W-Pos137 PRESSURE-INDUCED DISSOCIATION AND CONFORMATIONAL DRIFT OF TRYPTOPHAN SYNTHASE β_2 SUBUNIT. Jerson L. Silva, Edith W. Miles, & Gregorio Weber. Dept. of Biochemistry, University of Illinois, Urbana, IL 61801, & National Institutes of Health, NIADDK, Bethesda, MD 20205

The conformational drift is the limited change in structure, rather than complete unfolding, that occurs in oligomeric protein subunits after loss of intersubunit contact. Hydrostatic pressure in the range of 1 to 3 kbar provides a unique method of dissociation that does not affect by itself the tertiary structure and is rapidly reversible. The pressure induced dissociation of tryptophan synthase β_2 subunit was followed by the changes in the intrinsic fluorescence. The dissociation curves revealed a volume change ($\Delta V°$) of 160 ml/mol and a dissociation constant at atmospheric pressure of $4 \cdot 10^{-10}$M. The midpoint dissociation pressure was dependent on the protein concentration as expected for a dimer-monomer equilibrium. A large decrease of the total fluorescence intensity of pyridoxal-P was also found upon dissociation. After release of pressure, the dimeric structure was rapidly recovered (less than 5 min.) as judged by return of the original intrinsic fluorescence spectrum and by size elution HPLC. However, the activity and pyridoxal-P fluorescence were recovered slowly, indicating that an associated enzyme dimer with altered conformational properties was obtained. The pressure dissociation curves exhibited hysteresis corresponding to a loss of affinity between the altered monomers of $\approx$ 2 kcal/mol. The volume change for the dissociation of the apo-enzyme was significantly higher (270 ml/ml). This can be explained by a higher instability of the unliganded protein, becoming susceptible to larger conformational drift upon dissociation and consequently to additional compressibility effects. Supported by USPH:GM11223.

W-Pos138 **FLUORESCENCE LIFETIME AND ANISOTROPY STUDIES OF T4 LYSOZYME MUTANTS CONTAINING SINGLE TRYPTOPHAN RESIDUES,** Dan Harris, Lawrence McIntosh and Bruce Hudson, Institute of Molecular Biology & Department of Chemistry, University of Oregon, Eugene, OR 97403.

The 164 residue lysozyme of bacteriophage T4 contains two surface tryptophan residues (126 and 158) and a buried Trp at position 138. Variant forms in which two of these three residues have been replaced with tyrosine have been prepared and studied with time resolved fluorescence methods. The decay of the fluorescence of the single Trp-138 protein (W126Y/W158Y) requires at least two exponential components for an adequate analysis. The time dependence of the anisotropy at 10° is characterized by an r_o of 0.3 and a single correlation time characteristic of overall tumbling of the protein. Increasing the temperature results in a sub-nanosecond internal motion with increasing amplitude. At 45 and 50° there appear to be two rapid components, one too fast to resolve and one of a few hundred picoseconds. These results will be discussed in terms of the structure of this protein and molecular dynamics simulation calculations. The behavior of the surface tryptophans is quite different from that of Trp 138. These groups can be quenched with iodide. This aides in the interpretation of experiments on proteins containing all three Trp residues including those with substitutions at other positions in the protein. The substitution Ala 146 -> Thr is of particular interest because this replacement moves the neighboring Trp 138 residue.

W-Pos139 HARMONIC MODES AND CONFORMATIONAL SUBSTATES IN PROTEIN. Arthur S. Brill, Department of Physics, University of Virginia, Charlottesville, VA 22901.

Are conformational substates and harmonic modes independent ways of validly describing motional effects in proteins? A harmonic model that has metastable conformational states is described here. In it, conformational substates are associated with harmonic modes the natural frequencies of which are low compared with those of harmonic Brownian gates distributed along the normal coordinates of the conformational modes. Salient features of the model are heuristically pictured as those of a pendulum colliding with a barrier consisting of n identical thermally-activated gates, each of stiffness k and requiring contraction of at least x for the pendulum to pass. The activation energy for a conformational transition is then nE where $E = kx^2/2$. Systems (conformational modes), identical except for n, have equal transition rates at temperatures T_i which equalize $(.5)\ln(T_i) - n_i(E/k_B T_i - \ln(.5))$. Both the activation energy and the geometric factor $n\ln(.5)$ serve to raise T_i. In a more realistic picture the elementary barriers along a conformational mode are pairs of spring-mounted van der Waals spheres; the two members of each of the n pairs must pass each other for transition to occur; and ln(.5) becomes ln(f) where f, the fraction of energetically accessible orientation space for which each gate is open, is in the range $.5 < f < 1$. The spatial resolution and elementary excitation energy E of such processes can be consistent with observed displacements and minimum barriers.

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W-Pos140 THE SURFACE CONSTRAINT ALL-ATOM SOLVENT MODEL, Gregory King and Arieh Warshel, Department of Chemistry, University of Southern California, Los Angeles, California 90089-0482

A dynamical all-atom model for the simulation of ions in polar solvents is presented. The model concentrates on the key electrostatic aspect of solvent polarization. Constraint forces included in the model insure that the polarization and other properties of the surface molecules in systems with a small number of solvent molecules are similar to the corresponding properties in an infinite system. The model is expected to be very useful in overcoming the geometrical problems associated with using periodic boundary conditions in the simulation of systems containing ions. Extension of the model to electrostatic calculations in proteins is also considered.

W-Pos141 MOLECULAR DESIGN OF ARTIFICIAL ENZYMES, C.A. Venanzi, J.D. Bunce, and P.M. Canzius, Dept. of Chemical Engineering & Chemistry, New Jersey Institute of Technology, 323 King Blvd., Newark, NJ 07102

Enzymatic reactions are distinguished by substrate selectivity and high reaction velocity. Recently Cram and coworkers have synthesized cyclic urea compounds which mimic alpha chymotrypsin in these two features. We analyze the degree of structural preorganization of the artificial enzymes for their substrates by molecular mechanics calculations of the uncomplexed and complexed conformations of the mimetics for which x-ray data is not yet available. Using computer graphics and conformational analysis techniques, we model a cyclic urea mimetic suggested by Cram, but not yet synthesized, which has the potential of participating in the proton relay mechanism typical of alpha chymotrypsin. We calculate molecular electrostatic potential contour maps in order to determine the degree of electrostatic complementarity of the artificial enzymes for their substrates. In order to compare the steric and electrostatic interactions within the mimetic-substrate complex at the molecular level, we extend the analysis to alpha chymotrypsin and to a capped beta cyclodextrin mimetic of alpha chymotrypsin synthesized by Breslow and coworkers. Support to C.A.V.: National Science Foundation (CPE-8404363), ACS Petroleum Research Foundation (15665-GB4), and the State of New Jersey.

W-Pos142 ELECTROSTATIC POTENTIALS AND ION BINDING ON MYOGLOBIN, Bertrand Garcia-Moreno E., and Frank R. N. Gurd, Department of Chemistry, Indiana University, Bloomington, IN 47405.

The static accessibility modified Tanford-Kirkwood algorithm has been extended previously to include site-bound ions in the calculation of electrostatic properties of polyelectrolytes (Matthew et al., 1985, CRC Crit. Rev. Biochem. 18, 91). Ion binding sites are defined as ion-accessible regions of the protein-solvent interface where the electrostatic potential exceeds 2kT. Coordinates for two cation and three anion binding sites were obtained from maps of electrostatic potentials calculated around sperm whale aquo-ferrimyoglobin at a resolution of 0.25 Å. The dependence of the coordinates on pH was found to be minimal, suggesting that the ion binding properties of the protein are not determined by long range effects but rather through local interfacial characteristics. A wide distribution of association constants and fractional occupancies were calculated for the ion binding sites as a function of pH and ionic strength. Inclusion of site-bound monovalent ions (Na^+, Cl^-) in the calculation of the theoretical titration behavior of the protein and of individual titrating sites yields curves in close agreement with those obtained experimentally. The destabilizing effects of divalent cations (Ca^{++}) on the electrostatic interactions of Arg-45 suggest that opening of the channel to the heme pocket might be modulated through ionic interactions. Additionally, the entrance to the heme pocket was found to be surrounded by an extensive area of negative potential which would disfavor autoxidation, and affect interactions between the heme moiety and anionic ligands. (Supported by Public Health Service Research Grant HL-05556.)

W-Pos143 THE EFFECT OF LOCAL GEOMETRY ON ION BINDING TO PROTEINS. Antonio Raudino and David C. Mauzerall, The Rockefeller University, 1230 York Avenue, New York, NY 10021

It is known through the work of Manning and Zimm that the fraction of condensed ions, ie ions which remain on a charged surface as the surrounding volume expands to infinity, is a function of the geometry of that surface. This fraction ranges from zero for a sphere, through a finite fraction for a cylinder to unity for a plane. This suggests that a concave surface or cleft may hold more ions than the planar surface in which it is cut. An electrostatic calculation based on hemispherical concavity and the linearized Poisson-Boltzman equation supports this suggestion. The excess bound ions depends on the area of the concavity (at constant charge) and on the ionic strength. Conversely, there is a decrease in bound ions for a similar convexity. In the region of concavity size equal to the coulomb radius of water we estimate a charge of about one ion per concavity-convexity. These results are relevant to the common occurrence of clefts in protein structures, often containing charges. Changes in the size of such clefts could dramatically alter the binding of charged substrates. Likewise, a change in geometry of the highly charged surface of bacteriorhodopsin during its photocycle could cause the observed (Marinetti and Mauzerall) ion release and uptake and may be part of the ion pump itself.
This work was supported by the PHS grant GM 25693-07.

W-Pos144 ELECTROSTATIC EFFECTS IN DIFFUSION INFLUENCED REACTIONS OF DIPOLAR BIOMOLECULES - Scott H. Northrup, Marc S. Curvin, Paul Nichols, Jeffrey D. Smith, and Jeffrey O. Boles, Department of Chemistry, Tennessee Technological University.

The rates of many important reactive processes in solution are influenced or controlled by the diffusional encounter rate of reactants. An important example is the reaction of enzymes and their substrates. Molecular recognition of enzymes for substrates may in fact begin in the diffusional encounter stage of the reaction, with long ranged electrostatic forces and other factors selectively steering particles into favorable orientations for reaction. A computer simulation approach based on the Brownian trajectory method has been developed and employed to handle the arbitrarily complicated interactions present in real physical systems. This has been applied to the calculation of the diffusion controlled electron transfer rate between cytochrome proteins modelled as dipolar spheres with orientation-dependent reactivity. A dipole moment typical of cytochromes has little effect on the encounter rate of an orientation nonspecific reaction, even in cases where it produces an average attractive interaction potential. On the other hand, the dipole moment is found to have a significant enhancing effect on highly orientation-dependent reactions. A large perturbation in reaction rate is observed when the position of the dipole is varied relative to the electron transfer surface. This corresponds to the effect on reactivity of site-specific chemical modifications of cytochrome c as observed in recent experiments.
(Supported by NIH grants AM01403 and GM34248 and ACS/PRF grant 17051-B7.)

W-Pos145 EFFECTS OF TRIFLUOROETHANOL ON THE CHARGE EFFECT OF RNase S-PEPTIDE
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The S-peptide, residues 1-20 of RNase A, is unusual in exhibiting an intramolecular helical structure in water solutions at 0°C. At pH 4.5, the S-peptide is approximately 30% alpha-helical, with significantly less alpha helix at higher and lower pH's (1). We are utilizing the titration of the alpha-helix vs. trifluoroethanol (TFE) as a procedure to determine the stability of the alpha helix in a manner analogous to (but in the opposite direction of) urea or guanidinium chloride denaturation studies. Titrations of the S-peptide (residues 1-20 of RNase A), a C-peptide analogue (residues 1-13), and the P-peptide (residues 1-8) are being used to calibrate TFE as a probe for the stability of marginally-stable secondary structures.

Due to the effect of pH on the structural stability, careful control of pH in the TFE:water cosolvent is required. We use a standard procedure of using a dilute HCl solution to define the pH scale in each mixed solvent (2). In the presence of moderate concentrations of TFE, the charge effect, as measured by the pH dependence of the alpha helix content, is altered significantly: the stability of the helix no longer decreases as the pH drops below the optimal value in water solutions. The mechanism of alpha helix stabilization by TFE appears to operate selectively upon the charged groups in the molecule, suppressing the effect of the groups responsible for the loss of helix at low pH in water, presumably glu-2.

(1) P.S. Kim & R. L. Baldwin (1984) Nature, 307, 329-334.
(2) R. G. Bates, Determination of pH: Theory and Practice, c. 1973, John Wiley & Sons, Inc.

W-Pos146 PROTEOLYSIS AS A PROBE FOR MOTILITY IN MUTANT BACTERIOPHAGE T4 LYSOZYMES

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Relative susceptibility to proteolysis can be used to explore the effect of changing a single amino acid on the motility (flexibility) of a protein. In conjunction with studies on the effect of such substitutions on thermodynamic properties, three mutant T4 lysozymes (T157A, A126T, R96H) and the wild type (WT) have been incubated at 25, 35, and 45 C with equal weights of trypsin. The time course of the loss of activity was followed for up to 24 hours.

The relative order of susceptibility to trypsin at all three temperatures was A146T >> T157A > R96H > WT, which is the reverse of that for their relative activities, i.e. the closer the activity of a mutant is to WT, the less susceptible it is to trypsin.

At 25 C the activity of A146T is destroyed by trypsin in less than 2 hrs, while after eight hrs T157A retains about 85% of its original activity, and R96H and WT about 95%. At 35 C A146T activity is destroyed in less than 20 minutes while about 80% activity remains for T157A after 1 hr, R96H after 1.5 hrs, and WT after 8 hrs. At 45 C about 50% activity remains after 14 minutes for A146T, 20 minutes for T157A, 25 minutes for R96H, and 7-8 hrs for WT. Without trypsin present the activities of all remain unchanged for at least 24 hrs. These mutants were selected for temperature sensitivity at 43 C. However none are unfolded at this temperature as shown by melting temperatures and by these incubation studies. Indeed the activity at room temperature relative to the wild type seems to be enhanced by incubation at 45 C. However the sensitivity of the mutants to trypsin is much greater at this temperature, suggesting the molecules are more flexible.

W-Pos147 ROTATIONS OF TRYPTOPHAN RESIDUES IN LYSOZYME. Gerard Marriott,* Richardo Alcala, Enrico Gratton, Dept. of Physics & Biochemistry,* Univ. of Illinois, Urbana, IL 61801.

A multi-frequency phase and modulation fluorometer using the harmonic content of a mode-locked laser was used to determine the rotational motions of tryptophan in hen egg white lysozyme. A mode-locked argon-ion laser was used to synchronously pump a rhodamine B dye laser. The pulse train output was amplitude modulated using an acousto-optic modulator and then frequency doubled. The frequency was variable from a few kilohertz to 500 megahertz. Using the differential phase and modulation technique to measure fluorescence lifetime and anisotropy decay, we were able to determine rotational correlation times of a few tens of picoseconds. In a study of the temperature and viscosity dependence of tryptophan motion in lysozyme, which has two tryptophan residues responsible for about 90% of the fluorescence, we were able to determine at least two rotational motions. The first corresponds at 20°C in aqueous solution to rotation of the entire macromolecule having a rotational correlation time of 6 nanoseconds. This rotational motion obeys the Stokes-Einstein relationship. The other rotation corresponds at 20°C in aqueous solution to a faster, local motion where the rotational correlation time is 1 nanosecond. This rotational motion does not obey the Stokes-Einstein relationship. In these experiments we found the zero-time anisotropy was 0.31, i.e., the limiting anisotropy for tryptophan, and consequently we have accounted for all the rotational motions of the tryptophan ring. Through oxidation of Trp-62 we were able to demonstrate comparable rotational correlation times for the two tryptophans and that rotation occurs in a cone of 45° aperture. Supported in part by grants NSF PCM 84-03107 and NAVAIR Research Grant MDA 903-85-X-0027 (E.G. and R.A.) and PHS 5-R01 GM1223 (G.M.).

W-Pos148 ROTATIONAL MOBILITY OF THE TYROSINES OF CALMODULIN. R.F. Steiner, J.R. Lakowicz, and Ignacy Gryczynski, Departments of Chemistry and Biological Chemistry, University of Maryland, Baltimore, Maryland 21228

We report here the first tyrosine anisotropy decays obtained using a frequency-domain fluorometer. This instrument was employed to examine the rotational mobility of the two tyrosine groups of bovine calmodulin as a function of pH, Ca^{2+}-ligation, and temperature. Measurements were made over a frequency range from 3 to 165 MHz. For all conditions examined at least two correlation times were required to fit the data. The shorter of these was of the order of 1 ns, or less, and presumably reflects localized motion of the fluorophors. The longer correlation time represents the motion of a substantial portion of the molecule; its magnitude depends upon conditions, as do the relative amplitudes of the two rotational modes. The mobility of the tyrosines is greater at pH 6.5 than at pH 5.0. For all conditions the mobility is greater at 37° than at 5°. At pH 6.5 the mobility is substantially reduced by Ca^{2+}-ligation. At pH 6.5, 37°, in the absence of Ca^{2+}, there is little restriction upon the motion of the tyrosines.

W-Pos149 RESOLUTION OF SUBNANOSECOND ANISOTROPY DECAYS OF TRYPTOPHAN PROTEIN FLUORESCENCE USING FREQUENCY-DOMAIN FLUOROMETRY. Joseph R. Lakowicz, Ignacy Gryczynski, Henryk Cherek and Badri P. Maliwal, University of Maryland, School of Medicine, Department of Biological Chemistry, Baltimore, Maryland 21201.

We report the first anisotropy decays of protein fluorescence obtained using a frequency-domain fluorometer. The ultraviolet light source (300 nm) was a Argon-ion pumped ring dye laser equipped with a intracavity frequency doubler. The data, measured at modulation frequencies from 2 to 200 MHz, reveal the presence of substantial subnanosecond motions (0.1 to 0.2 nsec) of the single tryptophan residues in melittin and monellin. For melittin the data also indicate the presence of slower motions near 1 ns, which may be the result of concerted motions of several peptide units. Smaller amplitude motions, on a similar timescale, were also observed for the single tryptophan residue in Staphlococcal Nuclease. We demonstrate using N-acetyl-L-tryptophanamide in water that the method of frequency-domain fluorometry is capable of measuring correlation time as short as 50 psec This method can provide data for the direct comparison of measured anisotropy decays with those predicted from molecular dynamics calculations.

Additionally, we expanded the analysis to include measurement at multiple excitation wavelengths at which the fundamental anisotropies (r_0) are distinct. These GLOBAL data provide a increased ability to resolve closely spaced correlation times.

W-Pos150 SPECTROSCOPIC STUDIES OF AGGREGATION AND CONFORMATION OF RECOMBINANT BOVINE INTERFERON ALPHA I. S. J. Shire and J. F. Maher, Dept. of Pharm. R & D, Genentech, Inc. S. San Francisco, CA 94080

We have investigated the conformation of Bovine Interferon Alpha I using far UV circular dichroism, UV difference spectra and intrinsic tryptophan fluorescence (λ_{em}=336 nm, λ_{ex}=292 nm). Refolding and unfolding of interferon as a function of GuHCl concentration in succinate buffer is fully reversible, rapid and can be approximated by a two-state denaturation model based on the spectroscopic data. Heat precipitated protein can be resolubilized in 7 M GuHCl and after dialysis into 10 mM succinate buffer, pH 4.6 retains full biological activity. Furthermore, the unfolding and refolding of resolubilized precipitated interferon can be approximated as a two-state model and follows the same denaturation profile as unprecipitated monomer. The resulting free energy of unfolding determined by all spectroscopic methods in the absence of denaturant is 2.4 (± 0.4) kcal/mole. This is lower than that reported for other proteins but similar to that recently reported for the two domain protein, phosphoglycerate kinase. The estimated secondary structure from CD data is similar for monomer, resolubilzed precipitate and unfolded/refolded monomer. When the monomer was examined in the pH range of 3.5 to 7.5 by circular dichroism, similar spectra were obtained at the low pH's with a change in the magnitude of the CD data occurring at the higher pH's. These experiments strongly suggest that precipitated BoIFN Alpha I is a fully functional protein which precipitates either due to conformational changes and/or self association of the protein. However, separated dimers (covalent and noncovalent) have less than 2% of the monomer BoIFN Alpha I activity and yields significantly different secondary structure estimates from CD. The difference in the circular dichroism spectra may result partly from relative orientation of the monomer units rather than gross conformational changes and requires further investigation.

W-Pos151 ANALYSIS OF PROTEIN CD SPECTRA FOR SECONDARY STRUCTURE USING A SIMPLE MATRIX MULTIPLICATION. Larry A. Compton and W. Curtis Johnson, Jr., (Intr. by Louis Libertini), Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

A method is presented for the computerized analysis of globular protein secondary structure using circular dichroism (CD) spectroscopy. This method is based upon the generalized (Moore-Penrose) inverse matrix theorem and utilizes the singular value decomposition (SVD) of a set of CD spectra corresponding to proteins with known X-ray structure. The generalized inverse matrix method does not depend upon standard matrix diagonalization or inversion subroutines and therefore offers a quick, efficient means for the computation of the least-squares solution to large, unsymmetrical, and potentially unstable data arrays. Once obtained, the generalized inverse of our CD spectra basis set can be used to evaluate the secondary structure of an experimental CD spectrum of interest using simple matrix multiplication. In addition, secondary structure basis spectra analogous to the CD spectra of synthetic polypeptides are generated from the X-ray structure of our CD spectra set. These structure spectra are used to quantitatively analyze the independent structural elements within a protein CD spectrum.

W-Pos152 **^{1}H-NMR INVESTIGATIONS OF POLYOXYETHYLENE-BOUND OLIGOPEPTIDES: EFFECT OF SOLVENT AND BLOCKING GROUPS.** **Anthony A. Ribeiro* and Murray Goodman$^+$, *Department of Radiology, Duke University, Durham, NC 27710 and $^+$Department of Chemistry, University of California San Diego, La Jolla, CA 92093.**

Three series of side chain protected (α-OMe) glutamate peptides attached to a macromolecular solubilizing polyoxyethylene (POE) group and analogous compounds have been examined in detail using ^{1}H NMR spectroscopy. These peptides are soluble in both nonpolar solvents like $CDCl_3$ and highly polar environments like water. Chemical shift dependencies with respect to temperature and solvent, and line broadening effects of free radicals have allowed the determination of hydrogen-bonded residues based on the assignment of individual NH resonances through α-CH deuteration. The peptide form that is completely free of intermolecular hydrogen bonds and molecularly disperse in solution (the σ conformation) is identified at dilute conditions in $CDCL_3$ solution, and appears best conformationally interpreted as a series of seven-membered ring structures in the N-terminus. The NMR data are consistent with the onset of helical structure at the heptamer in trifluoroethanol and to a lesser extent, in water. The folded peptide forms contrast their disordered forms observed in DMSO. In the structure-supporting solvents, the POE-peptides with pGlu at the N-terminus showed considerably reduced stability of structure than those with the Boc or acetyl blocking groups. (Supported by NIH GM 18694 and NIH RR0711 and NSF GP23633.)

W-Pos153 LOW TEMPERATURE FLUORESCENCE DECAY STUDIES OF PROTEINS. M. VandeVen, J. Beechem, M.Han J. Schwartz, P. Neyroz, J. Rudzki, D. Walbridge, A. Maki and L. Brand. Dept. of Biology, The Johns Hopkins University, Baltimore, Md. 21218

The decay of the intrinsic fluorescence of several proteins was measured at liquid nitrogen temperature (77K) using the method of single photon counting. The excitation wavelength was 295 nm. and the time profile of the lamp had a pulse width (FWHM) of 2.6 ns. Data was obtained at emission wavelengths of 325, 345 and 367 nm. The data were analysed using the method of non-linear least squares. Samples were dissolved in 50% ethylene glycol-50% phosphate buffer. This mixture forms a transparant glass at 77 K. (The Staphylococcal nuclease sample contained 0.1 M sodium chloride in addition to 0.25 mM phosphate buffer.)

Monoexponential decays of 5.4 and 5.7 ns. were observed for L-tryptophan and N-acetyl tryptophanamide respectively. Staphylococcal nuclease (single Trp) exhibited a double exponential decay of 5.3 and 7.6 ns. Horse liver alcohol dehydrogenase (two Trp/ subunit) showed biexponential decay with τ_1 = 5.0 and τ_2 = 8.3 ns. Enzyme I of the PTS system showed biexponential decay with decay times of 4.9 and 9.3 ns. These results indicate that the molecular environment can influence the fluorescence of residues in proteins under conditions where molecular motions are minimized. Supported by NIH grant No. GM 11632.

W-Pos154 RADIATION DAMAGE IN GLYCOPROTEINS. M. J. McCreery, J. H. Miller, and E. Kempner. Letterman Army Institute of Research, Presidio, San Francisco, CA and NIADDK, National Institutes of Health, Bethesda, MD.

The functional unit size of glycoproteins as determined by radiation target analysis reflects the protein portion of the macromolecule without recognition of the contribution due to carbohydrate. This principle holds in every glycoprotein to which this method has been applied including the enzyme Invertase containing 50% carbohydrate and receptors comprised of as little as 2% carbohydrate. We have confirmed these observations in a synthetic glycoprotein enzyme, Glucose-6-phosphate dehydrogenase covalently bonded to agarose. Examination of the chemical transformations of radiation-induced free radicals observed in large oligosaccharides and simple model sugars suggests a rationale for this phenomenon. Those radicals whose formation is highly favored and whose fates play a major role in subsequent reactions occur at C1 and C5 in sugar residues. Intramolecular rearrangements of these species is very probable and leads to 1,2-radical migrations, ring cleavage at the lactol bridge, or scission of the glycosidic linkage. In this last process, free radical migration to the adjacent residue may occur but movement to a more distant residue is extremely unlikely. Thus the damage from a primary ionization occurring in the carbohydrate portion of a glycoprotein would be confined to a limited region with little energy spread into the polypeptide chain. A glycoprotein whose biochemical activity is independent of its associated carbohydrate therefore yields a target size reflecting only the peptide contribution.

W-Pos155 RADIATION INACTIVATION OF ASSIMILATORY NADH:NITRATE REDUCTASE FROM CHLORELLA: CATALYTIC AND PHYSICAL SIZES OF FUNCTIONAL UNITS. Larry P. Solomonson and Michael J. McCreery, Department of Biochemistry, University of South Florida, Tampa, FL 33612 and Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800.

Assimilatory NADH:nitrate reductase from Chlorella is a homotetramer which contains one of each of the prosthetic groups FAD, heme and Mo per 100 kDa subunit. At low protein concentrations this tetramer dissociates to a fully active dimer. To further elucidate the possible relationship between quarternary structure and activity, the functional size of nitrate reductase was determined by radiation inactivation analysis at high and low concentrations of enzyme where the principal physical species would be either tetrameric or dimeric, respectively. In both cases the size obtained by this method was 100 kDa suggesting that each subunit in the tetramer or dimer can function independently. These data confirm earlier results which indicated that the subunits are identical and that each contains a full complement of prosthetic groups. We also found that the functional sizes of the partial activities NADH:cytochrome c reductase, NADH:ferricyanide reductase, and reduced methyl viologen:nitrate reductase were fractions of the subunit molecular weight having target sizes of 58 kDa, 47 kDa, and 28 kDa, respectively. These results show that destruction of enzymatic activity on one portion of the subunit does not necessarily result in a loss of activity at a different site on the polypeptide. Apparently, the transfer of energy along the peptide backbone after a primary ionization may have greater restrictions than has previously been assumed.

W-Pos156 SOLUBILITY OF XENON-133 IN AMINO-ACID SOLUTIONS. HYDRATION OF AMINO ACIDS. Gerald L. Pollack and Jeffrey F. Himm, Physics and Astronomy Dept., Michigan State University, East Lansing, MI 48824-1116.

We have measured the Ostwald solubility (L) of ^{133}Xe at 25.0°C in aqueous solutions of the amino acids: alanine (0-1.8 M), arginine (0-0.9 M), glycine (0-2.1 M), hydroxyproline (0-2.3 M), lysine (0-2.8 M), and proline (0-3.5 M) as well as sucrose (0-2.25 M) and NaCl (0-5.25 M). Over the concentration ranges investigated L decreases, monotonically and approximately linearly, with increasing concentration for amino acids and sucrose. The effect on gas solubility of amino acid in solution can be large, e.g. for ^{133}Xe, we measured L(25.0°C) = 0.1060 in distilled water but L(25.0°C) = 0.055 for a 2.8 M lysine solution. The results can be used to calculate hydration numbers (H), i.e. the number of H_2O molecules associated with each solute molecule. The average values of hydration number ($\bar{H}$) obtained at 25.0°C are: 7.9 $\pm$ 0.6 for alanine, 0.2 $\pm$ 0.5 for arginine, 8.5 $\pm$ 0.6 for glycine, 4.5 $\pm$ 0.4 for hydroxyproline, 6.1 $\pm$ 1.1 for lysine, 2.0 $\pm$ 0.2 for proline, and 3.9 $\pm$ 0.7 for sucrose.

W-Pos157 SURFACE AFFINITIES IN PROTEIN SOLUTIONS MEASURED BY RED CELL AGGREGATION. J. Janzen, B. Kukan, D.E. Brooks and E. Evans. Department of Pathology, University of British Columbia, Vancouver, Canada V6T 1W5.

Aggregation of human erythrocytes in plasma has been associated with fibrinogen and the immunoglobulins. The aggregation is weak and is believed to be caused by reversible bridging adsorption between adjacent cell surfaces by these macromolecules. Dextrans of molecular weight greater than 40,000 daltons cause similar aggregation. Adhesion of uniform surfaces may be described in terms of surface free energy changes, adhesion being associated with a reduction in surface free energy. Estimates of the surface free energy potential in dextran solutions range from 2-22x10^{-3} erg/cm^2 (Buxbaum, K. et al., Biochem. 21:3235, 1982). Ideally, measurement of the surface concentrations of adsorbed macromolecules could be used to calculate the mean molecular free energy of adsorption. However, even without surface concentration estimates, comparison of changes in the surface free energy for the various protein components as a function of concentration allows identification of the essential components responsible for red cell aggregation in plasma and can be used to determine whether or not the effects of isolated components are additive. Preliminary experiments with plasma, serum and purified fibrinogen suggest that the major part of the free energy reduction is due to fibrinogen, but that other serum proteins also contribute. Surface affinity was measured by encapsulation of sphered red cells by flaccid red cells (Evans, E. and Buxbaum, K. Biophys. J. 34:1, 1981). The energy due to fibrinogen increased progressively between 2 and 8 mg/mL to a maximum of 5x10^{-3} erg/cm^2. In plasma, adhesion energies increased progressively with fibrinogen concentration. Supported by NIH grant #26965 and the Medical Research Council of Canada.

W-Pos158 THE VOLUME CHANGE OF COENZYME BINDING TO DEHYDROGENASES

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Horse liver alcohol dehydrogenase (HLADH) binds the coenzymes NAD^+ and NADH in strongly entropy driven reactions at 25 C, while for all other dehydrogenases thusfar studied the binding involves negative entropy changes and is thus enthalpy driven - entropy compensated. Unlike the other dehydrogenases, in addition, HLADH undergoes a large conformational change upon binding coenzyme, a change in which an open cleft closes down upon the ligand, shielding it from the solvent. When this occurs, the water that solvates both the coenzyme and the protein cleft is expelled, and we argue that its release provides the positive entropy change. This water also undergoes a change in packing density, which we detect as a rise in volume. The volume change is shown to correlate with the entropy driven nature of the reaction, as it does not occur in binding reactions that involve strongly unfavorable entropy changes, and it decreases with decreasing values of ΔS. Thus the binding of NADH, NAD^+, and ADP-ribose occur with entropy changes of 29.5, 12.1, and -2.2 cal/(mol K) and ΔV's of 95.7, 76.0, and 28.2 ml/mol respectively. The binding of NADH and NAD^+ to yeast alcohol dehydrogenase and of NAD^+ to glutamate dehydrogenase, in contrast, entail entropy changes of -10.7 to -38.9 cal/(mol K) and no volume changes within detection limits of ± 15 ml/mol. Volume changes thus appear to provide a unique "window" into events that transpire in the solvating water. Along with other data, their measurement may allow decomposition of the entropy change into various contributing terms. A methodological improvement is described that simplifies the direct measurement of volume changes.

W-Pos159 STRUCTURAL PROPERTIES OF REVERSE MICELLAR DROPLETS CONTAINING MYELIN BASIC PROTEIN.

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Microemulsion droplets of sodium bis (2-ethylhexyl) sulfosuccinate (AOT), water and isooctane constitute an excellent biomimetic system for myelin proteins. This is due in part to the very high affinity of myelin basic protein (MBP) for the water entrapped in the inner core of the droplets at low water to surfactant molar ratios. A prerequisite step for a better understanding of the behavior of MBP in these organized assemblies is a detailed knowledge of the system after protein incorporation.

For this purpose, simple geometrical models were developed and tested against experimental results obtained by quasi-elastic light scattering (QELS) and fluorescence recovery after fringe pattern photobleaching (FRAP). The obtained results are in good agreement with the proposed model and suggest the following picture of the system: (i) The protein is solubilized within the aqueous core of the droplets. (ii) The incorporation of MBP results in a redistribution of the water and the surfactant between "filled" and "empty" droplets. (iii) In the final state the system is characterized by the presence of two types of droplets of different hydrodynamic radii: protein containing ("filled") and protein free ("empty") droplets, the latter being identical in size to those present in the initial system.

The elucidation of the structural properties of these bioassemblies allows further investigations of the physicochemical and functional properties of the inserted myelin proteins and an extension to integral membrane proteins.

W-Pos160 SELF-ASSOCIATION OF RABBIT MUSCLE PHOSPHOFRUCTOKINASE: EFFECTS OF TEMPERATURE, PH, AND IONIC STRENGTH. Michael A. Luther, G.-Z. Cai, and James C. Lee, Department of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104.

Rabbit muscle phosphofructokinase (PFK) undergoes self-association in a complex manner. Sedimentation velocity studies have shown that the association and dissociation of PFK subunits can be characterized as rapid, and the oligomeric forms are in dynamic equilibrium. The overall mode of association is $M_1 \rightleftharpoons M_2 \rightleftharpoons M_4 \rightleftharpoons M_8 \rightleftharpoons M_{16}$. In order to further understand the thermodynamics of these multiple equilibria, the aggregation behavior of PFK was determined as a function of temperature, pH, and ionic strength by sedimentation velocity. Thermodynamic parameters, i.e., $\Delta G°$, $\Delta H°$, $\Delta S°_u$, and ΔC_p obtained from the temperature study show that the dimerization of PFK is characterized by negative entropy and enthalpy changes without a heat capacity change, whereas the tetramerization reaction is governed by positive entropy, enthalpy, and heat capacity changes. Low ionic strength favors the formation of dimers without a significant influence on the equilibrium constant for tetramerization which is enhanced by increasing the pH from 7.00 to 8.55. The summary of this study indicates that the formation of dimers and tetramers involves different types of interactions. (Supported by NIH Grants NS-14269 and AM-21489)

W-Pos161 THERMODYNAMIC AND KINETIC LINKAGES BETWEEN LIGAND BINDING AND FOLDING IN E. COLI ASPARTATE TRANSCARBAMYLASE. L. Sivak, S. Bromberg, M. Glackin and N. Allewell, Wesleyan University, Middletown, CT 06457; J. Matthew, E. I. Dupont Experimental Station, Wilmington DE 19898.

While linkages between ligand binding, subunit assembly and the R⇌T transition have been examined in detail, linkages between ligand binding and folding have not been explored in depth. A study of the effects of ligands on thermal unfolding by differential scanning calorimetry suggested that the bisubstrate analog PALA binds preferentially to folded c_3 and unfolded r_2, while ATP and CTP bind preferentially to folded r_2 and unfolded c_3 (Edge et al., Biochemistry, in press). Since thermal unfolding is irreversible, these conclusions need to be tested under conditions where folding is reversible. We have therefore begun to examine the kinetics and thermodynamics of denaturation in guanidine-HCl and urea. Both ATP and CTP reduce the rate of loss of activity of c_6r_6 by a factor of ~1000. This effect clearly depends upon nucleotide binding to r_2 subunits, since both nucleotides accelerate loss of activity by c_3 by a factor of ~1.5. Conversely, in renaturation experiments, both nucleotides accelerate restoration of activity to c_6r_6 by a factor of ~2.5, while slowing restoration of activity to c_3 by a factor of ~10. These observations are consistent with the conclusions drawn from the DSC study. The thermodynamics of unfolding in urea is being probed by ultraviolet difference spectroscopy, analytical gel chromatography, sedimentation equilibrium and circular dichroism. At neutral pH, unfolding of c_3 by 5M urea is blocked both by stoichiometric concentrations of PALA and 1 M NaCl. The effect of NaCl is consistent with the results of electrostatic calculations (Matthew et al., in preparation) which indicate that c_3 is destabilized by a positive potential energy surface lining the central aqueous cavity. This field presumably draws substrates into the cavity and promotes docking at the active site. Supported by NIH.

W-Pos162 CHARACTERIZATION OF RHODANESE*TETRACYANONICKELATE: AN ACTIVE SITE COMPLEX THAT SLOWS CONVERSION TO INERT CONFORMERS. Shiu Fung Chow and Paul Horowitz, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284.

Tetracyanonickelate (TCN) binds tightly at the active site of the enzyme rhodanese and displays characteristics expected of a transition state analog. The structure of the rhodanese*TCN complex has been characterized in spectral and physical studies using urea as a structural perturbant. UV difference absorption, sedimentation velocity ultracentrifugation, fluorescence, and circular dichroism data show no significant conformational differences between sulfur-free rhodanese (E) and the E*TCN complex. The urea-induced enzyme structural transition curves were noncoincident when different structural parameters were monitored. For E, the urea concentrations giving half-maximal change (C_m) were: C_m= 3.0 M for activity measurement; C_m= 2.8 M for protein intrinsic fluorescence intensity; C_m= 4.3 M for ellipticity at 220 nm; and C_m= 3.3 M for wavelength of fluorescence emission maximum. For the E*TCN complex, C_m was shifted to a higher urea concentration relative to that found for E when activity (C_m=3.6 M) and native protein fluorescence (C_m=3.6 M) were the measured parameters but not when the wavelength of the emission maximum and ellipticity were monitored. Furthermore, urea-induced rhodanese structural changes were time-dependent and TCN binding on E slowed enzyme inactivation that is associated with structural relaxations. These findings, that TCN affects structural relaxations in rhodanese, are of particular interest in light of the recent suggestion that the E*TCN complex mimics a normally inaccessible intermediate in catalysis. (Supported by NIH grant GM25177 and Welch grant AQ723).

W-Pos163 STUDY OF SULFHYDRYL GROUPS OF ENZYME I OF PTS. M. Han, D. Walbridge, R. LaForce, S. Shiber, N. Meadow, and L. Brand. Dept. of Biology, The Johns Hopkins University, Baltimore, MD. 21218 NIH grant No. GM 11632

The phosphoenolpyruvate:glycose phosphotransferase system (PTS) is responsible for the phosphorylation and concomitant transport of its sugar substrates across bacterial plasma membranes. Enzyme I (EI), HPr, and III^{glc} are involved in a series of phosphorylation reactions of the PTS. EI was purified from an overproducer strain of E. coli according to Weigel et. al. (N. Weigel et. al.,JBC,257,14461,1982). Titration of the SH groups of EI with didansylcystine inactivated the EI. When completely inactivated, fluorescence polarization studies showed that EI remains predominantly monomer. EI was titrated with 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) at pH 7.5 under denaturing conditions (with 8M urea and 6M guanidine HCl) at 23°C, and under non-denaturing conditions at 4°C and 23°C. Four SH groups were titrated under each of these conditions, in agreement with the number from DNA sequencing of E. coli (D. Saffen et. al.,unpublished observation). EI was progressively inactivated upon labeling with DTNB, and became totally inactive when all four SH groups were labeled, suggesting the involvement of the SH groups in the activity.

We also studied the kinetics of the DTNB reaction with EI. The preliminary results indicated that the rate was dependent on EI concentration; higher concentrations of EI (0.5 mg/ml) showed a psuedo-first order rate of .849 X 10^{-3}/s whereas lower concentrations of EI (0.05 mg/ml) showed a faster rate of 2.70 X 10^{-3}/s. The denatured EI increased the rate to 40.58 X 10^{-3}/s with 0.266 mg/ml of EI. This may reflect changes in the accessibility of SH in EI. The rate was also affected by the presence of HPr, Mg^{2+}, and PEP; HPr increased the rate, Mg^{2+} plus PEP decreased the rate.

W-Pos164 BIOPHYSICAL CHARACTERIZATION OF RECOMBINANT HUMAN ERYTHROPOIETIN PRODUCED IN CHINESE HAMSTER OVARY CELLS. Janice M. Davis, Thomas W. Strickland, David A. Yphantis*, and Tsutomu Arakawa (introduced by E. H. Braswell), Amgen, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320. *The Biological Sciences Group U-125, The University of Connecticut, Storrs, CT 06208.

Structural characterizations were carried out for recombinant human erythropoietin (EPO) produced in Chinese hamster ovary cells. The far UV CD spectrum indicates that EPO contains approximately 50% α-helix. The near UV CD spectrum shows strong positive bands at 290 nm and 282 nm, which can be assigned to the ^1L_b transition of tryptophan. This indicates that at least one tryptophan must be in a relatively rigid environment. These structural features, determined by CD, are identical to those for EPO isolated from urine (Lai, P., Everett, R., Wang, F., Arakawa, T., and Goldwasser, E., unpublished results). The fluorescence spectrum (room temperature) showed a maximum at 345 nm. The 0,0-band of phosphorescence (77 K) was at 410 nm. These spectra and fluorescence quenching experiments suggest that all luminescent tryptophans are solvent exposed. No tyrosine luminescence is observed. Sedimentation equilibrium experiments showed that CHO-derived EPO has a molecular weight of 31,500 ± 1,000, indicating a carbohydrate content of approximately 40%. Further structural characterizations are in progress and will be presented.

W-Pos165 SEDIMENTATION EQUILIBRIUM MEASUREMENTS OF RECOMBINANT DNA-DERIVED HUMAN INTERFERON-γ. David A. Yphantis and Tsutomu Arakawa. Department of Molecular and Cellular Biology, University of Connecticut, Storrs, CT 06268 and Amgen, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320.

The state of aggregation of interferon-γ (INF-γ) was examined using sedimentation equilibrium techniques near neutrality and under moderately acidic conditions. The INF-γ used was a recombinant DNA-derived human interferon-γ, expressed in E. Coli. This protein was not glycosylated and the N-terminal residues were met-gln. Short-column experiments in 0.1 M ammonium acetate at pH 6.9 and at loading concentrations from 0.37 to 1.5 g/l gave Z-average molecular weights twice those predicted from the known sequence. Some nonideality was seen. High-speed equilibrium experiments in 0.040 M imidazole buffer, pH 7.5, with initial concentrations of 0.14 to 1.4 g/l also showed IFN-γ to be a dimer with no observable dissociation to monomer but with significant Donnan nonideality. Complete dissociation to monomer was observed at pH 3.5 at 0.001 M ionic strength and at loading concentrations of 0.1 to 1 g/l. Extreme Donnan nonideality was evident. Addition of 0.1 M NaCl at pH 3.5 resulted in strong association of IFN-γ, with Z-average molecular weights over 1,200,000 observed in short column experiments with loading concentrations of 0.25 to 1 g/l. No reversible equilibrium was seen under these conditions and the smallest species detectable were at least decamers in size.

W-Pos166 UNFOLDING AND SELF-ASSOCIATION OF RECOMBINANT HUMAN INTERFERON-γ IN ACID T. Arakawa, Y.-R. Hsu and D. A. Yphantis* (intr. by T. M. Schuster). Amgen, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320 *The Biological Sciences Group U-125, The University of Connecticut, Storrs, CT 06268.

We have previously shown that acid treatment of recombinant human interferon-γ (IFN-γ) results in formation of an aggregated form the extent of which depends on the protein concentration. The present study was undertaken to determine the mechanism of aggregate formation. Purified IFN-γ in a dimeric form was dialyzed against 20 mM HCl or 20 mM sodium acetate, pH 3.5, which resulted in unfolding of the molecule. Sedimentation equilibrium showed that the protein at pH 3.5 is a monomer. When this was neutralized by dialyzing against 0.1M NH_4OAc, the same structure was restored. Addition of NaCl to 50 to 200 mM to an IFN-γ solution at pH 2 or pH 3.5 resulted in a different CD profile from the spectra obtained without salt. Sedimentation equilibrium analysis of the sample at pH 3.5 in the presence of 0.1M NaCl showed that the protein is extensively aggregated. Dialysis of this sample against 0.1M NH_4OAc showed that the protein maintains the aggregated state. Removal of 0.1M NaCl from the pH 3.5 sample by dialysis against the pH 3.5 buffer resulted in an identical structure to the one before NaCl addition. These results suggest that IFN-γ reversibly self-associates into large aggregates at low pH depending on salt concentration and that this self-association determines formation of the aggregate after acid treatment.

W-Pos167 TRANSIENT ELECTRIC BIREFRINGENCE OF RABBIT STRIATED MUSCLE MYOSIN OBTAINED FROM DIFFERENT SOURCES, Sonja Krause and Kusuma Thallam, Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180.

The birefringence relaxation time, and therefore the conformation in solution, of rabbit striated muscle myosin obtained from J. F. Koretz, Biology Dept., Rensselaer Polytechnic Inst. (sample A) and from the laboratory of W. F. Harrington, Dept. of Biology, The Johns Hopkins Univ. (sample B) are strikingly different. Both differ from values obtained by Bernengo and Cardinaud, J. Mol. Biol. (1982) 159, 501 (sample C), in high ionic strength phosphate buffer at pH 7, which were consistent with a myosin conformation that included a 90 degree bend between the light and heavy meromyosin portions of the molecule. Data on sample B, obtained in 10 mM pyrophosphate buffer at pH 8 to 9 both by Hvidt et al, Biopolymers (1984) 23, 1283, and in the present work, are consistent with the extended conformation of myosin. Data on sample A, obtained in this work, both in 2 mM and 10 mM pyrophosphate buffer at pH 9 and in high ionic strength phosphate buffer at pH 7, are internally consistent but do not agree with data on samples B or C. Because of the overlap of buffer systems and pH used for the different samples, the differences in data cannot be ascribed to the different buffer systems but are probably due to different impurities present in the different samples. The birefringence relaxation time of sample B decreases $\sim$ 32% as the temperature increases from 25°C to 40°C in 10 mM pyrophosphate buffer at pH 9, indicating a somewhat increased flexibility of myosin with increasing temperature.

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W-Pos168 INFLUENCE OF THE SIDE CHAIN PHOSPHATE ON THE BINDING OF REDUCED FLAVIN TO AZOTOBACTER APOFLAVODOXIN. D.E. Edmondson and G. Williamson (Intr. by D.B. McCormick) Dept. Biochem., Emory Univ., Atlanta, GA 30322

The effect of the side-chain phosphate on the affinity of Azotobacter apoflavodoxin for the various redox levels of flavin has been determined by redox potential measurements on reconstituted apoprotein complexes with FMN and with riboflavin (RFℓ). Both native and reconstituted FMN-apoprotein complexes have, at pH 8.0, one-electron couples of -203 mV (PF/PFH$\cdot$) and -500 mV (PFH$\cdot$/PFH$^-$). The same couples for the RFℓ-apoprotein complex were determined to be -277 and -251 mV, respectively. Approximately 23% of neutral RFℓ semiquinone is thermodynamically stabilized on 1-electron reduction of the RFℓ-apoprotein complex. The binding of the hydroquinone forms of riboflavin and of FMN to apoflavodoxin were also measured directly from the quenching of the tryptophanyl fluorescence of the apoprotein. The respective K_a values determined by this approach were in reasonable agreement with those calculated from the redox potential data. These show that the apoflavodoxin exhibits stronger affinities for the oxidized and neutral semiquinone forms of FMN than for riboflavin by factors of 10^3 and $>10^4$, respectively; however, the affinity for the hydroquinone form of FMN was only 3-10 times than for reduced riboflavin. These data suggest that electrostatic repulsion between the anionic side-chain phosphate and the anionic N(1) position of the flavin hydroquinone does not contribute to the reduced binding affinity of the apoflavodoxin for the reduced FMN relative to the oxidized form. (Supported by NIH Grant GM-29433).

W-Pos169 MOLECULAR AND STOCHASTIC DYNAMICS OF PROTEINS Walter Nadler and Klaus Schulten, Dept. of Physics, Technical University of Munich, 8046 Garching, Fed. Rep. Germany

The microscopic short-time dynamics of proteins resulting from molecular dynamics simulations (collaboration with Karplus et. al.) can be reproduced on a longer time scale by stochastic Langevin equations involving selected degrees of freedom. For this purpose one has to determine the local viscosity of the atomic motions by means of the corresponding velocity autocorrelation functions. The viscosity can be extrapolated to describe the 3-dimensional motion of subunits which contain many atoms. The extropolated viscosity yields a satisfactory description of the Moessbauer spectra of Myoglobin by Parak et. al. which account for the 1-100 ns dynamics of the heme group. However, on this time scale the heme group appears to be often trapped in conformational substates a feature which increases the effective viscosity of the group by a factor 100. Our comparision of the stochastic motion of single atoms in proteins was based on a solution (in terms of the velocity correlation function) of the Langevin equation for a harmonic potential. The analysis of the Moessbauer data involved the solution of the Langevin equation for a general 3-dimensional corrugated (many substates) potential surface in the limit of strong friction in terms of the Pade approximation of the Moessbauer line shape function reproducing correctly its low and the high frequency dependence.

W-Pos170 ANALYSIS OF ^{1}H NMR SPECTRA OF PROTEINS USING MULTIPLE QUANTUM COHERENCE. Mark Rance, Claudio Dalvit and Peter Wright, Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037.

Multiple quantum spectroscopy offers significant advantages for analysis of the overlapped ^{1}H NMR spectra of proteins. Such experiments are particularly important for proteins of molecular weight greater than 10,000, for which difficulties are experienced in establishing unambiguous spin system assignments using phase-sensitive COSY methods alone. We have successfully applied triple quantum-filtered COSY and double and triple quantum COSY experiments to plastocyanin (M_r 10,000) and myoglobin (M_r 18,000). The advantages of phase-sensitive spectra and the important role of remote connectivities in spin system analysis will be described. Considerable simplification and editing of one-dimensional spectra can be achieved through application of 3, 4 and 5 quantum filters. With the aid of multiple quantum NMR experiments, a great many spin systems can be unambiguously identified for proteins as large as myoglobin. Sequential assignment procedures then provide sequence-specific assignments. The new methods make feasible detailed ^{1}H NMR investigations of proteins of molecular weight as high as 18,000.

W-Pos171 MODIFICATION BY DICYLGLYCEROL OF THE STRUCTURE AND INTERACTION OF VARIOUS PHOSPHOLIPID BILAYER MEMBRANES. R.P. Rand, S. Das. Department of Biological Sciences, Brock University, St. Catharaines, Ontario, Canada.

We have measured the effects of incorporating diacylglycerol (DG) derived from egg phosphatidylcholine (PC) into PC, egg phosphatidylethanolamine (PE) and bovine phosphatidylserine (PS). In excess aqueous solution DG induces a multilamellar-to-hexagonal (L-H) structural transition in PE and PC that is temperature dependent. At 37°C it begins at about 3 and 30 mole percent DG respectively. In PC at lower DG concentrations a modified lamellar phase is formed; at about 70 mole percent DG a single primitive cubic phase forms. An L-H transition is induced by 20-30 mole percent DG in PS, dependent on ionic strength and degree of lipid hydration. Crystalline acyl chains appear at the higher DG levels. Calcium precipitates of DG/PS (1/1) mixtures have melted chains. Structural parameters were derived from the lamellar phases at sub-transition levels of DG in PE and PC. The area per polar group is increased but by contrast with cholesterol the polar group speading is not accompanied by an increase in bilayer thickness. DG does not affect the equilibrium separation of PC or PE bilayers in excess water. Measured interbilayer forces, as they vary with bilayer separation, show that DG at 20 mole percent does not effect closer apposition of PC bilayers at any separation. Spreading the polar groups may effect the binding of protein kinase C or the activation of phospholipases; the non-lamellar phases may be linked to the biochemical production of DG in cellular processes involving membrane fusion.

W-Pos172 KINETICS OF TRANSITIONS INVOLVING THE LAMELLAR, CUBIC, HEXAGONAL, AND FLUID ISOTROPIC PHASES OF HYDRATED MONOACYLGLYCERIDES MONITORED BY TIME-RESOLVED X-RAY DIFFRACTION. Martin Caffrey, Section of Biochemistry, Molecular and Cell Biology, Clark Hall, Cornell University, Ithaca, NY 14853.

A study of the dynamics of the various thermotropic phase transitions undergone by the hydrated monoacylglycerides, monoolein and monoelaidin, in the temperature range 0-120°C and over a range of salt concentrations has been undertaken. Measurements were made using time-resolved x-ray diffraction at the Cornell High Energy Synchrotron Source. The lamellar chain "melting", lamellar/cubic (body centered), cubic (body centered)/cubic (primitive), cubic (primitive)/fluid isotropic, cubic (primitive)/hexagonal, and hexagonal/fluid isotropic transitions were examined under active heating and passive cooling using a jump in temperature to effect phase transformation. All of the transitions in the heating direction were rapid occurring in $\leq$ 3 s. In the cooling direction however, the cubic (primitive)/cubic (body centered) transition was extremely slow with a transit time of 15-30 min and displayed a pronounced hysteresis.

In addition to the time-resolved measurements, data was obtained on the stability of the various phases at high salt concentrations. In the case of monoolein, high salt strongly favors the hexagonal over the cubic phase and slightly elevates the hexagonal/fluid isotropic transition temperature. With monoelaidin, the hexagonal phase which is not observed in the absence of salt, becomes the dominant phase at high salt concentration. In this case the cubic (body centered)/cubic (primitive) and lamellar/cubic (body centered) transition temperatures decrease, while that of the lamellar chain "melting" transition increases at high salt concentration.

W-Pos173 SOLID STATE ^{13}C-NMR OF ORIENTED MONOGLYCOSYLCERAMIDE-PHOSPHOLIPID BILAYERS. V.L.B. Braach-Maksvytis, Dpt Biochemistry, University of Sydney, NSW, 2006, Australia, and B.A. Cornell, CSIRO, PO Box 52, North Ryde, NSW, 2113, Australia.

We have prepared a series of monoglycosylceramides with different numbers of hydroxyl groups on the sphingosine base and a carbon-13 label at the carbonyl carbon. These monoglycosylceramides were dispersed at various mole ratios with egg yolk phosphatidylcholine, hydrated and dispersed on glass cover slips. Stacks of approximately 50 cover slips were sealed into glass tubes and studied by solid state cross polarization carbon-13 NMR as a function of orientation to the magnetic field. A similar study was performed using DMPC at temperatures below and above the phase transition temperature.

It was found that the use of aligned samples permitted the resolution of the chemical shift anisotropy derived from the phospholipid and monoglycosylceramide carbonyl resonances. The monoglycosylceramide carbonyl chemical shift anisotropy is interpreted in terms of the conformation and dynamics of the various monoglycosylceramides under the different conditions of concentration and temperature.

W-Pos174 STRUCTURE AND THERMOTROPIC PROPERTIES OF LACTOSYLCERAMIDES (LC) AND THEIR INTERACTION WITH DIPALMITOYL PHOSPHATIDYLCHOLINE (DPPC). R.A. Reed, and G.G. Shipley. Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

Differential scanning calorimetry (DSC) and x-ray diffraction have been used to study the properties of hydrated N-palmitoyl, N-stearoyl and N-lignoceryl dihydro LC (NPLC, NSLC and NLLC) and their interaction with DPPC. By DSC, NPLC exhibits 3 broad endothermic transitions on heating at 58, 64, and 75°C (total enthalpy 8.09 Kcal/mol). By x-ray diffraction, at 25°C a lamellar bilayer structure (d=61.4Å) with multiple wide angle reflections characteristic of an ordered hydrocarbon chain packing mode is observed. At 60°C, a polymorphic transition has occurred; the bilayer structure remains (d=62.9Å) but different wide angle reflections are observed indicative of a different, but still ordered, chain packing. At 83°C, the bilayer periodicity has decreased to 59.8Å and the broad reflection at $\sim 1/4.6Å^{-1}$ indicates formation of the melted chain L_α phase. Thus, NPLC exhibits both polymorphic and chain melting transitions. Similar behavior is exhibited by NSLC and NLLC, although an additional exothermic transition is observed at 69 and 75°C, respectively.

The binary systems NPLC-DPPC, NSLC-DPPC and NLLC-DPPC have been studied. In each case, (i) addition of ∿10 mol% LC removes the pre-transition associated with DPPC, (ii) addition of up to ∿25 mol% LC results in a slight increase of Tm to ∿ 43°C, (iii) at >25 mol% LC additional endotherms characteristic of an LC-rich phase are observed. Thus, similar to galactosylceramide (see Ruocco et al., Biophys. J. (1983) 43,91), lactosylceramides exhibit limited solubility in DPPC bilayers.

W-Pos175 IDENTIFICATION AND CHARACTERIZATION OF THE GLASS FORMING CRYOPROTECTANTS IN POPULUS Allen Hirsh, Tsuneo Takahashi, and Robert Williams, American Red Cross Biomedical R & D Laboratories, Bethesda, Maryland, USA

Complete resistance to freezing injury in Populus balsamifera is associated with the formation of intracellular glasses (Hirsh, Williams, and Meryman, Plant Physiology, Sept. 1985). The primary constituents of these aqueous glasses appear to be the tri-and tetrasaccharides, raffinose and stachyose. We show that, while aqueous solutions of these polysaccharides display glass transitions above -40°C, they are unstable to eutectic formation during devitrification. Populus cells, however, display no evidence of eutectic formation unless intracellular ice has formed during quick cooling but the melting pattern is then complex. Both Populus and aqueous solutions of high molecular weight polymers display complex thermal behavior when air dried whereas aqueous solutions of raffinose and stachyose do not, suggesting that intracellular proteins may be influencing thermal events during cooling and warming of the living cells.

W-Pos176 STRUCTURE AND DYNAMICS OF THE CARBOHYDRATE MOIETIES OF GLYCOLIPIDS: A ^2H NMR STUDY. Ian C.P. Smith, Jerzy B. Giziewicz and Harold C. Jarrell, Division of Biological Sciences, National Research Council, Ottawa, Canada K1A OR6.

The headgroup orientation and motional characteristics of 1,2-di-O-tetradecyl-3-O-β-D-glucopyranosyl-glycerol (DTGL) selectively ^2H-labelled on the glucose moiety have been investigated by differential scanning calorimetry and ^2H NMR. The glycolipid undergoes a major endothermic transition at 52°C which is attributed to the gel to liquid crystal phase transition. A smaller endothermic transition at 58°C, attributed to a lamellar to hexagonal mesophase transition by ^2H NMR, is confirmed by X-ray diffraction. In the lamellar phase the glycolipid headgroup undergoes axially symmetric motion and has an orientational order parameter, S_{mol}, of 0.45 which is significantly larger than that (0.31) reported for an analogous glucosylcerebroside. The headgroup is extended away from the bilayer surface. On entering the hexagonal mesophase the orientational order parameter for the sugar ring is reduced slightly to 0.38, but the ring undergoes a large reorientation with respect to the local rotation axis. The orientation of the headgroup is affected by the greater extension of the other surface residues in a phospholipid matrix relative to that observed for the glycolipid alone. Two orientations of the exocyclic hydroxyl group of the sugar were detected by ^2H NMR and are shown to have unequal populations.

W-Pos177 INTERACTION OF PHOSPHOLIPIDS AND TREHALOSE: A SOLID STATE NMR STUDY

Carolyn W.B. Lee, David J. Siminovitch, Martin J. Ruocco, Sunil K. Das Gupta, and Robert G. Griffin. Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139.

Trehalose, a non-reducing disaccharide, is known to expand phospholipid spacing in the bilayers of dry membranes. A postulated mechanism is that trehalose keeps the phospholipids in a liquid crystalline phase by replacing the water of hydration and interacting with the headgroups.[1] As a test of this hypothesis, solid state NMR studies were undertaken on dry mixtures of trehalose with DPPC at a concentration of 2:1 trehalose/DPPC (mole fraction). Both ^{2}H- and ^{31}P-NMR static techniques were employed. ^{31}P-NMR shows a rigid headgroup presumably due to hydrogen bonding between sugar OH groups and the phosphate headgroup of the lipid. An unusual intermediate exchange lineshape for 2[7,7-d_2]DPPC was noted by ^{2}H-NMR. This lineshape can be simulated by *trans-gauche* isomerization among four tetrahedral sites. These observations indicate that phospholipids in dry model membranes are not in a conventional liquid crystalline state; the chains are free to move but the headgroups are fixed by the sugar-lipid interaction.

1. Crowe et al., Science 223, 701 (1984).

W-Pos178 PRESERVATION OF DRY LIPOSOMES BY CARBOHYDRATES.

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We have shown previously that trehalose, a carbohydrate found at high concentrations in many organisms that normally survive dehydration, can prevent leakage of trapped solute from lyophilized liposomes (Crowe, et al., Arch. Biochem. Biophys 242, 1985). Trehalose achieves this effect by preventing fusion and lowering the phase transition temperature of the dry lipids. In this present study, we show that other carbohydrates can also prevent leakage of trapped solute from lyophilized unilamellar liposomes made from POPC:PS (9:1). These carbohydrates, mono-, di-, and trisaccharides and inositol, have varying effects on both the fusion of liposomes (as estimated by resonance energy transfer) and the retention of isocitrate during dehydration and rehydration. Although fusion was minimized by all the carbohydrates at between 0.2 and 0.4 g carbohydrate/g lipid (g/g), none of the carbohydrates tested were as effective as trehalose in preventing fusion of unilamellar liposomes. The exception to this was inositol, which had no effect in preventing fusion during freeze-drying and rehydration. Retention of isocitrate was maximized by most carbohydrates at between 0.8 and 1.0 g/g; only maltose was as effective as trehalose in preventing leakage during lyophilization and rehydration. Inositol showed some slight ability to prevent leakage at very high mass ratios; glucose and galactose were not very effective in preventing leakage. Calorimetric data are presented to explain the effect of carbohydrate on phase separation and phase transitions in dry liposomes. [Supported by National Science Foundation (grant PCM 82017538) and National Sea Grant (grant RA/41 to JHC and LMC).]

W-Pos179 UNUSUAL SOLUTION PROPERTIES OF THE STABILIZING AGENT PROLINE.

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Particular solutes accumulate in plants and animals during reduced water content induced by dehydration or freezing temperatures. We have previously studied the protective interactions of two such agents, the disacharride trehalose and the amino acid proline, with phospholipid bilayers. Proline is thought to participate in an alternate stack in bulk solution which may be important for its action in stabilizing biomacromolecules during reduced water states. In this work, we provide physical chemical evidence of unusual bulk solution properties of proline, including evidence for strong hydrogen bonding of proline in water. Differential scanning calorimetry of aqueous proline solutions show reduced enthalpy associated with the melting of bulk water and a reduction in the temperature of this phase change. As the enthalpy associated with the melting of bulk water is reduced, the enthalpy associated with a eutectic melt of proline at 252 K increases. This eutectic may be associated with the intermolecular stack of proline monomers. A partial phase diagram of proline is presented based on these results. Infrared spectroscopy data on the bulk solution properties of proline in D2O shows splitting of the COO^- asymmetric stretch in proline solutions of high concentration which may indicate participation of this group in the intermolecular stack of proline. Bulk solution effects of proline in aqueous dispersions of phospholipids are examined by FTIR (Fourier Transform Infrared spectroscopy). These experiments indicate that the COO^- group of proline and the phosphate group of the lipid head group may be important functional groups in this protective interaction. (Supported by grants R1A-62 from National Sea Grant and PCM 82-17538 from the National Science Foundation.)

W-Pos180 SURFACE COMPRESSIBILITY, EXPANSIVITY, AND TRANSITION PROPERTIES OF DMPC/CHOLESTEROL BILAYER VESICLES: MAJOR EFFECTS OF ENHANCED COHESION AND FLUIDIZATION. D. Needham and E. Evans, Pathology, University of British Columbia, Vancouver, B.C. V6T 1W5.

Micropipet aspiration of giant ($2x10^{-3}$ cm diameter) bilayer vesicles composed of dimyristoylphosphatidylcholine (DMPC):Cholesterol (CHOL) mixtures was used to regulate membrane tension levels and to provide sensitive detection of vesicle surface area changes. Vesicle area vs temperature plots showed that the area change associated with the DMPC gel-liquid phase transition decreased with cholesterol concentration and that the transition broadened and shifted to higher temperatures. The transition disappeared by 50 mol % CHOL with a resultant thermal expansivity of 1.5×10^{-3}/°C. Relative area changes at the transition were consistent with the formation of a 1:1 CHOL:DMPC complex, the area change being simply due to freezing of uncomplexed lipid. The bilayer area compressibility modulus for the 1:1 mixture was found to be comparable to that for gel state lipids (10^3 dyn/cm^{-1}); coupled with the significant increase in tension level for lysis (from 2 to 6 dyn/cm), these results indicate a highly cohesive surface. For concentrations above 12 mol %, however, the vesicles behaved in a liquid-like manner at temperatures well below the DMPC phase transition. Thus, cholesterol greatly enhances the bilayer surface cohesion but maintains a liquid-like structure. In addition, transition area changes observed for low cholesterol concentrations were consistent with tilted geometry for DMPC acyl chains.

W-Pos181 STRUCTURE AND THERMOTROPIC PROPERTIES OF HYDRATED N-PALMITOYL SPHINGOMYELIN (C16:0-SM) BILAYERS AND ITS INTERACTION WITH CHOLESTEROL AND DIPALMITOYLPHOSPHATIDYLCHOLINE (DPPC). P.R. Maulik, P.K. Sripada and G.G. Shipley. Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

N-palmitoyl sphingomyelin (C16:0-SM) was synthesized from bovine brain sphingomyelin following acid hydrolysis conversion to sphingosine phosphoryl choline (SPC), then reacylation of SPC using palmitoyl imidazole. A combination of differential scanning calorimetry (DSC) and x-ray diffraction have been utilized to investigate the structure and properties of C16:0-SM and its interaction with cholesterol and DPPC. DSC of hydrated multi-bilayers of C16:0-SM show reversible order-disorder transitions. Anhydrous C16:0-SM exhibits an endothermic transition at 75°C (ΔH= 4.01Kcal/mol). Increasing hydration progressively lowers the transition temperature (T_c) and increases the transition enthalpy (ΔH), until limiting values (T_c=41°C, ΔH=7.55Kcal/mol) are observed for hydration values >25 wt% H_2O. X-ray diffraction at temperatures below (T_c-29°C) show a bilayer gel structure (d=73.5Å; sharp 4.27Å reflection). Above T_c, at 55°C, a bilayer liquid crystal structure is present (d=66.6Å, diffuse 4.6Å). Addition of cholesterol to C16:0-SM bilayers results in a progressive decrease in the enthalpy of the transition at 41°C and no transition is detected at ≥50 mol% cholesterol. The x-ray diffraction data show no change of periodicity or wide-angle reflections at 29°C and 55°C when 50 mol% cholesterol is present. Thus, cholesterol inserts into C16:0-SM bilayers progressively removing the chain melting transition and changing the structural characteristic of the bilayer. DSC and x-ray diffraction data show that DPPC is miscible with C16:0-SM in both the gel and liquid-crystalline phases.

W-Pos182 KINETICS OF CHOLESTEROL EXCHANGE BETWEEN SMALL UNILAMELLAR VESICLES OF DIFFERENT PHOSPHOLIPID COMPOSITION. L. Bar, Y. Barenholz, P.L.-G. Chong and T.E. Thompson (Introduced by J.W. Ogilvie) Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908.

The kinetics of transfer of radioactive [^{3}H]cholesterol between small unilamellar vesicles was followed at 37°C. The exchange was performed using neutral donor vesicles containing 1, 10, or 20 mole % cholesterol in 1-palmitoyl, 2-oleoyl phosphatidylcholine (POPC), traces of the labelled cholesterol and [^{14}C]cholesteryl oleate as a nonexchangeable marker. As acceptors negatively charged vesicles were used containing the same mole percent of cholesterol, in POPC and 15 mole % of 1-palmitoyl, 2-oleoyl phosphatidyl glycerol as the negatively charged phospholipid. In order to minimize back exchange, a 10-fold excess of acceptors was used in all experiments. First order kinetics were observed with k=0.0117 min^{-1} in good agreement with previously reported results. Suprisingly, however, about 20% of the total cholesterol was non-exchangeable in the 8 h time frame of the experiments. The size of this pool was found to depend upon the type of phospholipid. Control experiments strongly suggest that this non-exchangeable pool is not the result of a transbilayer compositional asymmetry combined with a slow flip-flop rate. Similar results were obtained when dehydroergosterol (DHE), a fluorescent cholesterol analog, was substituted for [^{3}H]cholesterol. In addition the polarization of the non-exchangeable pool of DHE was found to be less than that of the transferable pool. The existence of a non-transferable pool must reflect the molecular organization of cholesterol in the donor vesicles. (Supported by USPHS Grant GM-14618 and HL-17576.

W-Pos183 PHOSPHATIDYLCHOLINE-7KETO CHOLESTEROL INTERACTIONS: II EFFECT OF PHOSPHOLIPID ACYL CHAIN LENGTH. H.C. Liu, W. Tamura-Lis and L.J. Lis, Department of Physics and the Liquid Crystal Institute, Kent State University, Kent, Ohio.

Oxygenated sterol compounds have recently been shown to interact differently with phospholipid bilayers and red blood cell membranes than does cholesterol. Part of the difference between mixtures of dipalmitoylphosphatidylcholine with cholesterol and with 7-keto cholesterol is observed in the number of lamellar phases formed at low sterol concentrations. Specifically, cholesterol/DPPC (1:19) mixtures produce two phases with different repeat spacings when observed as a function of water content by x-ray diffraction, whereas 7-keto cholesterol/DPPC (1:19) mixtures form a single phase. We have extended this work by examining mixtures of 7-keto cholesterol and disteroylphosphatidylcholine using x-ray diffraction. Two lamellar phases with different bilayer repeat spacings are observed at low sterol concentrations (1:19, 7-keto cholesterol/DSPC). These results indicate a difference in the miscibility of cholesterol and 7-keto cholesterol with phosphatidylcholines of different acyl chain lengths and may have implications for the compositions of domains in cell membranes.

W-Pos184 THERMODYNAMIC STUDIES OF THE SUBTRANSITION IN CHOLESTEROL/DPPC MIXTURES. S. Tristram-Nagle and John F. Nagle. Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.

Our differential scanning calorimetry using the Microcal MC-1 shows that mixtures of X mole fraction cholesterol and dipalmitoyl phosphatidylcholine (DPPC) in aqueous dispersions have subtransitions when $X \leq .09$. Our studies also show that very pure DPPC (main transition half width .08 ^{o}C) when incubated at 1 ^{o}C forms an unstable subgel phase with a time constant of about 50 hrs, and half width of melting of 1.7 ^{o}C. When scanned at 13 ^{o}C/hr after 26 hrs incubation, this phase melts at 15.6 ^{o}C. The unstable phase converts into an apparently stable subgel phase, with a time constant of about 700 hrs. This broader transition has a half width of 3.0 ^{o}C and melts at 21.5 ^{o}C, when scanned at 13 ^{o}C/hr.

In DPPC/cholesterol mixtures when $X \geq .02$, the two subgel phases are broadened and become indistinguishable. The kinetics of formation of the combined subgel phase is slightly faster than the subgel phase in pure DPPC, based on the total enthalpies. However, the kinetics of formation of the subgel phase in the mixtures is identical to pure DPPC based on the melting temperatures. The stable subgel phase in the cholesterol mixtures melts at 20.5 ^{o}C when scanned at 13 ^{o}C/hr. The total subtransition enthalpy after long incubation times at 1 ^{o}C (>500 hrs) decreases from 4.9 kcal/mole of DPPC at X = 0 to 3.2 kcal/mole of DPPC at X = .09. In contrast, the pretransition enthalpy at X = .09 is reduced by 90%. At higher concentrations of cholesterol ($.26 < X < .55$), the main transition is so broad that both the subgel transition and pretransition are not discernible.

W-Pos185 TRANSBILAYER MOVEMENT OF CHOLIC AND DEOXYCHOLIC ACIDS IN EGG PHOSPHATIDYLCHOLINE BILAYERS. **Donna J. Cabral, James A. Hamilton and Donald M. Small. Biophysics Institute, Housman Medical Research Center, Boston University School of Medicine, Boston, Massachusetts 02118.**

The location of small amounts (2-4 wt%) cholic acid (CA) and deoxycholic acid (DCA) in small unilamellar egg phosphatidylcholine vesicles at low pH was studied using ^{13}C-carboxyl enriched bile acids and ^{13}C NMR spectroscopy. At 37°C and pH 3.5, CA in its protonated form equilibrated between the inner and outer monolayers of the vesicle. The exchange rate between monolayers was sufficiently slow (<10 s^{-1}) to yield two carboxyl (C24) peaks, one from the outside (178.0 ppm) and the other from the inside (177.4 ppm). Spectra of DCA in vesicles at the same relative concentration and temperature showed one C24 peak at an intermediate chemical shift (177.7 ppm). Temperature-dependent spectra of DCA in vesicles showed two well-resolved carboxyl peaks for DCA at 10°C which coalesced at 27°C. The corresponding coalescence temperature for CA was 53°C. The estimated rate constant for transbilayer exchange at 37°C was approximately 100 times larger for DCA than for CA. After addition of a CA/DCA solution (1:1 w/w) to bile acid-free vesicles, spectra taken at 37°C showed three C24 peaks at the chemical shifts of the individual bile acids in vesicles at this temperature. Results for the three weight ratios examined (2, 3 and 4% bile acid) were similar. Thus, protonated CA and DCA exchanged between monolayers of small unilamellar phospholipid vesicles but the removal of one OH group increased the rate 100 times. Further, when both were present in the same vesicle the rate of transbilayer movement was independent for each bile acid.

W-Pos186 Phospholipid Domain Formation and Cholesterol/Lipid Interactions Modulated by Electrostatic Forces. W. Heckl, M. Lösche, D. A. Cadenhead and H. Möhwald, Physics Dept. E22, TU Munchen D-8046 Garching F.R.G. and Dept. Chemistry, SUNY at Buffalo, Buffalo, NY 14214, USA

Fluorescence microscopy of monolayers at the air/water interface is a powerful tool to study pressure induced crystallization of phospholipids. Using this technique we have shown that formation of solid domains and domain interaction is controlled by electrostatic forces. For the case of an acidic phospholipid such as dimyristoyl phosphatidic acid (DMPA) exhibiting two dissociable protons, these forces can be varied to a large extent via the ionic mileau of the subphase. Thus the charge density difference between fluid and solid phases can be varied between zero and one c over 50 $Å^2$. It was shown by Weis and McConnell that, due to the edge activity of cholesterol, spiral domains of solid dipalmitoyl phosphatidylcholine (DPPC) are formed. These domains seemed to be unique for the large choline headgroup and were not observed by us for other lipids except for DMPA at high pH ($\sim$11). In the latter case the large charge density causes head group repulsion and hence large molecular areas, resulting in a chain tilt. This effects a reduction in symmetry and allows for a preferential cholesterol attachment to one of the two crystal phases. Thus cholesterol/lipid interactions can be modulated by electrostatic forces which then affect the orientation of the hydrocarbon chains. Observations with a variety of phospholipids, in turn can be explained by the result that a titled chain configuration allows for maximum cholesterol/lipid interaction. During crystal growth one observes aggregation of domains for spiral phases. This attractive interaction is ascribed to the superpositioning of non-compensating in-plane dipole moments, giving rise to a ferro-electric state.

W-Pos187 FORCE MEASUREMENTS OF A THREE COMPONENT LIPID MULTILAMELLAR ARRAY.
J. M. Collins, Md. Nurul Amin, and E. J. Reber, Dept. of Physics, Marquette Univ. Milwaukee, WI 53233.

We have continued to study the modification of the bilayer interaction forces; repulsive hydration, attractive van der Waals, and repulsive electrostatic, in multilamellar arrays composed of mixtures of dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), and cerebrosides. X-ray diffraction was used to determine the structural parameters of the arrays, and the osmotic pressure technique of LeNeveu, et al. (Nature (1976):259) was used to measure the net repulsive force as a function of bilayer separation. We observe an increase in the strength of the van der Waals interaction, but not as great an increase as for the two component (DOPC/cerebroside) systems. The repulsive hydration force is unaffected. And the electrostatic force displays a more complex behavior.

W-Pos188 STRUCTURAL AND ELECTROSTATIC PROPERTIES OF PHOSPHOLIPID VESICLES STUDIED USING ^{1}H-NMR CROSS RELAXATION SPECTROSCOPY. **Zhen-Chen Xu, Jeffrey F. Ellena and David S. Cafiso, Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901.**

Two-dimensional ^{1}H-NMR cross-relaxation spectroscopy has been used in small sonicated vesicle systems to study the packing and conformation of the membrane phospholipids. Independent cross-relaxation *networks* are revealed on both the internal and external vesicle surfaces and reflect the expected packing constraints in these small structures. Headgroup, terminal-methyl interactions that are not the result of spin-diffusion are also revealed in these spectra. The time development of the cross-peaks in these spectra is a function of the distance between nuclei and can be used to localize *ligands* that are bound to the vesicle membrane. This spectroscopy has been used to examine the location of both positive and negative hydrophobic ions in vesicles. The pattern of intermolecular cross-relaxation suggest that these ions bind within the membrane-solution interface and that the negative hydrophobic ions bind slightly deeper than their positive analogues. This conclusion is also consistent with measurements made using paramagnetic derivatives of hydrophobic ions. These results provide important structural information that can be used in conjunction with electrical data on these ions to develop a detailed molecular picture of membrane electrostatics.

(This work was supported by a Camille and Henry Dreyfus Foundation grant and NIH grant GM35215 both to DSC).

W-Pos189 FREE ENERGY POTENTIAL FOR ADHESION OF NEUTRAL LIPID BILAYER VESICLES IN SALT SOLUTIONS: EFFECTS OF BILAYER CRYSTALLIZATION, LIPID COMPOSITION, AND IONIC STRENGTH. E. Evans and D. Needham, Pathology, Univ. of British Columbia, Vancouver, B.C. V6T 1W5

Micromechanical tests of giant (3×10^{-3} cm diameter) vesicle-vesicle adhesion in 0.1 M salt solutions were performed to study the natural affinity between neutral lipid membranes composed of phosphatidylcholine (SOPC), phosphatidylethanolamine (POPE), and cholesterol. Suction micropipets were used to control vesicle membrane tensions and thereby to regulate the extent of equilibrium contact area formation; the free energy potential per unit area of contact formation was determined from the tension-area data. The free energy potential is dominated by van der Waal's attraction but is limited in magnitude by the strong repulsive, hydration force. Results from symmetric SOPE:POPE vesicle tests showed that the adhesion energies were consistent with an ideal mixing relation for the repulsive hydration potential; energies for POPE vesicles were 3 to 4 fold greater than those containing SOPC. For ionic strengths from 10^{-4}-10^{-1} M, the adhesion energy remained constant. Addition of cholesterol to the SOPC membranes resulted in a significant increase (3 fold) in the adhesion energy which appears to be due to an increase in the van der Waal's attractive potential. Comparison of the adhesion energies above and below the acyl chain crystallization temperature showed a several fold increase in the adhesion energy for the low temperature phase. For asymmetric vesicle compositions, the repulsive hydration force can be represented by a surface potential given by the geometric mean of potentials for each composition, consistent with the recent model published by Gruen and Marcejla. Since there were no apparent effects of ionic strength, the origin of the van der Waal's attraction must lie (well within the Debye screening length) close to the membrane surface.

W-Pos190 THE ACTION OF MECHANICAL FLUCTUATIONS ON THE INTERACTION BETWEEN BILAYERS AND BETWEEN LINEAR MACROMOLECULES. E. A. Evans, University of British Columbia, Vancouver, BC, VT6 1W5; V. A. Parsegian, D. C. Rau, N.I.H., Bethesda, MD 20205

The prescient work of Helfrich has made us all aware of the possibility that mechanical fluctuations contribute to the repulsive forces observed between bilayers. His original formulation computed the magnitude of fluctuations and their consequent steric forces assuming no action of other interbilayer forces. We have modified his formalism to estimate fluctuations and their associated energies as variations from a pure elastic state governed by long-range non-steric (e.g., hydration, van der Waals, electrostatic) forces. We find that mechanical fluctuations should have little effect on the appearance of hydration forces between charged or electrically neutral membranes. Mechanical fluctuations do cause some expansion of a lamellar lattice of neutral bilayers allowed to equilibrate with an excess aqueous solution. For charged bilayers in the regime of electrostatic repulsion, fluctuations cause an expansion of the repulsive electrostatic double layer such as has been seen in direct measurements of forces between charged bilayers in multilayer assemblies and between parallel DNA double helices in condensed phases. From the spread of peaks in diffraction patterns from ordered arrays, we are attempting to correlate the rms fluctuations predicted by theory with those qualitatively apparent in scattering patterns used in force measurement.

W-Pos191 THE KINETIC MECHANISM OF ANION CATALYZED PHOSPHATIDYLGYCEROL TRANSBILAYER MIGRATION IMPLIES CLOSE CONTACT BETWEEN VESICLES AS AN INTERMEDIATE STATE
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We have investigated the variation in the rate of Mn^{2+}- or Cd^{2+}- catalyzed phosphatidylglycerol transbilayer migration (Lentz, Madden, and Alford, *Biochemistry* 21, 6799) as a function of phospholipid and anion concentration, over better than a hundred fold range of both parameters. The slope of a plot of the logarithm of the rate of trans-bilayer lipid migration versus the logarithm of lipid concentration was 1.7, indicating that lipid redistribution was second order in vesicle concentration. The observed variation in rate with Mn^{2+} concentration was not so simple but could be interpreted as reflecting saturation at very low ion concentration (less than 0.04 μM) and first order kinetics at higher concentration (from 0.04 μM to 0.002 mM). The rate of trans-bilayer redistribution increased substantially between 37 and 56°C. An Arrhenius plot of the rate was non-linear and yielded activation energies in the range of 18 to 58 Kcal/mol. Taken together, these studies can be interpreted in terms of a model for the intermediate state of lipid redistribution. This model assumes the existence of a bilayer-destabilized state associated with Mn^{2+}-mediated close contact between two vesicle bilayers. *Supported by USPHS grant GM32707.*

W-Pos192 CNS^- AND Br^- BIND "EXTENSIVELY" TO DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS.
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The influence of monovalent cation and anion salt solutions on the interactive forces between dipalmitoylphosphatidylcholine (DPPC) bilayers was investigated. X-ray diffraction was used to study the effect of monovalent salts on the structure and packing of DPPC bilayers, and osmotic pressure was used to determine the interactive forces between bilayers. Initial x-ray diffraction studies and DSC studies (B.A. Cunningham, et al (1985) Biophys. J. 47: 427) indicate that monovalent salts have no effect on bilayer packing but Li^+ and $Acetate^-$ bind to some degree to DPPC bilayers. The influence of one molar chloride salts (Na^+, K^+, Li^+, Cs^+, NH_4^+, and Ba^{2+}) and potassium salts (Cl^-, SO_4^{2-}, $Acetate^-$, Br^-, and CNS^-) on the forces between bilayers has been examined more extensively. Preliminary results from x-ray diffraction studies show that, in one molar solutions, Br^- and CNS^- bind extensively to the DPPC head groups as indicated by inferred increases in the inter-lamellar separations (d_W). The binding of Br^- and CNS^- to DPPC headgroups causes an increase in the electrostatic repulsive force between the bilayers. The interactive forces between these bilayers were also measured and analyzed.

W-Pos193 THE LIPID HEADGROUPS, A TWO-DIMENSIONAL PROTON RESERVOIR INVOLVED IN ENERGY TRANSDUCTION? Stephan Grzesiek and Norbert A. Dencher (Intr. by H. Otto). Biophysics Group, Dept. of Physics, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, FRG.

During measurements of passive proton/hydroxyl ion permeabilities across the membrane of sonicated small phospholipid vesicles (soybean PC and diphytanoyl PC) by means of the internally entrapped pH-indicator dye pyranine (Grzesiek and Dencher, Biophys. J. 47: 274a, 1984) a high contribution of the lipid headgroups to the buffering power of the vesicle interior was detected. Starting from very general considerations, any small pH-difference across the membrane should relax with a time constant proportional to the buffering power of the liposome interior. This linear relation was experimentally verified in pH-jump experiments by variation of the internally entrapped phosphate buffer concentration. It can be used to determine the buffering power of the lipids dynamically. Calculated buffering powers per lipid molecule of $6 \cdot 10^{-2}$ (pH-unit)$^{-1}$ (soybean PC) and $3 \cdot 10^{-2}$ (pH-unit)$^{-1}$ (diphytanoyl PC) agree reasonably well with results of normal titrations of vesicle suspensions in the presence of protono- and ionophores. For the soybean PC vesicles, this is equivalent to an internal phosphate buffer concentration of up to 110 mM. The buffering power of the lipids, which has not been considered in previous investigations, obviously influences any determination of permeability coefficients from relaxation time constants. Furthermore, the strong buffering power of the lipid headgroups should be of importance for energy transduction across biological membranes. The lipid headgroup region could represent a two-dimensional reservoir capable of storage (as demonstrated in our investigation) and rapid transfer (Teissié et al., PNAS 82: 3217-3221, 1985) of protons from proton delivering reactions to proton consuming ones (e.g., ATP-synthases).

W-Pos194 **CATION/MEMBRANE INTERACTIONS OF ISOLATED CARDIAC SARCOLEMMAL VESICLES.** Kenneth Leonards, Dept. of Physiology, UCLA Medical School, Los Angeles, CA 90024.

The interactions of Ca^{2+} with the sarcolemmal-glycocalyx complex of cardiac cells is thought to play an important role in the regulation of excitation-contraction (E-C) coupling in cardiac muscle. To help elucidate the molecular mechanisms involved in this regulation cation/sarcolemmal interactions were investigated by analyzing the aggregation behavior of isolated sarcolemmal vesicles, sonicated unilamellar vesicles (SUVs) made from sarcolemmal lipid extracts, and SUVs generated from combinations of synthetic lipids as a function of pH, cation concentration, and chemical modification. Our results demonstrate that Ca^{2+} is selectively bound to the sarcolemmal surface and that this binding is species (rat vs. canine) dependent. The pH dependence of Ca^{2+}-induced vesicle aggregation suggests that two types of reactive groups are involved in this process, one of which binds Ca^{2+} and the second which modifies this Ca^{2+} binding as a function of pH. This pH dependence is eliminated by fluorescamine labeling of the sarcolemmal vesicles. H^+ also induces sarcolemmal vesicle aggregation in a species dependent matter, with the "threshold" pH values being in the pH 5.0 to 6.0 range. Fluorescamine labeling has no effect on H^+ induced vesicle aggregation, suggesting that NH_2 groups are not involved. In contrast, the lipid SUVs do not aggregate as a function of $[H^+]$, until the pH is reduced to the range of 2.0-3.0 indicating that this H^+ effect is not due to phospholipids. Companion experiments conducted using the two types of lipid SUVs also demonstrate Ca^{2+}/phospholipid binding. However, these interactions were pH independent and unaffected by fluorescamine labeling, suggesting that the second type of reactive group, above, (which modifies Ca^{2+} binding to the first) may not be phospholipid in nature. (Supported by Laubisch and Tuchbrieter endowments and NIH grant HL 34517.)

W-Pos195 ^{1}HNMR STUDIES OF CATION EFFECTS ON THE ACYL CHAINS OF ACIDIC PHOSPHOLIPIDS IN MIXTURES WITH NEUTRAL PHOSPHOLIPIDS. Paul Meers, Cancer Research Institute, University of California, San Francisco, CA

In order to study the behavior of the acyl chains of individual phospholipids in a mixed composition membrane, ^{1}HNMR was used to observe unmodified natural acidic phospholipids in mixtures with deuterated-chain lipid. Dimyristoyl phoshatidylcholine-d_{54} (DMPC-d_{54}) was synthesized by deuteration of myristic acid and acylation of glycerophosphorylcholine. The effects of the cations Ca^{2+}, Cd^{2+} and La^{3+} were studied using small unilamellar vesicles produced by sonication of mixtures of DMPC-d_{54} with egg phosphatidate (PA) or bovine brain phosphatidylserine (PS). The intensity of the ^{1}HNMR resonances from the methyl and methylene acyl chain protons were monitored in these experiments. Slower or more restricted motion of the acyl chains leads to a broadening of the resonances which removes some of the intensity of the signal from the region of measurement. For instance, when dipalmitoyl phosphatidylcholine vesicles were taken below their main phase transition temperature, the acyl chain signals flattened showing a reversible decrease in intensity to almost zero. Aggregation of vesicles alone did not produce a significant change in the signal intensity. Irreversible changes in signal intensity occurred under conditions where fusion of the vesicles was expected. A particularly interesting finding in these studies was that La^{3+} was able to reversibly decrease the intensity of the acyl chain signals of 30 mole % PS. The intensity decrease correlated with aggregation of the vesicles. The DMPC signal decreased as much as the PS signal. The La^{3+} concentration at which these effects occurred corresponded to the lowest concentration at which lanthanide shift reagents were able to exert shifts of the DMPC headgroup protons in tne PS/DMPC vesicles. Electron microscopic observation of the La^{3+}-aggregated vesicles showed areas of flattened apposed membranes in contrast to vesicles aggregated by avidin which appeared as clusters of spheres and did not show a decrease in NMR signal intensity upon aggregation. The flattened membrane surfaces could be responsible for the decrease in motion observed for both the PS and DMPC acyl chains. Alternatively, a slowly moving or motionally restricted complex of La^{3+}, PS and DMPC could explain the results.

W-Pos196 COMPARATIVE STUDY OF THE INTERACTION OF ANTHRACYCLINES WITH LIPID BILAYERS USING HIGH SENSITIVITY DIFFERENTIAL SCANNING CALORIMETRY. P. P. Constantinides[1], N. Inouchi[2], T. R. Tritton[3], A. C. Sartorelli[1], and J. M. Sturtevant[2] (Intr. by C. Stevens). [1]Dept. Pharmacol. and Comprehensive Cancer Center, Yale Univ. Sch. Med., New Haven, CT 06510, [2]Dept. Chem., Yale Univ., New Haven, CT 06520 and [3]Dept. Pharmacol., Univ. Vermont Coll. Med., Burlington, VT 05405.

High sensitivity differential scanning calorimetry was used to study the thermotropic behavior of multilamellar vesicles of neutral and acidic phospholipids and mixtures thereof in the presence of adriamycin (ADR) and its lipophilic analogue, N-trifluoroacetyladriamycin-14-valerate (AD32). The observed effects with liposomes of neutral dipalmitoylphosphatidylcholine (DPPC) paralleled octanol/buffer partition coefficients. The cationic drugs, chlorpromazine (CPZ) and quinidine (QND), were more effective than ADR in altering the phase behavior of DPPC liposomes because of the low affinity of ADR for neutral phospholipids. AD32, at high drug levels, induced alterations in the thermotropic behavior of DPPC liposomes similar to those of CPZ. These alterations were not reversible upon cooling and reheating and may indicate the formation of an interdigitated phase. Calorimetric measurements on liposomes prepared from pure dipalmitoylphosphatidylglycerol (DPPG) or from binary mixtures of DPPG and DPPC showed that modulation of bilayer properties by ADR was greatly enhanced in the presence of negatively charged lipid headgroups, presumably due to electrostatic interactions. AD32 interacted differently from ADR with acidic bilayers at low drug concentrations, in a manner similar to that of its interaction with neutral bilayers. At high drug levels, both ADR and AD32 produced transitions with multiple peaks not exhibited by CPZ and QND which may be due to specific association of anthracyclines with DPPG. All 4 drugs produced only minor changes in the enthalpy of the main transition of the investigated lipids.

W-Pos197 PHYSICAL PARAMETERS AFFECTING THE BINDING OF HEMATOPORPHYRIN DERIVATIVE TO LIPOSOMES AND CELLS. Benjamin Ehrenberg, Eitan Gross, and Zvi Malik*. Departments of Physics and* Life Sciences, Bar Ilan University, Ramat Gan 52-100, ISRAEL. (Intr. by Dennis Koppel).

Hematoporphyrin derivative (Hpd) has been used in the last years as a photosensitizing agent in photoradiative therapy of cancer. We checked the effects of several physical parameters on Hpd binding to sonicated liposomes and bacterial cells. The extent of binding was evaluated from the characteristic fluorescence bands of aqueous and membrane bound Hpd. Using unilamellar liposomes we found that an increase in cholesterol content in the lipid caused a decreased Hpd binding. This effect was found to be caused by the changed bilayer microviscosity, since the binding/viscosity relationship was independent of the bilayer additive which was used to modulate the viscosity. Lowering the temperature enhanced Hpd binding indicating a strong energetic effect which overcomes the influence of the increased microviscosity. Hpd binding is independent of the magnitude of the membranes' surface potential, indicating clearly that the binding species is the uncharged $HpdH_2$ molecule. Using gram-positive and gram-negative bacterial cells, we found that the extent of Hpd binding to the cells' membranes was correlated with the observed light induced damage to the cells. Thus, the binding of Hpd to the cytoplasmic membrane is a prerequisite to its photosensitizing action. This could lead to the design of molecular derivatives with better binding properties to cancerous cells.

W-Pos198 ANESTHETIC INTERACTIONS WITH BOUND WATER IN MODEL BILAYER MEMBRANES. N. Phonphok,† S. Horvath,* J.W. Doane*† and P.W. Westerman,*†. *Department of Biochemistry/Molecular Pathology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and †Department of Physics and Liquid Crystal Institute, Kent State University, Kent, Ohio 44242.

More than a decade ago it was observed that ^2H NMR splittings of ^2H_2O in lecithin bilayers at low levels of hydration, undergo additional motional narrowing near the main phase transition. We have studied the effect on these observed spectral changes, of chloroform and ether dissolved in dimyristoylphosphatidylcholine (DMPC) bilayers. At a DMPC/water molar ratio of 1/10, concentrations of ether and chloroform that induce the anesthetic state in living organisms (lipid/anesthetic ≈10/1) cause further line narrowing of the ^2H_2O signal. ^2H NMR results from the same systems with the ^2H label on the ether or chloroform, show these substances to be ordered in the bilayer. At the same solute concentration no effects on ordering are observed in ^2H-labelled DMPC. With increasing solute concentration, the first observable effect occurs at ^2H-labelled sites in the glycerol backbone and choline headgroup regions of DMPC. These results indicate that chloroform and ether in DMPC bilayers spend significant time at the interfacial region and interact strongly with water which is tightly bound to the membrane. The effects of other anesthetic substances are being explored. (Supported by NIH Grant #27127.)

W-Pos199 EFFECT OF ABSCISIC ACID ON PHOSPHOLIPID BILAYERS.
William Stillwell* and Stephen R. Wassall#, Departments of Biology* and Physics#, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223

Our laboratory has been studying the effect of the plant hormone abscisic acid (ABA) on phospholipid bilayer membranes. The hormone is shown to greatly affect the gross bilayer properties of permeability and the aggregation of small unilamellar vesicles. Both processes require the presence of a second polar lipid incorporated into the phosphatidylcholine bilayers and are pH and temperature dependent. ABA does not appear to behave as a general bilayer perturbing agent as no ABA dependent change in acyl chain motion can be detected by ^{13}C-NMR or by ESR using spin labelled stearic acid. The failure of ABA to alter ^{31}PNMR relaxation furthermore implies that the hormone is not strongly interacting with the phospholipid head groups and no significant ABA induced perturbations of phase behavior can be detected by differential scanning calorimetry or by the ESR-Tempo spin label technique. These results indicate that although ABA does have a profound effect on bilayer permeability, it must be altering very small regions of the bilayer perhaps through channel formation.

W-Pos200 ADSORBED ALAMETHICIN ALTERS MEMBRANE VOLTAGE-DEPENDENT CAPACITANCE.
I. Vodyanoy, V. Vodyanoy, and J.E. Hall. Department of Physiology and Biophysics, California School of Medicine, University of California, Irvine, Irvine, CA 92717

We have measured the voltage-dependent capacitance of phosphatidylethanolamine-squalane membranes in the presence and absence of alamethicin (Biophys. J. **47**, 254a, 1985). Alamethicin and it's derivatives modulate voltage-dependent capacitance at voltages lower than the voltage at which membrane conductance is increased due to alamethicin channels. The magnitude of the voltage-dependent capacitance modulation depends on the aqueous alamethicin concentration. The modulation is described by the Langmuir adsorption isotherm in the following way:

$$\delta C = cNk[A]/(1 + k[A])$$

where δC is the change in voltage-dependent part of the capacitance at a given voltage, c is the change in capacitance per adsorbed monomer, k is the adsorption coefficient, $[A]$ is the aqueous concentration of alamethicin and N is the number of adsorption sites per unit area. The model yields a coefficient of adsorption of about 1×10^4 1/M. This value is consistent with our results from alamethicin desorption experiments and also with the assumption that only a part of adsorbed alamethicin monomers form channels. Plots of $1/\delta C$ vs $1/[A]$ show that c depends on the applied voltage.

This work was supported by NIH grant GM 30657-07.

W-Pos201 **BINDING OF DIVALENT AND TETRAVALENT CATIONS TO PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE, PIP_2.**
Maureen Toner and Stuart McLaughlin. Dept. of Physiology and Biophysics, SUNY at Stony Brook, Stony Brook, NY 11794.

In many cells the binding of a hormone to a receptor activates a phospholipase C, which splits PIP_2 into the second messengers diacylglycerol and inositol trisphosphate. We investigated the binding of some physiologically and pharmacologically relevant ions to this important lipid. We formed multilamellar vesicles from mixtures of egg phosphatidylcholine, PC, and phosphoinositide (PI, PIP, or PIP_2) in 0.1M NaCl, 0.1M KCl, or 10mM TMACl and measured the effect of Ca, Mg, spermine, or gentamicin on the electrophoretic mobility of the vesicles. We determined that the intrinsic binding constants of Ca and Mg to PI are about $10M^{-1}$. Similar results were previously obtained with two other monovalent lipids, phosphatidylserine and phosphatidylglycerol. Ca is about 10-fold more effective in reducing the zeta potential of a PC:PIP_2 than a PC:PI membrane. The binding of spermine and gentamicin to PIP_2 can be described by the Gouy-Chapman-Stern theory with a 1:1 intrinsic association constant of order 10^3M^{-1}: these cations bind 100 times more strongly to PIP_2 than to PI (Biochemistry, 1985, 24: 442-452). Although the concentration of free spermine in cells is not known, the total concentration can approach the millimolar range. Since the surface potential of most biological membranes is negative, it is possible that much of the PIP_2 within cells is bound to spermine. The nephrotoxic and ototoxic effects of gentamicin and the other aminoglycoside antibiotics (e.g. neomycin) could be related to their ability to bind specifically to PIP_2. Supported by NIH Grant GM 24971.

W-Pos202 LOCATION OF DRUGS IN MEMBRANE BILAYERS BY X-RAY DIFFRACTION. Chester, D.W. and Herbette, L.G. Univ. of Connecticut Health Center, Department of Medicine, Farmington, CT

1,4-dihydropyridine (DHP) calcium channel antagonists are potent effectors of voltage-dependent calcium channels in cardiac sarcolemma. A "membrane pathway model" has been evolked as the putative mechanism by which these antagonists bind to ("locate") specific receptors. This pathway involves at least 3 discrete steps; partitioning and orientation of the DHP within the bilayer, lateral diffusion, and receptor binding. Neutron diffraction showed that the DHP, nimodipine, was located at the hydrocarbon core/water interface of the sarcoplasmic reticulum membrane, consistent with its high membrane partition coefficient (K_p = 5,000). Bay P 8857, a nifedipine analogue (K_p = 152,000), is a potent calcium channel antagonist with an electron dense iodine atom covalently bonded at the 5-iodoethylester carboxylate substituent to the pyridine ring. X-ray crystal structures of different DHP's and molecular modeling calculations (see abstract by Rhodes, D.G. and Herbette, L.G.) suggest that the iodine atom could identify the position of the pyridine ring complex along the membrane profile axis. The equilibrium position of Bay P 8857 in both artificial (DPPC) and biological (cardiac sarcolemmal) lipid multibilayers was determined by lamellar meridional x-ray diffraction; Bay P 8857 resided at the hydrocarbon core/water interface, penetrating approximately 10A into the lipid bilayer. The precise location of this drug in the bilayer, is also being determined on an absolute electron density scale by difference electron density (bromine vs. iodine substitution) along the membrane profile axis. This location for these calcium channel antagonists in membranes may have implications as to the molecular mechanism involved in their binding to protein receptor sites. Supported by NIH HL-32588, HL-07420, Whitaker Foundation, and the American Heart Association.

W-Pos203 DIFFUSION OF DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONSITS IN SARCOLEMMAL LIPID BILAYERS. [1]Mason, R.P., [1]Chester, D.W., [3]Joslyn, A.F., [3]Triggle, D.J., [1,2]Herbette, L.G., and [2]Koppel, D.E. Departments of [1]Medicine and [2]Biochemistry, Univ. of Connecticut Health Center, Farmington, CT and Department of [3]Biochemical Pharmacology, SUNY at Buffalo.

A "membrane bilayer" pathway for dihydropyridine (DHP) calcium channel antagonist receptor binding in the cardiac sarcolemma has been proposed which involves drug partition in the bilayer, lateral diffusion and receptor binding. In support of this model, x-ray and neutron diffraction data indicate that these drugs are at the bilayer hydrocarbon core/water interface. We have used fluorescence redistribution after photobleaching (FRAP) to determine the diffusion coefficients of DiI and an active rhodamine "tagged" nisoldipine analogue in highly ordered extended sarcolemmal lipid multibilayers formed by the spin dry technique reported by Clark et al. (Biophys. J. 31:65-96, 1980). This technique allows us to examine very low drug concentrations in a preparation with precisely defined drug/lipid molar ratios. Lamellar meridional x-ray diffraction data indicate that these multilayers are highly ordered and direct correlation exists between "d" spacing and the degree of hydration of the multilayer. Electron microscopy and bright-field photomicroscopy demonstrate the presence of extended bilayer sheets and uniformity of these multilayers at different hydration states, consistent with the diffraction findings. Preliminary diffusion coefficients of these two probes were similar at high humidity (>80%) but differed significantly at lower humidities (66%). These data indicate that these drugs diffuse at a precise depth within the lipid bilayer at a rate similar to that of phospholipids, further evidence in support of the membrane bilayer model for receptor ligand interaction. Supported by NIH HL-32588, HL-07420, GM-23585, Whitaker Foundation, and the American Heart Association.

W-Pos204 INTRAMOLECULAR ENERGETICS OF DRUGS INTERACTING WITH LIPID BILAYERS. D.G. Rhodes and L.G. Herbette, Dept. of Medicine, U. of Connecticut Health Center, Farmington, CT 06032

Lipid soluble drugs which interact with membrane associated receptors may approach the active site to which they bind by a direct (aqueous) route or by an indirect (membrane) route, the latter involving partitioning of the drug into the membrane bilayer at some well defined position, orientation and conformation. The drug could then diffuse laterally to reach a binding site on the receptor protein. The latter mechanism has been suggested for several classes of drug compounds (e.g. 1,4-dihydropyridine calcium channel blockers). To evaluate the first step of the membrane approach, we have investigated the energetics of the partitioning process for several different drug structures. Measured bulk phase (octanol/water) partition coefficients were compared to those predicted by summing fragmental constants. Although the measured data were consistently larger, the rank order was conserved. Molecular electrostatic potentials were calculated for all atoms in these drugs. These data, taken with the crystal structures determined by x-ray diffraction, were used to calculate the intramolecular potential energy as a function of dielectic constant in homogeneous and interfacial (bilayer) environments. Most of these drugs showed significant (0.1-1.0 kcal/mole) changes in the intramolecular free energy when taken from aqueous to hydrophobic environments. Amphiphilic drugs and some non-amphiphilic drugs found the interfacial environment more favorable than the homogeneous environment, a result experimentally shown for some of these drugs by neutron diffraction. Calculations of minimum energy conformations at the bilayer interface may allow identification of specific molecular parameters may be identified for the design of more effective drugs which bind to specific receptor sites within the lipid bilayer. (Supported by NIH HL-32588, HL-07420, Whitaker Foundation and the American Heart Association.)

W-Pos205 ADSORPTION OF LOCAL ANSTHETICS ON PHOSPHOLIPID MEMBRANES. S. Ohki and H. Ohshima, Dept. Biophysical Sciences, State University of New York at Buffalo, Buffalo, N.Y. 14214

In order to elucidate various types of adsorption modes of local anesthetics in membranes, a study of local anesthetic adsorption on lipid membranes was made by measureing electrophoretic mobility of phospholipid vesicles in the presence of local anesthetics of various concentrations in vesicle suspension mediums.

The partitions of local anesthetics onto the membrane interface as well as into the membrane bulk phase from an aqueous solution were obtained. The amounts of local anesthetics adsorbed onto the phosphatidylserine membrane were much greater than those of the phosphatidylcholine membrane. It was deduced that the major factor for this greater adsorption was due to the enhancement of cationic local anesthetic concentration at the charged membrane surface. Divalent cations inhibited such surface adsorption of local anesthetics by reducing surface concentrations of local anesthetics where the surface potential of the negatively charged membrane surface was influenced by the presence of divalent cations in the solution.

Some modes of association of local anesthetics on nerve membranes are discussed with the results obtained in the above adsorption study.

W-Pos206 THE SIMULATION OF ESR AND NMR POWDER SPECTRA.
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J. Middlehurst and B.A. Cornell, CSIRO, PO Box 52, North Ryde, NSW 2113 Australia

Spin 1/2 chemical shift anisotropy broadened NMR spectra and I=1 nitroxide spin labelled ESR spectra have been generated simulating a variety of restricted motions. Unlike other published results of spectral simulation this approach employs a complete numerical simulation of the reorientation undergone by the interaction vector and is not limited to a particular class of dynamics. Examples will be shown of the agreement between these simulations and spectra obtained in this laboratory from macroscopically aligned and coarse dispersions of labelled lipids and the polypeptide gramicidin A'.

W-Pos207 DO TRANSPORT PROTEINS SHARE A UNIVERSAL MECHANISM? J. J. Falke, J. F. Wang, K. J. Kanes, & S. I. Chan, Dept. of Chemistry, California Institute of Technology, Pasadena, CA 94720.

The substrates of transport proteins differ greatly; for example Cl^- and glucose differ in charge, shape, size and bonding characteristics. Yet the anion and glucose transporters of the red cell membrane share important structural and mechanistic features. ^{35}Cl NMR linebroadening measurements and ^1H NMR NOE studies have been used to monitor the binding of Cl^- and glucose to the transport sites of band 3 and the glucose transporter, respectively. These novel NMR techniques provide resolution of inward- from outward-facing transport sites, thereby enabling tests of the nature of the Cl^- and glucose transport cycles. In each system side-specific inhibitors are shown to exist which bind only to one orientation of the transport site and which recruit all transport sites on both sides of the membrane to the inhibited orientation, indicating that each transport unit possesses a single transport site which is alternately exposed to opposite sides of the membrane. Further similarities between the Cl^- and glucose transport cycles are indicated by the observation that in each system substrate binding and dissociation are rapid relative to the translocation of bound substrate across the membrane. Moreover the sequences of these two proteins recently published by other laboratories indicate similarities in transmembrane structure. In short, a variety of evidence suggests that band 3, the glucose transporter and other transport proteins (excluding H^+ transporters) may share universal structural and mechanistic features. A model involving a mobile barrier which slides inside a channel past a fixed transport site will be discussed as an example of a structure which can be altered by evolution to yield similar transport proteins with different substrate specificities.

Support provided by NIGMS grant GM 22432 (SIC) and by an NSF predoctoral fellowship (JJF).

W-Pos208 **A MATHEMATICAL ANALYSIS OF LIPID CHARGE EFFECTS ON ELECTROPHORESIS, HYDROPHOBIC ION ADSORPTION, AND MEMBRANE CONDUCTANCES.** J.E. Schnitzer, Department of Cell Biology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

In an attempt to more realistically model from a molecular perspective the electrostatic charge effects on electrophoresis, hydrophobic ion adsorption, and specific ion bilayer conductances, a mathematical model was created dividing the lipid membrane into a hydrophobic, inner region and charged, hydrated polar outer regions. Recent studies give the region dimensions based on X-ray diffraction, NMR, and membrane capacitance measurements. Total ion/solution exclusion begins at the plane edge of the hydrocarbon region. The lipid head charges are assumed to be smeared uniformly over a finite thickness (0-25 Å). Dielectric variability, volume exclusion, and specific monovalent and divalent adsorption are included in the Poisson-Boltzman equation development. Comparison with experimental data and past models indicates improved description of charged hydrophobic ion adsorption (ex. ANS^-) to lipid membrane. Bilayer conductance data analysis predicts ion transport binding site localization at the pore entrance in the polar region which disagrees with past predictions placing the site up to 10 Å from the membrane surface. Model extension to electrophoresis maintains the hydrodynamic shear plane within the polar region and does not necessitate shear plane movement away from the surface (2-10 Å) with ionic strength diminution. This model analysis is predicated on an electrostatically induced polar region expansion with large surface charge and/or low ionic strength which is critical relative to the Debye length. Specific ion adsorption complicates the surface potential-charge relationship so that under special circumstances, a surface charge increase with concurrent potential decrease can be induced.

W-Pos209 MAGNETIC FIELD-INDUCED DRUG PERMEABILITY IN LIPOSOME VESICLES, R.P. Liburdy, T.S. Tenforde, Biology and Medicine Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720, and R.L. Magin, M. Niesman, Bioacoustic Laboratory, University of Illinois, Urbana, IL 61801

Liposomes vesicles maintained in a uniform static magnetic field release a chemotherapeutic drug (ARA-C, mw=243) at temperatures in the pre-phase transition region where these liposomes are not normally leaky. Drug release is rapid, and a maximum difference between treated and unexposed liposomes of 30% of the total maximal release of ARA-C occurs within one minute in a magnetic field. Dose-effect studies conducted between 10 and 75,000 Gauss (0.001 to 7.5 Tesla) reveal that this permeability effect has a sigmoidal dependence on magnetic flux density. The ED-50 is 150 Gauss, with a 95% confidence interval of 65-349 Gauss. Magnetic field exposures were conducted using a superconducting magnet with the liposomes maintained at +/- 0.08°C. For comparison, Samarium-cobalt permanent magnets induced a comparable drug release at 4000 Gauss. These results indicate that a static magnetic field of 150 Gauss or greater can increase passive transport in phospholipid membrane bilayers maintained at or near their membrane phase transition temperature. Lipid clustering which occurs at pre-phase transition temperatures may predispose membrane phospholipids to diamagnetic orientation in a magnetic field, and thereby trigger a phase transition enabling drug release.

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W-Pos210 EFFECT OF CHOLESTEROL ON OXYGEN TRANSPORT IN MEMBRANES. W. Karol Subczynski,[1,2] Akihiro Kusumi,[1] and James S. Hyde,[1] National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53222. [2]Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland.

We have previously defined the "oxygen transport parameter": $W = T_1^{-1}(Air) - T_1^{-1}(N_2)$, where the T_1's are the spin-lattice relaxation times of nitroxide radical spin labels in the presence and absence of oxygen (Proc. Natl. Acad. Sci. USA 79, 1854-1858 (1982)). In that work, W was measured in membranes for a variety of spin labels using the saturation-recovery technique. The parameter W depends on Heisenberg exchange between spin labels and molecular oxygen dissolved in the membrane. It is determined by the product of the concentration of oxygen and its translational diffusion constant. This work has now been extended to membranes (DMPC, EYPC, DOPC) containing 15, 27.5 and 50 mole % cholesterol. Spin labels 16-SASL (the nitroxide moiety at the 16 position of stearic acid, close to the center of the bilayer) and 5-SASL (close to the surface) were employed. In the fluid phase, the effects of cholesterol on oxygen transport as measured in the center of the bilayer are minimal (no change of W in DMPC; < 40% increase in EYPC and DOPC). However near the surface (5-SASL position) introduction of cholesterol results in a marked decrease in W in all membranes ($\sim$ x 3). W was measured in DMPC systematically as a function of both cholesterol concentration and temperature. Effects are complex below the main phase-transition temperature. As a summary we can say that cholesterol strongly inhibits transport of oxygen close to the membrane surface, but does not change (or may even increase) transport near the center of the bilayer.

W-Pos211 PROTON/HYDROXIDE CONDUCTANCE THROUGH LIPID BILAYER MEMBRANES. John Gutknecht, Physiology Dept., Duke University, and Duke Marine Lab., Beaufort, NC 28516.

Proton/hydroxide (H/OH) permeability through phospholipid bilayer membranes at pH 7 is about a million-fold higher than alkali/halide ion permeability, but the mechanism of H/OH permeability is unknown. In this study we measured H/OH conductance ($G_{H/OH}$) through planar bilayers made from decane or tetradecane solutions of bacterial phosphatidylethanolamine, egg phosphatidylcholine cholesterol (1:1) or diphytanoyl phosphatidylcholine. At neutral pH, $G_{H/OH}$ ranged from 10^{-9} to 10^{-8} S cm^2, with diphytanoyl PC having a significantly lower $G_{H/OH}$. The $G_{H/OH}$ values corresponded to H/OH permeabilities of 10^{-6} to 10^{-5} cm/sec, in reasonable agreement with previous vesicle studies. To find out whether $G_{H/OH}$ might be due to traces of fatty acids or other contaminants in the bilayer, we "washed" the membranes with bovine serum albumin (fatty-acid free, 0.2 mg/ml). Albumin reduced $G_{H/OH}$ by at least 10 fold, with a half time of about 5 min. Addition of long-chain fatty acids (myristic, palmitic, or phytanic, 10-20 mol percent) to the phospholipids increased $G_{H/OH}$ by about 10 fold. Albumin completely inhibited the fatty-acid induced $G_{H/OH}$. The water permeability (measured with THO) was about 1×10^{-3} cm/sec (after corrections for unstirred layers). Neither albumin nor the long-chain fatty acids had any effect on water permeability. Current-voltage curves were non-linear in both control and fatty acid containing bilayers. The voltage dependence of $G_{H/OH}$ was similar to that observed with weak acid proton carriers. Although the mechanism of H/OH transport is not known, the data are consistent with a proton carrier(s) mechanism, which could be due to traces of fatty acids, hydrolysis or oxidation products in the phospholipids. (Supported by NIH grant GM 28844.)

W-Pos212 EFFECTS OF SMALL ORGANIC NONELECTROLYTES ON THE PROTON PERMEABILITY OF LIPOSOMES. Gail L. Barchfeld and David W. Deamer, Department of Zoology, University of California, Davis, CA 95616

It is probable that the permeability of lipid bilayers to ionic and polar solutes is related to membrane associated water, particularly that present in transient defects which permit hydrated solutes to enter the non-polar phase of the bilayer. It follows that compounds capable of partitioning into the lipid bilayer and altering the state of water should affect permeability. To test this, the proton permeability (P +) of liposomes prepared by reverse phase evaporation (egg phosphatidylcholine, egg phosphatidic acid, cholesterol, 50:20:25 mole ratio) was measured by monitoring the decay of small pH gradients across the liposomes in the presence of alcohols and polyhydroxy alcohols. Methanol and n-propanol produced a modest enhancement of P +; ethylene glycol had no effect on P +; and 1,2-propanediol and glycerol reduced P + about three-fold. These results suggest that proton permeability is sensitive to the presence of water and hydroxyl groups in the bilayer, perhaps due to alterations in the hydrogen bond associations among the water molecules. Supported by the Office of Naval Research contract N00014-85-K-0242.

W-Pos213 DEPENDENCE OF TRANSBILAYER DIFFUSION OF PYRENE LABELED PHOSPHOLIPIDS ON HEAD GROUP COMPOSITION Reynold Homan and Henry J. Pownall, Baylor College of Medicine, Houston, TX 77030

Translocation of lipophiles across cell membranes is an important but poorly understood component of lipid metabolism. We have measured the rates of translocation (flip-flop) and intervesicular transfer of pyrene labeled analogs of phosphatidylcholine (PC), lysoPC, and phosphatidic acid (PA). The rates and activation energies for intervesicular transfer were strongly dependent upon the acyl chain length of the transfering species but were relatively independent of the identity of the polar headgroup. By contrast, the rates and energetics for translocation across single bilayers of PC were relatively insensitive to the acyl chain length or number but very sensitive to the identity and charge on the polar headgroup. At 65 deg and pH 7.4, the kinetics of flip-flop are exponential and increase in the order lysoPC < PC < PA. Decreasing the pH to 4 increased the rate of PA flip-flop by more than an order of magnitude. These results are consistent with a model that specifically includes the solubility of the lipophile in the hydrocarbon region of the bilayer. For amphiphilic molecules, the major barrier to translocation is the insolubility of the polar moiety, with the apolar part of the molecule being much less important. These observations are the opposite of those found for intervesicular transfer and suggests that the distribution of amphiphiles in cells, which occurs by a combination of these two processes, is a predictable function of the balance of hydrophilic and lipophilic groups.

W-Pos214 NIGERICIN INTERACTION WITH MONO-, DI- AND TRIVALENT CATIONS: Li^+, Ca^{2+} AND Pr^{3+}. E. Piedras, B. Rivera, R. Alva, E. Arzt, M. Toro, L. Toro, J. Cerbón* and S. Estrada-O. Dept. Health Sciences, Universidad Autónoma Metropolitana-I. *Dept. Biochemistry, Centro de Investigación y de Estudios Avanzados del IPN. México, D. F. México.

Nigericin has not been proved capable to transport lithium, calcium and praseodymium, through biological membranes or bulk phases. Nevertheless, spectroscopic evidence is given in this report for the complexation of nigericin with these three cations. Complexes were formed and analyzed by ^{1}H-NMR at 90 MHz and U.V. spectroscopy. The results were as follows: 1) Nigericin-Li^+ complex; ^{1}H-NMR spectrum shows alterations in the H_{21}, Me_{35} and $O_{11}M_{40}$ with respect to the nigericin free acid spectrum. 2) Nigericin-Ca^{2+} complex; ^{1}H-NMR shows the disappearance of a signal at 2.2 ppm and the appearance of a band at 1.2 ppm. On the other hand, the U.V. absorption spectrum shows three maxima at 216, 230 and 278nm, contrasting with a maximun peak at 208 nm of the nigericin free acid. 3) Nigericin-Pr^{3+} complex; ^{1}H-NMR spectroscopy shows the presence of the paramagnetic cation Pr^{3+} in the complex, since the ionophore spectrum is broadened and shifted. Moreover, the inorganic Pr^{3+} absorption peaks which occur in the visible region (446, 470 and 482 nm), disappear when this cation is complexed with nigericin. Also, the U.V. spectrum is broadened from 200 to 400 nm with a maximum at 222 nm. From the above results, it is clear that nigericin forms stable complexes with mono-, di-and trivalent cations such as Li^+, Ca^{2+} and Pr^{3+}.

W-Pos215 DIFFERENTIAL EFFECTS OF pH ON AQUEOUS AND MEMBRANE-BOUND COLICIN E1. J. O. Bullock, Dept. of Physiology, University of Missouri School of Medicine, Columbia, MO 65212.

Colicin E1 is a bacterial toxin which, though secreted as an aqueous protein, is spontaneously incorporated into membranes to form voltage-dependent ion channels. In planar lipid bilayers neither the number of open channels nor the rate of channel incorporation can be reduced by perfusion of the aqueous compartments, suggesting that large amounts of protein become strongly associated with some stationary phase in the chamber prior to forming a membrane channel. In membranes composed of diphytanoyl lecithin the channel is strongly anion-selective at pH 4. As reported by Raymond et al.,[1] raising the pH on both sides of the membrane from 4 to 5 has little or no effect on selectivity, but over the pH range from 5.5 to 8 the channel is converted to a nearly nonselective form. If, however, the protein is first introduced into the chamber at pH 5, the channels are nonselective. When the pH on the trans side of the membrane is lowered to 4 the channels already in the membrane and those which open subsequently become anion-selective. Restoring the trans pH to 5 does not alter the selectivity of extant or newly opened channels. The initial conditions are restored when the membrane is broken and reformed; nonselective channels are observed. These results suggest that at pH 4 the titratable groups which control the selectivity of colicin E1 are kinetically inaccessible on the protein associated with the lipid bilayer, but easily accessible on the aqueous protein and any protein associated with lipid monolayers. Further, it appears that the bound precursors of the open channels are associated with the bilayer proper and not with some other nonperfusable reservoir from which they can exchange with the bilayer via the aqueous phase.

[1] Raymond, L., S. L. Slatin and A. Finkelstein (1985). J. Membr. Biol. 84:173-181. (Supported by GM33012.)

W-Pos216 GRAMICIDIN MINIS HAVE REDUCED IMINE BLOCK RATES BUT NORMAL BLOCK DURATIONS. Greg Hemsley and David Busath. Section of Physiology and Biophysics, Brown University, Providence, RI 02912

We have examined the frequency and duration of blocks induced by guanidinium and other imines in single gramicidin channels. The channels formed spontaneously in lipid bilayers prepared using glyceryl monoolein in hexadecane (50 mg/ml) on the aperture (40 μm) of a polyethylene pipette with an electric potential of 150 mV. The bilayer was symmetrically bathed in 1.0 M KCl + 0.5 M Guanidine HCl (pH 5.7) or 1.0 M KCl + 0.5 M Guanidine Carbonate (pH 9.5). In both cases the addition of guanidine caused frequent complete blocks in single channel current. The blocks were reversible and did not affect mean channel lifetimes nor interblock currents, indicating that the channel is grossly unaffected by the guanidine. The blocks lasted about 2 ms on the average at 100 mV and became shorter at higher potentials, suggesting that the block is relieved by permeation through the channel. We attempted to use guanidine as a probe of the pore structure in channels which have abnormally low conductance. We found that minis had decreased block rates compared to gramicidin channels with standard conductance, suggesting that the entry of the guanidine into the channel is affected adversely in minis. The average block duration, on the other hand was unaffected (except in the very smallest of minis, 10-20% normal conductance, where it was increased). If block relief occurs by permeation, one simple interpretation of the unaffected block times in minis would be that mini channel interiors are normal. This suggests that the reduced cation conductance in minis may result primarily from inhibition of ion entry. (Supported by NIH GM33361).

ENZYMATIC METHYLATION INCREASES $^{45}Ca^{++}$ UPTAKE INTO LIPOSOMES CONTAINING MEMBRANE PROTEINS FROM CHICKEN RED BLOOD CELLS (CRBC). Takaomi C. Saido,* Satoshi Toyoshima and Toshiaki Osawa. Intr. by Jeffrey Marque. Faculty of Pharmaceutical Sciences, University of Tokyo.

CRBC, nucleated erythrocytes, are known to respond to concanavalin A stimulation with an early increase of Ca^{++} uptake that is suppressed by inhibitors of enzymatic methylation. To examine possible roles of enzymatic methylation in Ca^{++} regulation, the following investigations were performed. Cholate-solubilized membrane proteins from CRBC were reconstituted into phosphatidylcholine (PC)-liposomes by the dialysis-sonication method. The liposomes, for Ca^{++} uptake assay, were incubated with $^{45}Ca^{++}$ at 37°C, gel filtered to remove free outside $^{45}Ca^{++}$, and counted for trapped radioactivity. The presence of 1mg/ml S-adenosyl-L-methionine (AdoMet), a methyl donor in enzymatic methylation, increased Ca^{++} uptake into the liposomes twofold, and the increase was prevented by 1mg/ml S-adenosyl-L-homocysteine (AdoHcy), a specific inhibitor of AdoMet-dependent methylation. Similar effects of AdoMet and AdoHcy were observed on $^{45}Ca^{++}$ release from inside the liposomes, indicating that methylation affects Ca^{++} flux rather than adsorption. This AdoMet-induced Ca^{++} uptake has been characterized by (1) no effect of 1mM ATP, (2) pH optimum of 5.5, (3) distinct specificity for Ca^{++}, (4) Km for AdoMet of 0.38mM, (5) optimum cholesterol content of 40-50%mol/mol PC, and (6) optimum membrane potential of ca. -30mV. In the case of intact cells, preincubation of CRBC with AdoMet also caused an increase in $^{45}Ca^{++}$ uptake, which was inhibited by AdoHcy. These results suggest that enzymatic methylation of membrane component(s) may play a regulatory role in passive Ca^{++} transport through CRBC membranes.
*Presently in laboratory of W. W. Webb, Cornell University. Supported by Rotary Foundation.

W-Pos218 MONITORING OF RECONSTITUTION AND DISSOLUTION OF EGG PC VESICLES AND VSV G PROTEIN: APPLICATION TO MEMBRANE RECONSTITUTION.
Michel Ollivon, Ofer Eidelman, Anne Walter and Robert Blumenthal. Organisation Moleculaire et Macromoleculaire, C.N.R.S. Thiais, 94320, FRANCE and NCI, NIH, Bethesda, MD. 20892.
Reconstitution and dissolution of liposomes by detergent was studied using Egg PC and the detergent octylglucoside, with and without the Vesicular Stomatitus Virus G-protein. We studied the formation of lipid assemblies and of protein oligomers during dilution with octylglucoside from above to below its cmc. The different phases of bilayer reconstitution were characterized by fluorescence energy transfer between two lipid probes, by HPLC, and by turbidimetry. Using energy transfer we determined that the phase diagram of lipid detergent mixtures contains at least 3 discrete transitions which can be interpreted as discrete changes in the size of the phospholipid-detergent micellar structures. We determined the influence of phospholipid concentration and of temperature on the phase boundaries. By monitoring the "phase boundaries" at different phospholipid concentrations both by energy transfer and by turbidity, we could determine the molecular phospholipid to detergent ratios in the mixed micelles. The phase boundaries were strongly dependent on temperature. Turbidity as a function of detergent concentration at 2.3 mM Egg PC revealed several peaks, which were attributed to aggregation of mixed micelles. A new HPLC method based on gel exclusion (Ollivon et al, Biophys. Abstr, 1986) was used to determine integrity and size of bilayer aggregates at various detergent concentrations. Finally, liposome reconstitution in the presence of VSV G protein was monitored on-line by fluorescence energy transfer between lipid probes.

W-Pos219 SPONTANEOUS TRANSMEMBRANE INSERTION OF A MEMBRANE PROTEIN, BACTERIORHODOPSIN, INTO LIPID VESICLES FACILITATED BY SHORT-CHAIN LECITHINS. Norbert A. Dencher
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Mixing aqueous suspensions of long-chain lecithins (DMPC, DPPC, or soybean PC) with the short-chain lecithin diheptanoylphosphatidylcholine (20 mol% total lipid) in the presence of purple membranes (containing bacteriorhodopsin, BR, as the sole membrane protein) leads to spontaneous formation of reconstituted BR-lipid vesicles. Only in the case of soybean PC, but not with DMPC and DPPC, additional 30s sonication in a bath-sonicator is required for functional reconstitution of BR. Transmembrane insertion of BR into the lipid bilayer is verified by means of density gradient centrifugation, circular dichroism measurements, and vectorial proton-transport. BR is highly active as light-energized proton-pump. Steady-state values of up to 11 protons translocated per BR are observed. Reconstitution efficiency and general properties of the vesicles depend on the physical and chemical state of the long-chain lecithins. The majority of the soybean PC/diheptanoyl PC/BR vesicles have diameters between 35 and 100 nm, whereas the DMPC/diheptanoyl PC/BR vesicles are much larger ranging from 100 to 450 nm in diameter. The reconstituted vesicles seem to be predominantly unilamellar. Diheptanoyl PC has no harmful effect on BR, however, it influences the physical parameters of the lipid phase as can be expected for such a short-chain component at this relative high concentration. This rapid, easy, and gentle procedure might allow functional reconstitution of other membrane systems and isolated membrane proteins as well.

W-Pos220 ACYLATION OF THE MYELIN PROTEOLIPID : A PREREQUISITE FOR ITS SPECIFIC LOCATION IN THE OLIGODENDROCYTE PLASMIC MEMBRANE ? A. Beurrier, A. Alfsen and F. Lavialle

Fatty acid acylation of proteins -a post-translational modification- has been suggested to play specific roles in cellular processes such as protein sorting and insertion in membranes.

The protein moiety of the myelin proteolipid (PLA) -which accounts for almost half the total myelin proteins- was shown to contain 2-3 % (w/w) fatty acid linked by ester bonds to the protein. A comparative analysis of the acylated (PLA_a) and deacylated (PLA_d) proteolipid apoprotein has been performed by UV and FT-IR spectroscopy with the aim to clarify the role of fatty acids in the sorting process of PLA. At low pH as probed by UV spectroscopy (peak location, 278 nm/260 nm intensity ratio) PLA_d appeared more "structured" than PLA_a. At the neutral pH, the increase of ionic strenght induced a reversible precipitation of PLA_d. PLA_a was irreversibly denaturated in these experimental conditions. Both PLA_a and PLA_d conformations were then analyzed by FT-IR before and after insertion in DPPC multilayers. At pH 4.4, in a lipid environment, PLA_d exhibited a predominantly α helical conformation. Using water as solvent, features characteristic for β and random coil structures significantly increased in intensity. In contrast, PLA_a conformation was mainly an α helix when the molecule was solvated in H_2O. These results suggest that the aqueous medium would be specifically involved in the structurating effect of fatty acids on PLA. Similar experiments performed using membranes prepared with the lipid mixture extracted from myelin sheath will be presented. All these data will be discussed in terms of sorting process.

W-Pos221 NMR STUDIES OF THE INTERACTIONS OF METAL IONS AND POLYMYXIN B WITH LPS IN EITHER SDS MICELLES OR PHOSPHOLIPID VESICLES. H.D. Dettman, S.M. Strain and I.M. Armitage, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

Lipopolysaccharide (LPS), the major lipid of the outer membrane of gram negative bacteria, is the primary component responsible for the impermeability of the outer membrane to many detergents and lipophylic drugs. This barrier-function requires the interaction of the LPS with multivalent cations (ie. Mg^{2+}, Ca^{2+}) and can be disrupted by organic cations such as polymyxin antibiotics. The molecular details of the interactions with LPS by which the metal cations stabilize and the organic cations destabilize the outer membrane have not been clearly elucidated. We have used NMR techniques to characterise LPS interactions with metal ions and the drug, polymyxin B. The LPS from the heptoseless mutant of E. coli K12, D21f2, has been incorporated into either SDS micelles or phospholipid vesicles. Its structure consists of: Lipid A, a diglucosamine moiety substituted with acyl-chains and both mono- and di-phosphate groups; and two molecules of 2-keto-3-deoxy-octulosonic acid (KDO), in the core region. ^{31}P NMR of LPS in SDS micelles has shown that both the phosphate and diphosphate groups are involved with metal ion binding while ^{13}C NMR data gave inconclusive evidence for the involvement of the KDO carboxyl groups. The dissociation constants of metal ions and polymyxin B to LPS in SDS micelles were determined by the analyses of ^{23}Na NMR spectral lineshapes during competitive titration experiments; these K_D values will be compared to the binding constants obtained for LPS in unilamellar vesicles. Biosynthetic deuteration of the LPS acyl chains permitted ^2H NMR studies of the fluidity of the acyl chains during titrations with cations. These experiments yield greater insight into the mechanisms by which the metal ions stabilize and the polymyxins destabilize bacterial outer membranes. (Supported by NIH grant #AI20984 and NSERC Fellowship (HDD)).

W-Pos222 PURIFICATION, CHARACTERIZATION AND MANIPULATION OF GIANT UNILAMELLAR LIPID VESICLES D.J. Kulik, R.C. MacDonald and S.J. Kleene, Departments of Biochemistry, Molecular Biology and Cell Biology and of Neurobiology and Physiology, Northwestern University Evanston, IL

Methods for preparing very large unilamellar vesicles seldom yield homogeneous preparations. For many applications it is desirable to eliminate contaminating small and multilamellar vesicles. We have applied differential rate centrifugation through step density gradients to effect rapid and simple enrichment of giant unilamellar vesicles. We begin with GUVs prepared by the osmotic inflation method (Oku and MacDonald, Biochemistry, 22:855, 1983), and purify them by manipulating the density of the internal aqueous phase relative to that of the external medium. Vesicles, equilibrated with 0.15 M KCl, were mixed 1:1 with isosmotic sucrose, overlayed with 0.15 M $CsCl_2$ and centrifuged for up to several minutes at 1000xg. Unilamellar vesicles collect at the top at rate proportional to their size. Using this fraction, we have characterized the preparation method (reference above) with respect to trapping and retention of marker solute. In spite of the apparently disrupting conditions attending vesicle growth by osmotic inflation, purified GUVs retain significant proportions of a fluorescent aqueous phase marker and do so quite uniformly. Density manipulations also facilitate several useful biophysical applications of GUVs. They are easily collected in a close-packed layer at the air/water interface of a suspension having a hyperpycnic external phase. There, they may be impaled with patch-clamp pipettes by simply stabbing the pipette through the interface, allowing examination, with a mimimum of manipulation, of the electrical activity of channels incorporated into vesicles. Similarly, GUVs may also be collected on the undersurface of a cation-coated cover slip under conditions that that allow light microscopic monitoring of the response of the vesicles to substances in the perfusion medium.

W-Pos223 PLURONIC F127: AN EFFECTIVE AND BENIGN VEHICLE FOR THE INSERTION OF HYDROPHOBIC MOLECULES INTO MEMBRANES. Zenobia Lojewska and Leslie M. Loew, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032 (Introduced by: Ramadan I. Sha'afi).

Pluronic F127 is a high molecular weight (ca. 10,000 D) member of a family of amphiphilic polymers produced by BASF Corporation as agents for the formulation of aqueous gels. There have been reports by M. Ritchie and L.B. Cohen that it can be used to facilitate binding of drugs or stains to neuronal membranes. We have employed the membrane probe di-10-ASPPS to study the action of Pluronic F127 in a variety of membrane systems. The probe has a rod shaped chromophore with two decyl chains at one end and a fixed negative charge at the other; it binds very strongly to membranes with a characteristic increase in fluorescence quantum yield of 2 orders of magnitude. Binding to lipid vesicles can be accelerated by a factor of 10 in the presence of 0.05% Pluronic F127. Concentrations of up to 0.2% have no effect on the ability of the vesicles to support a diffusion potential, indicating that the amphiphile does not disrupt the membrane. The optimal results are obtained when a stock solution containing elevated concentrations of probe and Pluronic F127 are diluted into the lipid vesicle suspension. It appears that at least part of the reason for the enhanced rate of binding may be the ability of this high molecular weight surfactant to break up microcrystals or micelles of di-10-ASPPS while leaving the membrane bilayer intact. The details of the mode of insertion of the probe into the membrane are under investigation.
(We are grateful to BASF Wyandotte for the gift of a sample of Pluronic F127. Supported by USPHS grant GM35063.)

W-Pos224 ELECTRICAL PROPERTIES OF CHEMICALLY-GATED CATION CHANNELS FROM OLFACTORY EPITHELIUM HOMOGENATES. V. Vodyanoy and I. Vodyanoy. Dept. of Physiology & Biophysics, Univ. of California, Irvine, CA 92717

Electrical properties of ion channels from rat olfactory epithelium homogenates (OEH) were studied in Bimolecular Lipid Membrane Chamber (BLMC-2 Forward Technology Research Laboratory, Inc. N.J.). Two methods of reconstitution were used. (1) Chemosensitive membrane fragments were incorporated into planar BLM. (2) The vesicles with chemosensitive membrane fragments were attached to the planar BLM and the proton carrier SF6837 was added. The histogram of unmodified BLM conductance was characterized by a peak near 37 pS. A histogram of data from BLM treated with OEH does not show this peak and is instead characterized by two equally-spaced peaks (75 and 137 pS) with a separation of about 62 pS. The conductance of the OEH treated BLM is not a simple superimposition of the ionic pathways of the unmodified BLM upon the conductance of the reconstituted BLM. The shape of I-V curves was found to be not dependent on OEH concentration and composition of the bath media. The behavior in a many channel BLM reflected the properties of an individual channel, but did not appear to result from channel voltage dependence. Study of spontaneously fluctuating diethyl sulfide activated single channels showed that in presence of the odorant the mean open time of the channel increased , and significant deviation from random channel opening in time was observed. Analysis of autocorrelation functions of spontaneous and activated channel fluctuations revealed the existence of one more closed state of the channel. We hypothesize that this new state can be related to some internal second messenger. This idea is supported by our experiments in which the conductance of olfactory reconstituted membrane was directly modulated by c-AMP. Supported by U.S. Army Research Office grant DAAG29-85-K-01109.

W-Pos225 VESICLES CONTAINING FUNCTIONAL VOLTAGE DEPENDENT ANION CHANNEL (VDAC) CAN BE PURIFIED BY A TRANSPORT-SPECIFIC DENSITY SHIFT. A.L. Harris, A. Walter and J. Zimmerberg. Dept. Biophys., Johns Hopkins Univ. Baltimore, MD and LTB, NCI & LCB, NIADDK, NIH, Bethesda, MD.

The ability to enrich for functional channels of known permeability properties would be useful in reconstitution studies. We have applied the technique of a permeability-dependent density shift (e.g., Goldin and Rhoden, J. Biol. Chem. 253: 2575) to enrich for a large channel, voltage dependent anion channel (VDAC). Lipids with or without VDAC were solubilized in octylglucoside and urea buffer (459 mM urea, 10 mM KCl, 10 mM HEPES, 0.1 mM EDTA, 3 mM Na azide, pH 7.4) containing 10 mM calcein. Vesicles were formed on a gel filtration column. The vesicles were monodisperse with a peak diameter of 700 A as determined by elution profiles from a TSK 6000PW column. Fluorescence measurements showed that the +VDAC vesicles retained 58% less calcein/lipid than did the -VDAC vesicles. +VDAC vesicles alone, and mixtures of +VDAC and -VDAC vesicles were spun on iso-osmolar urea to sucrose density gradients. In theory, vesicles with functional VDAC will exchange sucrose for urea and become more dense. The +VDAC vesicles had two peaks, the major peak at higher density. The smaller, lighter +VDAC peak and the -VDAC vesicles ran at the same densities. The higher density +VDAC peak contained 80% less calcein/lipid than did the lighter +VDAC peak or the -VDAC peak. Presumably the vesicles in the lighter +VDAC peak did not contain functional VDAC channels. The shift to higher density of +VDAC vesicles was therefore specifically accompanied by loss of calcein. Addition of +VDAC vesicles to a bilayer chamber under conditions which promote vesicle fusion to planar bilayers resulted in channel activity with conductance, voltage dependence and kinetics characteristic of VDAC. This provides evidence that the permeability pathway for the density shift and the calcein loss was VDAC. We conclude that VDAC was incorporated into vesicular membrane by a gel-filtration method, and that vesicles containing functional VDAC channels were separable from those which did not by a transport-specific shift in density. We suggest that this technique may be useful for the enrichment of channels from impure material.

W-Pos226 CHANNEL-CLOSING ACTIVITY OF PORINS FROM ESCHERICHIA COLI DETECTED IN BLACK LIPID MEMBRANES. G. Xu[1], B. Shi[2], E. J. McGroarty[1] and H. T. Tien[2], Department of Biochemistry[1] and Department of Physiology[2], Michigan State University, East Lansing, MI 48824

The omp C and omp F porin proteins from Escherichia coli JF 701 and JF 703 were reconstituted into a planar black lipid membrane (BLM) system. More than 10^3 discrete electrical current increment events (CIE) and current decrement events (CDE) were recorded and analyzed. The conductance decrease is interpreted to reflect the closing of the channel-forming proteins. Variations in the experimental conditions before and during the insertion of porins into the BLM altered the frequency of the CDE. The ratio of the number of CDE to total (CDE and CIE) was found to be dependent on the transmembrane voltage, and varied with sample treatment prior to integration into the BLM. Low pH was shown to dramatically increase the percentage of CDE. At pH values between 3.1 and 3.5 two types of CDE were detected which may represent two types of closing events, a protein conformational change (fast process) and protein denaturation (slow process). This work was supported, in part, by Public Health Service grant GM-14971.

W-Pos227 EFFECTS OF DEFINED LIPID BILAYERS ON THE MORPHOLOGY OF RECONSTITUTED ACETYLCHOLINE RECEPTOR MEMBRANES. J.P. Earnest, R.M. Stroud, and M.G. McNamee. Dept. of Biochemistry and Biophysics, University of California at Davis, and Dept. of Biochemistry and Biophysics, University of California at San Francisco.

Examination by electron microscopy of reconstituted acetylcholine receptor (AChR) membranes reveals that both the lipid composition and the method of reconstitution affect intercalation of AChR into the lipid bilayer as well as size and shape of the reconstituted vesicles. AChR is affinity-purified from Torpedo californica in 1% cholate, combined with lipids at a lipid-to-protein molar ratio of 10,000, then reconstituted by equilibrium dialysis, cholate dilution, or gel filtration. Preliminary experiments suggest that cholesterol is required for correct intercalation of AChR into the lipid bilayer, while negatively charged phospholipids such as PA or PS may have a more specific effect on receptor function. In addition, while certain lipids do not incorporate AChR into vesicles using slow methods of cholate-removal such as equilibrium dialysis, these lipids will incorporate AChR using techniques which remove cholate more quickly, such as rapid dilution or gel filtration.

W-Pos228 LIPID SUBSTITUTION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR OF TORPEDO CALIFORNICA O.T. Jones, J. Eubanks and M.G. McNamee, Dept. Biochem. & Biophys., Univ. of Calif., Davis. CA 95616

An elegant reconstitution technique, originally applied by Warren et al. (Proc. Nat. Acad. Sci. 71, 622-626 (1974)), to Ca^{2+}+Mg^{2+}-ATPase has been employed to prepare complexes of acetylcholine receptor (AchR) in defined lipid environments. Lipid substitution is achieved by incubating purified AchR with a large excess of test lipid in the detergent cholate. The detergent acts as an inert equilibrating agent and facilitates exchange between AchR-associated and test lipid pools. The desired AchR-test lipid complexes can be separated from excess lipids and cholate by sucrose gradient centrifugation. Substituted complexes, prepared using a variety of synthetic lipids, were characterised with regard to structure, function and composition. The degree of substitution, as monitored by gas chromatography, was greater than 95%. The total lipid content of the complexes could be decreased by increasing the cholate concentration. Electron microscopy of the complexes, when prepared at a lipid to protein molar ratio of 100:1 revealed the presence of membrane sheets. The functional properties of the AchR were assessed by the ability of the receptor to undergo agonist mediated state transitions characteristic of the desensitization process. Not all lipids supported the affinity transitions. However, the effects of the lipids were reversible since the functional properties of all the membranes tested could be fully recovered by reconstituting the AchR back into lipids known to support AchR activity. The lipid substitution technique is rapid and convenient and should prove useful in studying AchR-lipid interactions.

W-Pos229 INTERACTIONS OF THE E. coli CATABOLITE ACTIVATOR PROTEIN WITH NONSPECIFIC DNA.
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The E. coli catabolite activator protein (CAP) displays very cooperative interactions with nonspecific DNAs which contain no special sites for CAP binding [Saxe and Revzin, Biochemistry 18, 255 (1979)]. In a solution containing CAP and, for example, a phage DNA, some DNA molecules will have many CAP dimers bound in a large cluster, while other DNA molecules are devoid of protein. Scatchard plots show characteristic "humps", but predict a cluster length of only about 10 CAP molecules, far less than what is observed. Electron micrographs of these complexes have been published [Chang et al., JMB 150, 435 (1981)]. Based on these pictures and other results in the literature, Salemme [PNAS 79, 5263 (1982)] proposed a model for the structure of CAP/DNA complexes which involves a solenoidal configuration for the DNA and includes interactions between protein molecules bound at nonadjacent regions of the nucleic acid.

We have reinvestigated nonspecific CAP/DNA binding using a variety of techniques, such as electron microscopy, centrifugation, protection from restriction enzyme digestion, and viscosity. The data are interpreted in terms of both expected structures and unusual configurations which may be present under a variety of ionic conditions and DNA/protein ratios.

W-Pos230 **ASSOCIATION-DISSOCIATION PROPERTIES OF E. COLI RHO PROTEIN IN TRANSCRIPTION TERMINATION**

J. Geiselmann, T. Yager, S. Gill, J.A. McSwiggen, W.A. Baase and P.H. von Hippel
Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229

Essential steps in the mechanism of action of the transcription termination protein rho of E. coli include binding to the nascent RNA transcript and a concomitant hydrolysis of ATP. It is not yet understood how these events (and possibly others) combine to cause transcript release.

The active form of rho appears to be a hexamer of 46.4 kD protomers. We are defining and analyzing the association equilibria involved in generating the active form of rho. Our strategy is to use the Svedberg equation ($S/D = M[1-\bar{v}\rho]/RT$) to obtain the molecular weight of each association intermediate.

Our results (at a rho concentration of 1 mg/ml) are as follows: (i) Under no solvent conditions tested (various Mg^{++} and KCl concentrations, also various concentrations of the denaturants $KClO_4$ and urea) can rho be driven to the fully dissociated form. (ii) At [KCl] > 0.19 M, rho exists as a 9.5 S species. (iii) Upon lowering [KCl] to 50 mM, the 9.5 S species is converted to an 11-12 S species. This process is reversible. (iv) Addition of Mg^{++} or ATP at 0.2 M KCl has no effect on the association state. (v) At 0.2 M KCl, addition of oligo$(rC)_n$ with n=23±1 causes the conversion of the 9.5 S form to the 11-12 S form. This can be reversed by raising the KCl concentration.

Currently we are working to relate these observations to additional studies of the RNA binding site size of rho (13±1 nucleotides/protomer) and of the dependence of ATPase activity on RNA length. We ultimately seek a detailed molecular picture of how rho functions in transcript release. [Supported by USPHS Research Grant GM-29158 (to PHvH) and USPHS Postdoctoral Fellowship GM-10227-02 (to TY).]

W-Pos231 INTERACTION BETWEEN GENE REGULATORY PROTEINS AND DNA: TIME-RESOLVED FLUORESCENCE STUDIES
J.C. Cook, S. Cheung, and P. Lu, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

Previous experiments from this lab in which cro repressor protein from λ phage was titrated with DNA have demonstrated the utility of steady-state fluorescence quenching of intrinsic tyrosyl fluorescence for monitoring cro protein-DNA interactions. However, similar steady-state experiments with E. coli lactose operon repressor and λ cII protein have not been useful in this respect. Because of the need for spectroscopic assays of DNA-protein interactions, we turned to time-resolved fluorescence experiments. We have examined three gene regulatory proteins: lac repressor cro repressor and cII protein.

Fluorescence lifetime measurements on these three proteins reflect the steady state results. Cro protein intrinsic tyrosyl fluorescence decay, which can be described by a bi-exponential equation, undergoes a reversible change in the proportions of the two components of the equation used to fit the experimental curve when operator DNA is added. In contrast, no significant changes are observed in the case of lac repressor and cII protein intrinsic tryptophyl fluorescence upon interaction with their specific target DNA sequences.

However, time resolved fluorescence anisotropy experiments with operator DNA labeled with ethidium bromide as the fluorophore have been more successful in reporting protein-DNA interactions. Fluorescence anisotropy decay kinetics undergo marked changes when the appropriate DNA binding proteins is added to the sample. This change in anisotropy decay kinetics corresponds to complex formation under a variety of conditions. (Supported by NIH grants).

W-Pos232 RESOLUTION OF THE INTRINSIC BINDING AND COOPERATIVE FREE ENERGIES FOR THE INTERACTION OF THE LAMBDA cI-REPRESSOR AND THE RIGHT OPERATOR, M. Brenowitz, D.F. Senear, and & G. K. Ackers, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

We have used the Quantitative DNase Footprint Titration Method (Brenowitz, Senear, Shea & Ackers, Meth. Enz., in press) to obtain individual-site isotherms (pH 7, 20°, 200 mM KCl) for binding of the bacteriophage lambda cI-repressor to the three-site wildtype right operator and to each of the six possible reduced-valency operators, in which mutation in one or two sites has diminished binding to those sites. The binding of the cI repressor to the three-site operator is defined by intrinsic binding constants for sites O_R1, O_R2, and O_R3, and two cooperativity terms which describe the pairwise interactions between adjacent liganded sites. Although it is possible in principle to evaluate all of the microscopic constants from wildtype data alone, these parameters are not uniquely resolved due to the inherent coupling of the binding expressions. With increasing cooperativity, the shapes of the individual-site isotherms reach asymptotic limits. At these limits, which are approached at very moderate values of the cooperative free energies, the cooperativity terms and the intrinsic binding constant for O_R2 are infinitely correlated. Unique resolution of the intrinsic binding and cooperative free energies was accomplished by the simultaneous analysis of isotherms for the wildtype operator along with a subset of the reduced-valency operators. This analysis assumes that when one or more sites are incapable of appreciable repressor binding, the intrinsic and cooperative interactions at the remaining sites are quantitatively unaltered. With individual-site binding data this assumption can be tested. The reduced-valency mutants (created by single base pair substitutions) and the wildtype operator produce an internally consistent set of individual-site isotherms; this observation strongly supports the validity of the assumption. (Supported by NIH Grant GM-24486).

W-Pos233 **FACTORS AFFECTING PROMOTER SEARCH BY ESCHERICHIA COLI RNA POLYMERASE.** Paul Singer and Cheng-Wen Wu, Dept. Pharmacol. Sciences, State Univ. of New York at Stony Brook, NY 11794

Our laboratory has developed a novel, highly sensitive rapid-mixing/photocrosslinking technique to study the kinetics of DNA-protein interaction. Earlier work from our laboratory focussed on the kinetics of promoter search by E. coli RNA polymerase (RPase) on linear phage T7 DNA and qualitatively demonstrated a linear diffusion along the DNA molecule as an important contributing factor to promoter binding. The quantitative interpretation of the data was limited due to the ambiguity arising from protein binding to the free ends of the DNA molecule. The present studies avoid this problem by using the circular plasmid pAR1319, a pBR322 derivative containing the T7 A2 early promoter and terminator.

At discrete times following the rapid-mixing of the RPase and pAR1319, the samples were cross-linked using a high intensity flash from a xenon arc lamp. The DNA was then digested into conveniently sized fragments. DNA fragments covalently linked to RPase were isolated via selective precipitation using SDS chilled on ice in the presence of K^+. After extensive proteolysis, the isolated fragments were ^{32}P end-labelled and separated on an agarose gel and quantitated. A series of such experiments was performed in a buffer containing 10 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.9) and KCl varying from 25 - 350 mM. The rate of specific complex formation, as measured by the number of cpm associated with the promoter containing fragment, varies non-monotonically with $[K^+]$, with a peak value of 1.5×10^8 $M^{-1}s^{-1}$ at 200 mM. Interestingly, the rate of non-specific binding appears to vary inversely with the specific rate. A computer simulation of the system, which solves the differential equations describing initial binding and linear diffusion, also predicts these salt dependences. Other factors affecting the kinetics of promoter search in this system, such as $[Mg^{++}]$ and DNA length and superhelicity, are currently under investigation. (This work was supported by NIH Grant GM 28069.)

W-Pos234 **QUANTITATIVE ANALYSIS OF SPECIFICITY DETERMINANTS IN THE RECOGNITION OF DNA OPERATOR SEQUENCES BY CRO REPRESSOR.** A. Sarai*, R.L. Jernigan*, J.G. Kim† and Y. Takeda†, *NCI, NIH, Bethesda, MD 20205, †Chemistry Dept., Univ. of Maryland Baltimore County, Catonsville, MD 21228.

To understand the mechanism of recognition of specific DNA sequences by proteins we have analysed quantitatively the factors responsible for the specificity determination in repressor-operator interactions. As a first-order approximation, based on the molecular model of Cro-operator binding, and by using a few parameters to represent the average free energies of H-bonds and hydrophobic interactions during Cro-DNA binding process, we make an interaction matrix for each combination of side chains and base pairs, and calculate the binding strengths for Cro to λ DNA. We find the Cro-operator binding to be highly specific. The calculated binding strengths for operators agree well with the measured binding constants, when the free energy parameters for charged and neutral H bonds and hydrophobic interaction are in ratios between 1 : 1 : 0.7 and 1 : 0.7 : 0.7. We have estimated free energies for each of these interactions to be 0.5 to 0.3 Kcal/mol. The nonspecific binding free energies calculated with these values agree with the experimental data. It appears that nearly half of the nonspecific interaction comes from the major groove interactions between Cro's α-helices and base pairs, and the rest from the interactions with the sugar-phosphate backbones and other sources. By gel analysis, we also find that Cro binds to most of the secondary binding sites predicted in the calculation. We are planning binding experiments with synthetic operator-like DNA's with systematic substitutions, as well as with nonspecific DNA's, to refine the interaction parameters, and to examine cooperativity effects in the interaction mode of Cro with DNA.

W-Pos235 RELEVANCE OF POTENTIAL HYDROPHOBIC AREAS ON DNA TO THE FORMATION OF SEQUENCE-SPECIFIC NUCLEOPROTEIN COMPLEXES. A. T. Ansevin, Dept. of Experimental Radiotherapy, Univ. of Texas System Cancer Center, Houston, Texas 77030.

Information about the structure of several well-studied repressor proteins suggests that critically placed hydrogen bonds help to determine the sequence specificity of complexes to operator DNA. However, it may be that H-bonding alone is insufficient to explain all of the specificity. Recent data of Pashley et al, (Science 229, 1099-1089, 1985) support the existence of long-range attractive forces between hydrophobic surfaces. In this case, significant orienting effects might occur between hydrophobic residues of proteins and any sizeable area of hydrophobicity on DNA. Model building indicates that hydrophobic character ought to be associated with the clustering of a hydrogen from a pyrimidine base and those from ribose along the edge of the wide groove. In the vicinity of sequences such as -T-T- or -T-C-T- an oval-shaped hydrophobic region might approach 100 square Angstrons in area. It is suggested that such a region would support sequence-specific protein attachment by: (1) attracting hydrophobic amino acid residues over distances as great as 20 A.u., (2) orienting the close approach of a "recognition helix" to the wide groove, (3) facillitating initial van der Waals contact of hydrophobic residues at the edge of the wide groove, and (4) supporting the subsequent formation of specific hydrogen bonds that otherwise would be unstable in an unsheltered aqueous solution. Other sequences might contribute smaller effects to operator-repressor associations.

W-Pos236 RAMAN SPECTROSCOPIC STUDIES OF RIBONUCLEASE A BOUND TO $d(pA)_4$ IN SOLUTION. Daniel M. Brown and Roger M. Wartell, School of Physics, Georgia Tech, Atlanta, Georgia 30332.

The complex formed by RNase A and the synthetic oligodeoxynucleotide $d(pA)_4$ in 0.1 M sodium cacodylate pH 7.0 at 25°C was studied by laser Raman spectroscopy. Spectra of 30 mM solutions of the protein and the oligomer were obtained separately in 0.1 M sodium cacodylate pH 7.0. Prominent Raman peaks in the spectrum of $d(pA)_4$ were observed at 728, 793, 1006, 1093, 1308, 1340, 1379, 1421, 1484, 1508, and 1578 cm^{-1}. On an equimolar basis, the intensities of most of the protein Raman bands were greater than those of the DNA oligomer bands. Raman bands in each spectrum were quantified by a computer analysis procedure which fits calculated peaks and a calculated background curve to the raw data. Peaks were compared between spectra using the 609 cm^{-1} cacodylate band as an internal frequency and intensity standard. Analysis of the spectra of the protein/oligomer mixture showed that $d(pA)_4$ adenine ring vibrations at 728, 1508, and 1578 cm^{-1}, as well as a S-S RNase A vibration at 515 cm^{-1} were relatively isolated and could be quantified without interference from other bands. A comparison of the normalized intensities at 728, 1508, and 1578 cm^{-1} showed that in the mixture, these bands increased by 29, 21, and 10%, respectively, relative to the free oligomer solution. These relative intensity changes of $d(pA)_4$ are consistent with unstacking of the adenines in the complex. The intensity of the S-S vibration at 515 cm^{-1} decreased by 57% in the complex, compared to the free protein solution. This indicates a conformational change involving disulfide bonds. Supported by N.I.H. Grant GM 33543.

W-Pos237 A SPECIFIC COMPLEX BETWEEN RIBOSOMAL PROTEIN S4 AND THE α MESSENGER RNA. David E. Draper and Ingrid C. Deckman, Dept. of Chemistry, Johns Hopkins Univ., Baltimore, MD 21218.

The *E. coli* ribosomal protein S4 is known to repress translation of its own gene and several other ribosomal protein genes in the α operon, as part of a general mechanism coordinating the levels of rRNA and r-protein synthesis. Using a filter binding assay and RNA transcripts prepared *in vitro*, we have detected and quantitated specific interactions between S4 and α mRNA fragments. We find that the complete recognition site is contained within the α mRNA leader, and that most of the binding free energy (90%) is derived from sequences upstream of the ribosome binding site. α mRNA and 16S rRNA compete for binding to S4 with about the same affinity ($\approx 2 \times 10^7$ M^{-1}), and presumably utilize the same S4 binding site.

An unexpected finding is that non-specific binding of S4 to RNA is rather strong and competes well with specific binding in approximately physiological salts ($K = 10^5$ to 10^6 M^{-1}). This affinity is large enough to strongly buffer the free S4 concentration *in vivo*.

It is not clear how S4 is able to effectively repress translation when specific recognition is so weakly affected by the ribosome binding site sequence. S4 may induce a transition between alternate RNA structures. Probable secondary structures for the α mRNA leader will be presented, based both on computer searches for optimum structures and chemical modification experiments.

W-Pos238 INTERACTION OF eIF-4A, eIF-4B, AND eIF-4F WITH MESSENGER RNA.
D.J. Goss[1], J.N. Dholakia[2], C.L. Woodley[2] and A.J. Wahba[2] [1]Department of Chemistry, Hunter College of CUNY, 695 Park Ave, New York, NY 10021 and [2]Department of Biochemistry, University of MS Medical Center, Jackson, MS 39216-4505.

The interaction of eucaryotic initiation factors eIF-4A, eIF-4B, and eIF-4F with mRNA is an important step in the selection of mRNA for translation. These proteins have been implicated in cap recognition and the melting of the secondary structure near the 5' terminus. Initiation factors eIF-4A and eIF-4B have been extensively purified. eIF-4B has been found to stimulate ternary complex [eIF-2·GTP·met-tRNA$_f$] formation and 40S initiation complex formation. We have used fluorescence intensity, fluorescence anisotropy, and circular dichroism to monitor the interactions of eIF-4A, eIF-4B and eIF-4F with mRNA and mRNA analogs. The fluorescent poly nucleotide, polyethenoadenosine, bound to all three proteins. The fluorescence enhancement upon binding of the proteins has allowed us to determine the approximate binding site size and equilibrium constants for binding. The cap analogs, m^7GTP and m^7GDP, are effective competitive inhibitors only for the binding of eIF-4F to polyethenoadenine. GTP does not affect binding. Globin mRNA, a capped natural mRNA, competes effectively with poly etheno A for binding sites of all three proteins.
Grant support: NIH GM25451, Research Corporation, and PSC-CUNY.

W-Pos239 TRITIUM EXCHANGE KINETICS OF YEAST RIBOSOMAL SUBUNITS. John C. Lee and Paul M. Horowitz (Intr. by Martin Meltz). Department of Biochemistry, University of Texas Health Science Center at San Antonio, TX 78284-7760

Tritium exchange kinetics of 60S and 40S ribosomal subunits from Saccharomyces cerevisiae have been studied using a rapid centrifugal ultrafiltration procedure. This assay uses commercially available disposable columns and microconcentrators. The tritium-labeled ribosome is separated from the tritiated solvent using a pre-packed gel filtration column. The labeled ribosome is applied to a microconcentrator and the exchange-out kinetics of the ribosome is measured by centrifugation and measurement of the amount of radioactivity present in the filtrate. With this method, the tritium exchange-out behavior of 60S and 40S ribosomal subunits have been determined. Within the first 20 min, about half of the tritium atoms are rapidly lost from the subunits. The exchange-out behavior can be represented by first order processes. The 60S ribosomes consist of at least two classes of slowly exchangeable protons with $t_{1/2}$ of approximately 30 and 225 min. The exchange-out behavior of 60S ribosomal subunits from yeast and wheat germ appear to be similar under these experimental conditions.
(Supported by NIH DE-07146 to JCL and NIH-GM-25177 and Welch Foundation AQ-723 to PMH.)

W-Pos240 MAGNESIUM ION-DEPENDENT EQUILIBRIA, KINETICS, AND THERMODYNAMIC PARAMETERS OF ARTEMIA RIBOSOME DISSOCIATION AND SUBUNIT ASSOCIATION.
Terry Harrigan and Dixie J. Goss (Intr. by Maria Tomasz) Dept. of Chemistry, Hunter College of CUNY, 695 Park Ave., New York, NY 10021

The influence of magnesium ion concentration on the equilibrium and kinetics of Artemia ribosome dissociation and subunit association has been studied by laser light scattering. Ribosomal aggregation was found to be reduced by addition of 0.1 - 0.05 mM spermidine and KCl concentrations of 100 mM. The ribosomes were found to be stable at low [Mg^{2+}] and the curves obtained for ribosome-subunit equilibrium were independent of the direction and origin of the magnesium ion titration. Thermodynamic parameters were obtained from the temperature-dependent equilibria and have been compared to those of wheat germ and E. coli type A ribosomes. The entropy term is negative (-49.0 cal/mol deg) favoring subunit dissociation, and contributes less to the free energy than the enthalpy term (-25.7 kcal/mol deg). The association rate constant was $3.7 \pm 0.2 \times 10^6$ $M^{-1}s^{-1}$ at 23.7° and the activation energy for the association reaction was 5.5 ± 0.2 kcal/mol. The dissociation rate constant was 0.022 s^{-1} and the activation energy for the dissociation reaction was 14 ± 1 kcal/mol. The reaction curves gave no evidence for sequential processes and were homogeneous.
Supported by: Research Corporation and the PSC-CUNY Research Award Program.

W-Pos241 THERMODYNAMICS OF THE BINDING OF E.COLI SSB PROTEIN TO S.S. NUCLEIC ACIDS. L. B. Overman and T. M. Lohman, Dept. of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.

The single strand binding (SSB) protein of E. coli, recently shown to have two binding modes to single stranded (s.s.) nucleic acids (Lohman and Overman (1985) J. Biol. Chem. 260, 3594) was examined over a range of salt conditions to determine the degree of cooperativity coincident with each mode. The cooperativity parameter (ω) was quantitatively measured under conditions ($\geq$0.20M NaCl) in which only the high site size mode (n=65±5 nucleotides/tetramer) exists by utilizing the fluorescence quenching of SSB upon polynucleotide binding. Equilibrium binding isotherms with poly(U) and poly(dA) yield ω=50±10, a value much lower than most previous estimates and, considering the large site size, implying only very small cooperative clusters. Agarose gel electrophoresis of SSB:s.s.M13 DNA complexes was used to obtain a qualitative estimate of cooperativity under conditions where the low site size mode (n=33±3 nucleotides/tetramer) predominates. At low salt (<0.20M NaCl) a nonrandom distribution is observed, indicative of high cooperativity. However, the high cooperativity complexes are not at equilibrium and eventually form low cooperativity complexes. This transient high cooperativity may be important in replication, a non-equilibrium process. Both modes may function selectively in replication, recombination and repair. The thermodynamics of the cooperative interaction of the high site size binding mode with poly(U) were examined. Under all conditions in which the high site size mode is the stable form, ω=50±10. The observed binding constant, K_{obs}, decreases with increasing [NaCl], ($\partial \log K_{obs}/\partial \log[NaCl]$) = -7.2 (pH 8.1, 25°C). At 0.25MNaCl $\Delta H°_{obs}$=-40 kcal/mol but is [NaCl]-dependent such that is approaches -14kcal/mol at 1M NaCl. The protein has at least two titratable groups important for binding since ($\partial \log K_{obs}/\partial pH$)≈-2 at high pH. A very dramatic anion effect demonstrates differential anion binding to the protein. (Supported by NIH-GM30498 and Welch A-898.)

W-Pos242 EFFECTS OF HISTONE HYPERACETYLATION ON NUCLEOSOME STRUCTURE AND STABILITY. Juan Ausio and K. E. van Holde, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

Using a new fractionation method, HeLa nucleosomes containing different levels of acetylation have been isolated. Fractions with 8-17 acetyl groups per nucleosome have been compared with control particles. At low to physiological ionic strength, the hyperacetylated nucleosomes exhibit a slightly lower sedimentation coefficient and a slightly modified circular dichroism spectrum when compared to controls. No evidence for major conformational differences can be seen. The thermal denaturation and nuclease sensitivity of the most highly acetylated particles are, however, markedly different from those of lightly acetylated nucleosomes.

In terms of stability toward high salt concentrations (0.3-0.7 M), the hyperacetylated and control particles behave identically. These combined observations suggest a model for the effects of hyperacetylation on nucleosomes. Supported by grant GM22916 from the PHS.

W-Pos243 STRUCTURE OF INTACT TOBACCO MOSAIC VIRUS AT 2.9A RESOLUTION. Keiichi Namba and Gerald Stubbs, Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

Intact tobacco mosaic virus has been studied by X-ray fiber diffraction methods. As reported in 1985, intersubunit electrostatic interactions are found in the virus which are completely different from those in the coat protein disk aggregate. This observation was based on a molecular model built from an electron density map at 3.6A resolution.

Among These interactions are two intersubunit pairs of cartoxyl groups, one between the subunits along the turn and another between the subunits in consecutive turns of the basic (1-start) helix. These carboxyl pairs account for anomalous pKs (7) observed in TMV, as well as proton and divalent cation binding which is obseved as the coat protein subunits form helical rod particles. The 20S nucleating aggregate, which is known from titration experiments to bind half a proton per subunit, is required to be a short helical aggregate in order to form the pairs of carboxyl groups between the turns of the helix. The other pair is in the disordered linear loop, which packs together and forms the wall of the central hole when the long rod particle is formed. This disordered inner loop is likely to be the site controlling the length of the helical aggregate, depending on the protonation of the carboxyl groups. Details of RNA binding were also observed, including both base-specific and non-specific elements.

In order to confirm these structural features, stereochemically restrained least-squares procedure has been applied to the fiber diffraction data. Refinement and extension of resolution up to 2.9A resolution has been done. The crystallographic R factor at the present stage of refinement is 13%.

W-Pos244 FILAMENTOUS BACTERIOPHAGE M13 PROTEIN COAT STRUCTURE:DETERMINATION BY X-RAY DIFFRACTION
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The structure of the M13 coat protein in the intact virus is currently being studied using X-ray fiber diffraction. The major coat protein of the class I filamentous coliphage [M13,f1,fd] is a 50 residue polypeptide which is predominantly alpha helical. The cylindrically shaped native virus is about 60Å in diameter and 9000Å long, consisting of a circular single-stranded DNA extending along the center of the virion and ensheathed by 2700 copies of the coat protein. The large anisotropy in the magnetic susceptibility of the particles makes it possible to form highly oriented fiber specimens in a 6 Tesla magnetic field. To obtain high quality diffraction patterns at neutral pH, we used a phage with a 4.3 Kb insert to increase the length of the particle by 60%. Half width of particle disorientation in these specimens is usually 1.5-2.0°.

X-ray diffraction data obtained using synchrotron radiation from the Cornell High Energy Synchrotron Source have been measured to 3.0Å resolution using angular deconvolution. Initial interpretation of this diffraction data to 7.0Å resolution has utilized cylindrically averaged Patterson functions (in collaboration with G. Stubbs, Vanderbilt U.) and model building to define the positions of the alpha helical segments in the protein coat. The organization of the structural subunits is rather different than is found in the structurally related class II filamentous bacteriophage, Pf1.

W-Pos245 PRELIMINARY STUDIES ON STRUCTURE AND ASSEMBLY OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS WATERMELON STRAIN. Sharon Lobert, Peter D. Heil, Keiichi Namba and Gerald Stubbs, Dept. of Molecular Biology, Vanderbilt University, Nashville, Tn. 37235

Analysis of preliminary data suggests some interesting differences between cucumber green mottle mottle mosaic virus watermelon strain (CGMMV-W) and tobacco mosaic virus (TMV), two members of the of the tobamovirus group of plant viruses. Using fiber diffraction methods, the radial electron density distribution of CGMMV-W was calculated. When compared with the radial electron density distribution of TMV, it indicates a possible difference in structural organization at a radius of 45-55 A. The amino acid sequence of the CGMMV-W coat protein was recently predicted from the nucleotide sequence. There are three new carboxylic acids in the left radial helix (Glu 124, Asp 126, and Glu 130). These charged groups are predicted to lie in the region 45-55 A from the viral axis.

In TMV, electrostatic interactions are believed to be important in the assembly of the virus. The carboxyl groups of Glu 95, 106, and Asp 109 at 20 A radius are thought to play a role in the promotion of viral assembly. These residues also form the single lead binding site found in TMV. In CGMMV-W,Glu 95 and 106 are conserved. Calcium titration studies of TMV indicate that TMV binds two calcium ions. The carboxyl groups at 20 A radius probably bind one calcium ion under physiological conditions. The second calcium binding site in TMV is thought to be formed by Asp 77 and Glu 50. In CGMMV-W these are not conserved.. These data together suggest that a second charged region, which may be involved in viral assembly and dissassembly processes in CGMMV-W, occurs at 45-55 A radius.

W-Pos246 COLLOIDAL CRYSTALS OF TOBACCO MOSAIC VIRUS AND ITS PROTEIN DISK AGGREGATE.
W.C. Phillips, D.L.D. Caspar, M. Cahoon and D.M. Salunke, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254.

X-ray diffraction patterns from hexagonal crystals of tobacco mosaic virus and from cubic crystals of the TMV protein disk show that the particles are rotationally disordered about their unique axes which are, themselves, arranged in regular lattices. The colloidal order in these crystals must arise from a balance of long-range forces since there are no regular patterns of intermolecular contacts between the particles. The hexagonal virus crystals, which we have grown from purified, monodisperse TMV, resemble the hexagonal crystalline inclusions observed in infected plants. The hexagonal lattice constants, measured for different crystalline preparations, range from 205 to 215 Å, thus, the 180 Å diameter virus particles are separated side-to-side by 25-35 Å of solvent. Light diffraction indicates that the thickness of the hexagonal layers is defined by the 3,000 Å length of the TMV particles. The colloidal cubic crystals of TMV protein which we have grown coexist with the well-characterized, molecularly-ordered orthorhombic form, and appear also to be composed of four-layer disk aggregates with 17 fold rotational symmetry. The lattice constant of the cubic disk crystal is 272 Å, corresponding to a 192 Å separation between disk axes which are oriented along the crystal three-fold axes. The fall-off in intensity of higher order Bragg reflections from both the virus and protein colloidal crystals indicates that the mean fluctuations from the average positions of particle centers in the lattices are in the range 5-10 Å. (This investigation was supported by PHS Grant CA15468 to D.L.D.C. awarded by the National Cancer Institute.)

W-Pos247 TOWARD COMPUTER AIDED SITE-DIRECTED MUTAGENESIS. A. Warshel and F. Sussman, Department of Chemistry, University of Southern California, Los Angeles, CA 90089

The manipulation of active sites of enzyme by site-directed mutagenesis has become a reality in recent years. Apart from the obvious commercial aspects of this approach it offers new breakthroughs in the fundamental undetstanding of enzyme activity. However, up to now there was no systematic way of predicting the effect of sequence changes on enzyme activity. In exploring solutions for this challenging problem we obtain very promising results using the recently developed EVB (Empirical Valance Bond)[1,2] method and the PDLD (Protein Dipoles Langevin Dipoles) method[1].

Our preliminary study focus on the recent "redesign" of trypsin by Craik et al. [3]. In simulating the catalytic reaction of the native and the mutant systems we succeeded to obtain an almost quantitative agreement with the very large observed effect. This enabled us to understand how the structural changes imposed by the mutation are translated into changes in enzyme activity.

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W-Pos248 THE RAS ONCOGENE PRODUCT P21 INHIBITS NADH-CYTOCHROME B5 REDUCTASE OF THE OUTER MITOCHONDRIAL MEMBRANE IN AN ATP-DRIVEN PROCESS. Martin Poe, Joseph K. Wu, Alan R. Bergstrom, and Karst Hoogsteen, Department of Biophysics, Merck & Co., Inc., Rahway, NJ 07065

When assayed by reduction of added cytochrome c by NADH, NADH-cytochrome b5 reductase [EC 1.6.2.2] in mitochondria isolated from rat liver was inhibited by picomolar concentrations of ras p21 oncogene products. This inhibition of the b5 reductase in the outer mitochondrial membrane also required ATP; half-maximal inhibition was seen with 350 nM ATP. The b5 reductase inhibition was time dependent; it took 90 to 120 sec to reach a steady level when mitochondria were treated with saturating levels of ras p21 and ATP. The b5 reductase inhibition could be blocked by preheating the ras p21 in boiling water or by prereaction of ras p21 with anti-(ras p21) monoclonal antibody. Complexes of ras p21 with GTP and nonhydrolyzable GTP analogs were much less potent at inhibition of the mitochondrial b5 reductase than the complexes with GDP. The inhibition by ras p21 plus ATP was not mediated by cAMP, as shown by direct use of cAMP. When assayed directly by ferricyanide reduction, the mitochondrial b5 reductase was not inhibited by ras p21 plus ATP. Microsomal NADH-cytochrome b5 reductase and NADPH-cytochrome b5 reductase were not inhibited by ras p21 plus ATP. It is proposed that restricted access of cytochrome b5 to phosphorylated b5 reductase is the mechanism of inhibition.

W-Pos249 THE INTERACTION OF THE E. COLI RECA PROTEIN WITH DUPLEX DNA WITH ATTACHED SINGLE-STRANDED TAILS. Sandra L. Shaner and Charles M. Radding, Department of Human Genetics, Yale University, New Haven, CT 06510.

The in vitro strand exchange reaction promoted by the E. coli recA protein is known to proceed via a polar mechanism. We have characterized differences in the interaction of recA with double-stranded (ds) DNA containing attached 5' or 3' single-stranded (ss) tails in order to identify the origin of that polarity. The DNA-dependent ATP hydrolysis activity of recA is known to be functionally essential for the occurrence of strand exchange. We have compared the ATP hydrolysis supported by 5' or 3' tailed duplexes with that supported by ssDNA. We find that the steady state rate of hydrolysis from tailed dsDNA, unlike that from ssDNA, is preceded by a lag. At constant tail length, hydrolysis rates are longer and lag times are shorter for a 5' tail than for a 3' tail. The effects on rates and lag times of ss tail length, total molecule length, [recA], [DNA], and [$MgCl_2$] have been examined. In addition, we have used a microfuge sedimentation assay to quantify the equilibrium amount of recA interaction with these tailed duplexes as well as to examine certain aspects of the kinetics of this interaction. We find that the nucleation of a sedimentable complex of recA with 3' tailed dsDNA exhibits a lag of 3 min. whereas nucleation of complexes with 5' tailed ds DNA does not. The extent of binding to 5' tails is greater than to 3' tails. This difference shows a dependence on ss tail length. The implications of these results for the in vivo roles of recA in recombination and recombinational repair will be discussed.

W-Pos250 CHEMICAL MODIFICATION OF HISTIDINES OF E. COLI RNA POLYMERASE: INVOLVEMENT OF HISTIDINES IN COORDINATION WITH THE INTRINSIC ZINC AT THE ACTIVE SITE. Felicia Y.-H. Wu and Abdulwahid W. Abdulwajid, Dept. Pharm. Sciences, State Univ. of New York at Stony Brook, N.Y. 11794

E. coli DNA-dependent RNA polymerase (RPase) consists of five subunits ($\alpha_2\beta\beta'\sigma$) and two Zn ions which are located in the β and β' subunits. Our earlier finding that Co(II)-Co(II) RPase could be oxidized to Co(III)-Co(III) RPase suggested the likelihood of metal coordination with the N atom of histidine. We have used diethylprocarbonate (DEP), a histidine acylating agent, to modify RPase, apoRPase and its subunits ($\alpha_2\beta$, β'). Incubation of RPase with DEP in different molar ratios (1/250 up to 1/1300) in 100 mM phosphate buffer (pH 6) resulted in loss of 60% enzyme activity in 3 min. Measurements of the formation of N-carbethoxyhistidine at 240 nm indicated that the inactivation of enzyme may be correlated to the modification of 3 histidine residues. The kinetics of enzyme inactivation was biphasic comprising of fast (3) and slow (ca. 20) reacting histidines. The inactive enzyme could be fully reactivated by incubation with 0.5 M hydroxylamine at R.T. for 60 min. No structural differences between native and modified RPase could be detected by fluorescence spectroscopy. Subunits α_2 β and β' were separately modified with DEP after removal of Zn ions. Preliminary results from the reconstitution of the unmodified and modified subunits with exogenous Zn (10^{-5}M) showed the incorporation of 0.8 and 0.5 mol Zn/mol $\alpha_2\beta$ subunits, and 0.7 and 0.2 mol Zn/mol β' subunit, respectively. Preparation of apoRPase and reconstitution of the DEP-modified apoenzyme with exogenous Zn ions are in progress. These results suggest that histidine residues may reside at the active site and some of them are involved in the coordination with the intrinsic Zn ions. (Supported by NIH GM 28057-04 and NSF PCM 8002858).

W-Pos251 EPR STUDIES ON THE ACTIVE SITE OF RNA POLYMERASE. Peter P. Chuknyiski, Joseph M. Rifkind and Gunther L. Eichhorn, Gerontology Research Center, National Institute on Aging, National Institutes of Health, DHHS, Baltimore, Maryland 21224.

The active site of E. coli RNA polymerase consists of an initiation and an elongation site, both bound to a metal, Zn(II) and Mg(II), respectively. Metals at both sites can be displaced by paramagnetic Mn(II), which can be used to probe the active site structure. Chatterji, Wu and Wu [J. Biol. Chem. 259, 284 (1984)] and Bean, Koren and Mildvan [Biochem. 16, 3322 (1977)] have determined metal-substrate distances by NMR at initiation and elongation sites, respectively, and further such studies have been carried out by Pillai in our laboratory. By determining the effects of the metals upon each other, the relationship between the two active site components can be determined. We have obtained separate EPR spectra for Mn on the initiation site and on the elongation site at different temperatures. The spectra and the relaxation properties are different for the two active site Mn ions. With Mn present in both sites there is a >90% decrease in the signal intensity without any appreciable broadening of the spectrum. These results indicate that the Leigh Theory for determining the Mn-Mn distance is applicable. We presently estimate this distance to be approximately 6 Å. The determination of this distance has led to the construction of a model of the active site of the enzyme, which has some interesting implications for the mechanism of RNA synthesis.

W-Pos252 THE INTERACTIONS OF CALMODULIN AND ITS TRYPTIC FRAGMENTS WITH SMOOTH MUSCLE MYOSIN LIGHT CHAIN KINASE AND ERYTHROCYTE Ca^{2+}-ATPase. H. Brzeska, W. Szyja, A. Wrzosek, and M.G. Sarzala. Nencki Institute of Experimental Biology, 02-093 Warsaw, 3 Pasteur Street, Poland. (Intr. by E.D. Korn).

The interactions of calmodulin and its tryptic fragments TR_1-C, TR_2-C and TR_3-E (corresponding to amino acid residues 1-77, 78-148 and 107-148, respectively) with smooth muscle myosin light chain kinase (MLCK) and erythrocyte Ca^{2+}-ATPase were studied. The possible contamination of the fragments with intact calmodulin was estimated to be less than 0.3% by the cAMP phosphodiesterase activity assay (Newton et al., J. Biol. Chem. 258, 4419-4426, 1984). The abilities of MLCK and Ca^{2+}-ATPase to bind to the calmodulin fragments were investigated using calmodulin fragment-affinity chromatography. The abilities of the calmodulin fragments to activate both enzymes were also measured. The maximal possible contamination of the peptides by calmodulin was shown to be too low to have had a significant effect on any of the assays. Both fragment TR_1-C and TR_2-C, coupled to sepharose gel, were able to bind Ca^{2+}- ATPase but only the TR_2-C fragment activated it. In the case of MLCK, only the TR_2-C fragment, coupled to sepharose gel, bound the enzyme, and none of the calmodulin fragments activated it. Fragment TR_3-E failed to bind or to activate either of the enzymes that were studied. These results indicate that the C-terminal half of the calmodulin molecule is involved in the interaction with MLCK and Ca^{2+}-ATPase but that the nature of the interaction is different for the two enzymes.

W-Pos253 LIGAND-INDUCED PROTON IONIZATION AS A CAUSE OF ΔC_p CHANGES IN THE FORMATION OF THE GLUTAMATE DEHYDROGENASE-NADPH COMPLEX. H.F. Fisher, P.M. Chalabi, and N. Singh U. of Kan. and VAMC, Kansas City, Missouri.

We have previously shown that the apparent large negative ΔC_p of formation of this binary complex can be completely accounted for by a temperature-induced shift between two different forms of the free enzyme (Nature, 292, 271, 1981). We now know that these two enzyme forms differ by a single proton whose ionization has a pK = 8.5, a ΔH° of 20 Kcal mol^{-1}, and a ΔS° of +25 eu. Since these parameters cannot be accounted for by the ionization of any known amino acid residue it is presumed that the loss of the proton must trigger a significant change in protein structure. Recently we have found a double anion binding site on this enzyme whose occupancy by two phosphate ions stabilizes this critical proton. Allosteric displacement of these anions by acetate lowers the pK of this enzyme group, decreasing the K_D of NADPH binding by as much as 50 fold.

The formation of complexes of pyridine-nucleotide dehydrogenases as a class exhibit a variety of unusual thermodynamic properties, including large negative ΔC_p's of binding observed here. The phenomena described above suggest a simple general explanation for this behavior.

W-Pos254 **ENERGETICS OF COENZYME AND LIGAND BINDING TO HORSE LIVER ALCOHOL DEHYDROGENASE,** **Maurice R. Eftink and Joanna Hu, Dept. of Chemistry, Univ. of Mississippi, University, MS 38677**

Horse liver alcohol dehydrogenase (LADH) interacts with the coenzyme NAD^+ and small inhibitors, such as pyrazole, in such a way that there is linkage between the binding of these two types of ligands. We have performed various thermodynamic (pH potentiometric, microcalorimetric, and equilibrium binding) studies to characterize the formation of binary and ternary complexes with LADH and to thus delineate the thermodynamics aspects of this linkage. The binding of NAD^+ to LADH is known to be pH dependent; the pK_a of a group on the protein shifts from ~9.2 to ~7.7 on forming this binary complex. The pH dependence of the apparent ΔH° for NAD^+ binding is consistent with this pK_a shift and the ΔH° for NAD^+ binding to the low pH form of the enzyme is about 0 kJ/mole. The formation of the binary complex of LADH with pyrazole shows essentially no pH dependence and the ΔH° of binding is about -37 kJ/mole. Proton uptake and apparent ΔH° data for the formation of the LADH·NAD^+·pyrazole ternary complex are consistent with a further downward shift in the pK_a of the ionizing group on the enzyme to a value $\lesssim 6$. The ΔH° for ternary complex formation with the protonated form of LADH is calculated to be -46 kJ/mole. Thus the enthalpy of coupling between these two ligands is relatively small (~-9 kJ/mole). Determinations of the binding constant of NAD^+ as a function of pyrazole concentration show, however, that there is a free energy of coupling for the binding of the two ligands of about -21 kJ/mole (for the protonated form of enzyme; even more negative for the unprotonated form). **This work was supported by NSF grant PCM-8206073 and DMB-8511569.**

W-Pos255 ^{57}FE MOSSBAUER STUDIES ON PHENYLALANINE-ACTIVATED PHENYLALANINE HYDROXYLASE. M. D. Davis, S. Kaufman, Laboratory of Neurochemistry, National Institute of Mental Health, A. Levy and J. M. Rifkind, Laboratory of Cellular and Molecular Biology, National Institute of Aging. Phenylalanine hydroxylase (PAH) catalyzes the conversion of phenylalanine (Phe) to tyrosine using tetrahydrobiopterin as a cofactor. PAH contains one mol of iron per mol of protein subunit. Although the role of iron in this enzyme is unknown, it is necessary for catalytic activity. As isolated, the iron in the enzyme is in the Fe^{+3} state and the enzyme is inactive. Activation of PAH can be achieved by reducing the iron to Fe^{+2} (this can be accomplished by incubation of the enzyme with cofactor, as well as with other reducing agents), and by incubation with Phe (the substrate which also serves as an activator). Wallick et al. (Biochem., 23, 1295-1302, 1984) have shown by epr spectroscopy that the environment of Fe^{+3} changes upon Phe activation of the enzyme. However, the iron in the active form of the enzyme, i.e. Fe^{+2}, is epr silent. We have exchanged ^{57}Fe for the naturally-occurring iron to make the enzyme suitable for study by Mossbauer spectroscopy, a technique that can monitor both Fe^{+2} and Fe^{+3}. The enriched ^{57}Fe enzyme shows no difference in its catalytic activity or its epr spectrum compared to the native enzyme. The Mossbauer spectrum of $^{57}Fe^{+3}$ PAH at 160°K yields an asymmetric doublet feature with an isomer shift of 0.41 mm/s relative to metallic iron and quadrupole splitting of 0.75 mm/s. The feature remains essentially unchanged by lowering the temperature to 100°K. This spectrum splits at 4.2°K with parameters that are difficult to calculate due to the reduction in the absorption intensities that accompanies the splitting. Addition of stoichiometric amounts of 6-methyltetrahydropterin (a cofactor analog) to reduce the iron yields a high spin Fe^{+2} with an isomer shift of 1.3 mm/s and a quadrupole splitting of 2.5 mm/s. The parameters of these components were altered by the binding of Phe to PAH. This result shows that the activation of PAH by incubation with Phe alters the environment of the iron in the functional Fe^{+2} enzyme.

W-Pos256 SPIN LABEL STUDIES OF THE ESSENTIAL SULFHYDYL GROUPS ENVIRONMENT IN CHICKEN LIVER FRUCTOSE 1,6 BISPHOSPHATASE Henry Zeidan, Atlanta University, Chemistry Department, Atlanta, Georgia 30314

The local environment of the essential sulfhydryl groups in chicken liver fructose 1,6-bisphosphatase has been investigated by ESR techniques using a series of iodoacetamide spin labels, varying in chain length between the iodoacetate and nitroxide free radical group. The ESR spectrum of spin labeled chicken liver fructose 1,6-bisphosphatase showed that the sites of labeling were highly immobilized when the enzyme was chemically modified by spin label iodoacetate, suggesting that the sulfhydryl group of the protein are in small, confined environment. From the change in the electron spin resonance spectra of these nitroxides as a function of chain length, we conclude that the sulfhydryl group is located in a cleft approximately 10.5 A° in depth.

W-Pos257 ACTIVATION OF LIPOPROTEIN LIPASE BY APOLIPOPROTEIN C-II IS PREDICTED BY THE sn-2 ACYL CHAIN LENGTH OF THE SUBSTRATE. L.R. McLean, A. Balasubramanian, & R.L. Jackson, (Intr. by M.A. Flanagan) Merrell Dow Research Institute and Dept. of Pharmacology, Univ. of Cincinnati, Cincinnati, OH 45215

Lipoprotein lipase (LpL) catalyzes the hydrolysis of triglycerides and phosphatidylcholines (PC) in plasma triglyceride-rich lipoproteins. Enzyme activity is increased in the presence of a 79 amino acid peptide, apolipoprotein C-II (apoC-II), associated with lipoproteins. The activation factor (AF, LpL activity plus apoC-II/ minus apoC-II) increases logarithmically with fatty acyl chain length of the substrate as a result of an increase in $\Delta S^{\ddagger}$. Mixed acyl chain PCs in Triton N-101 micelles were employed as substrates for LpL to test which acyl chain determines AF; the phospholipase A_1 activity of LpL was measured by pH-stat. AF increased monotonically with apoC-II conc. up to 1 μM at an enzyme conc. of 0.01 μM. Maximal AFs at 37°C for $C_{14}C_{14}$-, $C_{16}C_{14}$-, and $C_{18}C_{14}$-PC were 15.0, 16.0, and 15.0 and for $C_{14}C_{16}$- and $C_{16}C_{16}$-PC were 28.7, and 29.5. Thus, the sn-2 chain determines the activation factor. To test the importance of the lipid-binding domain of apoC-II, the peptide comprising residues 56-79 of apoC-II, which does not bind lipid was synthesized. The synthetic peptide and apoC-II increased LpL activity equally with PC/Triton N-101 micelles and sonicated PC vesicles with or without cholesterol above and below the phase transition temperature of the PC vesicles. These data are consistent with a model in which residues 56-79 bind to LpL thereby increasing the entropy of the transition state complex as a function of the sn-2 acyl chain length of the substrate or lysoPC product.

W-Pos258 ZINC IN CYTOCHROME OXIDASE: AN EXAFS STUDY. A. Naqui, L. Powers,* and B. Chance. Dept. Biochem.Biophys. Univ. of Penna.; ISFS Univ.CitySci.Ctr.,Phila.,PA & *AT&T Bell Labs,Murray Hill,NJ

Cytochrome c oxidase (CcO) is the terminal oxidase in the mitochondrial respiratory chain. It has been reported recently that CcO from beef heart contains 1 Zn and 1 Mg atom per functional unit (monomer) as well as 2 Cu and 2 Fe (1,2). We have searched for Zn content in 13 preparations of CcO from beef heart prepared in our laboratory by different methods and confirm that all these preparations contain Zn of reported ratio; i.e., Fe:Cu:Zn = 2:2:1.

However, the role of Zn in CcO is unknown. All our attempts to prepare a Zn-depleted enzyme that is otherwise intact have failed so far. To elucidate the structure of the Zn environment, we have used the X-ray absorption spectroscopy (both edge and EXAFS). Our preliminary results suggested that Zn is in a distorted tetrahedral environment. The ligands are mostly sulfur (S) with somewhat different distances. On the basis of studied Zn-enzymes, we propose a structural role for Zn in CcO. There are only 3 S groups in subunits I&II and they contain all redox centers. It is unlikely that these are any more "available" S ligands for Zn atoms in these two subunits. It is known that there are several (8 to 9) "unconserved" S groups in smaller subunits (III,V,VIa,VIb,VIIIc nomenclature of Buse)(3), so it is possible that Zn is bound to one or more of these subunits. This is also consistent with the finding that the bacterial terminal oxidase from PS3 does not contain Zn (N. Sone, personal communication. (1) Einarsdottir, O & Caughey W.S. (1984) BBRC 123:836-842; (2) Einarsdottir, O & Caughey, W.S. (1985) Fed.Proc. 44:1780; (3) Buse, G., Meineck, L. & Bruch, B. (1985) J. Inorg.Biochem.23:149-153. Supported in part by NIH Grant HL 31909.

W-Pos259 Purification And Characterization Of Human Synovial Fluid Phospholipase A_2. Abdel A. Fawzy and Richard C. Franson. Dept. of Biochemistry, Medical College of Virginia, Richmond, VA 23298 (Spon: R.C. Franson)

The accumulation of an extracellular calcium-dependent phospholipase A_2 (PLA_2) in synovial fluid from rheumatoid patients raises questions about the possible proinflammatory actions of this enzyme and its products. The purification and characterization of PLA_2 is described. Synovial fluid PLA_2 was purified 12,000-fold, in a yield of 37%, by sulfuric acid extraction and cation-exchange chromatography. At this stage of purification only minor contaminating bands ($< 5\%$) were apparent after SDS-PAGE and silver staining. The 2-acyl specificity of the PLA_2 activity was confirmed using 1-^{14}C-stearoyl-phosphatidylethanolamine as substrate. The purified enzyme was maximally active at neutral pH and had an absolute requirement for calcium. Molecular weight estimates by gel filtration and SDS-PAGE ranged from 12K-15K daltons. The activity of the purified enzyme was increased 3-fold by 155 mM NaCl (approximately 100 umole/min·mg protein) and activity was inhibited by retinal (IC_{50}=1uM). Retinoic acid, retinol acetate, and retinol were inhibitory but at higher concentrations. Thus, a potent extracellular PLA_2 is present in human synovial fluid and could contribute to the pathogenesis of this chronic inflammatory disease.

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