are also moderated by pH. We have employed a laser driven, time-resolved fluorescence spectrophotometer to: 1. obtain decay associated spectra (DAS) of wild type and mutant proteins vs. pH, 2. obtain anisotropy profiles and correlation times for each form, and 3. obtain decay profiles every few seconds during folding/unfolding reactions ("KINDK"). The loss of a native ~5.3 ns decay component and production of a ~1.8 ns term dominates these kinetic profiles.

M-Pos103

QUANTUM AND MOLECULAR MECHANICAL STUDIES OF 2-PYRIMIDINONES AS FLUORESCENT PROBES OF NUCLEIC ACID STRUCTURE. J.D.Petke, P.H.Lee, G.M.Maggiora, D.C.Rohrer, and R.E.Christoffersen, The Upjohn Co., Kalamazoo, MI 49001

The electronic spectrum of 2-pyrimidinone was computed using ab initio configuration interaction methods with an extended basis set. The calculations showed that the lowest spectral band contains a single intense transition at 310-315 nm. Further calculations on 5-amino-2-pyrimidinone showed a red shift of the band to 340 nm with no loss of intensity. Thus, 2-pyrimidinones with electron-donating substituents at the 5-position appear to be potential sources of fluorescense within a window of the nucleic acid spectrum, and it is proposed that such molecules selectively substituted for cytosine in polynucleotides may be used as fluorescent structural probes. Molecular mechanics studies of double-stranded DNA dodecamers in which one cytosine was replaced by 5-substituted 2-pyrimidinones in each strand showed little distortion of the helix, even though a hydrogen bonding site is lost.

M-Pos104

ANALAZING THE DECAY CONSTANTS IN PHASE MODULATION MULTIFREQUENCY FLUOROMETRY USING THE MAXIMUM ENTROPY METHOD (MEM). R.K. LIVESEY+,J.C. BROCHON *,J. POUGETx B. VALEURx / +: MRC-DAMTP Cambridge U.K. *: LURE Orsay France,x: CNAM Paris

MEM is used to recover the distribution of exponentials describing the fluorescence decay without any prior model.The chi-squared function is used to bound a solution. MEM can handle sharp,broad, and continous distributions of lifetimes The programm was tested by analyzing synthetic data with different magnetude of statistical error added.The widths of recovered distributions were related to the noise level,the frequency domain used and the number of data.The resolvability of closed distributions was studied from simulated data.MEM was applied to real data in analyzing either phase and modulation data simultaneously or phase and modulation data separately in order to test the influence of both statistical and systematic errors.

M-Pos105

SURFACE EXCITED SOLID STATE LUMINESCENCE SPECTRA OF LANTHANIDE -ATP-BISPYRIDYLAMINE COMPLEXES: VIBRONIC FEATURES

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The success of Cini et. al.(J. Chem. Soc. Dalton Trans. 1984,2467) in obtaining a stable solid complex of ATP with bispyridylamine and di--valent metal cations was exploited slight modifications to obtain the following complexes: Tb(III):ATP: bipy , Eu(III):ATP:bipy ,Eu(II): ATP:bipy ; whose spectral features were compared with those of Gd:P_2O_7 and Gd:$PO_3.CH_2.PO_3$, especially in the region of vibronic "satellite" transitions (cf. Timofeev et al. Opt. Spek. 1984, 56(3), 250, Stavola et al. J. Chem Phys. 74(8), 4228, 1981). These studies may help in determining such vital information as the change in force-constant ma--trix of P-O, O-P-O, P-O-P, etc. vibrations upon metal complexation in biologically relevant polyphos--phate systems.

M-Pos106

DYNAMICAL TEST OF DAVYDOV-TYPE SOLITON IN ACETANILIDE USING A PICOSECOND FREE-ELECTRON LASER

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Lewis Rothberg: Bell Labs
Wunshain Fann; Stanford Univ.
John Madey, Steve Benson: Duke Univ.
Shahab Etemad: Bell Core

Picosecond Infrared excitation experiments on acetanilide, an alpha-helix protein analog, indicate that the anomalous 1650 cm^{-1} band which appears on cooling of acetanilide crystals persists for at least 2 microsecon ds following rapid pulsed heating. The ground state recovery time is 15 picoseconds consistent with a conventional vibrational mode strongly coupled to the phonon bath. We therefore suggest that the unusual temperature dependent spectroscopy of ACN can be accounted for by a slightly non-degenerate hydrogen atom configurations in the crystal and not an unusual localized vibrational state.

M-Pos107

COLLAGEN MONOMERS UNDERGO A CONFORMATIONAL TRANSITION PRIOR TO ASSEMBLY TO FIBRILS. A. George and A. Veis. Northwestern University, Chicago, IL 60611 The assembly of collagen molecules into native fibrils can be accomplished *in vitro* in solutions at low ionic strength and neutral pH by raising the temperature above 30°C. Precipitation is not immediate but exhibits a distinct lag period. One proposal suggests that the lag phase is evidence for a conformational transition in the collagen monomer prior to assembly. Fourier Transform Infra Red Spectroscopy (FTIRS) is a very sensitive probe of the H-bonded states within the collagen triple helix. The carbonyl group spectrum (1700 to 1600 cm^{-1}) has been investigated in collagen H_2O solutions at 1 mg/ml under self assembly conditions from 4° to 34°C. The three clear bands at 1660, 1642 and 1630 cm^{-1} vary in relative intensity over this range. The 1660 cm^{-1} band, associated with the triple helix conformation, increases in intensity at 26°relative to both lower and higher T. From these data we hypothesize that the the triple-helix of the semiflexible collagen molecule is actually perfected during the lag phase, facilitating nucleation and intermolecular interaction, but that the helix must be distorted as the molecules are bent as they are incorporated into the fibrils. Supported by NIH- NIAMSD Grant AR-13921.

M-Pos108

A ZINC BINDING PEPTIDE BINDS TO NUCLEIC ACIDS.

Martha Delahunty, Richard Karpel and Michael Summers. Intro. by Maria Freire. Department of Chemistry and Biochemistry, UMBC; Baltimore, MD.

A fluorimetric method was used to study the interaction of a zinc-binding peptide with nucleic acids. The synthetic peptide corresponds to the first of two putative zinc-binding domains of the HIV nucleocapsid protein p7. The zinc binding domain is completely conserved in retroviral nucleocapsid proteins as well as in eukaryotic transposable elements. This sequence homology may reflect a shared requirement for interactions with nucleic acids. The 18 aa peptide used in these experiments has been shown by NMR methods to bind zinc tightly and stoichiometrically. Here, we examine the effect of zinc on the interaction of this peptide with nucleic acids by following the fluorescence enhancement of polyethenoadenylic acid (poly(ε A)) upon binding peptide. Zinc-bound peptide has a greater affinity for poly(ε A) than does the peptide alone. By competition experiments, we find that both single-stranded and double-stranded DNA compete for peptide binding with poly(ε A).

M-Pos109

A NEW DNA BINDING MODE FOR CAP

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In the absence of cyclic AMP, the Escherichia coli cyclic AMP receptor protein (CAP) binds without detectable sequence specificity to restriction fragments containing lac and crp promoter sequences. Under standard conditions (10mM Tris, 1mM EDTA, pH 8.0), our estimates of the equilibrium constant and cooperativity parameter for complex formation are 114,000 ± 1400 M^{-1} and 1.3 ± 0.8, respectively. Thus, this interaction lacks the substantial cooperativity previously reported for CAP binding to genomic DNAs. Using the electrophoresis mobility shift assay we find that complexes of increasing CAP content differ by a highly uniform mobility decrement. This result is most consistent with a binding mode in which little or no DNA bending occurs. The ability of CAP to distinguish between restriction fragments and genomic DNA, shown by the difference in binding cooperativity, suggests the existence of previously unsuspected DNA sequences or structures that modulate its binding cooperativity.

M-Pos110

DNA FOOTPRINTING AT HIGH PRESSURE

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Molecular Biophysics Research Department
AT&T Bell Laboratories

By the use of photoactive DNA cutting reagents, the interaction between DNA and proteins can be investigated at the single base pair level at elevated pressures. The perturbation induced by changing the pressure reveals regions of the protein-DNA complex that differ in their relative interaction strengths, thus providing a measure of the micro-association constants. High pressure footprinting data of the restriction endonuclease *Eco*RI with its recognition site will be presented and compared with thermodynamic and structural data for the DNA complex. A description of the technique and a discussion of its potential for investigating protein-DNA interactions will also be presented.

M-Pos111

VISUALIZATION OF T4 REPLICATION ACCESSORY PROTEIN COMPLEXES ON DNA BY CRYO-ELECTRON MICROSCOPY

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DNA replication in bacteriophage T4 is performed by proteins encoded in the T4 genome, which act in a multisubunit assembly. While the gene 43 product contains the essential polymerase activity, its function is dramatically enhanced by a set of "accessory" proteins, the gene 44, 62, 45, and 32 products. Together, they raise the polymerization rate, fidelity and processivity of the polymerase complex in an ATP-dependent manner, to levels which approximate those in vivo.

We have examined complexes formed by the accessory proteins bound to DNA by cryo-electron microscopy, in the absence of stains or other potential perturbants. Distinctive structures, approximately 90Å by 30Å, are clearly visible crossing the DNA strands at right angles. Their assembly and maintenance requires all four accessory proteins and ATP. The composition of these complexes and the arrangement of their components is currently under investigation.

M-Pos112

ROLE OF POLYAMINES IN THE INTERACTION OF GENE-REGULATORY PROTEINS WITH DNA: ESTROGEN AND PROGESTERONE RECEPTORS. Thresia Thomas and T.J. Thomas, Departments of Env. and Community Medicine and Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Hormonal regulation of gene expression involves the interaction of the hormone to its specific receptor protein and the recognition of the receptor by the hormone-response elements in the genome. To gain insight into the mechanism of receptor-DNA interactions, we studied the binding of estrogen (ER) and progesterone (PR) receptors to poly(dA-dC).-poly(dG-dT) in the presence of a natural polyamine, spermidine^{3+}. ER and PR allowed to bind to DNA-cellulose and then eluted with the polynucleotide. In the absence of spermidine, 1400 and 250 μg/ml of the polynucleotide was required to elute 50% of ER and PR, respectively from DNA cellulose. In the presence of 150 μM spermidine, only 65 and 50 μg/ml of the polynucleotide was required to elute 50% of ER and PR. We also found that the polynucleotide assumed a left-handed Z-DNA-like conformation in the presence of 150 μM spermidine. Control experiments poly(dA-dT).poly(dA-dT) that does not assume the Z conformation showed no enhanced binding of ER and PR in the presence of spermidine. Our results suggest that polyamine-induced conformational transitions may be a part of the mechanism of hormonal regulation of gene expression.

M-Pos114

CALCULATION OF MACROMOLECULAR CROWDING EFFECTS ON PROTEIN-DNA INTERACTIONS *IN VIVO*
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In *in vitro* assays protein-nucleic acid interactions are exquisitely sensitive to salt concentration changes. For *E. coli* grown in minimal medium, increased extracellular osmolarity results in a four-fold increase in cytoplasmic [K^+]. This increase has no effect on the extent of *lac* repressor occupancy of the *lac* operator or on the initiation rate of RNA polymerase at the λP_R promoter, even though such a change would abolish these interactions *in vitro* . In addition to increasing $[K^+]_{cyt}$, high external osmolarity results in a 56% increase in cytoplasmic protein concentration. Calculations of thermodynamic non-ideality due to excluded volume effects using scaled particle theory, assuming a reasonable distribution of sizes for cellular proteins and supramolecular assemblies, show that increases in protein activity coefficient due to increased macromolecular crowding could offset the decrease in affinity constant due to the increase in salt concentration. Hence, the extent of DNA binding would not be expected to decrease. We argue that this increase in excluded volume non-ideality is one of the physical chemical principles which allow the cell to maintain functional homeostasis in the face of changing cellular composition.

M-Pos113

DNA-BINDING CHARACTERISTICS AND THERMAL STABILITIES OF NATIVE AND MUTANT CRO REPRESSORS. James D. Baleja and Brian D. Sykes, Department of Biochemistry, Univ. of Alberta, Edmonton, AB, Canada T6G 2H7.

Cro repressor is a small dimeric DNA-binding protein from bacteriophage lambda. The X-ray structure of cro shows that α-helices of a helix-turn-helix motif of each monomer can interact with bases within successive major grooves of DNA. In the complex, either the DNA or the protein must bend in order to achieve a better topological fit and to explain chemical modification data. In the cro repressor, valine 55 of one monomer is near enough to valine 55 of the other monomer that, upon replacement of this residue by cysteine, an intersubunit cross-link forms without significantly perturbing the structure. Circular dichroism and ^{1}H-NMR spectra show that the folding of the covalent dimer of the variant (V55C) is nearly identical to that of the non-covalent dimer of wild-type cro. However, individual backbone amide proton exchange rates and thermal stability measurements indicate that V55C is less flexible. Upon protein-binding, chemical shift changes are induced for the base-pair imino protons of DNA which reflect binding stoichiometry and strength. The variant binds about 8 times more weakly than the native protein. Adjustments in protein structure, necessary to form a tight protein-DNA complex, are hindered by a loss in protein flexibility caused by the intersubunit cross-link.

M-Pos115

SPONTANEOUS PEPTIDE BOND FORMATION UNDER ORIGIN OF LIFE CONDITIONS
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Peptide bond formation occurs spontaneously in solutions containing iron(II), alanine, and the nucleotide base adenine. We found peptide formation to require all three components, and to be further enhanced by the addition of uracil. Based on the affinity of iron(II) for amino acids and nucleotide bases, and from information about the base-stacking characteristics of nucleotide bases in solution, we composed a model for a nucleotide base-iron(II)-amino acid complex which explains the formation of peptide bonds in solutions containing these components. The model is consistent with nonrandom polypeptide formation and information transfer, and may provide the missing link in the sequence from single component molecules to self-replicating forms.

M-Pos116

PEPTIDE BINDING TO POLY(dG-dC), A VIBRATIONAL CIRCULAR DICHROISM STUDY.

M.S. Gulotta, M. Diem' and D.J. Goss, Hunter College of CUNY, NY.

Many techniques have been used to examine peptide binding to DNA. Vibrational (ir) circular dichroism has already been used to describe peptide conformations as well as the helical structure of various polynucleotides and of DNA. Distinct spectral differences are seen for B and Z DNA. We have studied the effects of peptide binding to poly(dG-dC) using VCD and compared these effects to those seen in high salt solutions of poly(dG-dC). The spectra suggest that the helix remains in a B-conformation, however the positive peak of the bisignat couplet in the carbonyl region is greatly enhanced relative to the negative peak. We are currently developing a theoretical model to interpret these results. Grant Support: AHA, NSF86007070, NIH28619GM.

M-Pos117

SPATIAL DISTRIBUTION OF PROTEINS BOUND TO DNA DETERMINED BY TIME-RESOLVED FLUORESCENCE DEPOLARIZATION: APPLICATION TO DNA POLYMERASE.

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We demonstrate a new method for determining the spatial distribution of a protein bound to DNA in solution, based on the picosecond time-resolved fluorescence depolarization of an environmentally-sensitive dansyl probe covalently attached by a linker to a DNA base and positioned at a specific site in the major groove. Coverage of the probe by the protein increases its excited-state lifetimes and sterically restricts the internal reorientation of the probe. The bound protein is localized by the dependence of the coverage on the probe position on the DNA. Disorder of the complex produces a heterogeneous distribution of probe environments, resulting in a non-monotonic time-dependence of the depolarization. We have studied the complex of DNA and polymerase I (Klenow fragment), for which a crystal structure is unavailable. The complex was substantially disordered in solution. The effect of site-specific mutagenesis of the Klenow fragment on the mode of DNA binding has been examined.

M-Pos118

SEQUENCE-SELECTIVE BINDING OF DNA TO THE REGULATORY SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE.

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The regulatory subunit (R_{II}) of type II cAMP-dependent protein kinase binds the palindromic octanucleotide d(TGACGTCA) with concomitant decrease in the fluorescence of its Trp-226. Scatchard plots of the fluorometric data show that each subunit binds only 1 octanucleotide with K_d = 80 nm and 2 μM for $R_{II}(AMP)_2$ and R_{II} respectively. The K_d values of $R_{II}(cAMP)_2$ for non-specific octanucleotides and thymus DNA are 1 to 2 orders of magnitude higher. The complex formed by $R_{II}(cAMP)_2$ and d(CTCTGACGTCAGAG) can be cross-linked by treatment with Fenton reagent. Pepsin digestion of the cross-linked product followed by separation and sequencing of the peptides showed that the oligonucleotide was covalently attached to Ser-221 of R_{II}. The location of this cross-linked residue readily explains the effective quenching of Trp-226 fluorescence by DNA-binding. The presence of many hydrophobic groups as well as the positively charged sequence RRIIVKNNAKKRK in the vicinity of Ser-221 suggests possible modes of $R_{II}(cAMP)_2$-DNA interaction at the binding site (USPHS GM 41610).

M-Pos119

STRUCTURAL DETAILS OF DNA IN THE NUCLEOSOME.

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We have used the technique of hydroxyl radical footprinting to investigate the structure of DNA in the nucleosome formed with the 5S ribosomal RNA gene of *Xenopus borealis*. We find that the periodicity of cleavage by the hydroxyl radical is 10.0 nucleotides per repeat, from either end to within two helical turns of the dyad of the nucleosome. Around the dyad the cleavage periodicity changes to approximately 10.7 nucleotides per repeat. By cleavage of the same DNA molecule bound to a precipitate of calcium phosphate, we measured the helical periodicity of the 5S gene itself to be 10.4 base pairs per turn. These measurements were quantitatively evaluated by fitting of a sine wave to the densitometer scans of the cleavage patterns, and by Fourier transformation of the smoothed cleavage data. We also investigated the effect on DNA structure of forming a nucleosome on DNA designed to be highly curved. Implications of these measurements for the "linking number paradox", for the structural basis of phasing of nucleosomes on DNA, and for changes in structure suffered by DNA upon incorporation into a nucleosome, will be discussed.

M-Pos120

EFFECTS OF DNA, SOLVENTS AND A SINGLE-AMINOACID MUTATION ON THE PRESSURE-INDUCED DISSOCIATION OF ARC REPRESSOR Jerson L. Silva, Cristina F. Silveira and Leila P. Silva. Dept? Bioq. Universidade Federal do Rio de Janeiro, RJ, Brasil. Arc repressor is a dimeric DNA binding protein that represses transcription from the P_{ant} promoter of bacteriophage P22 (Vershon, A.K., Younderian,P., Susskind, M.M., & Sauer, R.T (1985) J. Biol. Chem. 260, 12124. The equilibrium association reaction of arc repressor was studied by pressure-induced dissociation and by dilution. The dissociation was measured by the decrease in the average energy of the the single tryptophan fluorescence. The dissociation constant (K_d) determined by pressure pertubation was the same of that determined from the dilution curve (K_d=30nM). The subunit interaction was about 2.7 Kcal/mol more stable in the PL8 mutant. Glycerol (30%) conferred a stabilization of 2.0 Kcal/mol. Arc repressor bound to the plasmid pBR322 was more stable 1.3 Kcal/mol than the unliganded protein. However, DNA did not confer additional stability to PL8 dissociation. These data suggest a large coupling btween subunit-subunit and protein-DNA interaction.Supported by FAPERJ CNPq, FINEP.

M-Pos121

The DNA Binding Properties of a Lac Repressor Mutant That is Deficient in Dimer to Tetramer Association. Amy Pickar, Elizabeth Jamison & Michael Brenowitz, Department of Biochemistry, The Albert Einstein College of Medicine, Bronx, NY 10461.

The binding of wildtype Lac repressor (Lac^+) and a mutant repressor deficient in the dimer to tetramer association (N. Mandal & S. Adhya, personal communication) to two sites on the DNA that are separated by 11 helical turns was investigated using quantitative footprint and mobility-shift assays. Binding of Lac^+ is highly cooperative. DNase I hypersensitivity of the DNA between the two binding sites is observed. This hypersensitivity is diagnostic of the formation of a "looped complex", ie. a single Lac tetramer bridging the two sites. The "looped complex" is 1.5 - 2.5 kcal/mol more stable than a tetramer binding to a single site.

In contrast, the binding of the Lac repressor mutant (Lac^m) is non-cooperative and there is no evidence of "looped complex" formation. Binding of Lac^m was best-fit by a model where monomers ⇌ dimers and dimer + DNA ⇌ dimer-DNA with $K_{dimer} << K_{dimer\text{-}DNA}$. The binding affinities of Lac^m and Lac^+ to single binding-sites are comparable. These results support the role of the dimer to tetramer equilibrium in mediating the formation of "looped complexes". (Supported by NIH Grant GM 39929).

M-Pos122

EFFECTS OF BASE MODIFICATIONS ON PROMOTER RECOGNITION BY T7 RNA POLYMERASE. Martha D. Jaworski & Craig T. Martin, University of Massachusetts, Amherst, MA

Specific contacts between T7 RNA polymerase and its promoter were probed by measuring the effects of base modifications (dT→dU or dC→5-methyl-dC) on transcription initiation kinetics. Positions of these substitutions were chosen to test and refine a model of polymerase-promoter binding in which RNA polymerase recognizes one face of duplex DNA, including a major groove and the two flanking minor grooves (1). The above substitutions result in an oligonucleotide promoter in which a single methyl group is either added to or removed from the major groove of the duplex DNA. Steady state kinetic assays reveal values of K_m ranging from 50 nM (native template) to 700 nM, with the largest effect occurring in the center of the proposed major groove recognition region (dT→dU, -6c). There were no significant changes in k_{cat} (10-20 μM/min), suggesting that specificity occurs at the level of binding and not catalysis. These results further define polymerase contacts with individual base functions in the DNA and show that methyl group interactions contribute significantly to specific binding by polymerase. Future studies with replacements of dG→dI and dA→2,6-diaminopurine will determine the role of minor groove amino groups in recognition by T7 RNA polymerase.

1. D. K. Muller, C. T. Martin, & J. E. Coleman, (1989) *Biochemistry 28*, 3306-3313.

M-Pos123

DIFFERENTIAL PROMOTER BINDING AFFINITIES OF MONO- AND DI- LIGATED ESCHERICHIA COLI CRP AS MONITORED BY FLUORESCENCE ENERGY TRANSFER AND POLARIZATION. Tomasz Heyduk and James C. Lee, Dept. of Biochemistry, St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104.

Structural and ligand binding studies have shown that CRP exhibits three conformational states - free CRP and two cAMP-dependent states. In order to obtain valid thermodynamic parameters to describe the linkages among cAMP and promoter bindings to CRP a simple fluorescence procedure to study DNA-protein interactions was established. A 32 bp DNA fragment of the lac promoter containing the primary binding site for CRP was labeled at the 5' end with a fluorescence probe. The binding of CRP to this fragment can be conveniently followed by monitoring the changes in anisotropy or fluorescence energy transfer from protein tryptophan residues to the DNA probe. The results clearly demonstrated a differential promoter binding affinities between the single- and double- ligated CRP. The association constants for binding of CRP-cAMP and CRP-$(cAMP)_2$ to the promoter are $7.6 \times 10^8\ M^{-1}$ and $< 1 \times 10^6\ M^{-1}$, respectively in 0.1 M KCl, pH 7.8, 23°C.

M-Pos124

^{19}F-NMR OF T7 RNA POLYMERASE-DNA INTERACTION. Fraydoon Rastinejad, Jean Ott and Ponzy Lu. Dept. Chemistry, University of Pennsylvania, Philadelphia, PA 19104.

By substituting 5-fluorodeoxyuracil (5-FdU) in place of thymine at specific sites in promoter DNA sequences, ^{19}F NMR signals monitor the local changes upon binding of T7 RNA polymerase and substrates. The 5-FdU substitutions do not impair promoter function. The data from six substitutions has provided an NMR footprint of the protein binding sites that can be compared with chemical footprints. The NMR data shows heterogeneity of chemical conformations in the promoter as it interacts with the enzyme. At positions -6 and -10 the largest perturbation of the fluorine chemical shift is observed, consistent with the chemical data. Adjacent to the transcription start-site, polymerase binding induces a large inhomogeneous broadening of the chemical shift, suggesting multiple small perturbations in the DNA. The addition of GMP, as a dead end initiation substrate analogue, imposes a reduction of the structural heterogeneity in this region, and causes observable changes elsewhere in the promoter. We are also using the triphosphate analogues 5-F-UTP and 5-F-CTP to study defined RNA molecules and their interactions with proteins by ^{19}F NMR.

M-Pos125

ON THE MECHANISM OF ASSEMBLY OF CHROMATIN IN VITRO. J. C. HANSEN & K. E. VAN HOLDE, DEPARTMENT OF BIOCHEMISTRY & BIOPHYSICS, OREGON STATE UNIVERSITY, CORVALLIS, OR

We have used analytical sedimentation to study the in vitro assembly of nucleosomes onto a tandemly repeated sea urchin gene construct. At a histone/DNA ratio (r)=1.1, a 29S particle containing 12 nucleosomes is formed after dialysis from 2M NaCl into 10 mM Tris/.25 mM EDTA buffer. However, at all r<1.1, we observe broad distributions of <29S. Using r=1.1, we observe a 19S particle after dialysis into 1M NaCl. By 0.6 M NaCl, 85% of the templates sediment at 26-29S, while the remaining 15% sediment at about 18S. In 0.3M NaCl, 15% of the material remains in the slower boundary, while the fast boundary sediments at 40S. These results indicate that in vitro nucleosome assembly is a complicated noncooperative process apparently involving subnucleosomal particle formation by 1M NaCl, followed by partial nucleosome assembly and particle folding in moderate salt, and climaxing with complete nucleosome assembly and particle unfolding in very low salt.

(SUPPORTED BY NIH GRANTS NOS. GM11719 AND GM22916)

M-Pos126

HUMAN TRIPLEX DNA-BINDING PROTEIN --- PARTIAL PURIFICATION AND CHARACTERIZATION. Kiyama, R. and Camerini-Otero, D. NIH/NIDDK/GBB, Bethesda, MD 20892.

Polypurine-polypyrimidine sequences (poly(dA)-poly(dT), for example), which can form triplex DNA under physiological conditions, are often found in recombination hot spots and the promoter region of genes, but their biological functions remain unknown.

Here, we present evidence for a protein(s) that binds to triplex DNA. We used a gel shift assay for the detection of the protein. Substrates for the assay are oligonucleotides $(dA)_{34}$ and $(dT)_{34}$ which can make a triplex DNA (TAT triplex) in the presence of Mg^{2+} at neutral pH. After purification of nuclear extracts from HeLa cells by conventional column procedures, we have obtained a fraction that contains a protein(s) that binds specifically to the TAT triplex substrate but not to the AT duplex. To purify the protein further we used a triplex DNA-affinity column.

To confirm that the protein binds to the triplex DNA, we recovered the band from a gel and showed that the bound oligonucleotides are in the triplex form. With the affinity-purified triplex-binding protein fraction we can show that the protein has a higher affinity to triplex DNA than to duplex DNA of the same sequence.

M-Pos127

ADDITIVE AND NONADDITIVE EFFECTS OF BASE-PAIR SUBSTITUTIONS IN *E. COLI* PROMOTERS, Bruce A. Beutel and M. Thomas Record, Jr., Departments of Chemistry and Biochemistry, University of Wisconsin-Madison

We have investigated the kinetics of interaction of *E. coli* RNA polymerase ($E\sigma^{70}$) holoenzyme with four synthetic promoters, which differ in sequence only at two nearest-neighbor positions (-33,-32) in the -35 region. Results of these *in vitro* studies have been compared with *in vivo* measurements of relative promoter strength. We are interested in the question of whether effects of promoter sequence substitutions are context-independent (additive) or context-dependent (nonadditive). *In vitro*, base-pair substitutions at the -33 and -32 positions affect rate constants for both the bimolecular and unimolecular steps of the association mechanism, but these substitutions have no effect on the dissociation rate constant. Effects on the unimolecular step(s) appear to be context-dependent, but the effects on the overall bimolecular association rate constant, as well as on the equilibrium constant for open complex formation, appear to be context-independent. We propose a model to explain our results in terms of additive and nonadditive effects of these sequence substitutions on the relative standard free energies of the reactants, intermediates, and open complex.

M-Pos128

Base Specificity of the SSB Protein-ss Polynucleotide Binding Mode Transitions. W. Bujalowski and T. M. Lohman, Department of Biochemistry and Biophysics, Texas A&M University, College Station TX 77843

The *E. coli* SSB protein forms multiple complexes with poly(dT), which differ by the number of nucleotides occluded per SSB tetramer; these are referred to as the $(SSB)_{35}$, $(SSB)_{56}$ and $(SSB)_{65}$ binding modes (Lohman & Overman (1985) *J. Biol.Chem.***260**, 3594; Bujalowski & Lohman (1986) *Biochemistry* **25**, 7799). We have now examined the salt-induced transitions among these different SSB tetramer - ssDNA binding modes for other ss polynucleotides, poly(rU), poly(rA), poly(dC) and poly(dA) (pH,8.1, 25°C). As we have previously shown for SSB-poly(dT) complexes, poly(rU), poly(dA) and poly(rA) form the $(SSB)_{35}$ binding mode at low salt concentration (≤ 5 mM NaCl). An increase in the salt concentration induces the transition to the $(SSB)_{56}$ binding mode. However, the existence of an intermediate binding mode, $(SSB)_{42}$, is observed; this mode was also observed in SSB-poly(dT) complexes at 37°C. In the case of poly(dC) the lowest site size binding mode observed was $(SSB)_{56}$. Studies of the binding of different oligonucleotides, $dC(pC)_{69}$, $dA(pA)_{69}$, $dC(pC)_{34}$ and $dA(pA)_{34}$ to the SSB tetramer reveals a strong dependence of the negative cooperativity among ssDNA binding sites upon the type of oligonucleotide. The correlation between the observed salt effect on the SSB-ss polynucleotide binding mode transitions and the binding of oligonucleotides to the SSB tetramer will be discussed.

M-Pos129

ENHANCEMENT OF THE INCORPORATION OF DNA IN RECONSTITUTED SENDAI VIRAL ENVELOPES. Christopher Di Simone and John D. Baldeschwieler, California Institute of Technology, Pasadena, CA, 91125.

Triton X-100 reconstituted Sendai viral envelopes have the ability to encapsulate nucleic acids. These vesicles have a radius of 100-200 nanometers while a 5000 base plasmid may have an effective radius of 70-140 nm. Under normal reconstitution conditions, less than 1% of the total available plasmid vector (5000+ bases) was encapsulated. Addition of certain basic proteins (polylysine, lysozyme, or protamine) led to a greater than ten fold enhancement in encapsulation (6-10% DNA encapsulated). Experiments were designed to avoid confusion between associated and encapsulated material; ^{32}P labeled DNA was found to associate with the viral vesicles even after DNAse cleavage.

M-Pos130

THE SPACING OF NUCLEOSOMES ON THE *XENOPUS LAEVIS* OOCYTE 5S GENE. John D. Brantley[1], and William Blass[2]. [1]Univ. of Kentucky. [2]Univ of Tennessee.

The oocyte 5S gene of *Xenopus laevis*, which exists as a set of tandemly repeated units, was solubilized by digestion with Hind III. The resulting population of multimers was labeled by hybridization with an oligonucleotide probe and streptavidin colloidal gold. Following dialysis into low salt spreading buffer, the samples were fixed with glutaraldehyde, spread on hydrophilic carbon coated grids, and shadowed. Labeled molecules clearly showed a gold particle at one end. The separation of the nucleosomes was measured from center to center by tracing the curve of the chromatin on a digitizing tablet. The distribution of measurements was smoothed and deconvoluted using the error distribution of the measurement process as the response function of the system modelled as a linear system. The resulting distribution shows several periodic peaks indicating that nucleosomes prefer to be separated by multiples of 5.4 nm, or about one half turn of the DNA helix.

M-Pos131

DISSECTION OF THE 16S rRNA BINDING SITE FOR RIBOSOMAL PROTEIN S4. Amalia Sapag, Jailaxmi V. Vartikar, Anne-Marie Downes, David E. Draper. Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218.

The ribosomal protein S4 from *E. coli* is one of the first to bind to 16S rRNA and is thus essential for the initiation of assembly of 30S subunits. We have undertaken the identification of specific features required in the 16S rRNA for S4 recognition on a 463 nt fragment (positions 39-500) which is the minimum domain capable of binding S4 with the same affinity measured for the full length (1542 nt) rRNA molecule. We have systematically deleted 11 hairpins found in the secondary structure in order to evaluate their individual contribution to the binding capacity. Some may be removed without altering intermolecular affinity, whereas excision of others results in a several fold decrease in the binding constant. A set of N- and C-terminal deletions in the S4 protein has also been prepared and is being tested for RNA recognition.

M-Pos132

Structural Studies of the Cro Binding Site, Free and Bound to Cro Protein.

Elisabeth Evertsz, Gerald Thomas, Warner Peticolas, University of Oregon, Chemistry Department

Raman spectra of the DNA binding site for cro repressor protein were obtained in the presence and absence of bound cro protein. The 17 base pair fragment is identical to the OR3 site except that the second base to the right of the center of pseudosymmetry is altered. Analysis of the spectrum of the free DNA indicates that the molecule exists in a B-like conformation with deviations from standard B-form occurring mainly in the bands assigned to A-T vibrations. The spectrum of the bound DNA was obtained by subtracting the spectrum of free cro from the spectrum of the complex which was estimated to be 90% bound. The DNA undergoes significant structural changes upon binding to the protein, most notable of these changes are a destacking of the G-C bases reflected by increases in the 1240, 1262, and 1320 cm^{-1} bands. A decrease in the 1361 cm^{-1} band that occurs has also been assigned to a destacking in guanine bases. The appearance of the 705 cm^{-1} band and the decrease and downshift of the 670 cm^{-1} band are consistent with the appearance of A-like character in the A-T region of the binding site when the protein binds, however the spectra indicate that the entire binding site remains in a distorted B-like conformation. Other shifts in both intensity and position cannot be assigned to characteristic changes in conformation and therefore must be attributed to the protein influencing the structure in a novel way.

M-Pos133

A SPECTROSCOPIC STUDY OF THE BINDING OF N-7- AND N-2-SUBSTITUTED CAP ANALOGS TO HUMAN PROTEIN SYNTHESIS INITIATION FACTOR 4E

S.E. Carberry[1], E. Darzynkiewicz[2], J. Stepinski[2], S.M. Tahara[3], R.E. Rhoads[4] & D.J. Goss[1]. [1]Hunter Coll. of CUNY, NY; [2]U. of Warsaw, Poland; [3]USC Sch. of Med., Los Angeles, CA; [4]U. of KY, Lexington, KY.

There is a correlation between the affinity of N-7- and N-2-substituted 5'-terminal mRNA cap analogs to protein synthesis factor eIF-4E and their potency as inhibitors of protein synthesis. The pH and ionic strength dependent binding of various N-7-substituted derivatives have been used to map the eIF-4E binding site. Differences in the binding of N-7-alkyl- and N-7-aryl-substituted cap analogs to eIF-4E appear to arise from favorable interactions of the phenyl ring with the guanosine moiety, which may explain the enhanced recognition of the aryl-substituted cap analogs by eIF-4E.

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M-Pos134

INVESTIGATION OF cAMP RECEPTOR PROTEIN SECONDARY STRUCTURE BY RAMAN SPECTROSCOPY.

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Raman spectroscopy was employed to examine the secondary structure of the cAMP receptor protein (CRP). Spectra were obtained over the range of 400 - 1900 cm^{-1} from solutions of CRP and from CRP:cAMP co-crystals. Estimates of the secondary structure distribution were made by analyzing the amide I region of the spectra (1630 - 1700 cm^{-1}). CRP secondary structure was essentially the same in pH 6 and 8. The amide I analyses indicated a structural distribution of 44% α-helix, 28% β-strand, 18% turn, and 10% undefined for CRP in solution. Raman spectra of CRP:cAMP crystals showed several differences from spectra of CRP in solution. Some differences were attributed to CRP structural differences. Analysis of the amide I region indicated 37% α-helix, 33% β-strand, 17% turn and 12% undefined. Changes in the amide III region and at 935 cm^{-1} also indicated secondary structure differences. A proposal that cAMP induces a shift in CRP structure that includes the conversion of α-helix to β-strand is consistent with the Raman data.

M-Pos135

CHROMATIN CONDENSATION IN THE FORESPORE COMPARTMENT OF SPORULATING CELLS OF BACILLUS SPECIES: VISUALIZATION BY FLUORESCENCE MICROSCOPY AND DIGITAL VIDEOIMAGING

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A key event in sporulation of Bacillus species is an unequal cell division, producing a smaller forespore and a larger mother cell. Using the DNA stain DAPI we find that at about the time of this division forespore chromatin becomes extremely condensed. This condensation requires products of spo0 genes and the spoIIAC gene but not other spoII or spoIII loci.

Analysis of DNA distribution in the sporulating cell by digital videoimaging has shown that the amount of DNA in the condensation spot increases with time until its value is approximately equal to the amount of DNA in the mother cell. After reaching this stage a partial decondensation of chromatin occurs but the amount of DNA in the forespore remains the same as in the mother cell.

From these results we infer that the number of chromosomes in the mature spore is the same as in the mother cell, and that there are significant changes in chromatin packing during bacterial sporulation.

M-Pos136

RESOLUTION OF HOLLIDAY JUNCTION ANALOGS BY T4 ENDONUCLEASE VII CAN BE DIRECTED BY SUBSTRATE STRUCTURE.

John E. Mueller[1], Colin J. Newton[1], Frank Jensch[2], Börries Kemper[2], Richard P. Cunningham[1], Neville R. Kallenbach[3] and Nadrian C. Seeman[3]. [1]Dept. of Biology, SUNY/Albany, NY 12222, [2]Institute of Genetics, University of Köln, FRG, [3]Dept. of Chemistry, New York University, NY, NY 10003.

Endonuclease VII is capable of resolving Holliday recombination intermediates. We have explored its substrate requirements by using immobile analogs of Holliday junctions that lack sequence homology. We find that junctions whose double helical arms contain fewer than nine nucleotide pairs are not resolved. Scission of substrates with arms symmetrically elongated produces cleavage of the lengthened arms. We use shamrock junction molecules formed from a single oligonucleotide strand and either end-labeled or internally labeled to confirm that the scission products are those of coordinated resolution cleavage, rather than nicking of individual strands/. The relationship of the long arms to the cleavage direction suggests that the portion of the enzyme that requires the minimum arm length interacts with the pair of arms containing the 3' portion of the crossover strands, on the bound surface of the antiparallel junction.

This research supported by GM-29554, CA-24101, GM-33346 (NIH) and SFB 74 (DFG).

M-Pos138

VARIABILITY IN RHO-RNA COMPLEXES SEEN BY CRYO-ELECTRON MICROSCOPY

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The E. coli transcription termination factor rho is a 47 kDa protein which binds nascent mRNA tracts and brings about their termination and release in an ATP-dependent manner. Hydrodynamic measurements have established that, at appropriate protein and salt concentrations, rho monomers associate to form hexamers, in equilibrium with subpopulations of smaller oligomers. Hexamers of rho have a latent ATPase activity which is activated on binding RNA.

We have visualized rho by cryo-electron microscopy, both in the absence and presence of oligomers of ribo-C. Images recorded in the absence of RNA reveal two classes of structures, doughnut-shaped hexamers and, more frequently, arcs of variable length. Addition of oligomers of ribo-C long enough to span at least two monomer binding sites results in a marked shift toward closed hexamers. Longer oligomers induce this structural transition even more effectively by cooperatively binding all six rho monomers.

M-Pos137

HIGH PRESSURE FLUORESCENCE STUDIES OF *lac* REPRESSOR REVEAL A ROLE FOR THE SUBUNIT INTERFACE IN REGULATING OPERATOR AFFINITY

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High pressure fluorescence studies were carried out on dansyl labeled *lac* repressor. The affinity between *lac* dimers was found to be 4.5 nM, implying the involvement of this equilibrium in measurements of apparent operator affinity. The tetramer was found to be destabilized at 21°C or at high pH. Inducer binding had no effect on the tetramer at 4.5°C, but resulted in a stabilization at 21°C. High pressure resulted in the dissociation of the repressor-operator complex at operator concentrations 10^5-fold over the dissociation constant, indicative of a large volume change for association. Finally, operator binding appears to decrease the affinity between dimers. These results bring to light the coupling between subunit interactions and ligand binding. Such interactions may be important in determining the level of repressor binding to multiple sites and pseudo-sites on looped DNA. (Supported by PHS-R29-GM39969 & PHS-R01-GM22441.)

M-Pos139

STRUCTURAL STUDIES OF THE BAMHI RESTRICTION ENZYME.

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Type II restriction enzymes are ideal for studying protein-DNA interactions because of their high sequence specificity and striking variety. Large, well-ordered BamHI crystals, diffracting to 2.3 Å, were obtained from PEG solutions. These crystals occur in two forms: monoclinic (space group C2, unit cell constants: a=76.24 Å, b=46.0 Å, c=69.4 Å and β=110.5°) and orthorhombic (space group $C222_1$, unit cell constants: a=46.7 Å, b=76.6 Å and c=143.6 Å). In both crystal forms there is one protein monomer per asymmetric unit. Currently, we are searching for heavy atom derivatives of the enzyme.

We are also attempting cocrystallization of the enzyme with a 12-bp DNA fragment containing the BamHI recognition site (5'-GGATCC-3'). We have recently obtained large, plate-like crystals, which we are exploring for the presence of DNA by X-ray and biochemical methods.

M-Pos140

THE DECAY OF FLUORESCENCE ANISOTROPY OF ETHIDIUM BROMIDE BOUND TO NUCLEOSOME CORE PARTICLES

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The binding of ethidium bromide to the DNA of nucleosome core particles is accompanied by a large increase in fluorescence lifetime from 1.6 ns to an average lifetime of about 22 ns, a change similar to that observed for the intercalative binding of ethidium to DNA. The ethidium lifetime can be further enhanced up to an average of about 39 ns by substituting deuterium oxide for water. These relatively long lifetimes permit one to measure the decay of the fluorescence anisotropy out to relatively long times (greater than 350 ns for the deuterium oxide). With such a decay one is able to clearly distinguish the relatively fast torsional motions of the DNA from the rotational diffusion of the particle. The effects of several solution-induced conformational changes on the recovered rotational correlation times will be reported, as well as what these changes in correlation times imply about shape changes in the nucleosome. We will also use deconvolutions of the early time data to analyze the restricted motions of the DNA on the particle. Supported by NIH grant GM-25663.

M-Pos141

VISUALIZATION BY ELECTRON MICROSCOPY OF HOLLIDAY JUNCTIONS WITH BOUND REC A PROTEIN

Sandra L. Shaner and Charles M. Radding, Dept. of Human Genetics, Yale Univ. School of Medicine, New Haven, CT.

The reciprocal exchange of single strands during recombination between two homologous DNA duplexes involves a branched intermediate known as a Holliday junction [Holliday (1964) Genet. Res. 5, 282]. Whereas many electron microscopic studies have visualized, in the presence and absence of bound protein, the reaction products of recA protein-promoted asymmetric strand exchange between a purely single-stranded (ss) DNA and a homologous duplex, the reaction products of symmetric strand exchange between two primarily duplex DNA molecules have been less well studied. Electron microscopy has been performed previously only on the deproteinized reaction products. Here, the Holliday structures formed as a result of recA protein-promoted reciprocal strand exchange between a 1325 bp duplex DNA with a 200 residue 5' ss tail and a partially homologous blunt-ended 2042 bp duplex were examined by electron microscopy in order to identify by direct visualization the location of bound recA protein.

M-Pos142

FLUORESCENCE MICROSCOPY AND CIRCULAR DICHROISM OF POLYLYSINE-DNA COMPLEXES

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We have used video fluorescence microscopy to study the condensation of calf thymus and T2 DNA induced by poly-D- and poly-L-lysine, and polyethyleneglycol (PEG). The fluorescent dye ethidium bromide was used in these studies. Large, polynemic, superhelical filaments of T2 DNA were observed in samples prepared with 0.5 µg/ml poly-L-lysine, and some filaments displayed several levels of supercoiling. These superhelices were stable and maintained their integrity after being mechanically disrupted. Toroids and spool-shapes were common in samples prepared with 5.0 µg/ml poly-L- or poly-D-lysine. Clusters of condensates were observed in samples with PEG. The structures observed were identical to those observed by electron microscopy. Circular dichroism (CD) spectra were obtained for solutions of calf thymus DNA with poly-L-lysine or PEG. A CD signal for DNA-PEG complexes could be obtained directly from the quantity examined microscopically (4.0 µl). For DNA-poly-L-lysine complexes larger quantities were necessary, and aliquots of these were examined microscopically. This application of fluorescence microscopy provides a direct method of correlating structure of condensate with CD spectrum. This technique will allow us to follow, in real-time, the condensation process and to study the kinetics and dynamics of *in-vitro* condensation of DNA.

M-Pos143

PROPERTIES OF FLUORESCENTLY LABELED CATABOLITE ACTIVATOR PROTEIN AND RNA POLYMERASE FROM E. COLI.

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We have fluorescently labeled CAP and RNA polymerase to gain information about the structure of these proteins in various types of transcription complexes. Each protein has, on average, a single covalently attached eosin group. We have (1) characterized the binding affinity of the labeled proteins for both the wild type and UV5 lactose promoters, (2) compared the kinetic properties of these complexes with those formed using unlabeled proteins, and (3) assessed the ability of the labeled proteins to participate in the transcription process. The data imply that the presence of the eosin group modifies but does not eliminate the ability of CAP or RNA polymerase to interact with promoter regions in a physiologically significant manner.

M-Pos144

Induced DNase I Hypersensitivity in O_R3 by Simultaneous cI Repressor Binding to O_R1 and O_R2.
Daniel Strahs and Michael Brenowitz. Department of Biochemistry, The Albert Einstein College of Medicine, Bronx, New York 10461.

The protein cI repressor (cI) of phage λ maintains the lysogenic state in infected *E. coli* cells by binding cooperatively to three tandemly repeated binding sites in the right operator of the phage λ genome. When cI repressor binds simultaneously to sites O_R1 and O_R2, transcription from the promoter P_R is blocked and transcription from the promoter P_{RM} is enhanced. cI is believed to repress transcription initiation at P_R by sterically blocking RNA polymerase (RNAP) binding and to enhance transcription initiation at P_{RM} by protein-protein contacts between cI bound at O_R2 and RNAP.

In vitro quantitative footprint titration studies of cI binding to O_R-containing restriction fragments show that DNase I hypersensitivity in site O_R3 is induced by the simultaneous, and cooperative, binding of cI to O_R1 and O_R2. The specificity of the hypersensitivity is shown by its absence upon binding cI to O_R1^-, O_R2^- and $O_R1^-2^-$ mutant operators. Modelling of the hypersensitivity as a function of O_R1 and O_R2 occupancy suggests that cooperative binding of cI can cause structural changes in the DNA. The implications of this result for the mechanism of cooperative binding and transcriptional enhancement at P_{RM} will be discussed.
(Supported by NIH Grants GM39929 and 5T32-GM-07128)

M-Pos145

INTERSUBUNIT COMMUNICATION IN cAMP RECEPTOR PROTEIN FROM E. coli.
Ewa Heyduk, Tomasz Heyduk, and James C. Lee. Dept. of Biochemistry, St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104.

Binding of cAMP to CRP exhibits a negative cooperative behavior. Therefore, there has to be communication between the subunits interface which is formed almost entirely by a long alfa-helix (C-helix). In search of a region of this helix responsible for intersubunit communication two proteolytic fragments of CRP were generated and purified. Ligand binding properties, conformational response to cAMP binding and global structural changes (using analytical gel chromatography) of these fragments were probed. The subtilisin fragment, which is devoided of 2/3 of C-helix, binds cAMP in a non-cooperative way and undergoes a slight expansion when complexed with cAMP. The chymotryptic fragment, which retains the whole C-helix, binds cAMP with high negative cooperativity and undergoes a more pronounced expansion. Intact protein, on the other hand, exhibits slight contraction upon binding of cAMP. These results showed that the region of C-helix between Leu 116 and Phe 136 is responsible for intersubunit communication, although it is not essential for dimer formation. They also suggest that ligand-induced conformational change in CRP, which leads to the expression of the biological function of the protein, can be a complex combination of changes in local (within a domain) structure, domain-domain and intersubunit interactions.

M-Pos146

THEORY FOR THE BINDING OF E.COLI SINGLE STRAND BINDING PROTEIN (SSB) TO SUPERCOILED DNA.
J. B. Clendenning and J. M. Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195.

A recent theory for the binding of unwinding ligands to supercoiled DNA is extended to include multisite coverage. This is applied to a model for binding SSB to supercoiled pBR322 in which the SSB binds in pairs to the complementary strands of a melted region. Partition functions are evaluated using an iterative linearized matrix method. Values for the stability and cooperativity constants for duplex DNA and the intrinsic binding constant, binding-site size, and cooperativity parameter for the protein on single-strand DNA are all taken from the literature. Binding data are well fitted by values within the reported ranges. Our results show that (1) SSB binding is driven largely by the reduction of superhelical strain upon melting 34 bp per bound SSB pair, (2) SSB clusters into a single contiguous stack on each plasmid, (3) the stack length remains nearly constant over the observed concentration range, so the binding is virtually all-or-none, and (4) the large cooperativity due to opening the DNA is effectively cancelled by the global anticooperativity that results from progressive unwinding, over the observed range. These results are in complete accord with several different experimental observations.

M-Pos147

A STRUCTURAL MODEL OF THE MITOCHONDRIAL VDAC CHANNEL. C.A. Mannella, X.W. Guo, H. Chen, Wadsworth Center, NYS Dept. of Health and Depts. of Biomedical Sciences and Physics, State Univ. of NY, Albany, NY.

The VDAC channel crystallizes in the plane of the outer membrane of Neurospora mitochondria by the action of phospholipase A_2. The geometry of the arrays can be explained by a model in which the channel has four binding sites on its periphery (A,B,C,D), which interact pairwise (A-B, C-D) with sites on adjacent channels. The crystal unit cell, which holds six channels, can accommodate one or two 30-kDa polypeptides per channel. If the channel is a monomer, most of the protein is needed to form the large transmembrane lumen, e.g. by folding into a beta-barrel as proposed by Guy (J. Bioenerg. Biomemb. 19:341). The backbone diameter of such a channel is 3.8 nm based on projected density maps obtained from cryoelectron microscopy of frozen-hydrated crystals. The sequence of the 20 amino acids at the N-terminus is consistent with an amphipathic helix. A model is proposed for a monomer channel in which gating and ion selectivity involve movement of this helical "arm" between the bilayer surface and the channel lumen. (Supported by NSF grant DMB-8613702.)

M-Pos148

LIPID-PROTEIN INTERACTIONS MODULATES ADENOSINE DEAMINASE ACTIVITY WHEN RECONSTITUTED IN LIPOSOMES: EFFECT OF CHOLESTEROL.

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Adenosine deaminase (ADA) is a malignency marker with a polymorphic structure: a small subunit, 45,000D, (SS-ADA) and an extrinsic membranal complexing protein, 220,000D, (ADCP). ADCP was reconstituted in liposomes (Caiolfa et al. (1989) Biophys J. 55:320a). Electron microscope micrographs verified our dynamic light scattering studies of liposome size distribution. Retention of SS-ADA specific activity is evident when bound to ADCP reconstituted in Asolectin or DMPC vesicles. Upon enrichment with cholesterol a 1.75 and 3 fold increase in activity was observed with Asolectin and DMPC liposomes, respectivly. Excessive incorporation of cholesterol, reduced SS-ADA activity in these vesicles. The quantitative differences observed between Asolectin and DMPC vesicles reflects differences in lipid-protein interactions. Our results are intriguing when compared with ADCP-SS-ADA interactions in solution which result rather in reduced ADA activity. These results may explain the observed reduction in SS-ADA activity upon cell transformation. ADCP in buffer and in vesicles modulates SS-ADA activity in an opposite fashion.

M-Pos149

CALORIMETRIC STUDIES OF THE INTERACTION OF AN AMPHIPHILIC MODEL PEPTIDE WITH PHOSPHATIDYLCHOLINE BILAYERS. Y. Zhang, R.N.A.H. Lewis, R.S. Hodges, and R.N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

High sensitivity DSC has been applied to study the effect of an amphiphilic peptide (Lys_2-Gly-Leu_{24}-Lys_2-Ala-amide) on the thermotropic behavior of a series of diacyl-PC's with different hydrocarbon chain lengths. At high lipid:peptide ratios (>25-30 lipids per peptide) the main transition endotherms can be resolved into two components, which we have assigned to lipid rich (narrow component) and the peptide rich domains (broad component). With an decrease in the lipid:peptide ratio there is a decrease in the transition temperatures of both components and a decrease in the total enthalpy change measured. We find that the peptide associated lipids make a significant contribution to the total enthalpy change. Moreover, the acyl chain length dependence of the thermotropic properties of the peptide associated lipids can be rationalized by a mismatch between the bilayer thickness and the length of the peptide.

(Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)``

M-Pos150

EFFECTS OF PHOSPHOLIPID ACYL CHAIN AND PH VARIATION ON THE STRUCTURE OF BACTERIORHODOPSIN IN RECONSTITUTED VESICLES. Wen-Cheng Huang and Barbara A. Lewis, Dept. of Chemistry, University of Wisconsin, Madison WI 53706.

Bacteriorhodopsin (BR) is an ideal model transmembrane protein. By performing solid state NMR on reconstituted vesicles made with delipidated BR and a range of lipids, we are exploring the interactions of BR with its lipid and ionic milieu. The goal is to elucidate the influences of hydrophobic and electrostatic forces on the structure of this prototypical membrane protein.

When delipidated BR is reconstituted into phosphatidylcholine (PC) vesicles, a blue-shifted species ($\lambda_{max} \approx 490$ nm) appears at alkaline pH. The apparent pK_a for this transition is affected both by lipid acyl chain length and unsaturation. Solid-state ^{13}C NMR studies of peptide-labeled BR in these vesicles are in progress. Using the approach previously described (Lewis et.al. (1985) *Biochemistry* 24: 4671), lipid and pH effects on the average peptide bond orientation (e.g., helix tilt angles) are determined.

(Supported by NIH GM38532.)

M-Pos151

EFFECTS OF HEADGROUP CHARGE ON THE ATPase ACTIVITIES AND Ca^{2+} BINDING PROPERTIES OF ISOLATED CARDIAC SARCOLEMMAL VESICLES

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In recent studies we demonstrated that the free fatty acids produced by PLA_2 treatment can combine with divalent cations, such as Ca^{2+}, to modify both Mg-ATPase and Na,K-ATPase activities in isolated cardiac sarcolemmal vesicles. To further determine the relative importance of headgroup charge in these interactions we examined the effects of various concentrations (0-100 μM) of a negatively charged fatty acid analogue (SDS), its neutral analogue (lauryl acetate), and a positively charged analogue (DDTMA) on the ATPase and Ca^{2+} binding properties of isolated cardiac sarcolemmal vesicles. Our results demonstrate that headgroup charge, changes in Ca^{2+} binding to the sarcolemmal surface, and inhibition of ATPase activity are closely correlated in these vesicles, and suggest that the surface properties of the sarcolemmal membrane may play a role in regulating Mg-ATPase and Na,K-ATPase function. (Supported by an AHA(GLAA) Grant-in-Aid (843-G1) the Laubisch Endowment, and a BRSG grant, UCLA)

M-Pos152

CALCIUM-INDEPENDENT BINDING OF PROTHROMBIN TO NEGATIVELY CHARGED MEMBRANES.

[1]Susan W. Tendian, [2]Nancy L. Thompson, & [1]Barry R. Lentz. *[1]Biochemistry Dept. & [2]Chemistry Dept., Univ. of North Carolina, Chapel Hill, NC 27599. Intro. by Francine R. Smith.*

A calcium-independent interaction of prothrombin with phosphatidylserine(PS)-containing membranes results in increased fluid phase acyl chain order as detected by the anisotropy of diphenylhexatriene in small unilamellar vesicles (SUV). Binding has been quantitated using the prothrombin-induced shift in fluorescence anisotropy. The dissociation constant obtained by this method for prothrombin bound to 30/70 PS/phosphatidylcholine (PC) SUV was $2x10^{-4}$ molar. The interaction was significantly reduced with equivalently charged phosphatidylglycerol (PG)-containing membranes and was negligible with pure PC membranes. The amino terminal third of prothrombin, fragment 1, displayed no such interaction with PS- or PG-containing membranes. The carboxy terminal 2/3 of prothrombin, prethrombin 1, bound to PS/PC vesicles in the same way as intact prothrombin, demonstrating that prethrombin 1 is the region containing the calcium-independent binding site. Total internal reflection fluorescence microscopy measurements confirmed that PS significantly enhanced the binding of fluorescein-labelled prothrombin to planar PC membranes deposited on fused silica surfaces. *Supported by USPHS(SCOR) Grant HL-26309 to BRL & NSF-PYI Grant DCB 8552986 to NLT.*

M-Pos153

INFRARED STUDIES OF LIPID-PROTEIN INTERACTION IN PULMONARY SURFACTANT.

Kim E. Reilly, Alan J. Mautone, and Richard Mendelsohn, Department of Chemistry, Rutgers University, Newark, NJ 07102.

SP-A, the major 29-36 kD class of surfactant protein, has been purified from bovine lung lavage and reconstituted into binary lipid mixtures of DPPC-d_{62}/DPPG. FT-IR melting curves of the lipid components revealed that high levels of SP-A induced an ordering of the phospholipid acyl chains. In contrast, a progressive disordering of the acyl chains and reduction in the cooperativity of their melting event was noted upon reconstitution of a mixture of the other surfactant proteins.

In addition to bulk transmission experiments, external reflection FT-IR was used to examine monolayer surfactant films in situ at the air-water interface, as a function of surface pressure. At surface pressures which correspond to large lung volumes in vivo, the surfactant acyl chains exist mostly in the ordered configuration. The surface film undergoes a weakly cooperative transition as function of surface pressure which mimics (in terms of acyl chain order), a broad thermotropic phase transition in native surfactant.

M-Pos154

THERMODYNAMICS OF CHOLERA TOXIN BINDING TO VARIOUS DERIVATIVES OF ITS CELL SURFACE RECEPTOR, GANGLIOSIDE GM_1.

M. Masserini, A. Schön, D. Xie and E. Freire, Department of Biology and The Biocalorimetry Center, The Johns Hopkins University, Baltimore, MD 21218

The thermodynamics of the association of cholera toxin to several derivatives of ganglioside GM_1 (native GM_1, Fucose-GM_1 and ganglioside GM_1 in which the fatty acid has been replaced by an acetyl group) incorporated into phospholipid vesicles has been measured by high sensitivity isothermal titration and differential scanning calorimetry. The enthalpy of association of the cleaved oligosacharide region of ganglioside GM_1 to cholera toxin in solution is -165 kcal/mole of toxin. On the other hand, the enthalpy of association of the intact toxin to membrane bound ganglioside is -100 kcal/mole and that of the isolated B subunit pentamer -110 kcal/mole. The difference of approx. +60 kcal/mole most likely arises from the direct interaction of the toxin with the membrane surface since the enthalpy changes do not exhibit a strong dependence on the head group or fatty acid modification of the ganglioside. (Supported by NIH grants NS-24520 and RR-04328.)

M-Pos155

STOPPED FLOW KINETICS OF GALA-LIPID INTERACTION. R. A. Parente, A. S. Verkman* & F. C. Szoka, Jr., Schools of Pharmacy & *Medicine, University of California, San Francisco, CA.

The rate of pH driven association of the synthetic amphipathic peptide, GALA, with unilamellar phosphatidylcholine (PC) vesicles has been investigated using stopped-flow fluorescence. Association was followed by fluorescence energy transfer from the N-terminal Trp of GALA to an NBD-lipid probe incorporated in the PC vesicles. An increase in fluorescence was measured when GALA associated with vesicles at pH 5. At 1 mM POPC the relaxation time for peptide-vesicle association was independent of [GALA] up to 40 uM and equaled 23±2 ms. The initial stages of lipid-peptide association can be described by the rate equation, $L + nP \rightleftharpoons L_nP_n$, where under conditions of [L] >>[P], the process is a pseudo first order reaction. When the pH of the premixed vesicle-peptide complex was raised from 5 to 7.5, the dissociation rate was an order of magnitude slower than the association rate.

The initial rate of GALA-induced leakage from vesicles was also studied on the millisecond timescale by stopped-flow fluorescence. A lag time in the initial rate of leakage was observed. This lag in the onset of leakage increases from 0.2 to 5 s as the lipid to peptide ratio is increased from 50:1 to 15000:1. These results are consistent with a model in which GALA rapidly associates with the vesicle and aggregates in the membrane to form a pore or channel.

M-Pos156

CHOLESTEROL ENRICHMENT INCREASES Ca^{++} AND K^+ FLUXES AND DECREASES MEMBRANE FLUIDITY IN ARTERIAL SMOOTH MUSCLE. T.N. Tulenko, M.M. Gleason and M.S. Medow. Medical College of Penna. Phila, PA. (Intro. by R.S. Moreland)

The cholesterol:phospholipid molar ratio (C/PL) in the plasma membrane regulates the physical state (fluidity) of the phospholipid bilayer. The relationship between membrane C content, and K^+ and Ca^{++} movements and fluorescence anisotropy were investigated in cultured rabbit aortic smooth muscle cells (SMC). SMC were incubated in media ± 3 types of C/PL liposomes: C-rich (2:1 C/PL), C-poor (0.5:1 C/PL) and C-free (0:1 C/PL) with constant PL (550 µg/ml media). Incubation with C-rich liposomes; 1) increased cellular C mass with no change in PL content resulting in an increase in C/PL ($p<.001$) and 2) increased SMC microsomal C mass with no change in PL content resulting in a increase in C/PL ($p<.05$). C enrichment increased basal Ca^{++} influx and Ca^{++} and K^+ effluxes. Net cellular C correlated with increased basal Ca^{++} influx ($r=.762$) and Ca^{++} and K^+ efflux rates ($r=.923$). C enrichment of SMC increased fluorescence anisotropy. These results suggest that the SMC plasma membrane cholesterol content is sensitive to enrichment and depletion, and that changes in either direction alter transmembrane Ca^{++} and K^+ movements and membrane fluidity.

M-Pos157

INTERACTION OF SYNTHETIC MAGAININS AND POLYMYXIN B WITH LPS AND OUTER MEMBRANES OF POLYMYXIN-RESISTANT AND -SENSITIVE STRAINS OF *SALMONELLA TYPHIMURIUM*. Fazale Rana*, Elizabeth Macias†, Malcolm Modrzakowski† and Jack Blazyk* (Intr. by P. Sullivan), Departments of *Chemistry and †Zoological and Biomedical Sciences, and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Lipopolysaccharide (LPS) in the outer membrane of *Salmonella typhimurium* is phosphorylated at numerous sites with either monophosphate or diphosphate monoesters or diesters. The ability of synthetic magainins and polymyxin B to bind to and disorder LPS in the outer membranes of polymyxin-resistant (SH5357) and -sensitive (SH5014) strains (gifts from E. McGroarty) is related to the nature of these phosphorylation sites. The effects of magainin 2, the more potent analog, [$Ala^{13,18}$]-magainin 2, and polymyxin B on the thermotropic behavior of LPS and isolated outer membranes from the two strains was monitored by FT-IR spectroscopy. The mole ratios of phosphatidylethanolamine to LPS in the outer membranes of each strain and the nature of the phosphate groups on LPS were determined by high-resolution ^{31}P NMR spectroscopy. The degree of lipid disordering and lipid composition are correlated with the bactericidal potency of the three peptides toward the two *Salmonella* strains.

M-Pos158

ROLE OF LPS IN THE INTERACTIONS OF MAGAININ WITH THE *S. TYPHIMURIUM* CELL ENVELOPE. Fazale Rana*‡, Elizabeth Macias†‡, Catherine Sultany+, Malcolm Modrzakowski†‡ and Jack Blazyk*‡, Departments of *Chemistry and †Zoological and Biomedical Sciences, ‡College of Osteopathic Medicine and +University Research and Instrumentation Center, Ohio University, Athens, OH 45701.

Smooth wild-type *Salmonella typhimurium* and three rough strains possessing R_a, R_{d1} and R_e chemotype LPS show susceptibility to the antimicrobial peptide, magainin 2, in the following order: $R_e > R_{d1} \geq R_a >$ smooth. The fluidizing effect of magainin on the outer membranes and lipopolysaccharides (LPS) of the four organisms was examined by FT-IR spectroscopy. The extent of disordering induced by magainin in the outer membranes is greatest in the smooth and least in the R_e strain. Heterogeneity of the LPS sugar phosphorylation sites, net charge on the LPS molecules and the relative amounts of LPS and phosphatidylethanolamine (PE) in the outer membranes of each strain were determined by high-resolution ^{31}P NMR spectroscopy. The concentration of LPS in the outer monolayer and the anionicity of LPS from the different strains appear to be responsible for differences in magainin binding. We postulate that LPS in the outer membrane may act as a molecular sponge for the cationic peptide, inhibiting its lethal attack on the plasma membrane.

M-Pos159

THE EFFECT OF SIGNAL PEPTIDES ON MEMBRANE LIPID ORGANIZATION

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To get insight into the possible role of signal sequences in protein-translocation we investigated the effect of several synthetic signal peptides on lipid structure in model membranes of phosphatidylethanolamine and phosphatidylglycerol mimicking the lipid composition of the *E. coli* inner membrane. Using ^{31}P NMR and freeze fracture electron microscopy it is demonstrated that addition of the signal peptide of the *E. coli* outer membrane protein PhoE to these model membranes even at very low concentrations (±1/3000 molar ratio of peptide to lipid) strongly promotes the formation of type II non-bilayer lipid structures. Furthermore, using a negatively charged mutant signal peptide, a correlation was found between the effect on lipid structure and *in vivo* functioning of the corresponding precursor protein. The changes in lipid structure upon addition of the PhoE wild type signal peptide and other signal peptides are found to be similar to those induced in the pure lipid system upon incubation at elevated temperatures.
It is proposed that signal sequences may act by inducing the local and transient formation of type II non-bilayer lipid structures that allow precursor proteins to cross the membrane.

M-Pos160

A QUALITATIVE AND QUANTITATIVE STUDY ON THE BINDING OF CYTOCHROME C TO A DIOLEOYLPHOSPHATIDYLCHOLINE MONOLAYER SPREAD AT THE AIR-WATER INTERFACE

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The monolayer technique has been widely used to study the lipid-protein interactions in biomembrane models. The binding of cytochrome c (cyt c) to different neutral and charged lipids has been previously reported by different authors. The general approach to characterize these systems is the measurement of adsorption kinetics. These experiments are generally time consuming and they give little quantitative information. We have undertaken the study of cyt c incorporation into a dioleoylphosphatidylcholine (DOPC) monolayer using a different approach. The lipid-protein system was characterized from surface pressure-molecular area isotherms obtained for a dynamically compressed-expanded film. From these measurements, surface pressure limits for adsorption/desoption are unambiguously determined. Furthermore, the free energy of incorporation, desorption and stabilization can be determined from those isotherms. A characterization of transferred DOPC-cyt c films by electron microscopy complete this study.

M-Pos161

FORMATION OF METARHODOPSIN II AND ACTIVATION OF G_v BY RHODOPSIN-CONTAINING DMPC VESICLES.

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Formation of meta II and rho* were measured independently in DMPC (T_m = 23C) and POPC (T_m = -2C) vesicles, using laser flash photolysis and G_v activation measurements, respectively. At 30 and 37C the meta I-meta II equilibrium constants are 0.4 and 0.75 in DMPC, 2.0 and 2.9 in POPC, respectively. The presence of G_v enhances production of meta II by binding to meta II in the absence of GTP. In POPC and DMPC, at 30 and 37C, the degree of enhancement corresponds to G_v binding to all available photolyzed rhodopsin molecules, but the apparent rate of binding in POPC is approximately 20 times greater than that in DMPC. In DMPC vesicles at 37C all the G_v-bound GDP exchanged for GMPPNP, a nonhydrolyzable GTP analogue, during a 5 minute exposure to room light, while at 5C less than 5% of the G_v had undergone exchange. In contrast, in POPC maximal exchange was obtained at both 5 and 37C. These results show that photoactivation of rhodopsin, incorporated into a DMPC bilayer, produces meta II and functionally competent rho*. However, the dependence of the functional coupling of rhodopsin and G_v on the bulk microenvironment provided by the phospholipid bilayer is demonstrated by the minimal activation of G_v below the T_m of DMPC. In sharp contrast, maximal activation was observed in the POPC vesicles at 5 and 37C; temperatures which are above the T_m of POPC.
(supported by NIH grant EY00548).

M-Pos162

CHOLESTEROL INCREASES THE STABILITY OF RHODOPSIN IN EGG PC VESICLES TO THERMALLY INDUCED UNFOLDING.

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Studies have been carried out to determine the effect of the addition of cholesterol to egg phosphatidylcholine (PC) vesicles on the thermal stability of an incorporated integral membrane protein. To this end calorimetric, spectrophotometric and spectropolarimetric measurements of rhodopsin's thermal unfolding in egg PC vesicles containing 0% and 30% cholesterol were made. Data from all three types of measurements concur and reveal a midtransition temperature 3 degrees higher for the cholesterol containing membranes. The maximum of the excess heat capacity function occurs at 72C and 68.5C respectively, for those membranes with and without cholesterol. The calorimetric enthalpy of unfolding is 130±10 kcal/mole for rhodopsin in both preparations consistant with similiar folded states and similiar unfolded states in the 0% and 30% cholesterol membranes. As the protein unfolding is irreversible, a kinetic study of the change in an observable is appropriate. Fitting the loss of rhodopsin's 500nm absorbance to a single exponential function yields rate constants of 0.020 min^{-1} for 30% cholesterol membranes and 0.040 min^{-1} for 0% cholesterol membranes at 65C. These results suggest an increased activation energy for the unfolding of rhodopsin in the cholesterol containing membranes due to the ordering effect of cholesterol on the PC acyl chains. (supported by NIH grant EY00548).

M-Pos163

HDL, APO A1 AND AMPHIPATHIC PEPTIDES STABILIZE LIPOSOMES COMPOSED OF DIOLEOYL-PHOSPHATIDYLETHANOLAMINE (DOPE) AND OLEIC ACID (OA). Dexi Liu and Leaf Huang, Dept. of Biochem., Univ. of Tennessee, Knoxville, TN 37996 and Jere P. Segrest and G.M. Anantharamaiah, Dept. of Med., Univ. of Alabama School of Med., Birmingham, AL 35294.

Small unilamellar liposomes composed of DOPE and OA are stabilized by incubation with normal human serum or plasma (Liu and Huang, Biochem (1989) 28, 7700-7707). Systematic studies on interaction of purified serum proteins with this type liposomes showed that albumin destabilizes liposomes by extracting OA from liposomes. However, HDL and, to some extent, VLDL showed a rapid stabilization activity against the lytic effect of albumin. Purified Apo A1, showed comparable stabilization activity as HDL. Furthermore, synthetic peptides resembling the amphipathic helices found in Apo A1 also showed strong stabilization activity. These data indicate that Apo A1 plays a major role in human serum or plasma for the liposome stabilization activity. The stabilization activity is probably through the interactions of the amphipathic helices of Apo A1 with the hydrophobic voids on the outer surfaces of liposomes. Supported by NIH grants AI25834, CA24553, and 5-PO1-HL34343.

M-Pos164

CHARACTERIZATION OF PHOSPHOLIPID-AMPHIPATHIC PEPTIDE COMPLEXES

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The synthetic, 20 residue, lipid-associating peptide LAP-20 (VSSLLSSLKEYWSSLKESFS) associates with dimyristoyl phosphatidylcholine (DMPC) to form complexes of defined stoichiometry and size. In solution, LAP-20 is a random coil, however, in the presence of DMPC, it acquires amphipathic helical properties and is capable of activating lecithin:cholesterol acyltransferase (Ponsin et al., 1986, *J.Biol.Chem. 261*:9202). We have prepared DMPC/LAP-20 complexes at a mole ratio of 100:1 with a 50 nm pore membrane extruder (Lipex Biomembranes, Inc.) Negative stain electron microscopy and dynamic light scattering measurements show that these particles are asymmetric in size and shape. In the complexes, the tryptophan fluorescence spectrum is blue shifted to 324 nm and the lifetime is shorter. Analysis of lifetime data by lorentzian distribution shows a decrease in the lifetime with increasing temperature. The distribution width correlates with the physical state of the DMPC. (PHS-P41-RR03155.)

M-Pos165

EFFECT OF PHOSPHATIDYLCHOLINE (PC) ON THE ORIENTATION OF 3-HYDROXYBUTYRATE DEHYDROGENASE (BDH) IN PHOSPHOLIPID (PL) VESICLES. Lauraine A. Dalton, J. Oliver McIntyre and Sidney Fleischer. Vanderbilt University, Nashville, TN 37235.

BDH is a membrane-bound enzyme with an absolute requirement for PL for enzymic activity. PC activates optimally and phosphatidylethanolamine (PE) activates partially (see McIntyre *et al.*, this volume). BDH has been derivatized at a single active-center sulfhydryl (SH1) with the spin label 2,2,6,6-tetramethylmaleimidopiperidinyl-1-oxyl (MSL). The nitroxide (NO) of MSL is 8 Å from SH1. MSL-BDH can be inserted unidirectionally into PL vesicles (BDH-V) containing diphosphatidylglycerol (DPG) and either PC or PE. Lineshapes of EPR and ST-EPR spectra of MSL-BDH were similar in the presence vs absence of PC, indicating comparable rotational mobility. Accessibility of the NO of MSL-BDH to paramagnetic Gd^{3+} was compared for BDH-V containing DPG and either PC or PE. Dipolar relaxation of NO by Gd^{3+} bound to DPG at the surface of the bilayer permits calculation of radial distance. For MSL-BDH-V in DPG/PE, the distance of closest approach of Gd^{3+} to the NO (≈9 Å) is much less than for MSL-BDH-V in DPG/PC [17 Å, Dalton et al., Biochemistry 26, 2117 (1987)]. The shorter distance between bound Gd^{3+} and NO in MSL-BDH-PE vs MSL-BDH-PC indicates that PC induces a conformational change in BDH resulting in a change in orientation of either the MSL with respect to BDH and/or BDH with respect to the PL bilayer. The nature of the PL determines the distance of the NO from the membrane surface but not the motion of BDH. [Tennessee Heart Assn. (JOM) and NIH DK 14632 (SF)].

M-Pos166

PHOSPHOLIPID INCORPORATION OF P^{32} INTO PRIMARY MYOTUBE CULTURES OF DYSTROPHIC MDX AND CONTROL MUSCLE. Judy E. Anderson, Department of Anatomy, University of Manitoba, Canada. R3E 0W3.

Membrane phospholipids (PLP) anchor cytoskeletal proteins, and have been used as a marker for fusion processes during myogenesis. The DMD gene product, dystrophin is thought to be critical in preventing sarcolemmal instability during muscle activation. However, dystrophin deficiency from mdx muscle does not prevent regeneration from early dystrophic degeneration. Incorporation of P-32 from orthophosphoric acid into PLP was studied from chloroform: methanol extracted primary cultures of muscle from newborn mdx and control mice, after the appearance of myofibrils in the myotubes. P-32 incorporation into mdx PLP was significantly increased into phosphatidyl ethanolamine and was decreased in lysophosphatidyl choline and phosphatidyl serine/phosphatidyl inositol fractions isolated from TLC plates as compared with the same fractions from control PLP.

Supported by the Manitoba Health Research Council

M-Pos167

CONFORMATIONAL DYNAMICS OF MELITTIN AND [Ala-14]-MELITTIN BY NMR AND AMIDE EXCHANGE, AND SIGNIFICANCE FOR MELITTIN'S MEMBRANE ACTIVITIES.

Christopher Dempsey, Renzo Bazzo and Iain D. Campbell (Intro by Steven Smith); Biochemistry Dept., Oxford University.

The contribution of the central proline residue to the conformational and dynamic properties, and membrane activities of melittin, has been determined using a synthetic analogue, [Ala-14]-melittin, in which the proline residue has been replaced by alanine. The pH-dependence of single amide exchange from [Ala-14]-melittin in methanol has been determined by ^{1}H NMR and compared with similar data for melittin [**Biochem.** 27 6893 (1988)]. The replacement of proline by alanine results in a marked stabilization of the melittin helix by ten-fold in the N and C terminal regions and by up to 100-fold in the central turn of helix containing Pro-14 of melittin. Local equilibrium constants for individual hydrogen-bond-breaking fluctuations are calculated and the data analysed in terms of models for helix fluctuations. The replacement of proline has marked effects on the haemolytic and voltage-dependent channel activities of melittin in membranes and these effects are interpreted in terms of the altered conformational and dynamic properties of the analogue.

M-Pos168

MODELLING OF ELECTROPORATION IN MEMBRANES BY THE NUCLEATION THEORY IN CONDENSED SYSTEMS.

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The rate of production of critical size pores resulting in membrane rupture in the presence of an applied transmembrane potential is modelled based on the theory of nucleation in condensed systems. The steady-state rate of critical pore formation was described by $I \sim O^* w N^*$; where O^* is the number of water molecules in contact with the critical pore, w is the molecular jump rate, and N^* is the equilibrium number of pores of critical size per unit membrane area (A_m). The time constant to cause the membrane rupture was estimated to be $\tau = 1/IA_m$. For artificial bilayers, the estimate of τ was in reasonable agreement with experiments. Irreversible mechanical breakdown time drops from ~ 25,000 ms at 200 mV to 0.024 ms at 240 mV. It was also shown that the dynamics of pore formation could be modelled only by including the transient behavior. At 240 mV, the time to reach 63% of the steady-state pore formation rate was about three orders-of-magnitude less than the estimated membrane rupture time. However, at a slightly higher membrane potential of 270 mV, the transient time is one order-of-magnitude longer than the rupture time and could not be ignored. The analysis from this work was intended to establish the basis for a more elaborate modelling of transient electroporation phenomenon in membranes using the principles of the transient nucleation theory.

M-Pos169

THE ROLE OF POLARIZATION ENERGY IN FREE ENERGY CALCULATIONS.

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A detailed implementation of the polarization energy and its derivatives into a molecular dynamics program (AMBER, version 3.3) is described. In order to examine the effect of the polarization energy on the calculated free energy differences using free energy perturbation method, we have computed the differences in free energy of solvation of normal alkanes, tetraalkylmethane, tetraalkylammonium ions and some closed shell ions in water. The calculated free energy differences are in reasonable agreement with the experimental values. The pattern of the free energy change computed is compared with the results of our earlier simulations where the polarization energy was not included in the calcu lation. It is found that, in the majority of the cases, the polarization energy contribution to the free energy change is additive.

M-Pos170

ROTATIONAL DYNAMICS OF Fc_ϵ RECEPTORS ON INDIVIDUAL 2H3-RBL CELLS STUDIED BY POLARIZED FLUORESCENCE DEPLETION.

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Polarized fluorescence depletion (PFD) has been used to measure the rotation of functional membrane proteins on individual, microscopically selected cells under physiological conditions. Combining long triplet-state probe lifetimes with the sensitivity of fluorescence detection renders measurable rotational correlation times ϕ from < 10 μs to > 1ms. ϕ for Fc_ϵ receptors ($Fc_\epsilon R$) on the surface of 2H3-RBL cells is 79.9 ± 4.4 μsec and 83.6 ± 6.7 μsec at 4°C when labeled with eosin conjugates of IgE and of F4 anti-$Fc_\epsilon R$ Fab fragments, respectively. These values agree with the known 100 kDa receptor size and with previous phosphorescence studies. When labeled with intact F4 antibody, ϕ for $Fc_\epsilon R$ increases about 2-fold to 170.8 ± 6.5 μsec, consistent with $Fc_\epsilon R$ dimer formation on the plasma membrane as determined independently. These studies demonstrate the utility of PFD measurements in assessing size and association of membrane proteins on individual cells. Supported by NIH grants AI-21873 and AI-26621 (BGB).

M-Pos171

PHOSPHORESCENCE QUENCHING OF PROTEINS AT ROOM TEMPERATURE BY ADDED SMALL MOLECULES. S. Papp, W. W. Wright, S. W. Englander, J. M. Vanderkooi & C. S. Owen. Dept. of Biochemistry & Biophysics, School of Medicine, Univ. of Pennsylvania, Philadelphia PA 19104 & Biochemistry Dept., School of Medicine, Jefferson Univ., Philadelphia PA 19103

Tryptophan phosphorescence lifetimes for proteins in solution at room temperature vary from about 20 usec to 2 sec. In a study of ten proteins (thermolysin, parvalbumin, ribonuclease T1, nuclease, alcohol and glyceraldehyde-3-phosphate dehydrogenase, aldolase, pronase, azurin, alkaline phosphatase), long lifetime correlates with rigid environments in alpha helix or beta sheets. Added small molecule quenchers can reduce lifetimes. Against a given protein, the quenching constants found for different quenchers (nitrite, ethanethiol, nicotinamide, azide) are remarkably constant. Through the sequence of proteins listed, the quenching constant varies from $\sim 10^6$ to 10^1 $M^{-1}sec^{-1}$, decreasing exponentially with the distance of the indole from the protein surface. Phosphorescence quenching may therefore be used to measure the distance of tryptophans in proteins from the aqueous surface without covalent modification of the protein. It is also interesting that the quenching mechanism appears to involve long range electron transfer. (Supported by NIH GM 34448 & DK 11295)

M-Pos172

HYDROPHOBIC AQUEOUS NANOCOLLOIDS AS MODEL FOR AGGREGATION OF PROTEINS

A. Blum (Intro. by D. A. Goldstein)

Water insoluble heterocyclic aromatic compounds can be distributed in aqueous medium to form a stable suspension of submicron, spherical particles. Such translucent suspensions have narrow monodisperse size distribution and are stable for more than one year. The effects of ionic strength, pH, temperature and hydrophobic interactions on the filtration, settling, aggregation and aging behavior of the nanocolloidal sols represents a close analogy to the behavior of protein solutions. The time-dependent transition from reversible to irreversible aggregates observed for nanocolloidal sols can mimic the non-specific interactions in protein aggregation. Due to chemical homogeneity of nanocolloidal particles surface there is good chance for better simulation of non-linear aggregation effects in computer dynamics. The addition of proper components allows for modelling of protein-lipid, protein-DNA and protein-sugar interactions. Use of nano-colloidal sols as experimental "testing boards" for computer simulations of protein interactions is proposed.

M-Pos173

FLUORESCENCE EMISSION AND ANISOTROPY DECAY OF THE (+)-anti-BENZO[A]PYRENE DIOL EPOXIDE ADDUCT IN POLY (dG-dC)•(dG-dC)

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(+)-anti-7,8-dihydrodiol-9,10-epoxy-benzo[a]pyrene (BPDE), a strongly carcinogenic metabolite of benzo[a]pyrene binds specifically to guanine in double stranded DNA. The fluorescence of the BPDE adduct in poly (dG-dC) is characterized by several lifetimes. Part of the population gives rise to excimer fluorescence with a different excitation profile compared to the monomer. Using synchrotron radiation as excitation we have studied the fluorescence emission and anisotropy decay properties of both monomer and excimer populations. The anisotropy decay curves may be described by only one decay time. This was found to be around 5 ns for monomer and 1 ns for excimer emission, indicating a more rapid mobility of the BPDE's bound as excimer forming adducts.

M-Pos174

MOLECULAR MECHANICS/DYNAMICS OF METAL ION BINDING: PARAMETER DEVELOPMENT FOR ALKALINE EARTH AND LANTHANIDE IONS.

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The recent success of the molecular mechanics/dynamics approach to the understanding of the structure, energetics and dynamics of proteins has prompted us to apply these techniques to metallobiomolecules. Non-bonded parameters have been developed for ions whose bonding is largely electrostatic. Our approach is to use the CHARMM program to stimulate (with full 3-dimensional periodicity) x-ray structures of complexes involving biologically relevant ligands and to perform molecular dynamics calculations on ions in solution with explicit TIP3P water molecules employing period boundary conditions or large droplets. Parameters are accepted or rejected on the basis of agreement between experimentally observed and calculated structural and thermodynamic properties. Applications of the results to calcium-binding proteins, eg. calmodulin, will be presented and implications regarding the use of lanthanide ions as surrogate probes for Ca^{2+} will be discussed. This research was supported in part by the NIH (GM23599).

M-Pos175

DISORDER IN LYSOZYME CRYSTALS. J.B. Clarage, M.S. Clarage, D.L.D. Caspar. Rosenstiel Basic Medical Sciences Research Center and Department of Physics, Brandeis University, Waltham, MA 02254.

Diffuse X-ray scattering in the halos about a crystal's Bragg reflections is determined by the correlations in atomic movements. We have simulated the diffuse intensity distribution for insulin and lysozyme in terms of exponential displacement correlation functions. In tetragonal lysozyme crystals the mean square atomic displacements are about twice that in the more tightly packed triclinic lattice, but the correlations in displacement are nevertheless very similar. About 90% of the disorder can be accounted for by internal movements correlated with a decay distance of about 6 Å; the remaining 10% corresponds to intermolecular movements that decay in a distance the order of size of the protein molecule. Displacement correlations do not decay inversely with distance as in an elastic solid, but rather exponentially, just as positional correlations in a liquid. This liquid-like disorder is similar to the disorder we have observed in two-dimensional colloidal crystals of polystyrene latex spheres, where repulsive interactions dominate.

M-Pos176

AVERAGE WATER STRUCTURE IN CUBIC INSULIN CRYSTALS. J. Badger and D.L.D. Caspar. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254.

A range of phenomena demonstrate the importance of solvent-protein interactions in biological processes, but little is known about the distribution of water molecules around proteins. Cubic insulin crystals provide a suitable experimental system for obtaining this information since they contain large solvent channels. The available X-ray diffraction data extend to 1.7 Å resolution and the protein model has been refined to R=0.20. By applying novel methods for electron density map refinement, analysis and graphical display, we have mapped the average electron density distribution in the solvent. This map shows that water molecules throughout the crystal (up to five water layers from the protein surface) are significantly more ordered than in pure liquid water. The solvent distribution appears to be representable by a small number of fluctuating water networks. Extended hydration around protein molecules could account for the "hydration force" that impedes molecular association and for anomalies in electrostatic interactions.

M-Pos177

THE STRUCTURE AND DYNAMICS OF PORCINE AND RAT INSULIN DIMERS.
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A molecular dynamics (MD) study has been carried out to evaluate structural fluctuations of porcine and rat insulin dimers *in vacuo* at 293K. The refined atomic coordinates of 2Zn porcine insulin (A conformation) at 0.15 nm resolution served as the initial structural model for MD simulations of porcine insulin and also as a basis for rat insulins after replacement of substituted amino acid residues. The MD averaged structures calculated from the final 50 ps portion of each trajectory showed averaged deviations of C^{α} atoms of 0.33 nm for the rat I-I homodimer and 0.15 and 0.165 nm for porcine and rat II-II insulin dimers, respectively. After 65 ps of MD simulations the rat I-I dimer exhibited large structural *hinge-bending* oscillations between monomeric units. This was not observed in the rat II-II dimer. The potential energy of the equilibrated rat insulin dimers at room temperature is about 250 kJ/mole higher than that of the porcine insulin dimer. The differences in potential energy of the different types of insulin dimers is being further investigated by the simulated annealing technique. (Supported by NIH #GM21400 and DK13914).

M-Pos178

CALCULATION OF PROTEIN CORRELATION TIMES FROM SOLVENT-ACCESSIBLE SURFACE AREA AND HYDRATION POTENTIALS
Arthur J. Weaver, Howard Hughes Medical Institute, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235-9050

A method for calculating protein correlation times (τ_m's) based on solvent-accessible surface area and hydration potentials (Wolfenden et al. 1981. *Biochemistry* 20: 849-855) is presented which successfully predicts τ_m's extracted from 13C NMR relaxation data using the model-free approach of Lipari and Szabo (1982. *J. Am. Chem. Soc.* 104: 4546-4559).

It has been shown that accurate determination of the side chain motional parameters (S,τ_e) by this method is dependent on τ_m values determined experimentally or by calculation (Weaver et al., *Biochemistry* in press). However, literature values for protein τ_m's show considerable variation. An independent determination of τ_m either experimentally or by calculation allows one to test the ability of the model-free approach to extract accurate τ_m's and, by implication, S and τ_e values for a given system.

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M-Pos179

DYNAMICS OF SHORT DNA DUPLEXES: AN EPR PROBE STUDY. Eric J. Hustedt, James J. Kirchner, Andreas Spaltenstein, Paul B. Hopkins, and Bruce H. Robinson, Department of Chemistry, University of Washington, Seattle, WA 98195.

We have recently developed a nitroxide spin label which is covalently attached to an analog of thymidine via an acetylene linkage. The spin labeled thymidine can be site specifically incorporated into DNA oligomers of a specific sequence using standard phosphoramidite chemistry. The acetylene linkage provides a spacing so that the probe does not interfere with the structure of the DNA, while limiting the independent motion of the probe.

Using this spin-probe, we have studied the dynamics of short DNA duplexes (up to 96 base pairs in length) as a function of temperature, viscosity, and duplex length. The EPR spectrum of a spin-labelled DNA duplex is sensitive to: 1) the global tumbling of the DNA duplex; 2) the length-dependent collective internal motions (bending and twisting) of the duplex; and 3) the independent motion of the spin-probe and/or the thymidine to which it is attached. Simulations of the EPR spectra of the spin-labelled DNA duplexes enable us to quantitatively determine the contribution to the lineshape from each of these three sources of motion. The simulations demonstrate that the global tumbling of the duplex can be predicted accurately in terms of the hydrodynamics of a rigid cylinder and that there is an additional length-dependent motion of the spin-probe which is due primarily to bending motions of the DNA duplex.

M-Pos180

ELECTROSTATIC SOLVATION FREE ENERGY : A COMPARISON OF CONTINUUM AND FREE ENERGY PERTURBATION METHODS

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Solvation is important to the stability and interactions in proteins and nucleic acids. Solvation energy can be obtained from free energy perturbation techniques (FEP) in which solute and solvent molecules are treated explicitly. Alternatively, the solvent can be described as a continuum, retaining the explicit solute representation. The solvation energy can then be obtained from finite difference solutions to the Poisson-Boltzmann equation (FDPB). A comparison of the FEP and FDPB methods for a dozen polar organic molecules shows good agreement, e.g., for MeOH the electrostatic component of solvation is -6.22(-6.88)kcal/mole, for acetamide -11.81(-10.77), for N,N-dimethyl-acetamide -6.5(-7.45). FEP results are in parentheses. Since the FDPB result entails only a thousandth of the computational cost of a FEP result, our results suggest that FDPB maybe a very efficient way of obtaining electrostatic solvation energies in proteins and nucleic acids.

M-Pos181

REFINEMENT OF LIPID BILAYER CRYSTAL STRUCTURES BY ENERGY MINIMIZATION. Garret Vanderkooi, Chemistry Department, Northern Illinois University, DeKalb, IL 60115.

Energy minimization calculations were carried out on the crystal structures of dilauryl phosphatidylethanolamine-acetic acid (DLPE-HA), palmitoyl lysophosphatidyl-ethanolamine (lysoPE), and dilauryl glycerol (DLG). The empirical energy was calculated as the sum of intramolecular and intermolecular terms. Analytical first derivatives of the energy were computed with respect to bond rotations, rigid body motions, and lattice constants. Minimization was carried out simultaneously with respect to all bond rotations (36 in DLPE-HA) and rigid body motions (12 in DLPE-HA), while maintaining the crystal symmetry and the experimental lattice constants. The DLPE-HA total energy decreased from -130 to -143 kcal/mol upon minimization, with a mean atomic displacement of 0.21Å. The lateral, head-head, and tail-tail contributions to the intermolecular energy were -71.2, -16.8, and -2.6 kcal/mol, resp. The head-head interbilayer electrostatic force is repulsive in DLPE if HA is omitted, but it is attractive if HA is present; for lysoPE, the head-head electrostatic force is attractive.

M-Pos182

DIELECTRIC CONSTANT OF PROTEIN CRYSTALS

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Dielectric constants are important constituents in calculating electrostatic properties of protein crystals. In general, an estimated interior low dielectric constant of ϵ_p=2-5, and a solvent high dielectric constant of ϵ_s=40-80 are currently used in the absence of experimental values. The dielectric substructure of protein crystals gives rise to interfacial polarization at the boundaries of protein and solvent. This manifests a dispersion with frequency of the overall capacitance and conductance of protein crystals. We will present measurements of the dispersion in capacitance and conductance to characterize the dielectric parameters of the substructure of myoglobin crystals. This method uses a four terminal high precision measuring system (a digital spectrometer) for the determinations of impedance and phase angle, at very low frequency.

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M-Pos183

A BIFURCATING ULTRAMETRIC DISTRIBUTED SYSTEM (BUDS) FOR SINGLE TRYPTOPHAN PROTEIN FLUORESCENCE

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Single tryptophan protein fluorescence displays a complex behavior, especially at low temperature. The fluorescence decay has been described by a continuous distribution of decay rates. The physical origin of the distribution has been attributed to conformational substates. Previous models have been useful in describing fluorescence decay over a wide temperature range. BUDS is proposed to explore the fluorescence behavior of single tryptophan residue proteins. BUDS introduces conformations (substates into the dynamics) through a hierarchy of conformational substates, and incorporates variations in protein structure through a distribution of hierarchical activation barriers. The key feature of BUDS is the non-exponential behavior of the fluorescence at low temperatures. BUDS is applied to both fluorescence lifetime and anisotropy of single tryptophan proteins. To get meaningful parameters from the model, fluorescence experiments of the protein are done over a wide temperature range. Both ligand bound and unbound proteins are investigated, as well as denatured forms of these proteins.

(Supported by PHS-P41-RR03155)

M-Pos184

CATALYSIS OF EUTECTIC CRYSTALLIZATION OF SUGARS BY LIQUID-LIQUID PHASE SEPARATION ON DEEPLY FROZEN POPULUS, Allen Hirsh, American Red Cross, Rockville, MD; Eric Erbe, USDA, Beltsville, MD; and Thomas Bent, COMSAT Corp., Clarksburg, MD.

Winter wood from Populus balsamifera v. virginiana, balsam poplar, survives cooling to liquid nitrogen at rates <5°C/hr. Analysis of the wood by differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) shows that liquid-liquid phase separations occur in intracellular fluids leading to sugar-rich domains with a high glass transition, approximately -30°C, and protein rich domains with a low glass transition, approximately -110°C. Cycling between 0°C and -70°C at 3°C/hr. increased injury progressively until total mortality was seen after 6 cycles. DMA analysis showed that this was concurrent with precipitation out of solution of the protective intracellular sugars. Cycling 6X at 3°C/hr between 0°C and -20°C or -20°C and -70°C was not injurious. These results lead us to postulate that an unusual seeding of the eutectic phase of the intracellular sugars takes place at very low temperatures in the sugar-dilute, protein rich phase of the intracellular milieu.

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M-Pos185

GLOBAL ANALYSIS OF DISTANCE DISTRIBUTION DATA FOR DONOR-ACCEPTOR PAIRS WITH DIFFERENT FÖRSTER DISTANCES.

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Global analyses of frequency-domain and steady-state fluorescence energy transfer measurements on D-A pairs with different Förster distances (R_o) were performed to yield increased resolution in the distance distribution data. Eight compounds were synthesized with R_os ranging from 6-32 Å. Each compound contained a tryptamine donor connected by a ten carbon alkyl chain to one of thirteen different acceptors. Both frequency-domain and steady-state energy transfer measurements were performed on these compounds. These results were compared with ene-to-end distance distributions which were predicted using a rotational isomer model and molecular dynamics computer simulations. Measurement of these compounds by steady-state and time-dependent methods should give additional information about the shape of the distance distribution. In addition, if the same distance distributions are recovered from these molecules, which have different acceptors and thus different transition dipole moment directions, then one can conclude that a κ^2 value of 2/3 is a good approximation for R_o calculations.

M-Pos186

DIFFUSE SCATTERING AND MOLECULAR MOTIONS OF TROPOMYOSIN

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Diffuse X-ray scattering is linked to the inter- and intra-molecular motions of tropomyosin within crystals. Diffuse scattering data along some of the major directions of the previously solved Bailey crystal form of tropomyosin have been measured. Various models for the three-dimensional motions of tropomyosin in the crystal have been tested by comparing their predicted diffuse scattering patterns with the experimental data. Thus the probable modes of motion have been determined. Work is in progress to study the perturbations in the diffuse scattering caused by the presence of troponin in these crystals. Structure determination and diffuse scattering studies are also being conducted on a more densely packed spermine-induced crystal form of tropomyosin.

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M-Pos187

LOW ENERGY SOLUTION CONFORMATIONS OF PIRENZEPINE DERIVATIVES.

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Research on effective pharmaceuticals for the treatment of Alzheimer's disease has recently focused on the muscarinic activation of the central nervous system. Specifically, selective post-synaptic M1 and presynaptic M2 receptor binding compounds are being studied. These two receptor types are best defined by pirenzepine and AF-DX 116, respectively. As experimental binding data for these derivatives becomes available, these results will be applied in determining structure-activity relationships.

This study consists of calculating low energy solution conformations of a series of sterically constrained pirenzepine derivatives formed by the systematic unsaturation of the pyradine ring. The method used is simulated annealing with the waters explicitly included. Calculations are performed using the program AMBER. The changes in the relative position of the ring nitrogen as a function of degree of unsaturation is examined.

M-Pos188

DYNAMIC LONG-RANGE ORDER IN HETEROGENEOUS BIOLOGICAL SYSTEMS: BOVINE MILK.

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Milk is a complex liquid secreted by epithelial cells of mammary tissue. It contains two unique supramolecular aggregates, casein micelles and plasma membrane-bounded lipid droplets suspended in a soluble phase, containing whey proteins, lactose, and ions. While the properties of the micelles and lipid have been studied independently, little attention has been directed at their kinetic interactions. Video-enhanced optical phase contrast microscopy of thin films of milk resolves casein micelles in rapid motion interspersed with the lipid droplets. Spatially filtered images reveal that the distribution of micelles is not uniform; micelles are clustered into aggregates with a persistence of 15-20 secs and fractal dimensions of 1.3-1.7. The aggregates have a uniform long-range interspace of 10-15 micrometers. Skim milk and purified casein micelles are also organized into these fractal aggregates in patterns with similar long-range order, suggesting that the lipid droplets do not play an essential structural role. These methods may be useful for analyzing dynamic weak interactions in heterogeneous biological media.

M-Pos189

PREDICTED THREE DIMENSIONAL STRUCTURES FOR NON-CRYSTALLINE PROTEINS

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Three very different classes of non-crystalline proteins are the collagen family, the milk caseins, and the serum apolipoproteins. 3D structures were constructed using the Sybyl-Mendyl molecular modeling programs. To minimize the probability of obtaining a spurious structure, initial structures incorporated the results of sequence based predictions and spectroscopically obtained estimates of secondary structures. All structures were refined with the Kollman Force Field Energy Minimization calculation. Van der Waals and electrostatic potential maps were generated from the refined structures, as well as geometric details including inter-residue distances and H-bond locations. Molecular dynamics calculations based on the refined structures showed relative mobilities of specific residues or chains. All structure showed agreement with global physical and biochemical information concerning the proteins.

M-Pos190

MOLECULAR DYNAMICS SIMULATIONS OF PEA LECTIN

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Once a three dimensional model of a protein is available from experimental techniques such as X-ray crystallography, this model may serve as the starting point of a theoretical investigation of that protein using the computational intensive technique of molecular dynamics (MD). This method involves solving the equations of motion with respect to time for each atom in the protein and generates a trajectory of the protein. One then has information about the kinds and amplitudes of motions available to that protein. The protein pea lectin, a dimer, has been refined in our laboratory to 1.7 Å resolution. A lectin is a protein which is characterized by its ability to agglutinate cells and precipitate complex carbohydrates . Lectins have been isolated from a wide variety of sources, but the best characterized lectins are those isolated from plant seeds. Pea lectin is isolated from the common garden pea, *Pisum sativum*. MD simulations are currently being performed on the protein pea lectin under various conditions: in vacuo (without any solvent molecules explicitly present), with a 4-layer shell of water molecules, and ultimately in a box of water using periodic boundary conditions.

M-Pos191

SIMULATION OF MACROMOLECULAR INTERACTIONS DURING SIZE-EXCLUSION CHROMATOGRAPHY: POSITIVE COOPERATIVITY CONTRIBUTION BY GEL MATRIX. F. J. Stevens, Biological and Medical Research Division, Argonne National Laboratory, Argonne IL 60439.

The interactions of ternary combinations of antibody and antigen during size-exclusion chromatography are relevant in competition experiments to determine relative epitope specificity and interaction kinetics. Peptide-mediated cross-linkage of antibodies in polyclonal antisera represents another ternary interaction that may be observed chromatographically. In the presence of the porous sequestering environment of a gel matrix, otherwise chemically and sterically independent interactions exhibit a characteristic of positive cooperativity; the formation of one antibody:epitope bond is dependent on the concentration of the second antibody. The formation of one complex decreases the solvent volume accessible to antigen and results in the presentation of the second epitope to the remaining antibody. Computer simulation is used to examine this phenomenon, which may have physiological analogs. This work supported by contract W-31-109-ENG-38 from the Office of Health and Environmental Research.

M-Pos192

MULTI-SCALE ISING MODEL FOR PROTEIN DENATURATION. **Peter Leopold (Intro. by Mauricio Montal), Lab. of Bio. Dyn. & Theo. Med. and Dept. of Phys., B-019, UCSD, La Jolla, CA 92093.**

In order to relate protein denaturation and the underlying geometry of secondary structure interactions, we have numerically investigated an Ising-like model of protein secondary bonds[1]. A key feature of the model is its reliance on X-ray crystal coordinates for defining the elemental hydrogen, cystine and salt bonds and hydrophobic contacts and their nearest neighbors. The free energy Hamiltonian $H(T) = -\Sigma(\varepsilon_i(T) - \alpha T)b_i - J\Sigma b_i b_j$ is numerically simulated for hydrated lysozyme and used to calculate the fractional folding <b> (Fig. A). Here, αT and $\varepsilon_i(T)$ are the entropic and enthalpic energies per broken bond b_i (where $b_i = \pm 1$), and J is the nearest-neighbor coupling constant. We then recast the problem in terms of an ordered, inhomogeneous Ising system. The nearest-neighbor bond distribution is best fit by a long-tailed Levy distribution (Fig. B) with exponent $\delta = 0.8$. Thus lysozyme is shown to possess a multi-scale secondary bond geometry that adequately exhibits denaturation and invites substructural fluctuation studies.

[1]Ikegami, A., *et.al.*, *Biophys.Chem.* 6:117–149(1977).