M-AM-Sym I-1

MORPHOLOGICAL PERSPECTIVES OF THE TRIAD JUNCTION. Clara Franzini-Armstrong, Dept. of Anatomy, U. of Pennsylvania, Philadelphia, PA.

Depolarization of T tubules is translated into a signal for calcium release from SR at triads. Structure gives specific clues to the mechanism of this transmission. The SR feet, composed of four identical subunits, are cytoplasmic domains of channels responsible for calcium release from the SR. Complete SR Ca release channels, including hydrophobic domains, are ryanodine receptors. Ryanodine blocks them in a partially open configuration. A second junctional protein occupies the T tubule membrane, in groups of four (jT tetrads). This protein is not directly identified, but it may be a dihydropyridine receptor. Feet span the entire T-SR gap in a location coinciding with that of jT tetrads. Thus a direct link may be provided between components of the two membranes, constituting the basis for a direct molecular interaction as proposed by Schneider and Chandler. Presumably the feet are the portion of the foot protein responsible for translating the T tubule signal into gating of release channels.

M-AM-Sym I-2

THE DIHYDROPYRIDINE SENSITIVE CALCIUM CHANNEL/VOLTAGE SENSOR IN SKELETAL MUSCLE. Hartmut Glossmann, Hans-Guenther Knaus, Joerg Striessnig and Fritz Scheffauer. Institute for Biochemical Pharmacology, Innsbruck, Austria.

The alpha$_1$-subunit of the channel contains allosteric interacting receptor domains for chemically distinct classes of drugs, i.e., for 1,4 dihydropyridines, phenylalkylamines, benzothiazepines, diphenylbutylpiperidines, piperazinylindoles, benzothiazinones as well as for naturally occurring ligands. These domains are already present on the skeletal muscle alpha$_1$ subunit of *cyprinus carpio* which we have purified, cloned and sequenced (Grabner et al., Naunyn-Schmiedeberg's Arch., in press). In contrast to the isolated rabbit or guinea pig alpha$_1$ which can be rapidly phosphorylated *in vitro* by cAMP-dependent protein kinase, the carp alpha$_1$-subunit is not phosphorylated in digitonin buffer. The *cytrinus carpio* alpha$_1$ also lacks the mammalian skeletal muscle Ser-687 phosphorylation site and, when isolated from membranes, is significantly greater than its mammalian counterpart, indicating different posttranslational proteolytic processing in fish vs mammals. By using photoaffinity labels and antibodies raised against photoaffinity ligands, we analyze the topology of the receptor domains on the mammalian alpha$_1$ (Striessnig et al., J. Biol. Chem., in press). For a new class of high-affinity ligands, which discriminate subtypes (i.e., between tissue-specific alpha$_1$ subunits), we could identify the binding domain(s) on the primary alpha$_1$ amino acid sequence. (Supported by FWF S 45/01, S 45/02, Dr. Legerlotz Foundation and Osterreichische Nationalbank.)

M-AM-Sym I-3

CALCIUM RELEASE MACHINERY OF SARCOPLASMIC RETICULUM (SR) FROM SKELETAL MUSCLE, HEART AND SMOOTH MUSCLE. Sidney Fleischer, Makoto Inui, Christopher C. Chadwick and Akitsugu Saito, Dept. Mol. Biol., Vanderbilt Univ., Nashville, TN; Lin Hymel and Hansgeorg Schindler, Dept. Biophys., Univ. of Linz, Austria; Andrew Marks and Bernardo Nadal-Ginard, Children's Hospital, Harvard Univ., Boston, MA; Joseph Wall, Brookhaven Nat'l. Lab., Upton, NY; Terry Wagenknecht, Michael Radermacher, R. Grassucci and Joachim Frank, Wadsworth Ctr., Albany, NY.

The Ca^{2+} release channel (CRC)/ryanodine receptor (RyRec)/junctional channel complex (JCC) from skeletal muscle (SkM) and heart have been isolated and identified morphologically as the foot structures in the terminal cisternae (TC) of SR and functionally as the CRC by reconstitution into planar bilayers. The size, determined by STEM, is 2.3 million. The JCC is a tetramer with four-fold symmetry. The three-dimensional structure of the JCC was determined using electron image reconstruction. The structure indicates the Ca^{2+} efflux pathway from TC of SR to the myoplasm. Recently, we have isolated the IP_3 receptor (IP_3Rec) from smooth muscle. It consists of an oligomer of an R_s 220 KD polypeptide. The structure of IP_3Rec by EM and its sedimentation behavior indicate a comparable size to the RyRec. Northern Blot coupled with S1 nuclease analysis using a cDNA probe from SkM encoding a portion of the RyRec shows a highly conserved region in the receptors from the three types of muscle. The isolated RyRec and IP_3Rec provide a molecular framework to study excitation-contraction coupling and stimulus-secretion coupling. (NIH DK 14632, HL 32711 and MDA.)

M-AM-Sym I-4

STUDIES OF THE E-C COUPLING MECHANISM IN INTACT SKELETAL MUSCLE FIBERS. S.M. Baylor, S. Hollingworth, M. Konishi, and P.C. Pape. Dept. of Physiology, Univ. of Pennsylvania.

Frog single twitch fibers, stretched to long sarcomere length and micro-injected with various substances, were studied on an optical bench apparatus. Changes in fiber optical and electrical properties were monitored in response to action potential stimulation in order to test hypotheses about the E-C coupling mechanism. In fibers injected with μmolar free ruthenium red, Ca^{2+} release from the sarcoplasmic reticulum (SR) was locally suppressed, in support of the conclusion from biochemical experiments that the physiological pathway for SR Ca^{2+} release is the ryanodine receptor/foot protein/Ca^{2+} channel. Low molecular weight heparin, at total concentrations in myoplasm as high as 4 mg/ml, had no effect on Ca^{2+} release, thus arguing against the hypothesis that $InsP_3$ serves as an essential chemical messenger for release. A similar, negative conclusion for an essential messenger role for Ca^{2+} or H^+ was also reached from experiments on fibers injected with fura-2 and phenol red, respectively. In light of these and other findings, evidence related to the 'mechanical' model for E-C coupling will be reviewed and discussed.

M-AM-Sym I-5

EXCITATION-CONTRACTION (EC) COUPLING IN SMOOTH MUSCLE. Andrew P. Somlyo and Avril V. Somlyo, University of Virginia.

Pharmacomechanical coupling plays a major role in EC coupling in smooth muscle. The pharmacomechanical release of Ca^{2+} from the SR by inositol 1,4,5 trisphosphate ($InsP_3$)] is mediated through a G-protein coupled phosphotidylinositol cascade. Ca^{2+}-release by agonists can be inhibited by the phospholipase C inhibitor, neomycin, while heparin blocks specifically both the $InsP_3$-sensitive channels of the SR and pharmacomechanical Ca^{2+} release. The kinetics of $InsP_3$-induced Ca^{2+} release are also compatible with its physiological role: following photo release of $InsP_3$ Ca^{2+}-channel opening occurs in the SR with a minimum delay of 30 ms (22°C) or less. Sensitization/desensitization of the regulatory/contractile apparatus to Ca^{2+} is another major component of pharmacomechanical coupling. Sensitization is mediated by G-proteins and is reflected in the agonist-dependent variability of the force/Ca^{2+} ratio. The different maximal contractions evoked by different agonists are related to differences in both $[Ca^{2+}]_i$ and modulation of Ca^{2+}-sensitivity. Supported by HL15835 to the Pennsylvania Muscle Institute.

M-AM-Sym II-1

STABILITY MUTANTS OF STAPHYLOCOCCAL NUCLEASE, **David Shortle, Dept. of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.**

The role of each of the 149 amino acid residues of staphylococcal nuclease in determining its structure and stability is being studied systematically by genetic methods. In the first phase of this analysis, each of the 40 large hydrophobic residues has been substituted with both alanine and glycine, and the resulting effects on the guanidine hydrochloride denaturation behavior have been quantitated. Several interesting patterns in the measured values of ΔΔGapp and in d[ΔGapp]/d[GuHCl] will be discussed.

In addition to amino acid substitutions, single and double insertions of alanine and/or glycine are being used to probe the folding information encoded in nuclease. On average, single amino acid insertions reduce the stability of the native state by approximately the same amount as an amino acid substitution of one of the residues flanking the site of the insertion. At a majority of sites analyzed (some in alpha helices and beta strands), two contiguous inserted residues are no more destabilizing than one.

M-AM-Sym II-2

FOLDING INTERMEDIATES IN DIHYDROFOLATE REDUCTASE. C. R. Matthews, E. P. Garvey, P. Jennings, S. Saalau and K. Kuwajimi.

The solution of the protein folding problem can be framed in terms of the identification of the structures of intermediate, partially folded structures which appear along the pathway. Although many of these intermediates are only transiently stable, recent developments in protein engineering and spectroscopy make possible an examination of their structures and properties. A case in point is dihydrofolate reductase. A combination of stopped-flow circular dichroism (CD) and fluorescence (FL) spectroscopies reveals the presence of two early folding intermediates. The CD species arise first and indicates that a substantial amount of secondary structure forms within 18 msec. The FL species appears next and corresponds to the burial of a specific Trp sidechain in a solvent-inaccessible pocket. The final stage in folding involves a large scale conformational change which results in the development of a cooperatively-folded structure. Amino acid replacements provide useful probes of residues which play key roles in these reactions.

This work was supported by the National Science Foundation, grant DMB-8705673.

M-AM-Sym II-3

MUTATIONAL ANALYSIS OF THE BPTI FOLDING PATHWAY David P. Goldenberg, Department of Biology, University of Utah, Salt Lake City, UT 84112

In order to test the roles of individual residues in the mechanism of protein folding, the effects of amino acid replacements on the disulphide-coupled folding pathway of bovine pancreatic trypsin inhibitor (BPTI) are being examined. Site-directed mutations were designed to disrupt interactions identified in the native structure. The substitutions destabilize the native protein relative to the unfolded state, but replacements at different sites have different effects on the stabilities of the various disulfide-bonded intermediates. These observations lend support to models of the folding process in which different regions of the polypeptide chain acquire structure in a preferred order, and provide a means of defining the order in which the various residues participate in stabilizing interactions. The analysis of these mutants also indicates that the contributions of individual interactions become much more pronounced as the protein folds from the unfolded state to the native conformation, presumably because the cooperativity between individual interactions increases.

To complement the studies with site-directed mutations, a novel genetic screen has been used to isolate a collection of variants of BPTI that are inactivated more rapidly than the wild-type protein. The mutations isolated in this screen cause amino acid substitutions throughout the central core of the native protein. However, the effects of substitutions at different sites are clearly distinguishable, and initial studies with these mutants provide evidence for two sub-domains within the native structure of BPTI.

M-Am-Sym II-4

PEPTIDE MODELS AND THE FOLDING OF BPTI

T.G. Oas, J.P. Staley, D.Y. Kwon, E.M. Goodman, R. Rukowski and Peter S. Kim.
Whitehead Institute for Biomedical Research
and Department of Biology, M.I.T.
Cambridge, MA 02142.

We have been using disulfide-bonded peptide pairs to mimic early intermediates in the oxidative refolding of bovine pancreatic trypsin inhibitor (BPTI). CD and NMR indicate that these peptide models form stable folded structure in aqueous solution even though they are quite small (30-40 residues). Because these peptides are highly soluble, it is possible to study their structure using 2D-NMR. Preliminary NMR studies indicate that two of the peptide models have native-like secondary and tertiary structure. The similarity of the NMR spectra of the peptide models and those of partially disulfide-bonded intermediates of native BPTI leads us to conclude that substantial native structure forms very early in the folding of BPTI. We also conclude that this early native-like structure is capable of directing the remainder of the folding pathway, including those steps involving non-native disulfide bonds.

M-Am-Sym II-5

STRUCTURES OF DISULFIDE STABILIZED PEPTIDES STUDIED WITH NMR

David E. Wemmer, Dept of Chemistry, University of California and Chemical Biodynamics Division, Lawrence Berkeley Laboratory

The structures of several small disulfide stabilized peptides have been determined using NMR spectroscopy The stabilization provided by the disulfides has been used to prepare hybrid sequence peptides which are useful for examining structure / function relationships, and for characterizing propagation of an α-helix. Using this approach we have probed the stop signal of the S-peptide from RNase-A. The NMR analysis shows that there is a gradual change in both average structure and dynamics along the peptide sequence.

M-PM-Sym-1

MITOCHONDRIAL ATP SYNTHASE: STRUCTURE, NUCLEOTIDE DOMAINS, AND GENETIC MODIFICATION. **Pedersen, Peter L., Garboczi, D.N., Mario Amzel, Ysern, X., Departments of Biological Chemistry and Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.**

The mitochondrial ATP synthase complex is comprised of two major units, one called F_1 and one called F_0. The x-ray structure of rat liver F_1 has been completed to 3.8Å resolution. Details of this structure will be presented together with any more recent information. Both the rat liver α and β subunits have been cloned and sequenced. Near full length cDNAs of both subunits have been overexpressed in E. coli and the corresponding proteins purified and characterized. Both subunits have been shown to bind the fluorescent probes TNP-ATP and TNP-ADP. In the case of the β subunit, site directed, random, and deletion mutations have been made in an attempt to map out the nucleotide binding domain(s). These studies have been complemented with experiments conducted on synthetic peptides up to 50 amino acids in length. The results should have direct relevance to structural-functional relationships in ATPases, as well as to our understanding of the architecture of nucleotide binding domains in general.

M-PM-Sym-2

COMBINED IMAGE RECONSTRUCTION AND MONOCLONAL ANTIBODY ANALYSIS OF E. coli ATP SYNTHASE. Edward P. Gogol and Roderick A. Capaldi, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The F_1F_0 ATP synthase is a multisubunit enzymatic complex which interconverts the electrochemical energy of a transmembrane proton gradient and the chemical energy of ATP. Neither the coupling phenomenon nor the highly cooperative catalytic mechanism is understood. We are exploring both questions by combining low-resolution structural analysis with immunochemical and biochemical methods. The subunit arrangement has been partially determined by electron microscopy and image analysis, and subunit positions within the structure have been identified by use of monoclonal antibodies. We are now trying to correlate variability in subunit locations with biochemically-defined states of the enzyme.

M-PM-Sym-3

STRUCTURE AND EVOLUTION OF VACUOLAR ATPases, Emma Jean Bowman, B. J. Bowman, and Lincoln Taiz, Dept. of Biology, Univ. of California, Santa Cruz, CA 95064. Intro. by Peter L. Pedersen.

Vacuoles of plants and fungi, like acidic compartments of other eucaryotic cells, contain a proton-translocating ATPase in their membranes. Amino acid sequences deduced for the two major subunits have shown that V-type ATPases are highly conserved enzymes, which are closely related to archaebacterial ATPases and less closely related to F-type ATPases. Electron microscopy of negatively-stained vacuolar membranes reveals structures generally similar to, but in detail different from, ATPases of mitochondrial membranes. Comparison of gene structures for components of F- and V-type ATPases shows similarities among fungal genes and gross differences between plant and fungal genes, suggesting that conservation of gene structures does not correlate with conservation of amino acid sequences. Evolutionary analyses of ATPase sequences support the three kingdom hypothesis and suggest eucaryotes arose from an archaebacterial-like ancestor.

M-PM-Sym-4

THE CYTOCHROME o COMPLEX OF ESCHERICHIA COLI by Robert B. Gennis, Departments of Biochemistry and Chemistry, University of Illinois, Urbana, IL 61801

The cytochrome o complex is one of two ubiquinol oxidases in the aerobic respiratory chain of E. coli. The enzyme is a coupling site, resulting in the translocation of $2H^+$ per e^- across the cytoplasmic membrane. Recent biophysical studies and sequence data from the cyo operon clearly show that this enzyme is closely related to the aa_3-type cytochrome c oxidases. Whereas the cytochrome c oxidases contain two heme a moieties plus Cu, the cytochrome o complex contains two heme b prosthetic groups plus Cu. Three of the five open reading frames within the cyo operon encode proteins homologous to the mitochondrial encoded subunits of the eukaryotic cytochrome c oxidase. Subunit I (cyoB product) can bind heme b in the absence of the other subunits. Fourier transform infrared spectroscopy has been used to demonstrate the cytochrome o complex contains a heme-copper binuclear center, much like that characterized in the cytochrome c oxidases. Site-directed mutagenesis studies are being directed at determining the amino acids which serve as heme or copper ligands.

M-PM-Sym-5

MOLECULAR BIOLOGY OF ACTIVE TRANSPORT: MEMBRANE TO MOLECULE TO MECHANISM. H.R. KABACK, HHMI/UCLA, LOS ANGELES, CA 90024-1570, USA.

The *lac* permease of *E. coli* is a prototype secondary transport protein, catalyzing symport of a single β-galactoside with a single H^+. The permease is a hydrophobic transmembrane protein, encoded by the *lacY* gene, that has been solubilized, purified to homogeneity, reconstituted and shown to be completely functional as a monomer. Spectroscopic techniques demonstrate that purified permease is about 80% helical, and hydropathy profiling suggests a secondary structure in which the polypeptide consists of 12 hydrophobic segments in α-helical conformation that traverse the membrane in zigzag fashion connected by shorter, hydrophilic domains. Support for general aspects of the model has been obtained from proteolysis and chemical labeling and from immunological studies. Most recently, *lacY-phoA* fusion analyses have provided strong support for more specific topological predictions of the 12-helix model. Site-directed mutagenesis is being utilized to probe the structure and function of the permease. Application of the technique suggests that Arg302 (helix IX), His322 (helix X) and Glu325 (helix X) may be sufficiently close to form an H-bond network that is important for substrate bind-ing and lactose-coupled H^+ translocation.

M-PM-Sym-6

RECENT WORK ON THE MECHANISM OF PROTON TRANSLOCATION BY BACTERIORHODOPSIN. H.G. Khorana, M.P.Heyn, M.Holz, M.Lindau, T. Marti, T.Mogi, H.Otto, L.Stern, and S. Subramaniam, Mass. Inst. of Technology, Cambridge, MA 02139 and Freie Universitat, Berlin. A variety of bR mutants with single amino acid replacements have been prepared by site-specific mutagenesis, expression in E. coli and folding of the mutants with all-trans retinal. Studies of the mutants have shown that Asp-212, Asp-85, Asp-96, and Arg-82 are important in proton translocation. Replacements of Asp-85, Asp-212 and Arg-82 affect the early part of the photocycle and they are involved in the release of the proton from the protonated Schiff base while Asp-96 is required for reprotonation of the M-intermediate. In overall mechanism, therefore, bR does not embody a proton (wire) conductance mechanism; instead, it functions as an enzyme in which at the active site a proton is transferred to and then released from the Schiff base and the deprotonated carboxylic groups serve as a proton acceptor or donor. Supported by grants from the NIH and ONR (HGK), Swiss NSF (T.Marti), Damon Runyon-Walter Winchell Cancer Fund (SS) and Deutsche Forschungsgemeinschaft (HPH).

M-PM-A1

HIGH-RESOLUTION STRUCTURE OF THE LAMBDA REPRESSOR-OPERATOR COMPLEX: IMPLICATIONS FOR PROTEIN-DNA RECOGNITION AND GENE REGULATION.
Carl O. Pabo, Howard Hughes Medical Institute and Johns Hopkins University School of Medicine, Department of Molecular Biology and Genetics and Department of Biophysics, 725 N. Wolfe Street, Baltimore, Maryland 21205.

The structure of the lambda repressor-operator has been solved and refined at 2.5 Å resolution and this talk will consider the implications this structure has for protein-DNA recognition and gene regulation. Other recent results that will be discussed include:

1) genetic and structural studies of repressor's N-terminal arm and
2) structural studies of the arc repressor from bacteriophage P22.

M-PM-A2

RECOGNITION OF OPERATOR SEQUENCES BY THE REPRESSOR AND CRO PROTEINS OF BACTERIOPHAGE 434.
Stephen C. Harrison, Howard Hughes Medical Institute and Harvard University, Department of Biochemistry and Molecular Biology, 7 Divinity Avenue, Cambridge, Massachusetts 02138.

High-resolution structures of a number of complexes between synthetic DNA operators and the repressor and Cro proteins of bacteriophage 434 reveal how these two proteins recognize the same sets of sites, but with different rank orders of affinity.

M-PM-A3

THE STRUCTURE OF THE ANTP HOMEODOMAIN FROM DROSOPHILA-COMPARISON WITH PROCARYOTIC REPRESSOR PROTEINS.
K. Wuthrich, Y.-Q. Qian G. Otting, M. Billeter, M. Muller* and W. Gehring*, Institut fur Molekularbiologe and Biophysik, ETH-Honggerberg, CH-8093 Zurich and *Biozentrum der Universitat Basel, Abt. Zellbiologie, Klingelbergstr. 70, CH-4056 Basel.

The three dimensional structure of the Antennapedia homeodomain of Drosophila was determined in aqueous solution by ^1H nuclear magnetic resonance (NMR) spectroscopy. A group of 19 conformers characterizes a well defined structure for the residues 7-59 of the homeodomain with an average root mean square distance for the backbone atoms of 0.6 Å relative to the mean of the 19 structures. The structure comprises a helix from residues 10-21, a helix-turn-helix motif similar to those found in procaryotic repressor proteins from residues 28-52, and a somewhat flexible fourth helix from residues 53-59, which essentially forms an extension of the presumed recognition helix 42-52. The helices enclose a structurally well defined molecular core of hydrophobic amino acid side chains. Comparisons with the three-dimensional structures of procaryotic repressor proteins showed that in spite of the near-identity of the helix-turn-helix motifs, the global structures of the individual proteins represent different molecular architectures.

M-PM-A4

THREE-DIMENSIONAL SOLUTION STRUCTURE OF A SINGLE ZINC FINGER DNA-BINDING DOMAIN.
P.E. Wright, M. Lee, G. Gippert, K. Soman, D. Case, Dept. of Molec. Biol., Res. Inst. of Scripps Clinic, La Jolla, CA 92037.

The three-dimensional solution structure of a zinc finger nucleic acid binding motif has been determined by NMR spectroscopy. Spectra of a synthetic peptide corresponding to a single zinc finger from the *Xenopus* protein *Xfin* yielded distance and dihedral angle constraints that were used to generate structures from distance geometry and restrained molecular dynamics calculations. The zinc finger is an independently folded domain with a compact globular structure in which the zinc atom is bound by two Cys and two His ligands. The polypeptide backbone fold consists of a well-defined helix, starting as α and ending as 3_{10} helix, packed against two β strands that are arranged in a hairpin structure. There are many basic and polar amino acid side chains on the exposed face of the helix that are probably involved in DNA binding. In the presence of zinc, the peptide binds non-specifically to DNA. The zinc plays an essential role in folding the finger motif; in the absence of zinc the peptide is largely unstructured and does not bind to DNA.

M-PM-A5

AN HIV ZINC FINGER: STRUCTURE AND NUCLEIC ACID BINDING STUDIES. Michael F. Summers, Dennis R. Hare, Terri L. South, Chemistry Department, UMBC, Baltimore, MD 21228, USA; and Hare Research, (D.H.), 14810 216th Ave., N.E., Woodinville, WA 98072, USA.

NMR spectroscopic results are reported for 1:1 Zn^{+2} and ^{113}Cd adducts with an 18-residue synthetic peptide containing the amino acid sequence of the first finger-like domain from the gag protein p55 of HIV. The peptide forms highly stable, stoichiometric adducts with Zn^{+2} and Cd^{+2}. The 3Cys, 1His metal binding mode was determined unambiguously for the ^{113}Cd adduct (^{113}Cd(p55F1)). The structure of the zinc adduct, determined using a new NMR-based distance geometry approach, exhibits a novel folding pattern with internal hydrogen bonding. The folding of residues C(1)-K(6) is identical to the folding observed in the iron domain of rubredoxin. Conservatively substituted hydrophobic residues comprise a potential nucleic acid interaction (recognition) site. Preliminary studies with oligodeoxyribonucleotides indicate that these residues indeed interact with specific nucleic acid residues in vitro.

M-PM-B1

Modelling Disorder in Protein Crystals By Simulated Annealing

John Kuriyan, Laboratory of Molecular Biophysics, The Rockefeller University, New York, NY. 10021

The success of the simulated annealing (restrained molecular dynamics) method in speeding up the refinement process leaves two major remaining problems. One is the estimation of the errors in protein structures and the other problem is the treatment of dynamics and disorder in X-ray refinement. Results of the application of simulated annealing to evaluating the results of high-resolution refinement, and searching for conformational disorder, will be discussed. A 300 ps simulation of crambin in the full crystal environment has been carried out. The results of the simulation are being used to develop simplified models for the refinement of thermal parameters.

M-PM-B2

REFINEMENT OF PROTEIN STRUCTURES BY SIMULATED ANNEALING. S. Almo, P. Bash*, A. Danishefsky, R. Davenport, E. Lolis, G. Petsko, B. Stoddard and Dagmar Ringe, Chem. Dept., MIT, Cambridge, MA & *Chem. Dept., Harvard Univ., Cambridge, MA

Brunger, Kuriyan and Karplus (Science 235, 458-460, 1987) introduced a new approach to protein structure refinement in which an X-ray pseudo-energy term is added to the empirical potential energy function used in molecular dynamics calculations. A protocol derived from simulated annealing is then used to drive the structure towards the best fit to the observed diffraction data. We have applied this method to X-ray structures of aspartate aminotransferase, iron superoxide dismutase, and triosephosphate isomerase. The method is fast, robust, and has a large radius of convergence (over 6A). Refinement from starting R-values over 40% to values below 20% is possible in a single annealing run without manual intervention. When the method is unable to correct a serious error in the atomic model, it gets the rest of the structure sufficiently correct that difference Fourier techniques, used in the proper way with unbiased coefficients, reveal the correct interpretation.

M-PM-B3

USE OF MOLECULAR DYNAMICS TO AID THE INTERPRETATION OF NOE DATA.

Carol Beth Post, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

Nuclear Overhauser effect (NOE) data on macromolecules are measured to obtain structural information in the form of interproton distances. Such information can be used with molecular dynamics or distance geometry to arrive at a three-dimensional structure consistent with the NOE data. The evaluation of distances from NOE data is carried out in most cases with the assumption of a single rotational correlation time, that is, with the assumption that the macromolecule is a rigid molecule without significant internal motion. There are, of course, internal vibrations. However, the extent to which either these high frequency, low amplitude motions, or transitions between conformational states, such as ones distinguished by dihedral angles, actually influence the observed NOE signal is not well determined from NOE data alone. One approach to assess the significance of internal motions on NMR relaxation rates is molecular dynamics. Using the ensemble of structures generated for the dynamics trajectory, it is possible to calculate the resulting effects on cross-relaxation rates of fast timescale vibrational motions or of multiple conformations with characteristic interproton distances. The results of such calculations will be presented.

M-PM-B4

Structure Determination from 2-and 3-dimensional NMR data by Dynamical Simulated Annealing

ANGELA M. GRONENBORN & G. MARIUS CLORE (National Institutes of Health, Bethesda, MD 20892)

Nuclear Magnetic Resonance (NMR) spectroscopy has evolved over the last decade into a powerful method for determining three dimensional structures of biological macromolecules in solution. Key advances have been the introduction of two and more recently three dimensional experiments, high field superconducting magnets and computational procedures for converting the NMR derived interproton distances and torsion angles into three-dimensional structures. The lecture will outline the methodology employed, describe the major NMR experiments necessary for the spectral analysis of proteins and illustrate the computational approaches used to date. The present state of the art will be demonstrated using a variety of examples and future developments will be indicated.

M-PM-B5

SUCCINCT MODELS FOR VERY LARGE NUCLEIC ACIDS, WITH APPLICATION TO SUPERCOILED DNA AND TO THE STRUCTURE OF THE RIBOSOME

Stephen C. Harvey, Robert K.Z. Tan and Arun Malhotra, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294

In order to model the structures of nucleic acids in the size range 100 - 10,000 nucleotides using the molecular mechanics approach, it is necessary to reduce the level of detail. While traditional methods treat every atom in the molecule, our succinct models use psudoatoms whose characteristics mimic the relevant physical properties we wish to study. The succinct model for DNA supercoiling describes the molecule down to the level of individual basepairs by representing each basepair as a plane composed of three pseudoatoms. The pseudobonds (springs) connecting each basepair have characteristics that give the model the same elastic properties as double stranded DNA. The structure of the ribosome is investigated using succinct models for the rRNA with one pseudoatom per nucleotide, like beads on a string. Models of the ribosome are built by the computer, which folds the RNA through the scaffold of ribosomal proteins, finding configurations that satisfy distance constraints from the secondary structure and from RNA-RNA and RNA-protein crosslinking experiments.

M-PM-B6

Structural and Dielectric Relaxation in the Photosynthetic Reaction Center of *Rhodopseudomonas viridis*

Klaus Schulten, Department of Physics, U. of Illinois, Urbana, IL 61801

We investigate the electrostatic and dynamic control of electron transfer processes SP $\rightarrow$ BPL $\rightarrow$ $Q_A \rightarrow Q_B$ in the photosynthetic reaction center of *Rh. v.* Electron transfer was modelled by altering chromophore charge distributions. Electrostatic contributions to the redox potentials due to all partial atomic charges, the inhomogeneous dielectric environment, bound ions near the protein surface and mobile ions were accounted for. Structural and dynamical properties at temperatures $T = 300^o, 200^o, 80^o, 10^o$ K have been examined including over 5 000 (Cray 2) and all 12 637 (Transputer-based parallel computer, all Coulomb forces) atoms. Fast (150 fs) protein relaxation after electron transfer has been found, the protein response resembling that of a polar fluid.

M-PM-B7

CALCULATING ELECTROSTATIC FORCES IN MOLECULAR DYNAMICS USING THE FINITE-DIFFERENCE POISSON-BOLTZMANN METHOD

Kim Sharp, Dept. of Biochemistry, Columbia University, 630 W168th St, NY NY 10032

A new way of calculating electrostatic forces in molecular dynamics (MD) that includes solvent and ion screening has been developed. It used finite difference solutions to the Poisson-Boltzmann equation (FDPB). This technique has proven to be a rapid and accurate way of calculating electrostatic effects, particularly those due to solvent and ionic strength, in a variety of protein and nucleic acid problems. The FDPB method is used to calculate the reaction field from the solvent and mobile ions during a MD simulation, avoiding the computational expense and uncertainty of trying to simulate the dielectric properties of the solvent using explicit ions and water molecules. The reaction field contribution to the forces is added to the conventional intramolecular Coulombic terms calculated with a dielectric of 1 (using the MD package XPLOR). For the ALA-dipeptide solvent screening shifts the conformational preference from the beta towards the alpha region of phi-psi space. In Crambin better fits to the crystal structure are obtained for charged amino-acid side chains.

M-PM-B8

EXPERIMENTAL VERIFICATION OF FLUORESCENCE ANISOTROPY DECAYS PREDICTED BY MOLECULAR DYNAMICS SIMULATIONS.

Axelsen, P.H., *Gratton, E., and F.G.Prendergast. Dept. of Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, MN, 55905; *Dept. of Physics, University of Illinois, Urbana, Il, 61801.

Molecular dynamics simulations predict that the binding of 2'GMP to ribonuclease-T1 causes an accelerated and more extensive anisotropy decay of TRP-59 relative to the free enzyme. Differential polarized phase fluorometry measurements were performed to confirm this prediction. Using modulation frequencies in excess of 3 GHz, rotational activity not attributable to rotation of the whole protein may be detected and is found to confirm the simulation results. Further analysis of the simulations show that 2'GMP increases the rotational activity of TRP-59 by facilitating collective motions of the protein matrix adjacent to TRP-59. This suggests that TRP-59 anisotropy measurements report both unhindered side chain rotations as well as large scale motions of the protein matrix.

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M-PM-C1

DOES ENDOCARDIAL ENDOTHELIUM MEDIATE FLOW-INDUCED AUTOREGULATION OF MYOCARDIAL RELAXATION?

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The endocardial endothelium (EE) modulates performance of subjacent myocardium (Brutsaert et al., Nov. 1986, Arch. Int. Physiol. 95:4, 1987). We have now investigated whether EE, similarly to vascular endothelium, could also mediate shear stress induced by superfusing flow.Cat papillary muscles were suspended in a specially designed miniature-bath and superfused with Krebs-Ringer (35°C; Ca++ 1.25 mM) at flow rates between 1.3 (stop-flow) and 13 ml/min and strictly conditioned for temperature, pCO2, pO2 and pH. In muscles with intact EE, a small positive inotropic effect was noted under stop-flow conditions; total isometric peak twitch tension increased quickly and significantly ($p<0.05$) to 103.34 ± 1.44% ($n=7$) with concomitant significant prolongation of the twitch but no change in maximal unloaded velocity of shortening (Vmax). After selective damage of the EE (1s in 0.5% Triton X-100), stop-flow caused no significant alterations in contractile performance.

These observations under strict control of temperature, pCO2, pO2 and pH thus suggested a small, stop-flow induced, EE-mediated positive inotropic effect. Shear stress induced trans-EE electrochemical changes or release of myocardial relaxing or contracting factors from the EE (Brutsaert, Ann. Rev. Physiol. 51:263, 1989; Shah et al., Eur. H. J. 10:333, 1989) may be involved.

M-PM-C2

MODULATION OF MYOCARDIAL RELAXATION BY FACTORS RELEASED BY ENDOCARDIUM

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In isolated heart muscle preparations selective endocardial removal results in a characteristic reduction of contraction duration and peak isometric tension without altering unloaded shortening velocity, thus differing from other inotropic interventions (eg, cyclic AMP, calcium, pH, frequency or hypoxia). In endocardium-intact ferret papillary muscle preparations, 8-bromo cyclic GMP, sodium nitroprusside, atrial natriuretic factor and substance P each induced similar changes in contraction to endocardial removal and were each associated with significant increases in myocardial cyclic GMP levels; though endocardial removal itself had no effect on myocardial cyclic GMP content. The effect of substance P on both contraction and cyclic GMP levels was prevented by endocardial removal or the EDRF inhibitor haemoglobin. Superfused, cultured porcine endocardial cells were shown to release both a humoral agent with properties identical to those of EDRF, and a stable substance which in endocardium-denuded ferret papillary muscles, reversed the mechanical effects of endocardial removal. These endocardial factors may thus mediate the contraction-abbreviating effect of substance P and the contraction-prolonging tonic effect of endocardium.

M-PM-C3

REGULATION OF CARDIAC MUSCLE RELAXATION.

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Recent studies by Brutsaert et al. (Circ. Res. 62:358-366, 1988) suggest that removal of endocardial endothelium abbreviates relaxation when compared to fibers with intact endothelium. Ferret right ventricular papillary muscle (0.25 Hz, 30°C, $[Ca^{2+}]_o$ 4.5 mM) cells were microinjected with the Ca^{2+}-regulated photoprotein aequorin. Developed force increased by 15% in response to L-phenylephrine (PE) 10^{-7}M with (±)-bupranolol 10^{-7}M. After exposure for 30 min. to PE 10^{-5}M to selectively remove endothelium (Meulemans et al., Circulation 76:IV-540, 1987), and subsequent washout, developed force did not increase in response to PE 10^{-7}M. Following washout of PE 10^{-5}M, twitch amplitude decreased slightly, with no change in contraction velocity compared to control before PE. Relaxation of isometric and isotonic twitches occurred some 50 ms earlier than in comparable twitches before PE. Light signals were decreased, yet their time course was not shortened. This is consistent with the hypothesis that myofibrillar Ca^{2+} sensitivity is permanently modified by high concentration PE-induced removal of the endocardial endothelium.

Support: Mayo Foundation and GM 36365.

M-PM-C4

THE VOLTAGE DEPENDENCE OF INWARD Na-Ca EXCHANGE CURRENT (I_{Na-Ca}) MEASURED AT ELEVATED $[Ca^{2+}]_i$ IN GUINEA PIG VENTRICULAR CELLS.

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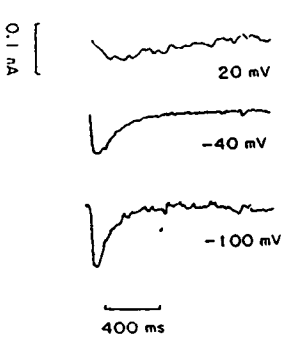
We used a rapid solution changer to isolate I_{Na-Ca}. Isolated myocytes were voltage clamped at -40mV (30° C) with suction micropipettes containing 145mM Cs, 0 Na, and 0 K (pH 7.1). Initially a cell was superfused in a stream of HEPES buffered solution pH 7.4 containing 0 Na, 145mM Li, 2.7mM Ca and 10mM caffeine (to prevent SR Ca sequestration). A 2 sec clamp pulse to 0mV caused a contracture. Relaxation and transient inward I_{Na-Ca} was induced 500 msecs after repolarization by rapidly moving an adjacent steam of solution containing 145mM Na (+10mM caffeine) over the cell. By activating I_{Na-Ca} at different potentials we were able to measure its voltage dependence. Outward currents were measured in the absence of Na_o and subtracted from current records obtained in the presence of Na_o. I_{Na-Ca} was the largest and decayed most rapidly at -100 mV (see Fig.). At +20mV peak I_{Na-Ca} was practically nulled and decayed very slowly (see Fig.). Slow transients presumably reflect the slow rate at which the exchange pumps down $[Ca^{2+}]_i$ at positive potentials. Extrapolation of the current voltage relationship indicates that in this example the current nulls at approximately +46mV. Assuming the null potential is also the reversal potential and further assuming a 3:1 exchange stoichiometry we infer that if $[Na+]_i \cong 8$ mM $[Ca^{2+}]_i$ in the vicinity of the exchanger was ≅2.8μM at peak I_{Na-Ca}.



M-PM-C5

REGULATION OF RESTING CALCIUM BY Na-Ca EXCHANGE IN GUINEA PIG CARDIAC MYOCYTES

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$[Ca^{2+}]_i$ was measured in enzymatically isolated guinea pig cardiac myocytes with Indo-1 under voltage clamp conditions. At rest, $[Ca^{2+}]_i$ of cells in 140 mM Na^+ and 2 mM Ca^+ was ≈100 nM. Depolarization to 0 mV resulted in an increase in $[Ca^{2+}]_i$ to ≈175 nM which rapidly declined to control levels ($t_{1/2}$ ≈1s). Under Na^+-free conditions resting $[Ca^{2+}]_i$ increased slowly and a similar depolarization resulted in a larger increase in $[Ca^{2+}]_i$ (≈250 nM) which very slowly declined ($t_{1/2}$ ≈10s) to a level considerably higher than control (≈225 nM). This increased level of resting $[Ca^{2+}]_i$ was only reversed when external Na^+ was replaced. Although the mechanism which causes the decline in $[Ca^{2+}]_i$ under Na^+-free conditions is unclear, it is likely that some (if not all) of the uptake is due to mitochondria as visual inspection revealed an increase in phase dense particles within the cell. These data illustrate that the activity of the Na-Ca exchange does contribute to resting calcium regulation within the mammalian cardiac myocyte and the contribution of a sarcolemmal Ca^{2+} ATPase may be small.

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M-PM-C6

MYOSIN LIGHT CHAIN KINASE PHOSPHORYLATION AND SMOOTH MUSCLE RELAXATION. J.T. Stull, Dept. of Physiol., UT Southwestern, Dallas, TX 75235

Ca^{2+}/calmodulin-dependent phosphorylation of myosin by myosin light chain kinase (MLCK) is a primary event for the initiation of smooth muscle contraction. Phosphorylation of purified MLCK in site A by protein kinases increases K_{CaM} (concentration of Ca^{2+}/CaM required for half maximal activation) and hence would decrease the sensitivity of MLCK to Ca^{2+} activation. Isoproterenol increases cAMP formation and relaxes tracheal smooth muscles contracted with carbachol. However, isoproterenol stimulates site A phosphorylation to a small extent (8%) and only at high concentrations. Thus, phosphorylation of MLCK is minimally involved in mediating smooth muscle relaxation and decreases in $[Ca^{2+}]_i$ are probably more important. Carbachol and KCl contract tracheal smooth muscle and stimulate more site A phosphorylation (50%) than isoproterenol. In contracting smooth muscle MLCK phosphorylation could play a role in desensitization of contractile elements to Ca^{2+}.

M-PM-C7

CALDESMON FUNCTIONS BY A MODIFIED STERIC BLOCKING MECHANISM L. Velaz, R.H. Ingraham, and J.M. Chalovich, East Carolina University School of Medicine, and Boehringer Ingerheim Pharmaceuticals, Inc. We have proposed that caldesmon inhibits the actin-activated ATPase activity of smooth muscle HMM by inhibiting the binding of the HMM•ATP complex to the productive site of actin (Hemric & Chalovich (1988) J. Biol. Chem. 263, 1868). This has been difficult to prove directly since caldesmon induces an inactive complex of actin and smooth HMM in which HMM is bound to caldesmon rather than to actin. We have eliminated the interaction between caldesmon and smooth HMM by digestion of caldesmon with chymotrypsin. This cleaved caldesmon inhibits the actin activated ATPase rate of smooth HMM and this inhibition is correlated with a decrease in the binding of HMM•ATP to actin. Therefore, caldesmon functions by inhibiting the binding of the myosin•ATP complex to actin regardless of the source of myosin. We have also isolated the myosin binding region of caldesmon and have performed a partial sequence. Comparison of this sequence with the derived sequence of caldesmon demonstrates, unequivocally, that the myosin binding region of caldesmon begins at the N-terminus and extends beyond the first cys residue.

M-PM-C8

ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF).

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The recent discovery of a factor(s) released from the endothelium referred to as EDRF has led to the demonstration of this as an important intercellular mediator controlling vascular smooth muscle tone. This biologically short-lived substance ($t_{1/2}$ ≈6 sec due to oxidative inactivation) not only relaxes vascular smooth muscle through activation of soluble guanylate cyclase but has also been shown to have inhibitory effects on platelet and white cell functions and to act as a neurotransmitter in the cerebellum. Evidence suggests that EDRF requires O_2 and Ca^{2+} for production and may in fact be composed of several distinct mediators generated in response to various vascular agonists. One of these may arise through the generation of nitric oxide from L-arginine mimicking the active moiety which produces the vascular relaxation of the nitrovasodilatory drugs. However additional studies demonstrate some functional and chemical features of EDRF which cannot be explained by nitric oxide alone suggesting that EDRF is either distinct from or may serve as a possible precursor for nitric oxide. The altered production of EDRF in various pathophysiologic conditions further highlights the importance of the active role of the endothelium in the vasculature.

M-PM-C9

RELAXATION OF VASCULAR SMOOTH MUSCLE BY ATRIAL NATRIURETIC FACTOR (ANF)

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ANF has a potent vasorelaxant activity. The relaxation occurs in arteries constricted by various vasoactive substances such as norepinephrine, angiotensin II, histamine and endothelin. Many target tissues are affected in versatile ways and ANF elicits copius amounts of cGMP production. To clarify the mechanism of ANF action, we studied ANF receptors. Affinity labeling experiments with ^{125}I-ANF revealed 135 kDa and 65 kDa receptors in various tissues. Vascular smooth muscles are rich in ANF receptors. Simultaneous purification of the two types of receptors from bovine adrenal revealed that the 135 kDa receptor has guanylate cyclase activity whereas 65 kDa receptor is considered to bind ANF less specifically and has clearance function. Both of these receptors are internalized and recycled. Thus, the intracellular messenger of ANF is considered to be cGMP. Hassid et al showed that ANF markedly reduces the baseline level of intracellular calcium concentration. This may be due to stimulation of calcium pump by cGMP dependent protein kinase.

M-PM-D1

ION CHANNEL FORMATION BY AMPHIPATHIC POLYPEPTIDES. EFFECTS OF SEQUENCE AND END GROUP MODIFICATIONS

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Hydropathy profiles of integral channel proteins seem to indicate that its partial sequences, which putatively span the bilayer core, consist of mainly hydrophobic amino acids and adopt helical structure. Experiments with natural and synthetic amphipathic polypeptides in planar bilayers reveal that most of the polypeptides only induce somewhat reproducible porous effects in the lipid system. However, a few exceptional polypeptides mainly of alamethicin-type structure exhibit low-noise stabilized conductance levels characteristic for biological ion channels. Data of open channel state stabilization in dependence on amino acid sequence and end group modifications of synthetic and alamethicin-type polypeptides is presented. Strong as well as weak voltage dependence in channel gating behavior is observed.

M-PM-D2

VOLTAGE-DEPENDENT GATING OF GRAMICIDIN CHANNELS INDUCED BY SINGLE AMINO ACID REPLACEMENT.

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Gramicidin single-channel current transitions result primarily from the reversible dimerization/dissociation of the channel-forming peptides, although a variety of subconductance states have been observed (Busath and Szabo, Biophys. J. 53:689, 1988). Here we show that gramicidin channels may exhibit rapid, voltage-dependent transitions between several well-defined conductance levels. Gramicidins in which Val^1 is replaced by a hexafluorine (F_6Val) form rectifying hybrid channels with [Val^1]gramicidin A (the conductance being higher in the F_6Val->Val direction). Using high-resolution (5 kHz) recordings we show that these hybrid channels exhibit two kinds of subconductance states. The fraction of time the channels are in the fully open state (f_0) varies as a function of potential: at 100 mV, f_0 for the low-conductance hybrid is ~0.8, while it is ~0.3 for the high-conductance hybrid; at 300 mV, f_0 is 0.98 and 0.05, respectively. The gating is presumably a result of field-induced alterations of the F_6Val dipole orientation, although this by itself will account for <10% of the observed gating charge, ~1/3 elementary charges.

M-PM-D3

GATING PHENOMENA IN COVALENTLY LINKED GRAMICIDIN CHANNELS

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Recently gramicidin A (GA) monomers were covalently coupled with linkers derived from SS- and RR-tartaric acid (Stankovic *et al.*, *Science*. 244:813-817. 1989). In contrast to the helix-maintaining SS-linker, the RR-isomer formed channels that exhibited a rapid flickering behavior. We hypothesised that the dioxolane ring of the linker could swing into the channel pore causing the flickers. Linkers having two additional methyl groups attached to the ring were expected to be too bulky to be accommodated inside the pore. Since these dimers did not exhibit this characteristic gating behavior, we conclude that the previously observed blockings must be tightly coupled to the specific structure of the linker ring and its match into the void between the two GA monomers.

In experiments designed to produce photo-gated ion channels, GAs were linked with 3,3'-asobis(phenylacetic acid). The aso group of this compound isomerises from *trans* to *cis* upon irradiation with light at 340 nm. Although it was expected based on computer simulations that this compound could not form unimolecular β-helices with the linker in the *trans* (dark) form, we found that the dark-adapted (24h at 45°C) form does form long-lived ion channels. The formation of intermolecular dimers is anticipated. Upon irradiation a new channel type is observed which disappears by dark-adapting the sample again. Thus these results represent the first example of an artificial ion channel which can be reversibly photo-modulated.

M-PM-D4

HOW CLOSING AN ION CHANNEL AFFECTS ITS VOLTAGE PROFILE. THE INFLUENCE OF GATES AND BLOCKERS.

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We present potential profiles along the axis of an aqueous pore due to externally applied voltages for a set of model channels as functions of channel structure and dielectric geometry. We contrast the voltage profiles in open and closed channels, specifically describe effects due both to external electrolyte and to permeant ions, and distinguish between behavior expected when the channel is closed by blockers, presumably polar, or by gates, presumably non-polar. We find important qualitative differences between ion selective channels and "sewer pipes." Large bore channels act as ideal conductors in which the total voltage drop occurs across regions of low dielectric constant. The voltage profile in narrow, ion specific, channels is strongly influenced by their relatively low dielectric surroundings (membrane and channel protein). Whether or not the channel is occupied by permeant ions, a significant fraction of the potential drop occurs in "conducting" regions. This effect is reduced, but not eliminated, if the pore contains electrolyte rather than only permeant ions of particular polarity.

M-PM-D5

PERMEANT ION EFFECTS ON GATING OF THE LARGE CONDUCTANCE Ca^{2+}-ACTIVATED K^+ CHANNEL FROM RAT SKELETAL MUSCLE. Susan D. Demo and Gary Yellen, Howard Hughes Med. Inst. and Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

We have studied the effects of permeant ions on the gating of the large conductance Ca^{2+}-activated K^+ channel from rat skeletal muscle. Rb^+ added to the external solution (20 mM) causes "block" of inward K^+ current, and also increases the probability that the channel is open (V_{MID} shifts -15 mV). Similar "block" by NH_4^+ (50 mM) *decreases* the open probability (V_{MID} shifts +15 mV). "Block" by Tl^+ (20 mM) does not affect the open probability. External Na^+, which is excluded from the pore, has no effect on gating (at 100 mM), suggesting that permeant ions affect gating not by an ionic strength effect but rather by interacting specifically with the pore.

External Cs^+ blocks the channel in a voltage-dependent fashion, and its effect on open probability is also voltage dependent. Cs^+ increases the open probability more at voltages that drive it into the channel. A simple model with a single parameter predicts the change in gating caused by Cs^+ from its blocking characteristics. These observations suggest that the effect of Cs^+ on gating occurs through Cs^+ occupancy of a permeation site and not through an allosteric mechanism.

M-PM-D7

ESTIMATING KINETIC RATE CONSTANTS FOR SINGLE CHANNELS USING SIMULATED TWO-DIMENSIONAL DWELL-TIME DISTRIBUTIONS: A GENERAL METHOD TO ACCOUNT FOR THE TRUE EFFECTS OF LIMITED TIME RESOLUTION AND NOISE.

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It is shown that the typical assumptions used in single-channel analysis (negligible noise and idealized filtering) can lead to large errors in estimates of kinetic rate constants as well as to non-unique models. To overcome these limitations an iterative simulation method is used. The single-channel current for a given kinetic scheme is simulated to incorporate the true effects of filtering and noise. The simulated current is then analyzed to obtain a two-dimensional dwell-time distribution of adjacent open-shut intervals. The likelihood that the experimental data (also expressed as a two-dimensional distribution) is drawn from the simulated distribution is calculated. This process is repeated until the rates which maximize the likelihood are found. The iterative simulation method can estimate rates with errors of <1-3%, exclude the false solutions which arise from an assumption of idealized filtering, and better distinguish among different kinetic schemes due to the correlation information in the two-dimensional distributions. Iterative simulation using simultaneous fitting of data obtained at several voltages or agonist concentrations can increase further the ability to distinguish models. Supported by the NIH AR32805 and the Muscular Dystrophy Association.

M-PM-D6

ACTIVATION OF TORPEDO ACETYLCHOLINE RECEPTORS EXPRESSED IN MOUSE FIBROBLASTS: SINGLE CHANNEL CURRENT KINETICS REVEAL DISTINCT AGONIST BINDING AFFINITIES.

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Single channel currents were recorded through *Torpedo* acetylcholine receptor channels stably expressed by clonal mouse fibroblasts (Claudio, et al., Science, 238, 1688-1694, 1987). Closed duration histograms were constructed from currents elicited by different concentrations of acetylcholine (ACh). The concentration dependence of closed durations is well described by a four-state linear scheme plus open channel block by ACh. Analysis of closed durations measured at low ACh concentrations reveals the rates doubly liganded receptors open (β), close (α), and lose bound ACh (k_{-2}). The rate ACh dissociates from singly liganded receptors, k_{-1}, is deduced from closed duration histograms obtained at intermediate ACh concentrations. With k_{-1}, k_{-2}, and a determined, the rates of ACh association, k_{+1} and k_{+2}, are estimated from closed duration histograms obtained over a range of high ACh concentrations. For measurements made at 22°C and in Ca^{++}-free solution containing 2 mM Mg^{++} the rates are as follows: β (22000/s), k_{-2} (70000/s), α (15000/s), k_{-1} (500/s), k_{+1} and k_{+2} (0.6 and 1 x 10^8/M/s). Owing to the different k_{-1} and k_{-2} values the dissociation constant for the first agonist binding site is 100-fold lower than that for the second site. Replacing half the extracellular Mg^{++} with Ca^{++} increases the efficiency of the receptor by increasing β (2-fold), decreasing k_{-1}, k_{-2}, and α (all 2-fold), without affecting k_{+1} and k_{+2}. Lowering the temperature from 22 to 12°C decreases k_{+1} (33-fold) and k_{+2} (60-fold).

M-PM-D8

HOMOGENEOUS MARKOV GATING OF SINGLE Ca CHANNELS DESPITE Ca-MEDIATED INACTIVATION

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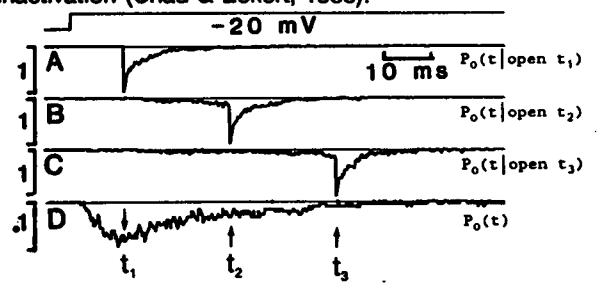
A major complexity in the description of Ca channel gating is introduced by Ca-mediated inactivation, a phenomenon whereby elevated intracellular [Ca^{2+}] speeds channel inactivation. Under such a regime, rate constants describing gating transitions should change according to prior channel activity. We tested for such inhomogeneous behavior in patch-clamp records of single L-type Ca channels of heart, obtained in the cell-attached mode with 110-160 mM [Ca] as charge carrier. Inhomogeneous gating as described above can be rigorously assessed by differences in the decay of conditional open probabilities ($P_o(t|\text{open at } t_i)$) calculated for different t_i during voltage steps (Sigworth, 1981). Panels A–C plot $P_o(t|\text{open at } t_i)$ that correspond to three time periods during activation of a single Ca channel (t_1, t_2, and t_3 in D, simple open probability P_o). Surprisingly, the time-course of decay is virtually identical among the various probabilities (A–C), despite different histories of activity before each t_i. Such a result argues that, despite Ca-mediated inactivation, Ca channel gating represents a homogeneous Markov process. While our findings simplify the description of Ca channel gating, the corollary requirement for hyperfast equilibration of Ca influx with enhanced inactivation appears to challenge current hypotheses for Ca-mediated inactivation (Chad & Eckert, 1986).



M-PM-D9

CHEMICAL MODIFICATION OF SQUID AXON K CHANNELS BY p-HYDROXIMERCURYPHENIL SULPHONIC ACID (PHMPS)

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External and internal exposure of voltage clamped and internally dialyzed squid axons to PHMPS, an S-S bridge reducing compound, produce major modifications in the kinetic parameters of the delayed rectifyer current without a significant change in selectivity. PHMPS produces a dramatic slow-down of the activation kinetics with a simultaneous decrease in the amplitude of the macroscopic current, probably due to a reduction in the number of active channels. Also, there is a marked increase in the delay of the macroscopic current, demonstrated by a change in the shape of the Cole-Moore shift curve and its maximum value. These kinetic effects of PHMPS can be reverted by treating the axon with 0.5 mM of DTT or 2-mercaptoethanol. The increase in the delay, together with the slow-down of the turn-on kinetics suggests that PHMPS is increasing the number of closed states of the channel. This has been confirmed by gating current and patch clamp experiments, where we found a slower rate of decay in the asymmetric currents and an increase in the accumulated first latency for the single channel experiments. The increase in the number of kinetically discrete states produced by PHMPS points to the importance of the S-S bridges as a factor that limits the freedom of internal movements in the channel molecule. Supported by MDA and NIH grant GM30376.

M-PM-E1

A functional region of the gastric H^+K^+ ATPase

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The gastric H,K ATPase is the enzyme responsible for H cytosolic for K extracellular exchange in the gastric parietal cell,resulting in net HCl secretion.There appear to be at least 2 subunits associated with activity of this enzyme,the α or catalytic subunit that is core glycosylated (ca 1000 amino acids) and a β subunit (ca 300 amino acids) containing complex carbohydrates.Hydropathy plots predict a membrane spanning sequence(M5) from amino acid #797 to #819 the major extracellular loop from amino acid #820 to #864 and membrane spanning sequence (M6) at amino acid #865 to #866 A covalent inhibitor of the H,K ATPase is omeprazole,which is an acid activated thiol reagent labelling only from the extracellular face.Trypsinisation and sequencing suggest that the reactive cysteines of the α subunits are cys #813 and cys #822 ,i.e. in M5 and immediately adjacent to M5.Following derivatisation,the enzyme is fixed in an E_2 like conformation.A photo-affinity, K competitive and K protectible reagent,Me-DAZIP* (an analog of SCH28080) that also reacts only from the luminal side, labelled the catalytic subunit at a sequence starting at amino acid leu#854 ,i.e. immediately adjacent to M6 and including M6.This class of reagent also induces the E_2 conformation of the enzyme and the presence of SCH28080 prevents inhibition by omeprazole.It appears therefore that the hydropathy plot correctly predicts the M5,M6 region and the intervening extracellular loop and that this region is involved in the determination of K transport and the E_2 conformations of the H,K ATPase.(NIH,VA support).

M-PM-E2

RUBIDIUM OCCLUSION OCCURS WITHIN MEMBRANE-EMBEDDED TRYPTIC FRAGMENTS OF RENAL Na/K-ATPase, R. Goldshleger, S.J.D. Karlish, W.D. Stein* and D. Tal. Weizmann Institute of Science, Rehovot and *Hebrew University, Jerusalem, Israel.

Trytic digestion of pig renal Na/K-ATPase in the presence of Rb and absence of Ca ions removes about half of the protein but leaves a stable 18KD membrane-embedded fragment derived from the alpha chain, and unchanged Rb occlusion capacity and apparent affinity for Rb ions. The beta chain is intact. Subsequent tryptic digestion in the presence of Ca or absence of Rb ions leads to rapid loss of the 18KD fragment and a strictly parallel loss of Rb occlusion.

Incubation of control enzyme with dicyclohexylcarbodiimide, DCCD, inactivates Rb (and Na) occlusion with protection by Rb ions (Shani-Sekkler *et al.* J. Biol. Chem. 263, 19331-19341, 1988), and is accompanied by incorporation of about 2 moles of DCCD per mole of phosphoenzyme at full inactivation of occlusion. Incubation of the selectively proteolysed membranes with DCCD leads to incorporation of DCCD with protection by Rb, occurring largely in the 18KD fragment. This finding should facilitate identification of carboxyl groups binding the cations.

This work appears to demonstrate that cation occlusion sites are embedded within trans-membrane segments, and are quite separate from the ATP binding site, on the cytoplasmic domain of the alpha chain.

M-PM-E3

THE "PUMP-LEAK" PARALLELISM IN NECTURUS ENTEROCYTES: PROPERTIES OF AN INWARDLY-RECTIFYING BASOLATERAL MEMBRANE POTASSIUM CHANNEL. S.G. Schultz, Department of Physiology and Cell Biology, University of Texas Medical School, Houston, TX 77225.

Previous studies have demonstrated the presence of an inwardly-rectifying, high conductance K^+ channel in vesicles isolated from the basolateral membranes of Necturus enterocytes and reconstituted into planar phospholipid bilayers, and have raised the possibility that these channels may be, at least in part, responsible for the parallelism between Na^+ pump rate and basolateral membrane K^+ conductance observed in this and other Na^+-absorbing epithelial cells (Costantin et al., Proc. Natl. Acad. Sci. USA, 86:5212-5216, 1989).

The results of recent studies indicate that pronase or trypsin added to the intracellular or extracellular compartments surrounding this reconstituted channel when in the open configuration markedly desensitize its voltage-sensing gating mechanism(s). However, when the channel in the closed or inactivated configuration, it is partially or fully protected from the proteolytic actions of these agents. Possible mechanical models of this channel will be discussed.

M-PM-E4

REGULATION OF Ca^{2+} TRANSPORT ACROSS THE ER MEMBRANE Shmuel Muallem; Department of Physiology; U.T. Southwestern Medical Center, Dallas, TX 75235

Ca^{2+} mobilizing agonists evoke free cytosolic Ca^{2+} ($[Ca^{2+}]_i$) oscillation in pancreatic acinar cells. Ca^{2+} oscillation requires maintained Ca^{2+} efflux across the plasma membrane and regulation of Ca^{2+} uptake and release by intracellular stores, probably the endoplasmic reticulum (ER). Ca^{2+} influx across the plasma membrane and into the cytosol occurs through an agonist-activated, H^+ and La^{3+}-inhibitable Ca^{2+} influx pathway. Ca^{2+} mobilizing agonists also activate the ER Ca^{2+} pump. This activation probably involves an acceleration of the conformational transition between the low (E_2) and high (E_1) Ca^{2+} affinity forms of the pump. Ca^{2+} release from the ER is largely mediated by Inositol-1,4,5-trisphosphate (IP_3). To obtain repetitive Ca^{2+} release and uptake across the ER membrane and thus Ca^{2+} oscillation, it is necessary to turn on and off the IP_3-activated Ca^{2+} channel. This appears to be mediated by a cytosolic Ca^{2+} binding protein. At 5μM Ca^{2+} the protein completely inhibits IP_3 binding and IP_3-mediated Ca^{2+} release. Only little inhibition is seen at 0.1μM Ca^{2+}. Regulation of this activity has the potential of inducing $[Ca^{2+}]_i$ oscillation.

M-PM-E5

DIFFERENT CHLORIDE CONDUCTANCES IN Cl-SECRETING EPITHELIA. R.A. Frizzell, W.H. Cliff, R.T. Worrell & A.P. Morris (Intro. by R.L. Shoemaker), Physiol. & Biophys., U. Alabama at B'ham, Birmingham, AL 35294.

We studied the properties of Cl conductances (gCl) activated by different classes of agonists using whole-cell patch-clamp. cAMP-mediated agonists activate an ohmic gCl that shows no voltage-dependence or DIDS sensitivity. Ca-mediated agonists elicit an outwardly-rectified gCl that activates at depolarizing voltages and is blocked by DIDS (K_i~50 μM). Cell swelling stimulates a gCl that inactivates at depolarizing voltages and is also DIDS-sensitive (K_i~5 μM). Changes in cell Ca do not mediate the swelling response. Thus, cAMP, Ca and cell swelling activate Cl conductances with distinctly different current-voltage relations, time- and voltage-dependent kinetics and blocker sensitivities. Conductances with these properties are present in several salt-secreting epithelia: the colonic cell lines T84 and HT29 and dog and human airway cells. Their single-channel basis will be discussed. [NIH, DK31091]

M-PM-E6

STRUCTURAL AND FUNCTIONAL SIMILARITIES BETWEEN PROKARYOTE AND EUKARYOTE Na^+ COTRANSPORT PROTEINS. Ernest M. Wright, Eric Turk, & Matthias Hediger, Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024-1751.

Na^+ cotransporters are a major class of membrane proteins responsible for active transport of substrates into enteric bacteria and eukaryote cells. Three have been cloned and sequenced: the E. coli melibiose and proline transporters and the mammalian glucose transporter. Glucose and proline transport are driven by Na^+ electrochemical potential gradients, while the melibiose transporter is driven by Na^+ or H^+ electrochemical gradients depending on the substrate. There is close similarity between glucose and proline transporters at the DNA (44%), primary sequence (53%), and secondary structure levels, but there is no homology between these proteins and the melibiose transport. We suggest that proline and glucose cotransporters share a common ancestral protein and that functional similarities between the glucose and melibiose transporters have arisen through convergent evolution. A similar structural homology occurs between mammalian facilitated glucose cotransporters and a number of bacterial sugar transporters.

M-PM-E7

PROPERTIES OF CHANNELS RECONSTITUTED FROM THE MAJOR INTRINSIC PROTEIN OF LENS FIBERS AND THEIR POSSIBLE ROLE IN MAINTAINING LENS TRANSPARENCY James E. Hall, George R. Ehring and Guido A. Zampighi. Dept. of Physiology and Biophysics, UC Irvine, Irvine CA 92717, and Dept of Anatomy, UCLA, Los Angeles CA,90024.

The lens of the eye contains numerous structures which resemble gap junctions. Some of these contain MIP, the major intrinsic protein of the lens and bear superficial resemblance to gap junctions. But immunocytochemistry at the electron microscopic level and label fracture demonstrate that, in at least some of these structures, MIP resides in only one of two closely apposed membranes. In these structures MIP cannot form a gap-junction-like channel. But MIP does form large slightly anion selective channels in lipid bilayers, and these channels turn off with increasing voltage of either sign.

On the basis of these two observations, we propose that MIP could play an important role in fluid transport and maintenance of lens transparency. The potential of extracellular space becomes more negative toward the center of the lens and that of intracellular space becomes more positive (Mathias and Rae. Cur. Eye Res. 4:421-430,1985). Thus membrane voltage decreases from outside to in, and voltage-dependent MIP channels would tend to be open at the center of the lens (low voltage drop), while those near the edge would tend to be closed (large voltage drop). The open channels would promote collapse of extracellular space at the center of the lens via Gibbs-Donnan absorption of fluid into the fibers.

M-PM-E8

MEMBRANE TRANSPORT REGULATION IN ENDOCYTIC VESICLES FROM MAMMALIAN PROXIMAL TUBULE. A.S. Verkman. CVRI, UCSF, San Francisco, CA.

The proximal tubule apical membrane undergoes rapid turnover by a cycle of endo- and exocytosis. An in vivo labeling procedure was developed to label endocytic vesicles from the apical membrane selectively with impermeant fluorophores. Rabbits or rats were injected with FITC-dextran or the Cl indicator SPQ. Fluorophores were filtered by the glomerulus and entrapped into endocytic vesicles. Stopped-flow experiments were performed on a microsomal pellet containing the labeled endocytic vesicles to measure water, urea and proton transport using FITC-dextran, and Cl transport using SPQ. Endocytic vesicles from proximal tubule contained a mercurial-inhibitable water channel, an NEM-inhibitable proton pump and a Cl channel. There was no measurable Na/glucose or Na/H transport. The Cl channel was activated by cAMP-dependent protein kinase. These results show that apical membrane transporters are retrieved selectively into endocytic vesicles and that the Cl channel is a target site for phosphorylation by protein kinase A. It is proposed that regulation of Cl channel activity is important for acidification of intracellular vesicles.

M-PM-E9

MECHANISMS OF OSMOTIC WATER TRANSPORT IN EPITHELIA. Luis Reuss, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Necturus gallbladder epithelial cells were loaded with the impermeant cation tetramethylammonium (TMA^+) by transient exposure to the pore-forming polyene antibiotic nystatin. TMA^+ fulfills the requirements for a marker for cell water volume; its intracellular activity was measured with K^+-selective microelectrodes (ca. 800 times more sensitive to TMA^+ than to K^+). Apical or basolateral solution osmolality was changed by altering the sucrose concentration, and tetrabutylammonium (TBA^+) was used as a marker to estimate the time course of the change in osmolality at the cell surface. TBA^+ activity was measured with an extracellular electrode. The acute changes in cell volume elicited by anisosmotic solutions were analyzed with a three-compartment model, and the apparent hydraulic permeability coefficients of apical and basolateral membranes were estimated to be 1.2 and $0.8 \cdot 10^{-3}$ $cm \cdot s^{-1} \cdot (osmol/kg)^{-1}$, respectively. Transcellular spontaneous net water flow requires osmolality differences of only 1-2 mosmol/kg across each membrane.

Supported by NIH grant DK 38588.

M-PM-F1

ANISOTROPIC ^2H SPIN LATTICE RELAXATION AS A PROBE OF DYNAMICS IN L_α PHASE LIPID MEMBRANES. J.B. Speyer, R.T. Weber, S.K. Das Gupta, and R.G. Griffin, Dept. of Chemistry and Francis Bitter Nat'l Magnet Laboratory, MIT, Cambridge, MA 02139.

Motion in L_α phase lipid is too fast to study by conventional ^2H-NMR lineshape analysis and are better examined by T_1 relaxation. The dependence of T_1 on the orientation of the lipid with respect to the applied magnetic field varies with the motions that dominate relaxation, and can be calculated from motional models. We have found this to be a very successful probe of lipid dynamics in unoriented liposomes of cerebroside, sphingomyelin, and lecithins, and in cholesterol containing membranes. The T_1's and partially recovered lineshapes from inversion recovery experiments in L_α phase liposomes of cerebroside have been simulated very successfully with a model of discrete jumps among nine sites -- three tetrahedral sites from gauche-trans isomerization coupled with 120° hops about the director. Correlation times for the two motions have been obtained for a number of sites in cerebroside. Lack of T_1 anisotropy is predictable at certain sites due to the serendipitous aspects of the motion and not due to orientational averaging arising from fast lateral diffusion.

M-PM-F2

SHORT- AND LONG-RANGE INTERACTIONS WHICH GOVERN THE SOLVATION OF LIPID BILAYER SURFACES. T. J. McIntosh, A. D. Magid, and S. A. Simon, Departments of Cell Biology and Neurobiology, Duke University Medical Center, Durham, N. C. 27710.

Using x-ray diffraction methods, we are measuring the magnitude of steric, solvation, and undulation pressures as a function of the distance between bilayer surfaces. At very small separations where apposing bilayers come into contact, steric hindrance is the dominant repulsive pressure and depends on the packing density of polar headgroups in the bilayer surface. At larger bilayer separations, the solvation pressure dominates. Experiments with charged, uncharged, and zwitterionic bilayers in polar solvents indicate: (1) the magnitude of the solvation pressure increases with increasing dipole potential and (2) the decay length of the solvation pressure depends on the packing density of solvent molecules. Compared to the solvation pressure, the undulation pressure is small for gel phase bilayers, but is large for thin, liquid-crystalline phase bilayers at large interbilayer separations. Thus, depending on the width and compressibility of the bilayer, thermally induced bending undulations can strongly influence the solvation of lipid bilayers.

M-PM-F3

ENTROPY-DRIVEN TENSION IN SINGLE MEMBRANES AND STERIC REPULSION BETWEEN MEMBRANES. E. Evans, Pathology and Physics, University of British Columbia, Vancouver, B.C. V6T 1W5

In recent years, it has become apparent that thin flexible membranes represent 2-D generalizations of linear-flexible polymers. Thus, thermal motions act to randomize surface conformations (crumpling transi-tions) whereas constraints posed by fixed capsule volumes or confinement adjacent to parallel stiff boundaries lead to steric effects (entropy-driven tension or long-range steric repulsion). For example, the tension in a vesicle is observed to increase initially as a soft exponential function of surface area expansion followed by a linear dependence on area expansion which demonstrates crossover from configurational entropy-dominated compliance to direct surface elastic compressibility. Similarly, regimes of vesicle adhesion to substrates are characterized by crossover from strong to weak binding, and ultimately "unbinding", which appears to be controlled by entropy effects. Sensitive micro-mechanical experiments are used to measure entropy-driven tension (and thereby curvature elasticity) in membranes and to examine the properties of the "unbinding" transition in the weak adhesion regime.

M-PM-F4

RELATIONS BETWEEN CURVATURE ELASTICITY, NONLAMELLAR PHASES, AND BIOMEMBRANE FUNCTION. S.M. Gruner, Physics Dept., Princeton University, Princeton, NJ 08544.

Much progress has been made over the last 5 years in understanding the physical basis of lamellar-nonlamellar lipid phase transitions. Lipid monolayer bending elasticity gives rise to a spontaneous curvature which is geometrically incompatible with minimization of competing free energies, such as those involved in optimal chain packing. These forces, which give rise to interfacially curved phases, such as H_{II} and cubic phases, are present in all liquid crystalline mesomorphs, including bilayers. Methods of measuring the energy of interfacial curvature are described. In lamellar bilayers, curvature energies range from 0 to about a kT/lipid molecule near a nonlamellar phase boundry. It is suggested that biomembranes store energy reversibly as an elastic curvature stress, that this energy modulates the activity of some membrane proteins, and that this provides a rationale for biomembrane lipid composition.

Work supported by DOE grant DE-FG02-87ER 60522.A000, NIH grant GM32614 and by ONR contract N00014-86-K-0396P00001.

M-PM-F5

THE STRUCTURAL ROLE OF CHOLESTEROL IN THE EVOLUTION OF MEMBRANES, Myer Bloom, Department of Physics, University of British Columbia, Vancouver, B.C., Canada.

Sterols such as cholesterol (CHOL) are found in the plasma membrane of all eucaryotic cells and are not present at all in procaryotic cells. CHOL evolved about 10^9 years ago when the earth's atmosphere was "polluted" by O_2. Considerations of lipid-protein and lipid-CHOL interactions lead us to propose that some essential mechanical properties of plasma membranes of eucaryotic cells were imparted by the lipid-CHOL interaction (M. Bloom and O.G. Mouritsen, Can. J. Chem. 66, 706 1988)). Experimental studies to check this hypothesis will be discussed.

M-PM-F6

1D, 2D and 3D DIFFRACTION METHODS IN STUDYING THE PROPERTIES AND INTERACTIONS OF GLYCOSPHINGOLIPIDS IN MEMBRANES. Robert A Reed, P.R. Maulik and G.G. Shipley. Biophysics Dept., Boston Univ. School of Med., Boston, Mass.

Glycosphingolipids (GSL) are a class of lipid which vary in complexity and occur in membranes. Gangliosides represent the most complex GSL, and are implicated in a variety of cellular functions, including acting as receptors for hormones, viruses and toxins. In particular, the ganglioside GM_1 is the cell surface receptor for cholera toxin (CT). One dimensional (1D) x-ray diffraction and calorimetry reveal that GM_1 undergoes a micellar transition ($T=23.5°C$, $\Delta H=1.7$ Kcal/mol) with changes occurring only in the wide angle region of the diffraction pattern. In mixtures with phosphatidylcholines, phase separation occurs at >30 mol% GM_1, exhibiting complex thermal and structural behavior at higher concentrations. The calorimetric and structural studies provide important information about the interactions of GM_1 in membranes, and are used to explore the receptor-ligand properties of membrane-bound GM_1. In supported lipid monolayers, GM_1 and CT-GM_1 complexes demonstrate rapid in-plane lateral diffusion ($\approx 10^{-8}$ cm^2/sec). Under appropriate conditions, GM_1-bound CT and CT B-subunit (CTB) form 2D crystals. Diffraction and image processing techniques show that CT has a pentameric character, reflecting the five-fold symmetry of CTB, and a central aqueous channel. 3D single crystals of CTB have been grown. Preliminary analysis indicates a monoclinic unit cell, $a=39.0Å$, $b=94.3Å$, $c=67.5Å$, $\beta=96°$, space group $P2_1$; one molecule per asymmetric unit. Recently, heavy atom derivatives were identified which should allow the structure to be solved to atomic resolution.

M-PM-G1

CYCLIC AMP-DEPENDENT PHOSPHORYLATION OF VOLTAGE DEPENDENT Ca CHANNELS IN INTACT SKELETAL MUSCLE CELLS.

C. M. Weilenmann, C. F. Chang, M. M. Hosey, Dept. of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

Dihydropyridine (DHP)-sensitive Ca channels are regulated by receptor-dependent events that appear to involve phosphorylation of channel components. *In vitro* studies have demonstrated that the α_1 and β subunits of skeletal muscle DHP-sensitive Ca channels are substrates for different protein kinases. We now report on the phosphorylation of these channels in *intact* skeletal muscle cells. Chick breast muscles were incubated in Kreb's solution with agents that elevate intracellular cAMP. DHP receptors were purified under conditions to prevent dephosphorylation. Using the technique of back phosphorylation, the purified channels were then subjected to *in vitro* phosphorylation with cAMP-dependent protein kinase. Incubation of the intact muscle with isoproterenol, IBMX or forskolin resulted in an inhibition of the *in vitro* phosphorylation of the purified α_1 peptide with no significant change at the level of β peptide. The results provide evidence that the α_1 subunit of DHP-sensitive Ca channels is phosphorylated in intact skeletal muscle cells and suggest that regulation of Ca channels in skeletal muscle occurs at the level of this protein.

M-PM-G2

PHOSPHORYLATION INCREASES THE VOLTAGE DEPENDENCE OF MIP CHANNELS ALREADY PRESENT IN LIPID BILAYERS.

G.R. Ehring, N. Lagos, G.A. Zampighi and J.E. Hall. Dept. of Physiology and Biophysics UCI, Irvine CA 92717,and Depts of Physiology and Anatomy, UCLA, Los Angeles CA,90024.

When reconstituted into dioleolyl phosphatidyl choline vesicles which are then added to both sides of a planar phosphatidyl ethanolamine (PE) lipid bilayer, MIP, the major intrinsic protein of lens fibers , forms voltage-dependent channels which turn off with increasing voltage of either sign. Though the average apparent gating charge is about 2 electronic charges, it varies between one and two from preparation to preparation. Preparations of bovine MIP from older animals tend to have weaker voltage dependence than those from younger animals. Recent experiments indicate that part of this variation is due to differences in the endogenous state of MIP phosphorylation. When dephosphorylated MIP is reconstituted into PE bilayers, the initial voltage dependence is less than that of native MIP from young calves. After channels from dephosphorylated MIP have appeared in the planar bilayer, addition of ATP, Mg^{2+}, cAMP and protein kinase A causes a dramatic increase in the voltage dependence over a period of hours. There are even long duration closures at 20 mV never seen with native MIP or dephosphorylated MIP. All the components of the phosphorylating cocktail are necessary for the increased voltage dependence. In particular, addition of everything except protein kinase A has no effect. (See also abstract of Lagos, et al) Supported by NIH grants EY05661, EY06075 and EY04110.

M-PM-G3

REDUCTION OF CARDIAC Ca^{2+} CHANNEL ACTIVITY BY INHIBITION OF PROTEIN KINASE C (PKC).

A.E. Lacerda, D. Rampe* and A.M. Brown. Baylor College of Medicine, Houston, Texas and *Merrell Dow Pharmaceuticals, Cincinnatti, Ohio.

Phorbol ester stimulation of PKC transiently stimulates and subsequently inhibits high threshold cardiac Ca^{2+} channel activity in cultured neonatal rat ventricular myocytes. One possible mechanism for the delayed inhibition is through a decline in PKC function. We have examined the role of PKC in maintaining basal Ca^{2+} channel activity by measurement of $^{45}Ca^{2+}$ uptake and Ca^{2+} channel current after treatment of cells with inhibitors of PKC. Staurosporine, H-7, sphingosine and preincubation with phorbol ester all produced inhibition of $^{45}Ca^{2+}$ uptake. Preincubation with 1μM phorbol ester for 1 or more hours reduced whole cell Ca^{2+} current by about 50%. This suggests that a portion of unstimulated cardiac Ca^{2+} channel activity is due to agonist-independent PKC function, analogous to the role of agonist-independent cAMP dependent protein kinase function in maintaining basal cardiac Ca^{2+} channel activity (Kameyama et al., Pflugers Arch. 405:285, 1985). Supported by AHA Texas Affiliate 89G0191 and NIH HL36930.

M-PM-G4

SINGLE CHANNEL ANALYSIS OF THE PHOSPHORYLATION OF THE SQUID AXON DELAYED RECTIFIER

Perozo, E., Jong, D-S. and Bezanilla F.
Dept. of Physiology UCLA, Los Angeles CA and MBL, Woods Hole, MA

In dialyzed and voltage clamped squid axons, the delayed rectifier current can be modulated in amplitude and kinetics by an ATP dependent phosphorylation process (PNAS 83:2743, 1986; JGP 93:1283, 1989). The 20 pS channel seems to be the main current carrier of the delayed rectifier current of the squid axon. Using the cut-open axon technique we have studied the activation and inactivation processes of this channel at the single channel level under nonstationary conditions. ATP changes inside the patch pipette were achieved by using caged ATP and a UV source focused at the tip of the pipette with a quartz fiber optic. After illumination, there was a shift in the steady state *inactivation* curve towards more positive potentials, as revealed by a decrease in the number of blank traces at holding potentials (HP) between -70 and -30 mV. On the other hand, at HP=-80 mV the open probability measured at test pulses between -60 and +20 mV decreased after illumination, showing a shift of the *activation* curve to more positive potentials. These results explain the effects of phosphorylation observed in the macroscopic currents recorded from whole axons and they are consistent with the idea that phosphorylation increases the density of negative charges near the voltage sensor of the channel. Supported by MDA and NIH grant GM30376. D-S. Jong was a Grass fellow.

M-PM-G5

MODULATION OF VOLTAGE -DEPENDENT Na AND K CHANNELS BY ISOFORMS OF PROTEIN KINASE C.

I. Lotan[#], N. Dascal[#], Z. Naor[*] & R. Boton[#], [#]Department of Physiology and Pharmacology, Sackler School of Medicine, and [*]Department of Biochemistry, Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel.

A role for protein kinase C (PKC) in modulation of voltage-dependent Na and K (A-type) channels has been proposed, but direct evidence for modulation of vertebrate channels is lacking. Moreover, it is not known whether PKC isoforms may have differential effects. We examined the effects of PKC isoforms on A- and Na channels expressed in Xenopus oocytes injected with chick brain RNA. Currents through the channels under consideration were recorded using the two-electrode voltage clamp technique. The endogenous PKC was "down-regulated" by a prolonged treatment with the phorbol ester, PMA. PKC isoforms were purified to homogeneity from rat brain. Intracellular injection of PKC II ($\beta 1+\beta 2$) and III (α) reduced both Na current (I_{Na}) current and the A-current (I_A) by 20 to 40%, after 20-40 min. PKC I (τ) under the same conditions reduced I_A, with no effect on I_{Na}. PKC did not affect the response to kainate. Inactivated or unactivated enzyme and vehicle alone had no effect. Our results strongly suggest that I_{Na} and I_A in vertebrate neurons are modulated by PKC, and that PKC subspecies may differently modulate certain excitability processes in neurones.

M-PM-G6

ATRIONATURETIC PEPTIDE (ANP) ENHANCES THE SELECTIVITY OF Na^+ CHANNEL TO Ca^{2+} IN MAMMALIAN CARDIAC MYOCYTES.

Lisa A. Sorbera & Martin Morad. Deptartment of Physiology, University of Pennsylvania, Philadelphia, PA, 19104.

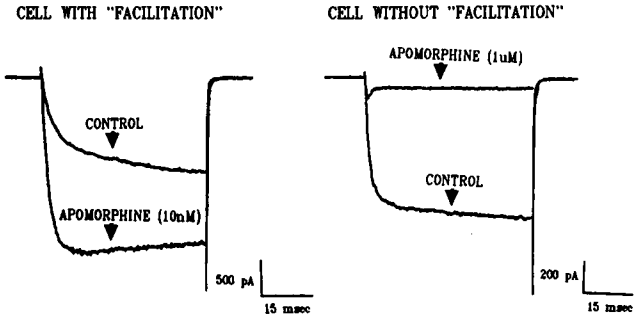
ANP is a vasoactive peptide which is released from the atrium in response to volume expansion. ANP also reduced I_{Ca} in frog ventricular myocytes, through an adenylate cyclase-dependent pathway (Gisbert & Fischmaeister, Circ. Res. 1988; 62:660). In isolated and whole cell clamped rat and guinea pig ventricular myocytes the effect of ANP on the I_{Na} and I_{Ca} was examined. ANP (100nM) decreased both I_{Ca} and I_{Na} rapidly. When Ca^{2+} was omitted from the external solution, ANP failed to suppress I_{Na}, suggesting that ANP effect was Ca^{2+} dependent. When external Na^+ was completely replaced by Cs^+ or n-methyl glucamine, ANP induced a rapidly activating and inactivating current with kinetics and voltage dependency similar to that of the Na^+ channel. In the absence of external Ca^{2+}, ANP failed to activate this current suggesting that Ca^{2+} was the charge carrier. Since 10uM TTX blocked this current, but the current was unaffected by 5mM Ni^{2+} or 10uM nifedipine, Na^+ channels were thought to carry this Ca^{2+} current. Our results therefore suggest that ANP induces a molecular transformation in the Na^+ channel making it more selective to calcium, without affecting its kinetics, voltage-dependency and pharmacological sensitivity. The combined suppressive effect of ANP on Na^+ and Ca^{2+} channels may render the secretory atrial tissues inexcitable and non-contracting. Thus, this mechanism may be involved in the feedback regulation of ANP secretion.

M-PM-G7

THE DOPAMINE AGONIST APOMORPHINE ACTIVATES "FACILITATION" Ca^{2+} CURRENTS AT LOW CONCENTRATIONS

C.R. Artalejo, M.K. Dahmer, R.L. Perlman, & A.P. Fox, The University of Chicago, Dept. of Pharm/ Phys.

Bovine chromaffin cells have two types of Ca currents. The "standard" component, was activated by brief depolarizations. The other component, called "facilitation", was activated by prolonged pre-depolarizations to very positive potentials, or by repetitive small depolarizations. These components are due to two separate types of Ca channels. The dopamine agonist apomorphine (10 nM) activated "facilitation" currents in lieu of any pre-depolarizations, as shown in the left panel. In cells that had no "facilitation" Ca currents, 10 nM apomorphine had no effect, but 1 μM suppressed the "standard" component. Dopamine release from the secretory granules may provide a feedback mechanism to regulate secretion, acting in opposite ways on the two types of Ca currents.



M-PM-G8

β-ADRENERGIC REGULATION OF CARDIAC CALCIUM CHANNELS: IONIC CURRENT AND GATING CURRENT.

Bruce P. Bean, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Effects of isoproterenol and 8-chlorophenylthio-cAMP were studied using whole-cell voltage clamp of bullfrog ventricular myocytes. Both agents greatly enhanced Ca channel currents elicited by small and moderate depolarizations but had little or no effect on the currents activated by very large depolarizations. When ionic current was blocked by Cd and La, non-linear charge movement could be recorded. Ca channel gating current was identified as the predominant (80-90%) component of non-linear charge movement by its inactivation in parallel with Ca channel ionic current and by its sensitivity to nitrendipine. Isoproterenol had little effect on the size or kinetics of Ca channel gating current even at potentials where ionic current was enhanced many-fold. This result suggests that under basal conditions, movement of the gating charge of Ca channels may be poorly coupled to opening of the channels - at least for submaximal depolarizations. β-adrenergic stimulation apparently increases Ca current by improving the coupling between the two processes.

M-PM-G9

METABOLIC ACTIVATION OF Ca2+-CHANNELS IN HUMAN PANCREATIC B-CELLS. E. Rojas, J. Hidalgo, P. B. Carroll, M-X. Li and I. Atwater. LCBG, NIH, Bethesda, MD.
In order to better understand glucose induced insulin relesase, we studied Ca^{2+}-channels in human B-cells prepared from collagenase digested islets. Single Ca^{2+} channel inward currents were recorded cell attached using 25 mM Ca^{2+}, TMA and TTx in the pipet. Results: In 0-glucose, infrequent channel openings of -0.34 ±0.02 pA (Vp=0 mV) were observed. Frequency of openings was relatively voltage insensitive. Conductance: 4.9 ± 0.2 pS. 11 mM glucose or alphaketocaproicacid (KIC) increased freqency of openings and induced action potentials. Mannoheptulose prevented channel activation by glucose but not by KIC. The channel was cation selective. It was not blocked by DHP or by high Ca^{2+} on the inner side. The data are compatible with channel activation by products of mitochondrial metabolism. We estimate there are 500 of these channels present in the membrane.

M-PM-G10

MODAL GATING OF L-TYPE CALCIUM CHANNELS. *Daniela Pietrobon and Peter Hess*, Dept. of Cell. and Mol. Physiology, Harvard Med. School, Boston, MA 02115.

The existence of modal gating in dihydropyridine (DHP) sensitive (L-type) Ca channels remains controversial and the mechanisms underlying transitions between modes are poorly understood. We have confirmed that in the absence of dihydropyridine (DHP) agonist drugs L-type channels from cardiac and PC12 cells can display periods of single channel activity with very high opening probability lasting for up to several hundred milliseconds. During such periods of mode 2 activity mean open times are prolonged more than 20 times and are similar to openings promoted by DHP agonists. Mode 2 activity of L-type channels is greatly enhanced following prepulses to positive potentials (>50 mV), both in the presence or absence of DHP agonists. The lifetime of the prepulse induced gating mode is also voltage dependent and decays with a time constant of ~60 ms at + 20 mV (with 110 mM Ba as charge carrier). The potentiating effect of positive prepulses is also observed in whole cell recordings, both in cardiac and PC12 cells. Thus the equilibrium between gating modes of L-type Ca channels is strongly voltage dependent and this voltage dependence is shifted towards more negative potentials by DHP agonists.

M-PM-H1 *Monday Evening Workshop*

CRYSTALLIZATION OF MEMBRANE PROTEINS

R. Michael Garavito, Dept. of Biochemistry, Univ. of Chicago, Chicago, IL, 60637

X-ray crystallography remains the most powerful method for determining the three dimensional structure of biological macromolecules at atomic resolution. Until a few years ago, it was not possible to obtain crystalline preparations of integral membrane proteins suitable for X-ray analysis. With the development of methods and new detergents for handling membrane proteins, crystals of several membrane proteins have been prepared. In this presentation, I will discuss the critical factors involved in the growth of membrane protein crystals with particular emphasis on choosing the most suitable detergent, minimizing the effects of lipids, and setting up the crystallization experiments. The primary lesson we have learned is that a membrane protein preparation must be homogeneous, in terms of protein and lipid composition, and monodisperse. Once a suitable preparation has been obtained, "standard" crystallization techniques can be used with good success.

R.M. Garavito et al. (1986) J. Crystal Growth 76, 701-709.

W. Kuehlbrandt (1988) Quart. Rev. Biophys. 21, 429-477.

M-PM-H2

ATOMIC STRUCTURES OF BINDING PROTEINS INVOLVED IN ACTIVE TRANSPORT AND CHEMOTAXIS: WHAT THEY TELL US ABOUT SITES FOR INTERACTION WITH A VARIETY OF SUBSTRATES AND WITH MEMBRANE PROTEIN COMPONENTS

Florante A. Quiocho

Howard Hughes Medical Institute and Department of Biochemistry and of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030

In recent years our laboratory has been engaged in the determination of very high resolution X-ray structures of seven periplasmic binding proteins each with substrate specificity for L-arabinose, D-galactose/D-glucose, maltodextrins, leucine/isoleucine/valine, leucine, sulfate and phosphate. These proteins, which constitute about a third of the entire family of proteins, serve as initial receptors for high-affinity active transport systems or permeases. The galactose-binding protein and maltodextrin-binding protein also act as chemoreceptors for chemotaxis. In addition to the fast-reacting tight-binding substrate (or nutrient) site, the proteins that participate in both transport and chemotaxis have two other distinct sites or sets of sites: one set for interacting with the plasma-membrane bound protein components that actually pass nutrient from the periplasm to the cytoplasm and another set for interacting with the cytoplasmic transmembrane signal transducer in order to trigger taxis toward chemical attractants. The atomic interactions between binding proteins and substrates have been clearly elucidated and the other sites for interacting membrane components have been identified in some binding proteins.

M-PM-H3

FUMARATE REDUCTASE AS A MODEL FOR MEMBRANE PROTEIN OVER-EXPRESSION.

Joel H. Weiner, Dept. of Biochemistry, Univ. of Alberta, Edmonton, Canada T6G 2H7.

The electron transfer enzyme fumarate reductase (FR) has been used to examine factors influencing the high level expression of membrane proteins. FR, encoded by the *frd* operon, is composed of 4 non-identical subunits: FrdAB form an extrinsic catalytic dimer and FrdCD comprise an intrinsic anchor dimer. Expression of FR is positively regulated by Fnr, fumarate and an alanine dependent attenuator mechanism. Transformation of *E. coli* by a multicopy plasmid encoding the *frd* operon results in massive overproduction of the enzyme such that it accounts for up to 90% of membrane-associated protein. This does not displace other membrane enzymes but it does interfere with coupled reactions such as NADH oxidase. Overproduction results in an increase in total membrane protein and phospholipid (PL) but the lipid:protein ratio remains near 0.4. To accommodate the extra PL and protein a tubular organelle (a complex of PL and helically ordered FR) erupts from one pole of the cell and fills much of the cytoplasm. Amplification of FR is not dependent on PL synthesis as the two can be uncoupled when phosphatidyl ethanolamine becomes limiting, yet ordered protein arrays of FR accumulate. Clones of the *frd* operon encoding only FrdAB express high levels of a soluble FR dimer but clones encoding only the intrinsic subunits do not accumulate FrdCD in significant amounts due to apparent proteolysis, indicating that FrdAB are required to protect the anchor subunits. Formation of similar tubular organelles is seen following over-expression of other membrane proteins including transhydrogenase and glycerol-P acyltransferase but is not seen following over-expression of DMSO reductase or AmpE. Attempts to co-clone poorly expressed membrane proteins into *E. coli* over-expressing FR have not resulted in enhanced expression.

M-PM-H4

ISOLATION OF INTEGRAL POLYTOPIC MEMBRANE PROTEINS IN SOLUBLE FORM

Paul D. Roepe[1] and H. Ronald Kaback
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Recently (Roepe and Kaback, P.N.A.S. 86, 6087) we have isolated and characterized a metastable soluble form of the lac permease from an overproducing strain of E. coli. Soluble permease appears after the membrane is saturated with newly synthesized permease, has little associated phospholipid, and binds ligand while in solution. Moreover, it can be reconstituted into phospholipid vesicles that catalyze lactose counterflow or active transport in response to a membrane potential. Preliminary data suggests this form of the permease may offer a less difficult route to crystallization of the polytopic integral membrane protein. Application of the techniques described to the isolation of other integral membrane proteins in soluble form will be discussed.

[1]P.D.R. was supported by a Jane Coffin Childs postdoctoral fellowship

M-PM-I1

EFFECTS OF MUTATIONS ON THE THERMODYNAMIC PROPERTIES OF PROTEINS

Julian M. Sturtevant, Yale University, New Haven, CT (Intro. by Ernesto Freire)

An important means for improving our understanding of the forces which stabilize protein structure is the study of the thermodynamic changes resulting from known replacements of amino acid residues in proteins. Two forms of study will be discussed: (1) the effects on the thermodynamics of unfolding of proteins as determined by differential scanning calorimetry (DSC); and (2) the effects on the thermodynamics of protein ligand interactions as evaluated by isothermal titration calorimetry. DSC studies on mutant forms of T4 lysozyme will be reviewed. The titration of the S-protein derived from ribonuclease A with modified forms of the S-peptide will be reported.

It should be emphasized that the interpretation of the results of such studies is fraught with difficulties. For example, the usually large heat capacity changes in protein reactions result in significant temperature variations in other thermodynamic properties. This obviously complicates attempts to correlate the thermodynamic changes with changes in other data such as structures derived from x-ray crystallography.

M-PM-I2 *Monday Evening Workshop*

MULTIFREQUENCY CALORIMETRY OF PROTEINS.

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M-PM-I3

COLD DENATURATION OF PROTEINS

P.L.Privalov, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

Theoretically predicted protein denaturation at low temperatures has been studied experimentally by supercooling aqueous solutions below 0°C using various techniques, including scanning microcalorimetry. It was shown that cold denaturation is a very general phenomenon specific for globular proteins. It results from strong temperature dependence of the enthalpy and entropy of protein denaturation caused by hydration of internal groups upon unfolding of a compact native structure. Thermodynamic analysis of this effect shows that the Gibbs energy of hydration of internal non-polar groups is never positive: it is large and negative at low temperatures and decreases to zero upon temperature increasing to 130°C. Therefore hydration of non-polar groups cannot be considered as a factor stabilizing compact protein structure. This fact leads to reconsideration of the concept of hydrophobic interaction in which the dominant role is likely to be played not by hydration of non-polar groups but by their van-der-Waals interaction.

M-PM-I4

CALORIMETRIC STUDIES OF MULTIDOMAIN PROTEINS

John Brandts. University of Massachusetts, Amherst, MA 01003.

M-PM-J1 *Monday Evening Workshop*

Mapping the Protein Binding Sites of Erythrocyte Ankyrin for Band 3, Spectrin, and the Na/K ATPase. V. Bennett, L. Davis, and J. Davis, Department of Biochemistry and Howard Hughes Medical Institute, Duke Medical Center, Durham, NC. A Mr=89,000 N-terminal fragment of erythrocyte ankyrin (89K domain) containing 22 tandem 33 amino acid repeats (Lux S.E., John, K.M., and Bennett, V. (1989) Clin. Res. 37:547A) associates with band 3 in RBC membranes with equal affinity and increased capacity compared to native ankyrin. A proteolytic fragment of the 89K domain entirely comprised of repeats retains ability to bind to band 3. One or more of the repeats of the 89K domain are therefore involved in association with band 3. Spectrin-binding activity of ankyrin is located in a Mr=62,000 domain. Deletion of a protease-sensitive region comprising 70 amino acids at the N-terminus of the 62 K domain reduced by 10-20 fold the ability of the domain to associate with spectrin. The N-terminal 70 amino acids of the 62K domain thus are likely to play an important role in association of ankyrin with spectrin. Band 3 and the Na/K ATPase associate with distinct sites on ankyrin. The ankyrin-binding activity of these integral membrane proteins thus is likely to have evolved by convergent evolution.

M-PM-J2

FUNCTIONAL ASPECTS OF SYNAPSIN. Martin Bähler, The Rockefeller Univ. New York, NY 10021.
The synapsins are a family of synaptic vesicle-associated phosphoproteins. They bind both to synaptic vesicles and to cytoskeletal elements. The binding of synapsin I to synaptic vesicles and to F-actin will be described. Both of these interactions are complex and are regulated by phosphorylation of synapsin I. Synapsin I represents a point of convergence between signal transduction pathways which generate the second messengers cAMP and calcium. A variety of physiological stimuli have been shown to alter the phosphorylation state of the synapsins. The synapsins may therefore regulate the positioning of synaptic vesicles in the presynaptic nerve terminal and thereby regulate neurotransmitter release.

M-PM-J3

MOLECULAR PROPERTIES OF A MEMBRANE ASSOCIATED MINIMYOSIN: BRUSH BORDER MYOSIN I. Mark S. Mooseker, Dept. of Biology, Yale University. The actin bundle within each microvillus of the avian intestinal brush border (BB) is tethered laterally to the membrane by bridges composed of a 110kD protein complexed with multiple molecules of calmodulin (CM). Results from several laboratories will be outlined which have shown that this complex is a mechano-chemically active myosin of the single-headed, tail-less myosin I class. The heavy chain of BB myosin I (BBMI) consists of a ~90kD myosin head domain homologous in primary structure to other myosin S_1 domains and a C-terminal 30kD, non-alpha-helical domain which contains the CM binding sites. Several lines of evidence will be summarized that indicate that the C-terminal domain, together with its associated CM light chains, may regulate the functional properties of this motor. For example, in vitro motility of BB myosin I-coated beads requires Ca++, but this Ca++ sensitivity is lost if a C-terminal specific antibody is used to couple BBMI to the bead. Like Acanthamoeba myosin I, BBMI binds with high affinity to anionic phospholipid vesicles; this interaction is through site(s) on the C-terminal domain of the heavy chain.

M-PM-J4

STRUCTURE AND FUNCTION CORRELATES FOR VINCULIN: AN AMPHITROPIC, CYTOSKELETAL PROTEIN. Susan W. Craig, Chelly Coutu, Barry Byrne, Yolanda Kaczworoski, and Candy de Berry. Dept. Biological Chemistry. Johns Hopkins Medical School, Baltimore, MD.

Several observations have been made that may lead to molecular understanding of the dynamics of vinculin assembly at the membrane and cytoskeleton-associated plaques of adherens junctions. Among these are the *in vitro* interaction of vinculin with acidic phospholipids, phosphorylation of vinculin *in vitro* and *in vivo*, and tissue-specific expression of both isoelectric and molecular weight variants of vinculin. To understand the structural basis for these observations and their relationship to the cellular properties of vinculin, we first determined the 1° structure of vinculin and the genetic basis for expression of vinculin and its muscle-specific isoform, meta-vinculin. There is a single gene that codes for both vinculin and meta-vinculin, and the 2 forms arise by alternative splicing of mRNA. The 1° structure of vinculin shows that there is a proline-rich (45%) and acidic residue-rich (25%) region that separates an NH_2-terminal 90 kd, acidic domain (pI_{calc} 5.4) from a carboxy-terminal 30 kd, basic domain (pI_{calc}=9.7). This feature suggests a structural basis for the interaction of vinculin with acidic phospholipids and a mechanism for shuttling vinculin between cytoplasm and membrane-associated junctional plaque. Experimental tests of the structural predictions will be presented.

M-PM-K1

NEW DEVELOPMENTS IN TIME RESOLVED FLUORESCENCE SPECTROSCOPY: ULTRA-HIGH FREQUENCY, PARALLEL ACQUISITION, AND ARRAY DETECTORS

E. Gratton. *UIUC, Lab. for Fluorescence Dynamics, Physics Dept., 1110 W. Green St., Urbana, IL 61801.*

Frequency domain fluorometry provides an alternative to correlated single photon counting fluorescence spectroscopy. Using microchannel plate detectors and the harmonic content of high repetition mode-locked lasers, measurements can be performed at frequencies as high as 10 GHz. Since all frequency harmonics are detected simultaneously, it is also possible to measure several frequencies at one time, increasing the overall instrument performance. One of the advantages of the frequency domain method is the conversion of the electrical signal to a low frequency domain, where signal processing can be accomplished using digital techniques. Also using a modulated image intensifier, the optical signal can be frequency converted to low frequency before it reaches the detector. This process allows the use of very slow detectors with a large number of elements, such as diode-array and CCD cameras. Examples of applications of novel methods will be presented. (PHS P41-RR03155)

M-PM-K2 *Monday Evening Workshop*

SOLUTION STRUCTURE DETERMINATION USING TIME RESOLVED FLUORESCENCE ENERGY TRANSFER: MILLISECOND MOTION PICTURES OF PROTEIN FOLDING.

Joseph M. Beechem, Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.

Fluorescence energy-transfer can determine intramolecular distance(s) in proteins over distances of 5-50Å (complementing 2-D NOE). There have been three major technical problems associated with this technique: 1) non-discrete donor-acceptor distances, 2) diffusion of the donor-acceptor pairs during the lifetime of the excited-state, 3) angular orientation changes. Recent work of Beechem & Haas (Biophys. J. 55:1225, 1989) have solved problems 1 & 2 (above). A plan of action for solving problem 3 has been outlined but is not yet complete (Beechem & Haas, Biophys. J. 55:124a, 1989). Even though problem 3 has not yet been completely solved, a new class of fluorescence energy-transfer experiments are being designed (and instrumentation is being assembled) which has the potential to provide structural information on sparesly populated (3-10%) transient (1-10 millisec) conformational species along protein folding (or other reaction) pathways. The key feature of this spectrometer involves a stopped-flow sample chamber, high repetition laser light excitation and parallel multi-anode microchannel plate detection and data storage. In this manner, picosecond time-resolved fluorescence decays can be collected in ~ 10 millisec. These experiments are not designed to duplicate other solution structure methods, but rather take advantage of properties unique to fluorescence spectroscopy/analysis. Support: JMB is a Lucille P. Markey scholar in biomedical science.

M-PM-K3

NEW EPR METHODS IN BIOPHYSICS

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The last few years have seen important new advances in EPR which have the possibility of revolutionizing the applications of EPR in biophysics. These include the following new methods developed in our laboratory: a) Two-Dimensional and Fourier Transform EPR; b) Far-Infrared EPR (with 1 mm. waves); c) Dynamic EPR Imaging of Diffusion; d) Powerful computational algorithms for spectral simulation. These new methods will be surveyed, and their implications for biophysical studies will be discussed.

M-PM-K4

ELECTRON SPIN ECHO (ESE) STUDIES OF METALLOPROTEINS AND ENZYMES,

HANS THOMANN, EXXON RESEARCH & ENGINEERING;

In the past decade, the ESE envelope modulation (ESE-EM) technique has matured into a quantitative spectrocopic probe of the coordination structure of active sites in metallo-proteins and enzymes. Advances in microwave hardware, in methods for data reduction, and in theoretical analysis have contributed towards this development. Current ESEEM and new pulsed Electron Nuclear Double Resonance (ENDOR) techniques based on ESE methods will be described. Applications to copper proteins (stellacyanin, hemocyanin); copper based enzymes (nitrous oxide reductase, cytochrome c oxidase); iron-sulfur proteins (ferredoxins); and iron-sulfur based enzymes (hydrogenases, nitrogenases) will be presented.

M-PM-K5

APPLICATIONS OF X-RAY ABSORPTION SPECTROSCOPY TO THE CHARACTERIZATION OF METALLOPROTEINS: POTENTIAL AND LIMITATIONS.

J.E. Penner-Hahn (Intro. by A.H. Beth). Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055.

Over the last ten years, x-ray absorption spectroscopy (XAS) has come to be a widely used technique for investigating the immediate structural environment of the metal sites in metalloproteins. The information which can be obtained from x-ray absorption, both in the extended x-ray absorption fine structure (EXAFS) region and in the x-ray absorption near edge structure (XANES) region will be reviewed. Different approaches to data analysis will be surveyed with emphasis on their relative ease of use, applicability, and reliability. The potential of polarized measurements for enhancing the information content of XAS will be discussed. Recent results will be described in order to highlight both the potentials and the potential limitations of the technique.

Supported in part by the NIH (GM-38047) and the Camille and Henry Dreyfus Foundation.

M-PM-L1

COMPARISON OF CORRESPONDING PROTEIN STRUCTURES DETERMINED BY NMR SPECTROSCOPY IN SOLUTION OR BY X-RAY DIFFRACTION IN SINGLE CRYSTALS.

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ETH-Hönggerberg, CH-8093 Zürich, Switzerland

The introduction of nuclear magnetic resonance (NMR) spectroscopy as a second method for protein structure determination (K. Wüthrich, "NMR of Proteins and Nucleic Acids", Wiley, New York, 1986) besides the well established X-ray diffraction technique with single crystals enables for the first time direct comparisons between corresponding protein structures in crystals and in solution. Such comparisons showed both close similarity between the structures in the different environments and significant differences in the molecular architectures, depending on the protein type studied. In many instances they also provided interesting insights into the potentialities and limitations of the two methods for structure determination. Examples to be discussed are cytochrome b5, the polypeptide hormone glucagon, metallothionein, the a-amylase inhibitor Tendamistat, and a group of procaryotic and eucaryotic DNA-binding proteins.

COMPARISON OF SOLUTION & CRYSTAL STRUCTURES OF PROTEINS & PROTEIN FRAGMENTS

M-PM-L2 *Monday Evening Workshop*

EVIDENCE FOR A NATIVE-LIKE HELIX IN THE ISOLATED C-PEPTIDE OF RNASE A. Robert L. Baldwin, Biochemistry Dept., Beckman Center, Stanford Univ., Stanford, CA 94305. The C-peptide provides an example of how an isolated peptide fragment can assume a structure close to the one it has in an intact protein. The results emphasize the role of local interactions in protein folding and the hierarchical nature of the folding process. The evidence is of three kinds. (1) Substitution experiments reveal the presence of two pairwise, helix-stabilizing interactions which correspond to structures found in the X-ray structure of RNase A, a $Glu2^-$ ...$Arg10^+$ salt bridge and a Phe8 ... $His12^+$ ring interaction. (2) NMR experiments provide independent evidence for these interactions. (3) Helix stop experiments show that the peptide helix is localized much as it is in intact RNase A. The nature of the C-terminal helix stop will be discussed. These findings are the work of Katie Armstrong, Robert Fairman, Susan Marqusee, John Osterhout, Andrew Robertson, David Schultz and Kevin Shoemaker, and in collaboration with Eunice York and John Steward at Denver and Jane Dyson and Peter Wright at La Jolla.

M-PM-L3

SOLUTION STRUCTURES OF FUNCTIONAL PEPTIDE FRAGMENTS OF ENZYMES. Albert S. Mildvan. Dept. Biol. Chem., The Johns Hopkins Medical School, Baltimore, MD 21205.

Synthetic peptides, based on amino acid sequences at the active sites of enzymes which retain the ability to bind substrates contain helices and some turns but are partially unfolded in solution. In the complete enzymes, the remainder of the protein thus provides structural support for the active site. A peptide based on residues 1-45 of rabbit muscle adenylate kinase binds MgATP with an affinity, and in a conformation similar to those found on the complete enzyme. NMR studies at 250 and 500 MHz indicate that this peptide is floppy and that 42% of the residues are in secondary structures corresponding to those of the crystalline enzyme (Biochem. 24, 4680; 27, 3588). A peptide consisting of residues 728-777 of DNA polymerase I binds both dNTP substrates and duplex DNA with affinities ~10^2-fold weaker than the complete enzyme (J.Biol. Chem., in press). 2D NMR studies at 600 MHz indicate that 46% of the residues are in secondary structures consistent with those in crystalline enzyme and reveal differences in tertiary structure. In contrast, a peptide consisting of residues 6 to 48 of Staphylococcal nuclease, containing all of the liganding residues for the essential Ca^{2+} and most of the active site residues shows little ability to bind divalent cations or substrates and little structure in solution. This region of the crystalline enzyme shows antiparallel β structure and loops, but no helices.

M-PM-L4

SOLUTION VERSUS CRYSTAL STRUCTURE: PLASTOCYANIN AND THIOREDOXIN. P.E. Wright, H.J. Dyson, G.P. Gippert, J.M. Moore and D.A. Case, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Three-dimensional solution structures have been determined by NMR for two proteins, plastocyanin and thioredoxin, and compared to the protein X-ray crystal structures. NMR structures were obtained for French bean plastocyanin, the X-ray for protein from poplar leaves. Despite amino acid substitutions at 21 of the 99 residues, the structures are remarkably similar, even at the level of surface side chain conformations. The largest differences occur in a region in which there are both multiple amino acid substitutions and crystal packing contacts. The NMR structure determined for reduced *E. coli* thioredoxin, which does not crystallize. Structural differences observed in the region of the active site appear to be associated with reduction of the disulfide bridge present in the oxidized thioredoxin of the crystal structure. Additional structural differences may be attributed to the binding of the Cu^{2+} ion necessary for crystallization.

M-PM-L5

COMPARISON OF SOLUTION AND CRYSTAL STRUCTURES OF STAPHYLOCOCCAL NUCLEASE.

D.A. Torchia, D.M. Baldisseri, H.B.R. Cole and S.W. Sparks, NIDR. L.W. Kay and A. Bax, NIDDK, NIH, Bethesda, MD 20892.

We have assigned the backbone nmr signals of the staphylococcal nuclease/pdTp/Ca^{2+} ternary complex. These assignments together with measurements of an extensive number of NOESY cross-peaks and NH/αH J couplings provide much information about the enzyme structure in solution. I will compare various aspects of the protein solution structure, derived from the nmr data, with the refined crystal structure of Loll and Lattman. In addition I will show that measurements of heteronuclear chemical shifts in solution and in crystals are a useful means of comparing protein structures in the two states. Finally, I will discuss the dynamical aspects of nuclease structure that have been derived from measurements of nuclease spin relaxation times and hydrogen-deuterium exchange rates.

M-Pos1

KINETICS OF THE LAMELLAR AND HEXAGONAL PHASE TRANSITIONS IN PHOSPHATIDYLETHANOLAMINE: A TIME-RESOLVED X-RAY DIFFRACTION STUDY USING A MICROWAVE-INDUCED TEMPERATURE-JUMP.

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The kinetics of the $L_{\beta'}/L_{\alpha}$ and L_{α}/H_{II} phase transitions in fully hydrated dihexadecylphosphatidylethanolamine (DHPE) has been studied. Low-angle time-resolved x-ray diffraction (TRXRD) was used to monitor progress of the transitions and microwave energy at 2.45 GHz was used to increase the sample temperature rapidly through the phase transitions. Both transitions were examined under active heating and passive cooling and were found to be repeatable, reversible and to have an upper bound on the time required to complete the transition of ≤ 2 s. Regardless of the direction both transitions appear to be two-state with no accumulation of intermediates to within the sensitivity limits of the TRXRD method.

M-Pos2

DIACETYLENIC LIPID MICROSTRUCTURES: CHARACTERIZATION BY X-RAY DIFFRACTION AND CALORIMETRY.

Martin Caffrey†, Alan S. Rudolph* and Jacqueline L. Hogan†.

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* Biomolecular Engineering Branch, Naval Research Laboratories, Washington DC 20375-5000.

Thermotropic and lyotropic mesomorphism in the polymerizable lecithin, 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine, and its saturated analogue 1,2-tricosanoyl-*sn*-glycero-3-phosphocholine has been investigated by wide- and low-angle x-ray diffraction of both powder and oriented samples and by differential scanning calorimetry. Previous studies have shown that the hydrated lipid forms novel microstructures (tubules and stacked bilayer sheets) in its low temperature phase. The diffraction results indicate that at low temperatures fully hydrated tubules and sheets have identical lamellar repeat size ($d_{001} = 66.4$Å) and crystalline-like packing of the acyl chains. A model for the polymerizable lecithin with 1) fully extended all-trans methylene segments, 2) a long-axis tilt of 32° and 3) minimal chain interdigitation seems most reasonable on energetic grounds, is consistent with the diffraction data (to 3.91Å resolution) and is likely to support facile polymerization. Chain packing in the lamellar crystalline phase is hydration independent. The conformation of the saturated lecithin appears to be similar to that of other saturated lecithins in the lamellar gel phase.

M-Pos3

KINETICS OF THE Ca^{2+}-INDUCED DISORDER/ORDER PHASE TRANSITION IN DIOLEOYLPHOSPHATIDYLSERINE (DOPS) MONITORED BY TIME-RESOLVED X-RAY DIFFRACTION (TRXRD).

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The kinetics of the lamellar disorder-to-order phase transition in dry and in fully hydrated DOPS was monitored by small-angle TRXRD. The transition was initiated by a rapid jump in Ca^{2+} concentration from 0 to ≥ 0.1M $CaCl_2$ effected by mechanical means. Following the Ca^{2+}-jump, the diffuse low-angle scattering at 53.5Å from hydrated DOPS converts to a sharp reflection at 50.0Å within 8s and then slowly relaxes to an equilibrium value of 51.8Å in the following 1.5 min. With dry DOPS, the Ca^{2+}-jump triggers hydration *and* a Ca^{2+}-induced phase transition. Following the jump, the diffuse peak at 40Å converts to a sharp lamellar reflection at 51Å within 10s. Subsequently, a second lamellar reflection at 72Å emerges which slowly disappears to leave a single lamellar crystalline phase (d = 51.9Å).

M-Pos4

A CENTRALIZED DATABASE FOR THERMODYNAMIC DATA ON LIPID MESOMORPHIC PHASE TRANSITIONS AND MISCIBILITY

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The wealth of lipid mesomorphic phase transition temperature (T_t) and enthalpy data, traditionally scattered throughout the literature, has now been compiled on a microcomputer database. The database comprises roughly 4000 transition temperatures and enthalpies and is easily searched for these data, along with accompanying database fields, such as method used for T_t determination, aqueous phase composition and pH, and bibliographic information. A mesophase nomenclature system has been devised to allow accurate and uniform identification of the many mesophases reported in the literature. The database runs on either a Macintosh or a PC and has also been produced in hardcopy form. Standard data reporting sheets and data abstracting guidelines have been developed so that, in the future, workers may report their data directly to the database, thereby ensuring further database completeness and veracity.

M-Pos5

EXAMINATION OF THE STRUCTURES OF MEMBRANE BOUND GLYCOLIPIDS BY ORIENTED SAMPLE NMR METHODS.

Charles R. Sanders II and James H. Prestegard

The conformation and dynamics of the dissacharide headgroups of glycolipid analogs, β-dodecyl melibiose and β-dodecyl lactose, were examined by interpreting the deuterium NMR spectra arising from the deuterated analogs in structural terms using AMBER (S. S. Weiner et. al. J. Comp. Chem. (1986) 7 230-252) and the "NMR Pseudoenenergy Approach" (P. Ram and J. H. Prestegard, J. Am. Chem. Soc (1988) **110** 2383-2388). The different glycosidic linkages between the terminal galactose and the β-alkyl glucoside of the two molecules (α-1-6 for the melibioside, β-1-4 for the lactoside) was found to result not only in significant differences in the relative configurations of the two sugars comprising the head groups of the two but also in different orientations for the headgroups as a whole with respect to the membrane interface. The affect of the presence of both positively and negatively charged amphiphiles in the phospholipid matrix upon the structures of the glycolipids was also examined.

M-Pos6

LIGHT INDUCED MODULATION OF ELECTRIC OSCILLATIONS IN A LIPID MEMBRANE SYSTEM.

H. Nakanishi and K. Seki, Advanced Res. Lab., Toshiba R & D Center, 1 Komukai Toshiba-cho, Saiwai-ku, Kawasaki 210, JAPAN.

We propose a simple biomimetic model of the retina which converts the light signals to electric signals through the structural change of the membrane.

The photoresponsive membrane was prepared by impregnation of the lipid mixture, 4'-n-octylazobenzene-4-oxybutyric acid(AZ) and monoolein, to a porous polyester filter. To measure the electric oscillation across the membrane, Pt electrodes immersed in 0.1M KCl solution were fixed at both sides of the membrane.

The frequency was found to be decreased by UV irradiation and regained by VIS irradiation, corresponding to the cis-trans reaction of the azobenzene group. Similar change was observed for the membrane resistance and capacitance. Therefore it was clarified that this reversible change of the frequency was due to the change of the membrane structure initiated with the photoreaction.

This work was supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

M-Pos7

A NUCLEAR MAGNETIC RESONANCE STUDY OF DIACETYLENIC PHOSPHOLIPID LIPOSOMES.

G.L. Jendrasiak, Radiation Oncology, East Carolina University, and M. Nagumo, Code 6090, Naval Research Laboratory, Washington, DC.

A nuclear magnetic resonance (NMR) study of 1,2-bis (10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine liposomes was made. At 50°C, the proton spectrum is a high resolution spectrum similar to that from egg phosphatidycholine SUV's. The proton peaks arising from both the first and second methylene groups of the hydrocarbon chains are split. The choline head-group peak is also split and anion addition indicates that this splitting arises from head-groups interior and exterior to the SUV, as has been found for other SUV systems. The phosphorus NMR signal suggests an isotropic lipid phase. Upon lowering the temperature to approximately 3°C, the high resolution NMR spectrum is maintained. The methylene proton peak splittings disappear as the temperature is lowered, consistent with infrared spectroscopy measurements made by others. The choline group splitting increases as the temperature is lowered suggesting that the SUV's are decreasing in size. Previously reported dynamic light scattering measurements show a decrease in size consistent with the increase in the choline signal splitting.

M-Pos8

SOLUBILITY OF INERT GAS IN BLOOD PLASMA, BLOOD SUBSTITUTE, AND LIQUID PERFLUOROCHEMICALS.

Richard P. Kennan, Gary T. Holm, and Gerald L. Pollack, Department of Physics and Astronomy, Michigan State University, East Lansing, MI 48824.

We report measurements of the Ostwald Solubility (L) of the inert gas xenon in the following liquids: (a) recovered blood plasma, (b) a perfluorotributylamine emulsion (Oxypherol,TM) which can transport and exchange oxygen in living systems, (c) perfluorotributylamine, (d) three perfluoroalkanes (n-C_6F_{14}, C_7F_{16}, C_8F_{18}), and (e) water. Experiments were carried out using the gamma-emitting isotope Xe-133. Measurements of L(T) are reported for blood plasma in the approximate range 20-37°C, for water and perfluoroalkanes from 5-50°C, and appropriate temperature ranges for the other liquids. Results will be analyzed and discussed in terms of modern statistical mechanics and thermodynamics, including additivity of components, interactions in the liquids, and enthalpies and entropies of solution.

Supported by ONR-NMRDC Contract No. N00014-88-K-0287.

M-Pos9

SIZING OF PHOSPHOLIPID-BILE SALT MIXED MICELLES BY SIZE-EXCLUSION HPLC. J. Wylie Nichols and Justyna Ozarowski. Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

We have demonstrated the usefulness of size exclusion-HPLC (TSK 5000PW column) for determining the size of phospholipid (PL)-bile salt (BS) mixed micelles. To avoid altering the equilibrium between aqueous soluble and micellar BS the column was pre-equilibrated with buffer containing the aqueous soluble concentration of the BS. Globular proteins of known size and small unilammelar vesicles (size determined by QELS) were used to calibrate the column. Micelle sizes determined for several different BS-PL mixtures were consistent with those obtained by other techniques (e.g., QELS, NMR, and SANS, small angle neuron scattering). We found that the measured dependence of mixed micelle size on the PL to BS ratio could be modeled equally well by a new model based on the elongation of PL-BS rods with BS-rich caps, which is consistent with recent SANS data (Hjelm et al. [1988], J. Appl. Cryst. 21:858-863) as the currently accepted mixed disk model in which bile salts and phospholipids interact to form a mixed bilayer disk which is surrounded by bile salts (Mazer et al. [1980], Biochemistry 19:601-615). Supported by USPHS Grants DK40641 and GM32342.

M-Pos10

LYSOPHOSPHOLIPIDS ASSOCIATE WITH SUBMICELLAR CONCENTRATIONS OF BILE SALTS. David G. Shoemaker and J. Wylie Nichols, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322

We used the environmental sensitivity of the fluorescent probe N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1-acylphosphatidyl-ethanolamine (N-NBD-lysoPE) to monitor its association with taurodeoxycholate (TDC) and cholate (C) in solution. The aggregation and micellization of the bile salts were monitored by light scattering. Increased fluorescence yield was used to measure TDC association with a homologous series of N-NBD-lysoPE's at submicellar concentrations. Half maximal increase in fluorescence yield was obtained at the following [TDC] (mM): 1.4, laurylPE (LPE); 1.0, myristoylPE (MPE); 0.55, palmitoylPE (PPE); 0.41, stearoylPE (SPE). Maximal fluorescence in all cases was obtained at [TDC] below the CMC (2.8 mM). The analogous [C] were (mM): LPE, 6.4; MPE, 3.9; PPE, 2.2; SPE, 1.0. Again, maximal fluorescence always occurred below the CMC (12 mM). The free fluorophore, NBD, did not associate with submicellar bile salt concentrations. These results indicate bile salt and lysophopholipid associate at concentrations where both species exist predominantly as monomers. Supported by USPHS Grants DK 40641 and GM 32342.

M-Pos11

RESOLUTION OF κ^2 (ORIENTATION) DISTRIBUTIONS IN RIGID STEROID COMPOUNDS VIA FREQUENCY-DOMAIN FLUORESENCE ENERGY TRANSFER MEASUREMENT. J. Lakowicz, W. Wiczk, M. Fishman (Univ. of Maryland School of Medicine) and R. Haugland (Molecular Probes, Inc.)

Two similar steroids in which a fluorescent donor (N-methylindole, fused to the C2:C3 bond) and an energy transfer acceptor (carbonyl) are separated by the length of the steroid rings were prepared. In one ("more rigid") the carbonyl is fused directly to C17 (-17-one) but the other is 17-acetyl.

Transfer in the more rigid compound was found to be more efficient than in the latter, where the acceptor dipole has rotational (κ^2) freedom and slight distance variation. The measured data are interpreted using transfer efficiency calculations which incorporate donor/acceptor orientation and distance probability distributions based on geometry, calculated (CHARMm) energy, and the positions of the dipoles with respect to the indole and carbonyl moieties.

M-Pos12

THERMODYNAMIC CHARACTERIZATION OF THE INFLUENCE OF Ca^{2+} ON DILAUROYLPHOSPHATIDYL-ETHANOLAMINE. J. Phiri, D. Patterson, and J.M. Collins, Marquette University, Milwaukee, WI, L.J. Lis, The Chicago Medical School and VA Medical Center, N. Chicago, IL, and W. Tamura-Lis, University of Nebraska Medical Center, Omaha, NE.

The thermodynamic properties of dilauroylphosphatidylethanolamine(DLPE) were determined in water and low molarity $CaCl_2$ solutions using DSC and X-ray diffraction. In all cases, the subgel phase was induced as the initial bilayer phase. The Ca^{2+} interaction with DLPE appears to be biphasic. Low molarity $CaCl_2$ solutions caused the main phase transition to become less thermodynamically distinct with low transition enthalpies. However, in the presence of 50mM $CaCl_2$, DLPE bilayers underwent two phase transitions, as was observed for DLPE in water. Static x-ray diffraction patterns collected at various temperatures indicate that the transitions described calorimetrically were reversible. Real time x-ray diffraction studies provide information about the nature of the two DLPE phase transitions observed in water and 50mM $CaCl_2$.

M-Pos13

INTERACTIONS OF OLEOYL-COENZYME A WITH PHOSPHOLIPID BILAYERS AND ALBUMIN. John G. Boylan and James A. Hamilton, Biophysics Department, Boston University School of Medicine, Boston, MA 02118.

Binding interactions of [1-^{13}C] oleoyl-coenzyme A (OCOA) with egg phosphatidylcholine (EYPC) and bovine serum albumin (BSA) were studied by ^{13}C NMR. Carbonyl chemical shifts were different for aqueous OCOA micelles, OCOA bound to EYPC, and OCOA bound to BSA. A single peak was observed for micellar OCOA, with a concentration-independent (1-20 mM) chemical shift (201.5 ppm). One major peak (200.1 ppm) was seen from both cosonicated mixtures of OCOA/EYPC and preformed EYPC vesicles mixed with micellar OCOA. 3-4 major peaks (197.0-203.5 ppm) were observed for OCOA bound to BSA at molar ratios (OCOA:BSA) $\geq$ 1:1. When OCOA was added to BSA with bound (1-^{13}C) oleic acid (OA), the distribution of OA in its various binding sites on BSA changed. Similarly, the binding distribution of OCOA to BSA changed when OA was added to mixtures of OCOA and BSA. Because of the thiol linkage, the chemical shifts of OCOA in the systems studied are far downfield from other carbonyl-containing intermediates or products of fatty acid metabolism are and thus readily distinguishable in complex systems.

M-Pos14

ORIENTATIONAL ORDER AND MAGNETIC ALIGNMENT OF DPPC BILAYERS CONTAINING PALMITOYL-LYSOPHOSPHATIDYLCHOLINE. Mikael Jansson, Robin L. Thurmond, Theodore P. Trouard, and Michael F. Brown. Department of Chemistry, University of Arizona, Tucson, AZ 85721.

Mixed bilayers of 1-palmitoyl-*sn*-glycero-3-phosphocholine (PaLPC) and dipalmitoylphosphatidylcholine (DPPC) have been investigated by ^{2}H and ^{31}P NMR spectroscopy. The two mixed systems studied were PaLPC-d$_{31}$/DPPC (1:3) and PaLPC/DPPC-d$_{62}$ (1:3), each in a 50 wt.% H_2O dispersion. At temperatures below the main phase transition the mixed PaLPC/DPPC bilayers appear to coexist with PaLPC micelles. These micelles disappear at temperatures above the transition, where bilayers in the liquid-crystalline state are formed. The orientational order of the alkyl chains of the PaLPC component of the mixed system is essentially identical to that of the DPPC component, but is less than in the pure DPPC system. Magnetic alignment of the mixed liquid-crystalline bilayers was also observed in which the director axis was perpendicular to the magnetic field.

Work supported by grants GM41413 and EY03754 from the NIH.

M-Pos15

A HEXAGONAL-LAMELLAR-HEXAGONAL DOUBLE TRANSITION OF DIOLEYLPHOSPHATIDYLETHANOLAMINE (DOPE). CHARACTERIZATION UNDER OSMOTIC AND THERMAL PERTURBATION.

Klaus Gawrisch, V. Adrian Parsegian, NIH
R. Peter Rand, Nola Fuller, Brock University
Mark W. Tate, Damian Hajduk, Sol M. Gruner, Princeton Univ.

Changes in surface topology of lipid assemblies have often been postulated to occur in cell membrane processes. Our three laboratories have independently observed the transformation of DOPE, at fixed temperature, successively from an inverted hexagonal form to a lamellar phase then, contrary to dogma, back to hexagonal with progressive dehydration. Lower temperatures favor the lamellar form. By osmotic stress, we measure directly the work needed to effect phase transformation. This work turns out to be remarkably small, less than one kT per lipid molecule, and consonant with the temperature sensitivity and small enthalpy of the transition. The small amount of energy required for transformation suggests that changes in surface topology can be sensitive to many kinds of small perturbation such as the introduction of charged lipids, the binding of cations or anions, varying polar group charge through changes in pH. If one invokes transient changes in the surface continuity as part of a biological process such as membrane fusion, one can now imagine how lipid systems can be tuned to make this an energetically reasonable event.

M-Pos16

MEMBRANE DIPOLE POTENTIALS AND HYDRATION FORCES: CAUSE OR EFFECT?

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Ever since the identification and characterization of hydration forces as the dominant interaction between membranes or macromolecules approaching contact, there has been a serious need to know the properties of the molecular surfaces that establish hydration. It is thought that the presence of oriented dipoles at the membrane surfaces gives rise to a "dipole potential". A recent proposal (Biophys. J. 55:248a) asserts the existence of a correlation between the square of the "dipole potential" measured on monolayers at an air-water interface and the coefficient of the hydration force between bilayers of the same lipid. There are many possible dipoles in a bilayer membrane:boundary water, phospholipid carbonyl groups, zwitterionic polar groups may play a role.We therefore investigated the hydration of DPPC and its ether analogue DHPC (same head and tails, sans carbonyl) with NMR and small angle x-ray scattering. We expect a difference in dipole potential of 100 mV based on literature values for similar lipids. Experiments are in progress to measure the exact potentials from transbilayer conductance of lipophilic ions.Despite the expected difference in the dipole potentials, we found no detectable difference in the repulsive hydration force,the headgroup orientation, or the mobilities.Water ordering does not appear to be the result of a preexisting electric dipole potential.Water molecules in the first hydration layer are ordered by hydrogen bonding with the lipid headgroups.It would appear the change in water arrangement enforced by these headgroups is the source of the hydration force and that the "dipole potential" can come in good part from oriented water molecules.

M-Pos17

LYSOPHOSPHATIDYLCHOLINE CRITICAL MICELLE CONCENTRATION MEASURED BY PHOSPHORUS-31 NMR IN THE PRESENCE OF LANTHANIDE IONS. V.V. Kumar and Wolfgang J. Baumann, The Hormel Institute, University of Minnesota, Austin, Minnesota 55912.

The ^{31}P NMR spectra of lysophosphatidylcholines (lysoPC) in aqueous medium are shifted downfield by Pr^{3+}. However, the observed downfield shifts for the longer-chain lysoPC's (C_{12}lysoPC to C_{18}lysoPC), which exist in the aggregated state, were more than five times greater than those observed for the shorter-chain lysoPC's (C_6lysoPC, C_8lysoPC), which exist as monomers. The effect can be utilized to determine lysoPC critical micelle concentration (CMC) based on the break in the downfield chemical shift-versus-lysoPC concentration plot. The CMC values we determined (C_8lysoPC, 60 mM; C_{10}lysoPC, 6 mM; C_{12}lysoPC, 0.5 mM) are in good agreement with those measured by other physical techniques. The much larger downfield shift exerted by Pr^{3+} on lysoPC micelles than monomers can be ascribed to differences in phosphocholine bonding and in head group conformations in the two dispersion states. (Supported by NIH Grant HL08214 and the Hormel Foundation)

M-Pos18

POLYMORPHIC PHASE BEHAVIOR OF A HOMOLOGOUS SERIES OF 1,2-DI-O-ACYL-3-O-(α-D-GLUCOPYRANOSYL)-*SN*-GLYCEROLS. A. Sen and S.W.Hui, Membrane Biophysics Laboratory, Roswell Park Memorial Institute, Buffalo, N.Y. 14263 & D.A. Mannock, R.N.A.H. Lewis and R.N. McElhaney, Department of Biochemistry, University of Edmonton, ALberta, Canada.

X-ray diffraction methods were used to characterize the thermotropic polymorphism of a homologous series of 1,2-di-O-acyl-3-O-(α-D-glucopyranosyl)-*sn*-glycerols. Below chain melting temperatures these compounds form lamellar L_β phases which after suitable low temperature annealing form highly ordered, lamellar L_c phases. The diffraction patterns obtained from the L_c phase were similar for all of the compounds studied, suggesting that the unit cell structure is invariant. This assumption makes the assignment of phase to the diffraction lines, and the calculation of electron density profiles, possible. The results of these studies indicated that the L_c phases of these lipids are poorly hydrated structures with the hydrocarbon chains inclined at 35° to the bilayer normal. Above chain melting temperatures, the diffraction patterns of these lipids changed to those characteristic of either lamellar liquid crystalline (L_α) phase ($N \leq 12$), inverted cubic (Pn3m) phase (N = 13 to 16), or inverted hexagonal (H_{II}) phase (N = 17 to 20).

M-Pos19

DIDODECANEPHOSPHATE (DDP) COMPOUNDS ALLOW INVESTIGATION OF THE CONTRIBUTION OF PHOSPHATE GROUPS TO THE INTERACTION OF BIMOLECULAR LAYERS. Y. Fang and R.P. Rand, Biological Sciences, Brock University, St. Catharines, Canada.

In water, the tetramethylammonium salt of DDP forms lamellar phases, over a large concentration range. Its unusually high order is indicated, for example, by the formation of a 210 Å lamellar phase with six orders of sharp X-ray reflections. That order decreases with increasing ionic strength. Osmotic stress of this lamellar phase, at 22°C, yields a force vs bilayer separation that can be described by electrostatic repulsion from 300 Å to 50 Å, and by a hydration repulsion from about 30 Å to 10 Å. The system forms three dimensional crystals with further dehydration. There are surprising changes from frozen to melted and back to frozen hydrocarbon chains as the multilayers are osmotically stressed. Whether this results from a very sensitive dependence of chain melting on surface charge density is being pursued. The sodium salt of DDP forms completely dehydrated multilamellar phases in excess water, indicating little dissociation of this salt.

M-Pos20

PHOSPHORYL TRANSFER BY ADENYLATE KINASE COUPLED TO GLYCOLYTIC FLUX IN SKELETAL MUSCLE

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Rates of adenylate kinase (AK)-catalyzed phosphoryl transfer were measured in intact rat diaphragm equilibrated with [^{18}O]water. In non-contracting muscle AK activity is limited to 1/1000 of the estimated V_{max} (cellfree), apparently because of restricted ADP availability, and is localized in subcellular compartments that increase in size and/or number with contractile frequency. Contraction also causes frequency-dependent increments in AK velocity (22-fold at 4 Hz) as does oxygen deprivation (30- to 50-fold). These enhanced rates of AK activity, equivalent to processing all the cellular ATP and ADP in approximately a minute, occur when levels of ATP, ADP and AMP are maintained near their basal steady states. Over a range of contractile frequencies, during O_2 deprivation or when oxidative phosphorylation is uncoupled there is a stoichiometric relationship between the rates of lactate formation and AK catalyzed phosphoryl transfer. These results conform to a proposed scheme whereby the AK system functions to transfer, as β-ADP, high energy phosphoryls generated by glycolysis and thereby coordinates ATP use with synthesis. (GM 28818)

M-Pos21

TUMOR MITOCHONDRIAL HEXOKINASE: cDNA CLONING, AMINO ACID SEQUENCE AND OVEREXPRESSION IN E. COLI. OF AN ACTIVE, BINDABLE FORM,

Krishan K. Arora, Maurizio Fanciulli and Peter L. Pedersen, Dept. of Biological Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

Previous studies from this laboratory have shown that in rapidly growing tumor cells exhibiting high glycolytic rates, the hexokinase activity is markedly elevated and is also bound in large amounts to the outer mitochondrial membrane. In our most recent work, we have isolated a full length cDNA clone encoding tumor hexokinase from a highly glycolytic mouse hepatoma cDNA expression library. This clone has been completely sequenced and overexpressed as an active hexokinase enzyme in E. coli. The overexpressed enzyme binds to the outer mitochondrial membrane in the presence of MgCl2. With the overexpression system in hand, it should now be possible to test directly using molecular genetic tools current views about putative catalytic, regulatory, and membrane binding domains of hexokinase, as well as views about the evolution of eukaryotic forms of this enzyme. [Work supported by Grant CA 32742 from the NIH to PLP].

M-Pos22

NOVEL FLUOROGENIC SUBSTRATES FOR ASSAYING RETROVIRAL PROTEOLYTIC ACTIVITY BY RESONANCE ENERGY TRANSFER

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The 11kD protease (PR) encoded by the human immunodeficiency virus 1 (HIV) is essential for the processing of viral polyproteins and the maturation of infectious virus, and is therefore a target for the design of selective AIDS therapeutics. To facilitate the identification of novel inhibitors of HIV PR as well as to permit detailed studies on the enzymology and inhibition of HIV PR, we developed a continuous assay for its activity based on intramolecular fluorescence resonance energy transfer (RET). The fluorogenic substrate for HIV PR undergoes a 40-fold increase in quantum yield upon proteolytic cleavage. Incubation of recombinant HIV PR with the fluorogenic substrate results in a time-dependent increase in fluorescence intensity which is linearly related to the extent of substrate hydrolysis. We also designed an internally quenched fluorogenic substrate for the related protease from avian myeloblastosis virus (AMV). Because of its simplicity, rapidity, and precision in the determination of reaction rates required for kinetic analysis, this method offers many advantages over the commonly used HPLC- or electrophoresis- based assays for peptide substrate hydrolysis by retroviral proteases. Examination of the known structures of aspartic proteinases suggests that the design of fluorogenic peptide substrates and RET-based assays should be applicable to many members of this large family of related viral and eucaryotic enzymes.

M-Pos23

ENZYME CATALYSIS AT LOW WATER CONCENTRATIONS AND HIGH TEMPERATURES. THE ATPase OF SUBMITOCHONDRIAL PARTICLES IN ORGANIC SOLVENTS.

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The ATPase of bovine heart submitochondrial particles in a system composed of toluene, phospholipids and 15% Triton X100 (v/v) (TPT) hydrolysed ATP when the concentration of water was above 0.5% (v/v); the rate of hydrolysis increased as the concentration of water was raised to 6.0%. In the TPT system at water concentrations below 2.0%, the half-life of the enzyme at temperatures of 70° to 85°C was orders of magnitude higher than in all water media. Apparently the enhancement of catalytic rates by water is due to a higher conformational mobility of the enzyme, but water also decrease of enzyme thermostability. Through experiments at various temperatures and water concentrations, it was found that in the TPT system with 1.6% water, the enzyme could catalyze ATP hydrolysis at 91°C for as long as 40 min. This work was supported by grants from CONACyT (México) and the Organization of American States.

M-Pos24

EXAMINATION OF POSSIBLE SUBUNIT STRUCTURE OF THE TRANSVERSE TUBULE Mg^{2+}-ATPase.
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There exists a Mg^{2+}-ATPase localized in the transverse tubule in skeletal muscle, the function of which has yet to be elucidated. This enzyme was previously demonstrated to be structurally distinct from the (Na,K)-ATPase and the sarcoplasmic reticulum Ca^{2+}-ATPase (Kirley, J. Biol. Chem. 263, 12682-12689 (1988)). The Mg^{2+}-ATPase contains a 100 KDa ConA and WGA reactive glycoprotein. Most preparations also contain other proteins, including a 45 KDa protein. In order to investigate the possible subunit structure of this enzyme, various purification schemes were investigated and cross linking experiments were performed. Some results indicated that the 45 KDa protein may be a subunit of the Mg^{2+}-ATPase, since it was present in amounts nearly equal to the 100 KDa protein. The glycosylation states of the 100 KDa (α) and 45 KDa (β) putative subunits were also examined. The experimental results will be discussed with respect to the ease of inactivation of the Mg^{2+}-ATPase by detergents. Supported by National Institute of Health grant RO1 AR38576.

M-Pos25

ELECTROSTATIC CONTROL OF ACETYLCHOLINESTERASE REACTIVITY by H. A. Berman and K. Leonard, Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, New York 14260

One of the distinguishing features of acetylcholinesterase (AchE) is its high net negative charge, which is distributed asymmetrically at the active center and at a peripheral anionic site (PAS) remote from the active center. This paper examines the functional significance of this negative charge distribution, and focuses on *covalent* reactivity of the active center towards charged and uncharged substrates and irreversible organophosphorus inhibitors. The kinetics of reversible antagonism of covalent reaction at the active center by active center- and PAS-directed ligands are straightforward and display linear behaviour when measured with respect to cationic substrates and inhibitors. The salient finding is that antagonism is absent or complex, displaying non-linear behaviour, when measured against uncharged substrates and inhibitors; in these cases the propensity for allosteric ligand interactions is greatly muted. Taken with localization of AchE in the ionic environment of the synaptic cleft, these studies reveal a functional role for the enzyme net negative charge in *regulation* of catalytic activity, and provide an electrostatic mechanism through which the peripheral anionic site mediates this regulation.

M-Pos26

QUANTITATIVE ANALYSIS OF PHOSPHOLIPASE A_2 ACTIVATION MECHANISMS. J. D. Bell, T. R. Heimburg and R. L. Biltonen. Depts. of Biochemistry and Pharmacology, University of Virginia, Charlottesville, VA 22908.

The hydrolysis of dipalmitoylphosphatidylcholine large unilamellar vesicles by the monomer aspartate-49 phospholipase A_2 from Agkistrodon piscivorus piscivorus is a complex function of time. Initially, the rate of hydrolysis is very slow and gradually increases with time as the enzyme slowly becomes activated. When a specific mole fraction of the lipid has been hydrolyzed, the catalytic rate of the enzyme suddenly increases 100 to 1000-fold. This rapid activation appears to result from a cooperative change in the structure of the vesicle bilayer which can be monitored with the fluorescent probe trimethylammonium diphenylhexatriene. We have derived mathematical models of three hypotheses describing the mechanism of phospholipase activation and the coupling to the membrane structure change: activation by 1) enhanced binding, 2) shift of a preexisting equilibrium, and 3) a kinetic process requiring the membrane surface. We have compared computer simulations of these models to actual time courses of vesicle hydrolysis and concluded that the kinetic activation model is most viable. The role of enzyme dimerization is also considered.

M-Pos27

PRELIMINARY CHARACTERIZATION OF CLONED HUMAN RENIN AND PRORENIN EXPRESSED IN YEAST AND CHO CELLS. T.F. Holzman*, C. Chung, D. Egan, E. Gubbins, A. Rueter, L. Yang, T. Pederson, G. Krafft, G. Wang.

Renin is an aspartyl proteinase produced, in vivo, as the proenzyme, prorenin. Renin catalyzes the cleavage of the N-terminal decapeptide from angiotensinogen to form angiotensin I. Angiotensin I is subsequently converted to the octapeptide angiotensin II which functions as a vasoconstrictor and Na^+ regulator. A thorough understanding of the mechanism of action of renin and its protein biochemistry is essential to the design and characterization of renin inhibitors as anti-hypertensive agents. Recombinant DNA technology was used to express Human Renin in Sacchromyces cerevisiae and Human Prorenin in CHO cells. In this work we describe the purification of analytical and preparative quantities of Renin and Prorenin and preliminary characterization of these proteins for determination of their three dimensional structure.

M-Pos28

STUDIES OF SINGLE AND DOUBLE MUTANTS AT THE ACTIVE SITE OF STAPHYLOCOCCAL NUCLEASE (SN). D.J. Weber, E.H. Serpersu, A.S. Mildvan. Dept. of Biol. Chem., Johns Hopkins Medical School, Baltimore, MD 21205.

The $10^{4.8}$-fold reduction in V_{max} of the R87G mutant of SN with DNA as substrate has been ascribed to the loss of transition state stabilization and general acid catalysis, while the 10^{3}-fold decrease in V_{max} of the D21E mutant has been ascribed to partial occlusion of the binding site for the attacking water molecule (Biochem. 28, 1289, 1987). V_{max} of the double mutant R87G + D21E is decreased by a factor of only $10^{4.2}$ suggesting antagonistic structural effects of the R87G and D21E mutants. Depending on the properties examined, the effects of the single mutations interact differently in the double mutant. Mn^{2+} and Ca^{2+} affinities of the double mutant parallel those of the wild type and D21E enzymes, while pdTp affinities in binary and ternary complexes are 10^{2}- to 10^{3}-fold weaker with the double mutant than with wild type or with either single mutant. The dinucleotide, pdTdA is bound more weakly by the single mutants than by wild type, and the effects of these mutations are multiplicative in the double mutant. A stepwise mechanism involving attack of water on phosphorus followed by protonation of the leaving group by Arg-87 is unlikely, since no enzyme-bound phosphorane intermediate (<1% of [enzyme]) was found under steady-state conditions on the R87G mutant by ^{31}P NMR at 243 MHz.

M-Pos29

ADDITIVE EFFECTS OF MUTATIONS OF CATALYTIC RESIDUES ON THE KETOSTEROID ISOMERASE (KSI) REACTION. A. Kuliopulos, P. Talalay, and A.S. Mildvan. Depts. Biol. Chem. and Pharmacol. Johns Hopkins Medical School, Baltimore, MD 21205.

KSI catalyzes the isomerization of Δ^5-3-ketosteroids to Δ^4-3-ketosteroids by a conservative tautomeric transfer of the 4β-proton to the 6β-position using Tyr-14 as a general acid and Asp-38 as a general base (Biochem. 28, 149, 1989). Kinetic isotope effects on k_{cat} and k_{cat}/K_M, on deuterating the 4β position of the substrate and the aqueous solvent, establish concerted general acid base-catalysis (Xue et al., Abs. 198th A.C.S. Mtg., 1989, ORGN-59), consistent with the anti-orientations of Tyr-14 and Asp-38 with respect to the enzyme-bound substrate. The product of the fractional k_{cat} value resulting from the Y14F mutation ($10^{-4.7}$) and the D38N mutation ($10^{-5.6}$) is comparable ($10^{-10.3}$) to that of the double mutant Y14F + D38N ($\leq 10^{-9.8}$), indicating that total enzymatic activity results from the independent functioning of Tyr-14 and Asp-38 in the same chemical step. Such simple additivity of the effects of mutations on k_{cat} occurs when the mutated residues act concertedly, i.e., in the same chemical step. While completely inactive, the double mutant enzyme tightly binds steroid substrates and substrate analogs and holds a spin labeled steroid in an orientation indistinguishable from that found on the wild type enzyme.

M-Pos30

Investigation of Angiotensinogen-Renin Interactions by a Continuous Fluorescence Assay.

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Renin is an important enzyme that catalyzes the hydrolytic conversion of the plasma glycoprotein angiotensinogen to angiotensin I. Subsequent hydrolysis by angiotensin converting enzyme (ACE) produces the potent vasoconstrictor octapeptide angiotensin II. In this paper, we describe experiments directed towards elucidation of the interactions of the natural substrate angiotensinogen and its hydrolysis product des-angiotensinogen with renin. Our results indicate that des-angiotensinogen interacts with renin to form a catalytic system with different kinetic properties than purified human renin or recombinant human renin, with respect to hydrolysis of synthetic peptide substrates. We also describe studies of renin inhibitors with renin and with the renin-des-angiotensinogen system. These experiments utilized a recently developed assay involving a fluorogenic renin-specific peptide substrate that permits continuous monitoring of renin hydrolytic activity.

M-Pos31

REACTION KINETICS AND THERMODYNAMICS OF NAD-NADASE IN THE TEMPERATURE RANGE OF 4 C to 37 C BY STOPPED-FLOW MICROCALORIMETRY

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The reaction of NAD-NADSE has been extended to the temperature range of 4C to 37C. Various buffers and pH's have been used in order to examine various possible reaction temperature range. Since the free energy is negative, the entropy is positive and such reactions are often termed "Entropy Driven". This reaction would appear to be a classic example.

M-Pos32

PREPARATION AND CHARACTERIZATION OF CYTOCHROME c COVALENTLY MODIFIED WITH EDTA. Donald J. Hirsh and Gary W. Brudvig, Department of Chemistry, Yale University, New Haven, Connecticut 06511.

Derivatives of cytochrome c have been synthesized by forming an amide bond between one of the carboxylic acid groups of EDTA and the terminal amino group of a surface lysine using the carbodiimide, EDC. The protein-bound EDTA moiety retains the high affinity of free EDTA for di- and trivalent metal ions. Singly-labeled derivatives of cytochrome c have been isolated by ion exchange chromatography. The EDTA-labeled derivatives scavenge even trace quantities of metal ions. This necessitated the addition of a specific divalent (or trivalent) metal ion to the ion exchange eluant to insure that differences in retention time were a function of the site of covalent modification of the protein and not which metal ion was bound. A derivative labeled at Lys-13 has been identified by tryptic hydrolysis and reverse phase HPLC. Elution in the presence of excess Cu(II) yielded the Cu(II)-EDTA derivatives. After dialysis to remove nonspecifically-bound copper the derivatives were found to contain Cu(II) in a 1:1 ratio to heme iron. These derivatives are being used to study dipolar interactions between the Cu(II) and heme iron. [Work supported by NIH grant GM36422.]

M-Pos33

STRUCTURAL STUDIES OF THERMOLYSIN-INHIBITOR COMPLEXES WITH SOLID-STATE NMR: MEASUREMENTS OF ^{31}P-^{15}N BOND LENGTHS AND CHEMICAL SHIFTS

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^{31}P and ^{15}N chemical shifts and ^{31}P-^{15}N bond lengths have been measured with solid-state NMR techniques in two inhibitors of thermolysin, ZG^{P}LA and ZF^{P}LA, both as lithium salts and when bound to the enzyme. we observe large changes in ^{31}P chemical shifts upon inhibitor binding to the enzyme, but the ^{15}N shift changes are small, indicating that the nitrogen atom is not protonated. Our NMR distance measurements indicate no change in P-N bond length upon inhibitor binding to thermolysin, and we report r_{P-N}=1.68 Å for ZF^{P}LA and r_{P-N}=1.60 Å for ZG^{P}LA, both as free inhibitors and when bound to thermolysin. Finally, these NMR data suggest that the large change in ^{31}P chemical shift seen upon ZF^{P}LA binding to thermolysin reflects its ability to form a tight-binding complex with the protein.

M-Pos34

P-(HYDROXYMERCURI)BENZENESULFONATE (PMPS) OR Cu^{2+} LABILIZES ONE ZINC BUT INCORPORATES ONE MERCURY OR COPPER INTO *E. COLI RNA POLYMERASE (RPase).* *M.C. Brenner and F.Y.-H. Wu. (Spon: C.-W. Wu), Dept. Pharmacol. Sci., SUNY at Stony Brook, NY 11794.* *E. coli* RPase ($\alpha_2\beta\beta'\sigma$) contains 2 Zn ion/mol RPase, located in the β & β' subunit. Titration of RPase with 10-40 equivalents of the sulfhydryl reagent, PMPS, labilizes one of the two Zn ions to EDTA removal, yielding a fully active Zn_1-RPase after treatment with dithiothreitol (DTT) to remove bound PMPS (Giedroc & Coleman (1986), *Biochemistry* 25, 4969). In light of the high affinity of the native enzyme for both Zn ions, this was an unexpected result. We have found, however, that incubation of RPase with 35 equivalents of PMPS followed by dialysis against 10 mM EDTA removes one of the two Zn ions as reported, but subsequent dialysis against 10 mM DTT for up to 48 hr yields Zn_1Hg_1-RPase with full enzymatic activity, suggesting incorporation of Hg at the vacated Zn binding site. In analogy with the PMPS treatment, dialysis of RPase against 0.1 mM Cu^{2+} gives a Zn_2Cu_{17}-RPase which yields a Zn_1Cu_1-RPase after dialysis against 10 mM EDTA with as much as 80% enzyme activity. The subunit location of the incorporated Hg or Cu ion is under investigation.

M-Pos35

X-RAY ABSORPTION SPECTROSCOPY (XAS) AND ANOMALOUS SMALL ANGLE SCATTERING STUDIES (ASASS) OF RNA POLYMERASE (RPase) **F.Y.-H. Wu[1,2], L. Powers[3], J.C. Phillips[4], W.-J. Huang[1], & R.B. Sinclair[3]. [1]Dept. Pharmacol. Sci., SUNY at Stony Brook, NY. [2]Inst. Biomed. Sci., Academia Sinica, Taipei, R.O.C. [3]CTR. for Bio-catalysis Sci. & Tech., Dept. Chem. & Biochem. Utah State Univ., Logan, UT. [4]Nat'l. Synchr. Light Source, BNL, NY.** *E. coli* RPase ($\alpha_2\beta\beta'\sigma$) contains 2 Zn ions, located in the substrate (β) and template (β') binding subunits. Our earlier UV-Vis & NMR spectroscopic, & chemical modification studies showed that metal ions have a distorted tetrahedral (Td) geometry with His, Cys and/or H_2O as ligands. Combination of XAS and ASASS allows a unique approach to determine the organization of these metal metal active sites. XAS studies with Zn-Zn RPase (0.5 mM) show that the ligands of Zn are N and/or O and S in a distorted Td geometry in agreement with earlier studies. Removal of Zn from the β' site and selective metal substitution allowed investigation of each site individually. The Zn-Zn separation was not observed in the XAS data suggesting that it is > ~ 4 Å. ASASS provides a method for determining this distance as well as the position of these sites relative to the protein. Preliminary data (780 ~ 10 Å region) suggest that both contributions are observed.

M-Pos36

ANALYSIS OF THE CALCIUM REQUIREMENT FOR PHOSPHOLIPASE A_2-CATALYZED HYDROLYSIS OF LIPID VESICLES. B.K. Lathrop, R.L. Biltonen. (Intro. by G.G. Romero) Depts. of Biochemistry and Pharmacology, University of Virginia, Charlottesville, Virginia, 22908.

The requirement of calcium for phospholipase A_2-catalyzed hydrolysis of small unilamellar vesicles of dipalmitoyl-lecithin has been examined under conditions where PLA_2 is instantaneously activated. At low ionic strength (50 mM KCl), the concentration of Ca^{+2} required to obtain half-maximal initial velocity was about 700 μM for PLA_2 from Agkistrodon piscivorus piscivorus (AppD49) and about 200 μM for porcine pancreatic PLA_2. However, in the presence of 10 mM $MgCl_2$, only 22 μM Ca^{+2} was required. The apparent dissociation constants for Ca^{+2} in solution are 0.3 mM and 1.6 mM for the pancreatic and AppD49 enzymes, respectively, and the binding affinity for Ca^{+2} does not change for AppD49 when bound to a non-hydrolyzable vesicular surface. Therefore, enhanced affinity for Ca^{+2} under catalytic conditions must reflect a change in the enzyme related to activation following binding to the lipid substrate surface. Analysis of the hydrolysis time-courses and the dependence of the initial velocity on $[Ca^{+2}]$ and $[Mg^{+2}]$ indicates a dramatic reduction of product inhibition by high concentrations of divalent cations.

M-Pos37

POSSIBLE ROLE OF STRUCTURAL FLUCTUATIONS IN ENZYME REACTIONS: A MICROVISCOSITY APPROACH. Kingman Ng and Andreas Rosenberg. Department of Laboratory Medicine and Pathology, University of Minnesota, Minnesota, USA.

Transition state stabilization has been shown to be the energetic throttle for rate enhancements and substrate specificities of enzyme reactions. Structural fluctuations of the enzyme molecule have also been proposed to play a role in catalysis. The extent of which these fluctuations are coupled to the reaction coordinate is however not known. We have tested the possibility of such coupling using viscosity as a variable. Microviscosity represents a stochastic type of dissipation of fluctuation forces and thus should attenuate the rates if such forces are coupled to the reaction coordinate. We will present data on the viscosity dependence of hydrolysis of amides and esters by subtilisin BPN' using a variety of cosolvents. Acylation and deacylation as rate limiting steps show very different viscosity dependence suggesting that effect of fluctuation forces can only be observed in steps following the formation of stabilized transition states. (Supported by NSF/DMB-8704740)

M-Pos38

A RAMAN SPECTRASCOPIC STUDY OF PYRUVATE BOUND TO LACTATE DEHYDROGENASE AND ITS R109Q AND D168N MUTANT. Hua Deng , Jie Zheng, John Burgner, Tony Clarke, John Holbrook and Robert Callender. Physics Department(H. D., J. Z.and R. C.), City College of City University of New York , New York New York 10031, Department of Biological Sciences(J.B.) Purdue University, West Lafayette, IN 47907 and Department of Biochemistry(T.C. and J.H.) Universty of Bristol, Bristol BS8 1TD England. (intro. by Donald Sloan)

Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate by the direct transfer of a hydride ion from its cofactor, NADH, to pyruvate's carbonyl carbon. We have shown in Raman studies (PNAS 86, pp. 4484-4488, 1989) that the carbonyl bond of the substrate is strongly polarized , which is largely responsible for LDH's catalytic power. It has been found that the R109Q and D168N mutants also catalyze this reaction but at a reduced rate. We find that a reduced reaction rate is correlated with a smaller polarization of the carbonyl bond of the substrate in the D168N mutant compared with wildtype LDH. However, the even greater reduced reaction rate in the R109Q mutant does not corespond to an even smaller carbonyl bond polarizition, as might be expected based on a simple model. Other Raman data suggest that the R109Q mutant may substantially affect the structure of the active site, more than the simple replacement of a charged residue for a non-charged residue.

M-Pos39

RAMAN STUDIES OF THE PYRIDOXAL COENZYME IN ASPARTATE AMINOTRANSFERASE AND ITS Y225F MUTANT. Jie Zheng, Hua Deng , Jon Goldberg, Jack Kirsch, and Robert Callender. Physics Department(J. Z., H. D. and R. C.), City College of City University of New York , New York New York 10031, and Department of Biochemistry, (J. G. and J. K.), University of California at Berkeley, Berkeley, California 94720.

Aspartate aminotransferase(AAT) catalyzes transamination from aspartate to ketogrutarate. Pyridoxal phosphate is the coenzyme for this enzyme and forms a Schiff base linkage with lysine 258. The Schiff base linkage of the wild type has a pKa of about 7. On the other hand , the absorption maximum of the Y225F mutant shifts from 430 nm at low pH to 390 nm at high pH with a pKa of about 9. The absorption maximum of theY225F mutant at high pH is different from that of the wildtype AAT, but is the same as that of pyridoxal phosphate in solution as an aldehyde. This suggests that an aldehyde may be formed in Y225F mutant at high pH. We have used Raman spectroscopy to resolve this problem. Raman spectra of pyridoxal phosphate in widetype and Y225F mutant have been obtained by difference techniques and will be presented and discussed. Based on these results, various models of the interactions between pyridoxal phosphate and aspartate amino transferase will be discussed.

M-Pos40

INDICATOR METABOLITE ESTIMATE OF CYTOSOLIC FREE Mg^{2+} CONCENTRATION (Mg_f) IN PERFUSED HEART. RT Mallet, YH Kang, R Bünger (Spon: JK Kelleher). Physiol., USUHS, Bethesda, MD 20814

Mg_f influences cellular Ca^{2+} homeostasis and various enzymatic reactions in heart, but is difficult to measure directly. We estimated Mg_f using the known Mg^{2+}-sensitivity of the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase equilibrium, $K_{GAPDH}{\cdot}K_{PGK}$, in nonworking, spontaneously beating guinea pig hearts. Hearts, perfused with oxygenated (95% O_2:5% CO_2) Krebs-Henseleit bicarbonate buffer (pH 7.4), metabolized 5 mM glucose + 5 U/l insulin at 1 mM Ca^{2+} and physiological 0.6 or excess 6 mM perfusate Mg^{2+}. Cytosolic [ATP]/([ADP][P_i]) (~ATP, mM^{-1}) was estimated from [CrP]/([Cr][P_i]) (~CrP, M^{-1}) and intra-cellular pH measured in freeze-clamped hearts using Mg^{2+}-sensitive creatine kinase equilibrium (K_{CK}). Exper-imental $K'_{GAPDH}{\cdot}K'_{PGK}$, calculated as ($K_{CK}$/ K_{LDH})· ~ATP· ([lactate]/[pyruvate] (=L/P))·([3-phosphoglycerate]·22/ [dihydroxyacetone-P] (=3PG/DAP)) at 0.05-4 mM Mg^{2+} was compared with true $K_{GAPDH}{\cdot}K_{PGK}$ values (Eur J Biochem 84:421, 1978) using an iterative maximum likelihood (ML) computer program. The Mg^{2+} at which experimental and true $K_{GAPDH}{\cdot}K_{PGK}$ were equal was taken as the ML estimate of Mg_f. Data are means ± SE, n = 5-8; *: $p < 0.01$

[Mg^{2+}]	L/P	3PG/DAP	~CrP	H^+/K_{CK}	~ATP	Mg_f
0.6mM	4.6±0.4	36±5.9	480±48	61	30±3.1	0.4
6 mM	22±3.2*	8.7±1.6*	454±47	128	58±5.0*	3.1

Thus, 6 mM Mg^{2+} produced increases in estimated Mg_f (approx. eightfold) and ~ATP, but reciprocal changes in L/P vs. 3PG/DAP. Supported by NIH HL36067.