W-Pos240

MULTIDIMENSIONAL ANALYSIS OF PROTEIN FOLDING ENERGETICS. Dong Xie, Vinod Bhakuni and Ernesto Freire. Biocalorimetry Center and Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

The folding/unfolding transitions of apo-α-lactalbumin and lysozyme have been determined as a function of both temperature and GuHCl concentration using high sensitivity calorimetric techniques. These experiments have allowed us to define the energetics and thermodynamic folding mechanism for these two proteins. In the absence of GuHCl both proteins obey the two-state mechanism and undergo folding/unfolding transitions characterized by a ΔHvh/ΔH close to unity. Upon increasing the concentration of GuHCl, the ΔHvh/ΔH ratio remains constant for lysozyme but increases monotonically for lactalbumin. The increase in ΔHvh/ΔH with GuHCl for lactalbumin is primarily due to a large decrease in the calorimetric enthalpy with only a slight change in ΔHvh. These results indicate that in the case of lactalbumin GuHCl triggers the formation of a third state (molten globule?) uncapable of undergoing a calorimetrically detectable unfolding transition. Analysis of the GuHCl-temperature stability surface suggests that this state is formed by the association of two GuHCl molecules to the protein with a Kd of 0.42 $M^{-1}$. The remaining GuHCl sites only contribute to a slight destabilization of the native conformation. This is manifested by a downwards shift in Tm of only 5°C after increasing the GuHCl concentration up to 1M compared to a shift of 10°C for lysozyme. These studies have allowed us to develop a generalized algorithm directed to the analysis of temperature - GuHCl or temperature-ligand protein stability surfaces. This analysis allows calculation of the population of protein states along the stability surface. (Supported by NIH Grants RR-04328 and GM-37911.)

W-Pos241

## Molecular Dynamics Analysis of Protein Comformations in the Vicinity of the Native State

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(Intro by Colin A. Wraight)

By subjecting the native structure of *T4 Lysozyme* to artificial expansion forces during molecular dynamics simulation a series of partially unfolded states were generated. The behaviour of these conformations after removal of the artificial forces was analyzed. We find that the molecular force field used in the simulations is accurate enough to refold the correct secondary and tertiary structure of the protein backbone, provided the degree of unfolding is small. When the refolding simulation is modified to include an artificial attractive potential between backbone atoms, reasonably accurate refolding can be achieved at much greater distances from the native state. The protein structure of Lysozyme was selected for its well known crystal structure. The simulations were carried out using our molecular dynamics program MD on a Connection Machine.

W-Pos242

**A BRANCHED CHAIN MODEL OF PROTEIN FOLDING AND LIGAND BINDING: Exhaustive Simulations of Conformation Space.** S. Bromberg and K. A. Dill, Dept. of Pharm. Chem. UCSF, San Francisco, CA 94118.
A versatile model representing proteins with side chains or with bound ligands is being developed. Exhaustive simulations exploring all of conformation space and "branch space" have been performed. **Side Chain Model:** Almost nothing is currently known about the role played by side chains in the statistical thermodynamics of protein folding. While proteins have side chains which branch out from the backbone, current exhaustive lattice simulations treat polypeptides as a simple linear string of beads. Classical mean field theory (Flory P. J., 1953, *Principles of Polymer Chemistry*, Cornell U. Press, Ithaca, N.Y.) treats only local effects of bulky side chains on polymer conformations. The branched chain model explores local and non-local (excluded volume) effects of side chains or bound ligands as a function of the compactness of the protein. Due to excluded volume, side-chains lose degrees of freedom and contribute an opposing entropy when proteins fold to compact native states. The side chain model has been devised to address the following questions: What is the contribution of side chains to the entropy of unfolding? How do main chain and side chain contacts differ in native and non-native compact states? How do random probabilities govern the contacts among side chains? We begin with an unbranched chain whose conformations are defined by a self-avoiding walk on a square lattice. Branches are defined to occupy one lattice site adjacent to main chain residues. Distributions of conformations as a function of radius of gyration, number of topological contacts, and number of main chain residues have been analysed to formulate theory. Simple expressions for the distributions of populations have been derived. **Ligand Binding Model:** Ligand binding may also be treated by the introduction of single lattice unit branches. As do sidechains, bound ligands restrict conformation space of compact chains more severely than open chains. In this way some bound ligands may contribute an entropy opposing folding. By comparing distributions of conformations of chains with and without ligands, the ligand binding model addresses the following questions: What fraction of ligand bindings lead to conformational change? How does this fraction depend on the relative ligand binding energy, on position of the liganded residue, on sequence and on chain length? How is binding specificity determined? Distributions have been generated as a function of radius of gyration, so that expressions may be derived for comparison with experiment. Due to direct and indirect consequences of the combinatorics of distributing a given number of ligands over a given number of binding sites, more complicated expressions are needed to describe the full range of ligand binding behaviors than for the side chain model.

W-Pos243

## Pattern-based Prediction of Protein Structural Class

Saurugger P.N., Metfessel B.A., Chaudary L., Foo Q.

Since the classical experiments by Anfinsen (1971), who showed that ribonuclease could be denatured and refolded without loss of enzymatic activity, it is accepted that the primary sequence of a protein contains sufficient information to determine its three-dimensional structure in a given environment. The problem of predicting the three-dimensional structure from the amino acid sequence of a protein, called the protein folding problem, is still one of the large quests in structural biology.

Despite the vast array of primary sequences encountered, proteins fold into conformations that can be described by a small number of structural classes. We consider the correct prediction of the structural class of a protein an important step in the prediction of the three-dimensional structure of a protein (Saurugger and Langsetmo, 1990). Nakashima (1986) showed that the structural class of a protein is partially determined by its amino acid composition. Our working hypothesis is that there exist patterns in the amino acid sequence which determine the structural class the protein will assume under native conditions.

Patterns are developed using ARIADNE (Webster et al. 1987) or a neural network based on the neocognitron (Fukushima 1988) architecture. This network architecture was shown to be able to recognize patterns even if they are shifted along the sequence, or if the patterns are distorted. In addition, the neocognitron can be trained to recognize patterns even without knowledge of the actual structural class of the protein.

Preliminary results show that patterns exist which do discriminate between different structural classes of proteins.

**W-Pos244**

A COMPARISON OF DIFFERENT EQUILIBRIUM MODELS FOR THE THERMAL UNFOLDING OF A LIGAND-BOUND PROTEIN, SERUM RETINOL BINDING PROTEIN. Donald D. Muccio Fred Fish, and D. Vincent Waterhous, Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294.

Serum retinol binding protein (SRBP) contains retinol noncovalently bound to the protein. Thermal unfolding curves of this protein derived from far-UV CD spectroscopic measurements are asymmetric about the midpoint temperature. The unfolding curves using near-UV CD data also contain this asymmetric transition as well as a smaller pretransition.

In order to evaluate these data for thermodynamic information, we have used several models. These include: (1) NR = U + R; (2) NR = IR = U + R; (3) NR = N + R = U + R, where NR is the native holo-protein, N is the native apo-protein, U is the thermally unfolded protein, IR is an intermediate, and R is retinol. Each of these models give characteristically different profiles. However, these differences become less obvious when the spectroscopic probes are considered to not only report information about the thermodynamic transitions, but also contain temperature dependence, either linear and/or quadratic, for the stable species.

A comparison is made between the fits of experimental data with each of these models. In order to test the uniqueness of the fit, synthetic data derived from one of these models or more simple N = U and N = I = U models were fit by the other models. It was shown that the differences were systematic but the magnitude of these trends could be easily hidden by random noise.

**W-Pos245**

RANDOM COILS TO COILED COILS: THE FOLDING KINETICS OF NONPOLYMERIZABLE TROPOMYOSIN. Jianming Mo, Marilyn Emerson Holtzer, and Alfred Holtzer, Department of Chemistry, Washington University, St. Louis, MO 63130. The kinetics of assembly of nonpolymerizable tropomyosin (NPTm) coiled coils from unfolded chains was studied by Stopped Flow Circular Dichroism (SFCD). The backbone CD at 222 nm shows that about 70% of equilibrium helix content forms in a fast phase (<0.04s, the SFCD dead time), the remaining structure forming in a slow, observable phase. The slow phase is first order ($k^{-1}$ = 1.67s at 20°C). Crosslinking at Cys-190 increases the helix content formed in fast phase to about 85%, but leaves the rate constant of slow phase unchanged. The rate constant of the slow phase is reduced by about one order of magnitude by the reduction of ionic strength from 0.6M to 0.026M, but the fast phase remained undetectable even at the lowest ionic strength. [Supported by NIH and MDA.]

**W-Pos246**

KINETICS OF HETERODIMER FORMATION IN TROPOMYOSIN COILED COILS. Tadashi Kato, Sumio Ozeki, Marilyn Emerson Holtzer, and Alfred Holtzer, Department of Chemistry, Washington University, St. Louis, MO 63130. Mixtures of beta tropomyosin ($\beta\beta$) and sulfhydryl-blocked beta tropomyosin (:$\beta\beta$:) coiled coils at 32, 34, 36, and 38°C are assayed for formation of $\beta\beta$: heterodimers as a function of time. Under the conditions employed, the principal species are dimers and the equilibrium fraction ($h_\infty$) of heterodimer is very close to 0.5. We find that the fraction of heterodimer is given by $h = h_\infty[1 - \exp(-k_1 t)]$ and that $k_1$ is independent of concentration. Of the three most likely mechanisms (dissociation-reassociation, dissociation-displacement, and double displacement), dissociation-reassociation is most consistent with the data, as previously reported for the formation of $\alpha\alpha'$ tropomyosin heterodimers [Ozeki, S., Holtzer, M.E., and Holtzer, A., Biophysical J., 443a (1990)]. Additional data for the formation of $\alpha\alpha'$ heterodimers at temperatures between 34-42°C are also presented. We find that equilibrium is reached much faster for the formation of $\beta\beta$: than for $\alpha\alpha'$ heterodimers, e.g., near physiological temperature (36°C) $k_1 = 1.7 \times 10^{-3}$ ($4.2 \times 10^{-5}$) $sec^{-1}$ for $\beta\beta$: ($\alpha\alpha'$) species. Values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ determined from plots of $\ln k_1 T^{-1}$ vs $T^{-1}$ using Eyring theory are 160 (100) kcal•mol$^{-1}$, and 430 (250) cal•K$^{-1}$•mol$^{-1}$, respectively, for the $\alpha$ ($\beta$) system. [Supported by NIH and MDA]

**W-Pos247**

RANDOM COILS TO COILED COILS: THE FOLDING KINETICS OF $\alpha\alpha$-TROPOMYOSIN SUB-SEQUENCES. Jianming Mo, Marilyn Emerson Holtzer, and Alfred Holtzer, Department of Chemistry, Washington University, St. Louis, MO 63130. The kinetics of folding from random coils to 2-chain coiled coils of $\alpha\alpha$-tropomyosin sub-sequences was studied by Stopped Flow Circular Dichroism (SFCD). Sub-sequences included chains comprising residues 11-127 ($_{11}Tm_{127}$), 142-281 ($_{142}Tm_{281}$), and 1-189 ($_{1}Tm_{189}$). $_{1}Tm_{189}$ and $_{11}Tm_{127}$ folded into the native coiled coil structure from random chains within the dead time of the instrument (<0.04s). In contrast, the folding of segment $_{142}Tm_{281}$ consisted of two phases, a fast phase, within which 40% of the equilibrium helix content formed, and a first-order slow phase ($k^{-1}$ = 2.7s at 20°C) in which the remaining structure formed. Crosslinking at Cys-190 increases the helix content formed in fast phase to 90% of the equilibrium value, but the remaining structure still forms slowly. [Supported by NIH and MDA.]

**W-Pos248**

THE KINETICS OF UNFOLDING OF A MULTIDOMAIN PROTEIN IN GUANIDINIUM CHLORIDE: THE CASE OF HUMAN RENIN. R.A. Poorman, D.E.Epps, *D. Brems, J.L. Sarcich, S. Plaisted, W.C. Krueger and F.J. Kézdy, The Upjohn Company, Kalamazoo, Mich. and *The Eli Lilly Company, Indianapolis, Indiana.

The thermodynamics and kinetics of guanidinium chloride (Gdn) denaturation of human renin (HR) were studied by spectroscopy, chromatography and enzyme activity assays. The unfolding in Gdn is consistent with a pathway of a fully reversible step followed by a slow, irreversible denaturation according to the scheme:

$$N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} U_1 \overset{k_2}{\rightarrow} U_2 \rightarrow D$$
$$\downarrow A$$

The rate constants $k_1$, $k_{-1}$ and $k_2$ were evaluated by monitoring at different Gdn concentrations the time dependence of the loss of intrinsic fluorescence (FS) of HR and the irreversible loss of enzymatic activity. Only $k_{-1}$ is Gdn-dependent and the order of the reaction is -17 ± 3. Thus, the reversible unfolding of native HR (N) is not catalyzed by Gdn but rather, Gdn stabilizes $U_1$, the reversibly unfolded form of HR. $U_1$ is then slowly transformed into $U_2$, a species which retains all of the CD ellipticity but only 60% of the fluorescence of N. $U_2$ does not yield enzymatic activity upon removal of Gdn. The formation of $U_2$ is most likely the result of self-association of the Gdn-stabilized unfolded form. 6 M Gdn dissociates this partial aggregate into a fully unfolded monomer (D) stabilized by Gdn. Attempts to refold $U_2$ leads to the formation of A, an aggregate which has both partial CD and FS, but no catalytic activity.

**W-Pos249**

RECEPTOR AFFINITIES AND CD ANALYSES OF DISULFIDE ANALOGS OF INSULIN-LIKE GROWTH FACTOR I

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Insulin-like growth factor I (IGF-I) is a protein hormone with anabolic effects which may have therapeutic utility in treating cachexia. The protein has a molecular mass of 7.6 kd (70 amino acids) and has three disulfide bonds connecting cysteine residues 47 and 52, 6 and 48 and 18 to 61. These correspond to the invariant insulin disulfides A6-A11, A7-B7, A20-B19, respectively.

To determine the role of these disulfide bonds in folding and specific binding to the IGF-1 receptor (Type I), we have made analogs using site-directed mutagenesis. These analogs were purified, their relative secondary structures examined by circular dichroism, and relative affinities for the receptor determined.

Modification of individual disulfide bonds produced conformational perturbations observable by CD and decreases in the receptor affinity of two to three orders of magnitude.

(1) The analog in which the Cys47-Cys52 bond was replaced with two alanine residues, [Ala 47, Ala 52]-IGF-I, retained only 1/3000 of the affinity ($3 \times 10^{-7}$M) of the natural sequence, which had an observed affinity of $1.1 \times 10^{-10}$M. This large change in affinity was paralleled by a large change in the near UV CD-observable structure, but showed little change in the far UV.

(2) IGF-I in which the Cys6-Cys48 disulfide was replaced by substitution of serine at positions 6 and 48 had a competition-based affinity of $3.3 \times 10^{-8}$M, 1/300 that observed for the natural molecule, but ten times stronger than the [Ala 47, Ala 52]-IGF-I. This analog had small changes in both the near and far UV CD.

(3) IGF-I in which all cysteines were removed, [Ala 18, 47, 52, 61, Ser 6, 48]-IGF-1, did not compete with $^{125}$I-IGF-I for the receptor at concentrations up to $3 \times 10^{-7}$M and showed only small signals attributable to folding and secondary structure upon CD analysis.

**W-Pos250**

ANTIBODY ASSISTED PROTEIN FOLDING. J.D. Carlson*, P.S. Kim#, M.L. YarmushΔ. Dept. of Chem. Engg. & Biology*, M.I.T.#, Cambridge MA 02138. Dept. of Chem & Biochem Engg.Δ Rutgers University, Piscataway, N.J. 08855.

Currently, many proteins of mammalian origin are produced in large quantities in recombinant micro-organisms. Often, these complex proteins misfold and aggregate in organisms lacking the post-translational processing capabilities of mammalian cells. Many studies have attempted to elucidate conditions under which these proteins can be denatured and renatured in vitro to their native conformation, generally focusing on environmental conditions, with varying success. Our studies on the S-Protein fragment of Ribonuclease A (RNase A) show that the addition, during refolding, of monoclonal antibodies specific to the native structure of the protein, increases the yield of native refolded S-Protein.

Under a constant redox potential of 1.5 mM oxidized glutathione and 1.5 mM reduced glutathione, reduced, unfolded S-Protein refolded to 15% of native activity. Addition of a stoichiometric concentration of one anti-RNase A monoclonal antibody (MAb) increased the recovered activity to 27% and an anti-S-Protein MAb increased the yield to 54% of native activity. Four other anti-RNase A MAbs and non-specific mouse antibodies failed to increase the yield of refolded native S-Protein.

Results show the increase in recovered activity was directly related to MAb concentration and the process was saturable with excess antibody. This suggests that the antibodies are assisting refolding through direct interaction at specific epitopes. This approach may offer a general scheme for assisting the refolding of reduced proteins by the use of specific affinity ligands. This work may also yield information about critical steps and residues involved in protein folding. Work is currently underway to further understand the nature of, and to optimize, monoclonal antibody assisted protein refolding.

**W-Pos251**

***IN VITRO* FOLDING OF PRO-α-LYTIC PROTEASE IS TEMPERATURE SENSITIVE**

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The serine proteases comprise two distinct evolutionary families distinguishable by sequence and structural homology to either trypsin or subtilisin. The extracellular bacterial serine proteases share the common feature of a large pro-region; the role of the pro-region in folding has been previously demonstrated for a bacterial representative of each evolutionary family. Alpha-lytic protease, a member of the trypsin family, originates as a preproenzyme before being secreted extracellularly by the Gram-negative soil bacterium, *Lysobacter enzymogenes*. The protease exhibits temperature sensitivity for expression in *E. coli* that parallels the viable growth temperature profile of the native *L. enzymogenes* host. At permissive temperatures in *E. coli*, the mature (proteolytically processed and active) protease is secreted into the media, and at nonpermissive temperatures the inactive proenzyme accumulates in the outer membrane. We have now isolated and refolded the proenzyme from *E.coli*, and demonstrate that the folding process itself is temperature sensitive *in vitro*.

W-Pos252

## MUTATIONS THAT AFFECT FOLDING SELECTED AS SUPPRESSORS OF AN EXPORT DEFECT

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Folding mutations of ribose-binding protein were selected as suppressors of a defect in export from *E. coli*. Our model of export proposes a kinetic partitioning between productive translocation and folding of the precursor protein into an export incompetent state inside the cell. This cytoplasmic folding of a precursor can result from a mutation in the leader sequence which disallows efficient export. Our model predicts that a mutation in the mature portion of the precursor protein that slows the folding of the polypeptide could suppress the leader mutation by allowing more time for the precursor to interact with the export apparatus. Suppressors of a leader sequence mutation of ribose-binding protein, isolated in the laboratory of Dr. Chankyu Park, enhanced the export of ribose-binding protein and the ability of the cells to grow on ribose. This study reports the characterization of two suppressors whose mutations were located in the mature portion of ribose-binding protein; suppressor 1 has valine at postion 36 changed to a glutatmic acid and suppressor 2 is altered at position 27 from an alanine to a threonine. As predicted by the model of kinetic partitioning, the mutational alterations of the suppressor proteins were found to slow the folding as compared to the wild-type ribose-binding protein. Suppressor 1 altered the unfolding reaction as well. Both suppressors were shown to have energies of stabilization lower than the wild-type protein. Both suppressor proteins were able to bind to ribose though suppressor 1 bound ribose with an affinity ~25 fold lower than the wild-type protein. This work was supported by a grant from NIH(GM29798).

W-Pos253

## PARTIALLY UNFOLDED STRUCTURE OF BPTI INDUCED BY A SINGLE AMINO ACID SUBSTITUTION OUTSIDE THE HYDROPHOBIC CORE

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Initial proton NMR studies of BPTI mutant Y35G, in which Tyr35 is substituted by glycine, indicate that the mutant protein is partially unfolded. Assignments of the fingerprint resonances in COSY/NOESY spectra have been made. The spectral characteristics of the hydrophobic core resonances are very similar to wild type. The amide protons in the region around the substitution have a much narrower chemical shift dispersion than wild type, suggesting a very flexible local structure. NOESY crosspeaks for the hydrophobic core resonances are similar to wild type. No long-range NOE's are observed for resonances for the NH's in the loops which enclose the region around Tyr35 in the wild type. Hydrogen exchange rates are faster for all NH's in the mutant compared with wild type. In particular, the amide protons in the loops are rapidly exchanging on the order of surface NH exchange rates. In addition, the hydrophobic core region, which has little change in structure as shown by circular dichroism and NOESY crosspeaks, has much faster exchange rates than wild type protein. This agrees with thermodynamic data obtained by calorimetry which shows remarkable destabilization in the mutant. Protease digestion experiments, which are sensitive to backbone accessibility show that the mutant is much more readily proteolyzed than wild type protein. Taken together, our results indicate that in Y35G, the secondary structure in the hydrophobic core is essentially retained while the loops are essentially unfolded.

W-Pos254

## *ESCHERICHIA COLI* THIOREDOXIN MUTANT K57G: THE EFFECT OF K57 ON THE REDOX MECHANISM.

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*E. coli* thioredoxin (MW 11,900) is a small, stable, redox active globular protein. Its crystal structure has been solved to 1.68 Å [Katti, et al., *J. Mol. Biol.*, (1990) 212, 167-184]. The single disulfide located between Cys 32 and Cys 35 catalyzes diverse intracellular redox reactions. The carboxylic acid side chain of aspartic acid 26 is buried near the active site disulfide. Previous studies of the site-directed mutant D26A showed that Asp 26 has an anomalous pK of 7.4 in the wild type structure which accounts for the destabilizing effect of Asp 26 in the naturally occuring protein [K. Langsetmo, unpublished data]. This is reflected in the observation that the D26A mutant protein is stabilized by ~5 kcal/mol at pH 8.5 compared to wild type [cf. Langsetmo et al., *Current Research in Protein Chemistry*, (1990), 449-56]. The fact that Asp 26 is conserved throughout all thioredoxins suggests that its anomalously titrating carboxylic acid side chain is important to thioredoxin catalytic function. Molecular dynamics simulations suggest a salt bridge between Asp 26 and Lys 57 may act to stabilize the buried Asp 26 [K. Langsetmo, unpublished data]. To examine the role of Lys 57 in wild type thioredoxin activity and folding, we have constructed a site directed mutant replacing Lys 57 with a glycine. In the absence of Lys 57 we postulate that the carboxylic acid side chain of Asp 26 in its hydrophobic surrounding is so destabilized that it will move to become more accessible to solvent. In this case we expect the disulfide equilibrium to shift toward the reduced form. We have obtained expression of K57G in an overproducing *E. coli* system. Initial results suggest that K57G is folded but undergoes aggregation which could be due to the presence of reduced forms.

W-Pos255

## A Double Mutation in Dihydrofolate Reductase Simplifies the Folding Mechanism.

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The folding mechanism of the wild type dihydrofolate reductase (DHFR) has been proposed to involve a series of native, intermediate, and unfolded forms which fold through four parallel channels (Touchette et al.(1986) Biochemistry 25,5445). The complexity of the model precludes an analytical solution relating relaxation times and microscopic rate constants. Recently, we constructed a DHFR double mutant (C85S/C152E) which was found to have simplified folding kinetics. Kinetic studies of unfolding and refolding reactions at various final urea concentrations suggest that the folding mechanism of the C85S/C152E DHFR is well described by a four state sequential model:

$$N \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} I_1 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} I_2 \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} U$$

By simulating the observed relaxation times as a function of urea concentration, all of the microscopic rate constants and their urea dependences were determined. The urea dependence of the amplitudes of the unfolding and the slowest refolding phases shows that the optical properties of $I_1$ and $I_2$ must be native-like and unfolded-like, respectively. The apparent fraction of unfolded protein at equilibrium as a function of urea concentration is well described by these microscopic rate constants and the estimated optical properties of the intermediates, strongly supporting the sequential folding model for this mutant DHFR. This work was supported by NSF grant DMB 9004707.

W-Pos256

## Reversible Thermal Unfolding of a Double Mutant of Dihydrofolate Reductase

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Although the urea and guanidine hydrochloride induced unfolding of dihydrofolate reductase (DHFR) is reversible, the thermal unfolding of the wild type protein is not. Thus, it has not been possible to obtain a full range of thermodynamic parameters for the folding reaction. Presuming that this irreversibility is due to chemical damage to the two free cysteine residues, the C85A/C152S double mutant was constructed and its thermal unfolding properties examined.

C85A/C152S DHFR undergoes a thermal unfolding transition as detected by a sigmoidal decrease in ellipticity at 222 nm. The transition is well described by a two state model with a van't Hoff enthalpy of 35.81 kcal/mol and has a midpoint of 51.4° C. The process was shown to be fully reversible by complete recovery of the ellipticity at 222 nm and greater than 95% of the enzyme activity. The stability as assayed by the urea induced unfolding reaction was nearly identical to the wild type DHFR (M. Iwakura, unpublished results) showing that the two mutations do not significantly perturb the free energy difference between the native and unfolded forms.

The C85A/C152S DHFR appears to be an excellent system in which to make quantitative measurements of the thermodynamic parameters for the unfolding reaction and to study the effects of mutations on these parameters.

This work was supported by NSF grant DMB 9004707.

W-Pos257

## INSERTION MUTAGENESIS: A NEW TOOL FOR MODIFYING THE STRUCTURE AND FUNCTIONAL ACTIVITY OF PROTEINS

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Five different single amino acids (Ala, Gly, Leu, Pro, Asn) and two different dipeptides (Gly-Gly, Ala-Gly) were inserted into peptide bonds 60/61 and 61/62, located in the middle of the first alpha-helix of staphylococcal nuclease. The 14 insertion mutant proteins have stabilities in the absence of denaturant, $\Delta G_{H_2O}$, that are very similar to a set of proteins substituted at positions flanking the inserted peptide bonds (i.e., 60, 61, or 62). However, the insertion mutant proteins have specific activities that are 100 to 1000 fold lower than wild-type enzyme, whereas the comparison set of substitution mutant proteins display enzymatic activities comparable to wild-type. Similarly, the circular dichroism spectra of the insertion mutants show clear evidence of reduction in native alpha-helical content, whereas the spectra of the substitution mutants are essentially identical to wild-type. These data strongly suggest that the native structure of nuclease has been significantly altered by amino acid insertions in ways that do not occur with substitutions, even though the stability consequences for the two mutant types are nearly the same. Evidence for structural modifications at other sites in nuclease also indicates that insertions have a much greater propensity for inducing significant perturbations in native structure than do substitutions. Given that, on average, an insertion of a single alanine of glycine residue is no more destabilizing than a substitution of an alanine or glycine at one of the positions flanking the inserted peptide bond, we suggest that single residue insertions should be considered the mutations of choice for introducing new conformations and new functional activities in proteins.

W-Pos258

## A SIX RESIDUE INTERNAL DELETION IN STAPHYLOCOCCAL NUCLEASE SLOWS THE REFOLDING RATE BY ~1000-FOLD.

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We have investigated the folding kinetics of a staphylococcal nuclease mutant in which the six residues 114 to 119 (inclusive) have been deleted. When the refolding reaction of the acid denatured mutant is monitored by tryptophan fluorescence and circular dichroism at 222 nm the rates are found to be decreased by approximately three orders of magnitude compared to that of wild-type nuclease. Instead of time constants of 55 milliseconds and 350 milliseconds values of approximately 100 and 500 seconds are observed. Slow refolding is observed whether the protein is denatured by acid, heat, urea or guanidine hydrochloride. The folding reaction is fully reversible and the protein moderately stable, with a free energy of denaturation, $\Delta G_{H_2O}$, of 2.2 kcal/mole compared to 5.5 for wild-type. Double jump experiments in which the mutant is rapidly denatured with either acid or GuHCl and then allowed to renature show similar slow folding kinetics, suggesting that proline isomerization in the denatured state is not responsible for the slow rates. Furthermore, the six residue deletion includes the only *cis*-proline in wild-type (proline 117). The protein's tertiary structure apparently is relatively unchanged. The enzymatic activity of the mutant nuclease is only down by a factor of 200 relative to the wild-type enzyme and the circular dichroism spectra is virtually identical to wild-type.

W-Pos259

## STAPHYLOCOCCAL NUCLEASE MUTANT V66K HAS THE SIDE CHAIN OF THE MUTANT LYSINE FULLY BURIED IN THE HYDROPHOBIC CORE.

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The crystal structure of the staphylococcal nuclease mutant, in which valine 66 is replaced by lysine (V66K), has been solved at 1.95 Å resolution. Unlike lysines in previously reported protein structures, this residue appears to bury its side chain in the hydrophobic core without salt bridging, hydrogen bonding or other form of electrostatic stabilization. This observation is, to our knowledge, unprecedented. Solution studies of the free energy of denaturation, $\Delta G_{H_2O}$, show marked pH dependence and clearly indicate that the lysine must be deprotonated in the folded state. V66K is highly unstable at neutral pH but only modestly less stable than wild-type at high pH. The pH dependence of $\Delta G_{H_2O}$ in combination with similar measurements for wild-type allowed us to determine the $pK_a$s of the lysine in both the denatured and native forms. This, in turn, enables the calculation of the $\epsilon$-amine's relative change in free energy of solvation between solvent and the protein interior and the dielectric constant of the protein interior. Lysine is frequently found with the methylenes of its side chain partly buried but is nevertheless considered a hydrophilic, surface residue. It would appear that the high $pK_a$ of lysine, which gives it a positive charge at physiological pH, is the primary reason for its almost exclusive confinement to the surface of proteins. When deprotonated, this amino acid type can be fully incorporated into the hydrophobic core.

**W-Pos260**

CONTRIBUTIONS OF PROLINE, ALANINE, AND GLYCINE RESIDUES TO THE FOLDING AND STABILITY OF STAPHYLOCOCCAL NUCLEASE.

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To examine the contribution of every amino acid residue to the stability and folding of staphylococcal nuclease, single alanine and glycine substitutions are being constructed at each of the 149 residue positions in this small protein. As part of this project, the twelve alanine, nine glycine and six proline residues in staphylococcal nuclease have been mutated, and the free energies of denaturation as well as the rate of change of free energy with respect to guanidine hydrochloride concentration (mGuHCl) have been determined. Somewhat surprisingly, the stability loss accompanying replacement of proline with alanine or glycine at all six positions was either quite small ($\Delta\Delta G < 0.9$ kcal/mole) or the substitutions were found to be stabilizing ($\Delta\Delta G \cong 1.0$ kcal/mole). At all positions, replacement of alanine with glycine or valine and replacement of glycine with alanine or valine lowered stability, in some cases by 4-6 kcal/mole or more. Correlation of these data with various parameters of the wild-type native structure were examined. In addition, the differences in stability between alanine and glycine substitutions at the 12 wild-type alanine sites versus the 39 large hydrophobic residues (Biochemistry 29, 8033, 1990) have been analyzed.

The role of prolines in protein folding kinetics has been a subject of widespread interest. Slow rates observed in the refolding of various proteins have often been attributed to proline isomerization. We have constructed a completely proline-minus nuclease using either alanine or glycine (choosing whichever was more stable as a single mutant) at each position as well as several mutants with one or more proline residues present. The folding and refolding of these proline-minus mutants are being quantitated and compared to the rates of the wild-type protein in order to assign the different relaxation time constants to individual proline residues.

**W-Pos261**

EVIDENCE FOR COLD DENATURATION OF A MUTANT OF STAPHYLOCOCCAL NUCLEASE A. Maurice R. Eftink, Department of Chemistry, University of Mississippi, University, MS 38677

The unfolding of globular proteins at low temperature is a predicted thermodynamic phenomena, but is difficult to observe. Evidence will be presented for cold denaturation, at pH 5 to 7 and in the absence of denaturants, for nuclease-conA S28G, a hybrid mutant of nuclease A (Hynes et al Nature 339, 73-76). This mutant has a greatly reduced stability ($\Delta G^{\circ}_{unfolding} \simeq 1.2$ kcal/mole at 20°), compared to the wild type ($\Delta G^{\circ}_{unfolding} \simeq 5.8$ kcal/mole). Steady-state and time-resolved fluorescence measurements reveal unfolding at both low and high tempertaures ($T_m$s of ~ -5° and 30°C). The data can be described in terms of a two-state unfolding transition with $\Delta H^{\circ}_{UN} = 45.8$ kcal/mole, $\Delta C_{p,UN} = 2.68$ kcal/mole·deg and a temperature of maximum stability of 13.5°C. (There is some uncertainty in the $\Delta C_p$ value due to the necessity of assuming base line behavior.) Analytical gel filtration chromatography shows the separate elution of unfolded and folded forms near 0°C, indicating that the unfolded form has an expanded structure and that the transition is slow on the chromatographic time scale. Additional studies will be presented that compare the denaturant and pressure induced unfolding of the hybrid mutant with the wild type nuclease A. This research was supported by NSF grant DMB 88-06113.

**W-Pos262**

**Cold unfolding in T4 lysozyme: variants with engineered disulfide bridges.**

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Several disulfides bridges have been engineered into T4 lysozyme (after removal of the natural cysteines : Matsumura et al., PNAS 86, 6562-6566, 1989). Three of these bridges (connecting cysteines at positions 3 and 97, 9 and 164, 21 and 142) have been shown to increase the thermal stability of the protein. Combinations of these bridges were recently prepared, two combinations with double disulfide bridges (3-97 with 9-164 in one construct and 9-164 with 21-142 in the other) and one combination with all three bridges. These multiply bridged mutants showed even greater increases in thermal stability and stability toward denaturants. Cold unfolding is a common feature of all these proteins in presence of GdnHCl at pH 2.0.

Analysis of cold unfolding data by means of a two-state model (with $\Delta$Cp independent of temperature) permits the determination of the thermodynamic parameters, for the folding-unfolding reaction, in a much wider range of temperature (relative to simple melting curves). In particular it is possible to determine with great accuracy a value for the $\Delta$Cp of the unfolding reaction. Goals of our work are to find the effects of single, double or triple disulfide bridges on the $\Delta$Cp of denaturation and possibly to see the effect of the denaturant concentration (GdnHCl) on $\Delta$Cp. We also analyze the cold unfolding curves in terms of a model in which $\Delta$Cp is allowed to vary linearly with temperature.

The accuracy of this method should allow determination of the contribution of single site mutations to the $\Delta$Cp of unfolding.

**W-Pos263**

Solvent Stabilization of Proteins Against Thermal Unfolding

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Methylamine compounds are concentrated by a number of organisms for the apparent purpose of protecting their proteins against certain environmental stresses. We have found that members of this class of protectants such as sarcosine and betaine provide extraordinary protection for ribonuclease against thermal unfolding. In particular, sarcosine is able to provide an increase in melting temperature (Tm) of 20°C at 7.5 M concentration. This amounts to over 100,000 fold stabilization against thermal unfolding while preserving reversibility, two-state behavior, and catalytic activity of the enzyme. It is also of interest that, despite a Tm increase of about 20°C, the calorimetric enthalpy of ribonuclease remains the same as it was in the absence of sarcosine. Thus, the apparent heat capacity change over the temperature range of stabilization is zero. This latter effect is a curious feature of unknown origin.

Sarcosine also causes a Tm increase of over 20°C for HEW lysoyme. But in contrast with ribonuclease, lysozyme thermal unfolding is not as reversible and the cooperativity of thermal unfolding increases as a function of sarcosine concentration. The difference in thermal unfolding behavior for ribonuclease and lysozyme is likely to involve differences in the hydrophobic character of the two proteins in relationship to the ability of sarcosine to solubilize hydrophobic residues.

This work was supported by The Biotechnology Research Development Corporation.

W-Pos264

THERMAL DENATURATION OF PLASTOCYANIN

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The thermal stability and folding of plastocyanin (PC) were studied in order to determine how PC functions in the lumen of the thylakoid. The oxidized form of PC had a $T_m$ of ca. 61 °C as determined by the decreases in the 597 nm absorption band and 255 nm circular dichroism (CD) band which monitor the copper center as well as by changes in the secondary structure as determined by far-UV CD. Native and denatured forms of PC were separated using ion exchange FPLC. Only a single denatured form of oxidized PC was observed after FPLC. Thus, its denaturation can be described by a two-state mechanism (N <—> D) in which native PC unfolds to produce a single, denatured form with no observable intermediates. Removal of oxygen increased the $T_m$ to 65 °C. The $T_m$ for reduced PC was 71 °C as determined by changes in the copper center and secondary structure. However, the near-UV CD band at 280 nm, which monitors tertiary structure, showed complex behavior. The magnitude of this band increased with increasing temperatures up to 72° C and decreased again at still higher temperatures. These results were confirmed by the FPLC experiments which showed two denatured forms of PC (designated forms 1 and 3 from the order of elution on the FPLC; Form 2 consists of native, reduced PC). Addition of reductants such as ascorbate or dithionite increased the $T_m$ and decreased the amount of Form 3 observed. Removal of oxygen increased the $T_m$ to >100 °C. Reversal of denaturation was observed for Form 3 but not Form 1 upon incubation in the presence of dithionite and 0.6 mM $CuSO_4$. It is postulated that reductants and anaerobicity increase the stability of reduced PC by either preventing the formation of Form 3 or promoting the refolding reaction. The data are consistent with the following scheme.


W-Pos265

EFFECTS OF ACTIVE-SITE LIGANDS ON THE HEAT-INDUCED, REVERSIBLE PARTIAL UNFOLDING OF DODECAMERIC GLUTAMINE SYNTHETASE (GS) FROM *E. COLI*.

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Heating induces reversible (two-state) transitions in two types of domains of GS in which exposure of Trp residues occurs at ~2° lower temperatures than that of Tyr residues [Biochemistry 28, 6281-6294, 1989]. No dissociation of GS occurs during the partial unfolding processes and >93% of GS activity is recovered after cooling. Differential scanning calorimetry (DSC) shows a single endotherm which can be approximated as the sum of two (two-state) transitions and the $\Delta H_{cal}$ value is about equal to the sum of $\Delta H_{vH}$ values obtained for Trp and Tyr exposures. The DSC data indicate that the cooperative unit is the dodecamer (rather than each subunit) and that interactions betweeen domains occur. For GS (622000 $M_r$) at pH 7.0 in 100 mM KCl and 1.0 mM $MnCl_2$, the maximum $C_P$ is at 51.6 °C ($T_m$) and $\Delta H_{cal}$ = 210 kcal(mol dodecamer)$^{-1}$, which is ≤ 10% of $\Delta H_{cal}$ for complete unfolding. The L-Glu analogue L-Met-(SR)-sulfoximine (MSOX) stabilizes both GS domains and increases $T_m$. The increase in $\Delta H_{cal}$ produced by MSOX is attributable directly to ligand dissociation from $GS_u$: $GS_f{\bullet}L_{12} \rightarrow GS_u + 12L$, where the subscripts f and u refer to low- and high-temperature forms, respectively, and $\Delta H_{diss}$ = 13.8 kcal/mol for MSOX dissociation at high temperatures could be estimated. Isothermal titration calorimetry has shown that MSOX does not bind to $GS_u$ and at pretransition temperatures, $\Delta C_P = -200$ cal/deg•mol for MSOX binding. The value of $\Delta H_{cal}$ is not increased by 150 mM L-Gln whereas $T_m$ is increased 7°; these effects are due to the binding of L-Gln to both $GS_f$ and $GS_u$ with $GS_f$ having a higher affinity than $GS_u$ for Gln. ADP also binds to $GS_u$ and appears to decouple some intramolecular interactions during partial unfolding reactions. Thus, the binding of different active-site ligands to GS promotes diverse effects on the energetics of intra- and inter-domain interactions in this dodecameric enzyme.

W-Pos266

THERMODYNAMIC STABILITY OF OXIDIZED AND REDUCED *E. COLI* GLUTAREDOXIN; RESIDUAL STRUCTURE IN THE DENATURED STATE.

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*E. coli* glutaredoxin (MW 9700) catalyzes intracellular redox reactions utilizing a disulfide/dithiol enzymatic mechanism involving the active site sequence -Cys-Pro-Tyr-Cys-. It is functionally related to the thioredoxin family and is expected to share similar three-dimensional structure [Eklund, H., Cambillau, C., Sjöberg, B.-M., Holmgren, A., Jörnvall, H., Höög, J.-O., & Brändén, C.-I. (1984) *EMBO J. 3*, 1443-1449]. We constructed an overexpression system in which glutaredoxin production is controlled by temperature-sensitive expression of the phage T7 promoter and are studying the structure and stability of the oxidized and reduced forms (grx-$S_2$ and grx-$(SH)_2$, respectively). Secondary structure calculated from CD data agrees with that predicted from the three-dimensional model of Eklund et al. Our studies of GuHCl-induced denaturation (monitored by fluorescence) and heat-induced denaturation (monitored by CD) show that the reduced (dithiol) form is slightly more stable than the oxidized (disulfide) form. Spectroscopic studies of the denatured protein suggest that it may retain substantial residual structure. In thioredoxin, the oxidized form is far more stable than the reduced [Kelley, R. F., Shalongo, W., Jagannadham, M. V., Stellwagen, E. (1987) *Biochemistry 26*, 1406-1411]. Failure to observe the expected entropic stabilization due to disulfide ring formation lends additional support to the suggestion that denatured grx-$S_2$ and grx-$(SH)_2$ contain residual structure. It also implies that the redox potentials of the disulfide bond are similar in unfolded and folded glutaredoxin. In addition to glutaredoxin a second gene product is observed in our recombinant system; this species, which we call glutaredoxin N, is glutaredoxin extended the sequence Met-Arg-Arg-Glu-Ile- at the N-terminus.

W-Pos267

DEVELOPMENT OF THERMAL GRADIENT GEL ELECTROPHORESIS AS AN ANALYTICAL TECHNIQUE.

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The characterization of complex folding/unfolding transitions in multidomain proteins requires knowledge of the: a) overall thermodynamics; b) number of intermediate, partially folded states; c) individual domain stability and cooperative interactions; and d) identification of relevant structural states. While calorimetric techniques allow a precise determination of thermodynamic parameters, they do not permit a precise identification of folding/unfolding intermediates. This identification usually requires the use of complementary techniques. One such technique is thermal gradient gel electrophoresis (TGGE). TGGE can be described as a native electrophoresis gel in which a thermal gradient is superimposed on the sample perpendicular to the direction of the electric field. Thermally induced conformational transitions are reflected as changes in the rate of migration of the protein. TGGE is capable of revealing the presence of intermediate states. Up to now the analysis of TGGE gels has been strictly qualitative even though these gels contain thermodynamic information. In the simplest case of a single two-state reversible transition, the fitted parameters are the intrinsic mobility of the sample and its temperature coefficient, the Tm and enthalpy of the transition, and changes in the mobility coefficients upon unfolding. These parameters can be obtained from the observed temperature dependence of the migration pattern. The presence of partially unfolded states results in the appearance of additional bands within the transition region, thus allowing a direct visualization of intermediate states. (Supported by NIH grants RR-04328 and GM-37911)

**W-Pos268**

THE SYNTHESIS AND REFOLDING OF 115-NITROTYROSYL AND 115-AMINOTYROSYL RIBONUCLEASE A: PROBES FOR MONITORING THE CIS/TRANS ISOMERIZATION OF PROLINE-114. R. G. Biringer and B. Puntambekar (Chemistry Dept., San Jose State University, San Jose, CA 95192-0101)

Chemical modification of specific amino acid residues with spectroscopic probes provides a means by which folding about discrete regions of a protein can be monitored. The thrust of this investigation centers around monitoring the cis/trans isomerization of Pro-114 in ribonuclease A via nitrated and aminated derivatives of the neighboring Tyr-115. We have synthesized, purified to homogeneity, and characterized derivatives of ribonuclease A in which Tyr-115 and both Tyr-115 and -76 have been nitrated and aminated. The absorbance monitored folding of the nitrated derivatives have been reported previously and show no kinetic phase which can be associated with proline isomerization. Proton nmr of the nitrated derivatives under native conditions show a single resonance for the δ-proton on each of the nitrated rings whereas thermal and guanidine denatured spectra show two resonances for this proton. Peak area measurements reveal that the dual nature of this resonance is attributable only to Tyr-115. These results suggest that the resonances reflect the isomerization state of the neighboring proline and indicate a 45:55 ratio of the isomers in the thermally unfolded state and a 62:38 ratio in the guanidine unfolded state. The folding of urea and thermally unfolded aminated ribonucleases were monitored at 10°C(pH 5.5) and -15°C(pH*6, 35% methanol) respectively and showed triphasic kinetics under all conditions. The slowest phase observed for each give rate constants which are consistent with that expected for proline isomerization. The amplitudes associated with the slowest phases represent 50% of the total expected when urea was used to unfold the protein and 57% for the thermally unfolded protein and thus corroborate the proton nmr results. Supported by NIH GM42190-01.

**W-Pos269**

A CONFORMATIONALLY MODIFIED AND IRREVERSIBLY INACTIVATED FORM OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IS GENERATED BY PARTIAL UNFOLDING. Jeff Plomer and Ari Gafni (Intro.by M.J. Coon), Institute of Gerontology, The University of Michigan, 300 N. Ingalls, Ann Arbor, MI 48109-2007.

The equilibrium denaturation of glucose-6-phosphate dehydrogenase (G6PD) from Leuconostoc mesenteriodes by guanidine hydrochloride (GuHCl) was studied using enzyme activity, light scattering, and tryptophan fluorescence. G6PD, which is a homodimer of Mr = 103,700, was incubated with increasing concentrations of GuHCl in 50mM TrisHCl, pH 7.8, 25 C for 24 hours prior to making measurements.

Enzyme activity is lost in a single phase transition while light scattering is increased between 1.0 and 1.4M GuHCl. Tryptophan fluorescence is quenched in two phases. The first phase coincides with the loss of enzymatic activity. The second phase is complete by 2.5M GuHCl. These transitions are not affected by enzyme concentration or by the presence of saturating concentration of substrate (NADP+ or glucose-6-phosphate).

Kinetic experiments at 1.4M GuHCl reveal that activity and first phase fluorescence decay with similar first order rate constants ($k_{activity}$ = 0.094 min.-1, $k_{fluorescence}$ = 0.122 min.-1). Light scattering increases little on the same time scale. Over 24 hours, however, light scattering elevates indicating that inactive, conformationally modified G6PD slowly aggregates to higher molecular weight species.

The percent enzyme activity recovered upon dilution of GuHCl depends on the concentration of denaturant used. Practically no activity is regained when 1.2 to 1.6M denaturant are used. Light scattering measurements showed that dilution in fact causes G6PD to aggregate and precipitate out of solution. However, G6PD denatured with GuHCl concentrations higher than 1.6M did allow increasing recovery of activity and less aggregation/precipitation. We are currently exploring conditions which will minimize aggregation and maximize recovery of active enzyme.

This study indicates that a conformationally modified and irreversibly inactivated form of G6PD is generated by partial unfolding by 1.0 to 1.4M GuHCl. Conformational modification may expose hydrophobic patches which allow G6PD to aggregate. GuHCl concentrations above 2.5M cause complete unfolding thus allowing G6PD to fold and associate properly upon dilution.

**W-Pos270**

COOPERATIVE FOLDING DOMAINS IN CHOLERA TOXIN B SUBUNIT Vinod Bhakuni, Dong Xie and Ernesto Freire. Biocalorimetry Center and Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Cholera toxin is a globular protein composed of two protomeric species A ($M_r$ = 26,000) and B ($M_r$ = 11,600 per monomer). The toxin binds specifically through the B-subunit pentamer to ganglioside GM1 present on the plasma membrane of most eukaryotic cells. This association is believed to cause a conformational change in the protein that facilitates the exposure and subsequent membrane penetration of the A-subunit. We have performed a systematic characterization of the conformation and thermal stability of cholera toxin B-subunit as a function of guanidine hydrochloride and pH using differential scanning calorimetry, fluorescence spectroscopy, isothermal titration calorimetry and protease digestion. At pH 7.5 and in the absence of GuHCl the thermal unfolding of the B-subunit pentamer is characterized by a single peak in the heat capacity function centered at 77°C and characterized by a ΔH of 328 kcal/mole of B-subunit pentamer and a $\Delta H_{VH}/\Delta H$ of 0.3. The initial effect of pH and GuHCl results in a decrease in the calorimetric enthalpy for the transition with no significant effect on the van't Hoff enthalpy. The transition enthalpy decreases in a sigmoidal fashion with pH, with an inflection point centered at pH 5.3. The fluorescence intensity of tryptophan in the B-subunit decreases to about half of the original intensity upon lowering the pH from 7.5 to 5.0, or upon increasing the GuHCl concentration to 2M. In the case of GuHCl the change is biphasic having a first inflection point at 0.5 M GuHCl and a second one at 3M GuHCl. Isothermal titration calorimetric studies of the B-subunit as a function of pH also report a transition centered at pH 5.3 and characterized by a ΔH of 30 kcal/mole of B-subunit pentamer at 25°C. Below this pH the enthalpy change for the unfolding transition is reduced to approximately 100 kcal/mole of B-subunit pentamer. Trypsin digestion studies show that the B-subunit is four times more susceptible to digestion at pH 5.0 than at pH 7.0 and that digestion results in two fragments of ~7kDa and ~5kDa. (Supported by NIH grants NS-24520 and RR-0432.)

**W-Pos271**

THERMAL UNFOLDING AND AGGREGATION OF HUMAN COMPLEMENT PROTEIN C9. K. Lohner and A.F. Esser, Lab. for Structural Biology, Health Science Center, University of Florida, Gainesville, FL 32610.

The thermotropic behavior of the Ca-binding protein C9 was investigated by high-sensitivity DSC. In physiological buffers (pH 7.2, 150 mM NaCl) C9 underwent three endothermic transitions ($T_{m1}$ = 32°C, $T_{m2}$ = 48°C, $T_{m3}$ = 53°C) and one exothermic transition above 64°C that correlated with protein aggregation. The calorimetric enthalpies of the three endothermic transitions were 45, 60, and 161 kcal/mol with cooperative ratios ($\Delta H_{cal}/\Delta H_{vH}$) close to unity. The total calorimetric enthalphy for the unfolding process was in the range of 260 to 280 kcal/mol under all conditions. The aggregation temperature was strongly pH dependent changing from 60°C at pH 6.6 to 81.4°C at pH 8.0 whereas none of the three endothermic transitions were significantly affected by pH changes. They were, however, sensitive to addition of calcium ions; most affected was $T_{m1}$ which shifted from 32°C to 35.8°C in the presence of 3 mM calcium, i.e., the normal blood concentration. Kosmotropic ions stabilized the protein by shifting the endothermic transitions to slightly higher temperatures whereas inclusion of chaotropic ions (such as choline), or removal of bound calcium by addition of EDTA, lowered the transition temperatures. Previous studies had indicated the formation of at least three different forms of C9 during membrane insertion or during heat polymerization and it is suggested that the three endothermic transitions reflect the formation of such C9 conformers. Choline, which is present at high concentrations on the surface of biological membranes, and calcium ions have the ability to shift the transition temperatures of the first two transitions isothermally to be either close to or below body temperature. Thus, it is very likely that C9 is present in vivo in a state akin to a "molten globule" when bound to a membrane surface and we propose that this facilitates membrane insertion and refolding of the protein into an amphiphilic conformation. (Supported by NIH RO1 AI-19478)

**W-Pos272**

NMR STRUCTURAL COMPARAISON OF TWO DNA DODECAMERS CONTAINING THE RECOGNITION SITES FOR EcoRI AND BamHI RESTRICTION ENDONUCLEASES.
Michelle Fausel and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06457

Sequence-specific variations in the conformation of DNA have been suggested to play a role in site recognition by proteins through an indirect readout mechanism. In the present work, we have investigated the solution conformations of 5'-d(CGCGAATTCGCG)-3' and 5'-d(CGCGGATCCGCG)-3' dodecamers which contain the recognition sites for EcoRI and BamHI endonuclease, respectively (underlined in the sequence). Proton resonance assignments were obtained from 2D nuclear Overhauser enhancement (NOESY) and 2D-correlated experiments. Eight phase-sensitive NOESY spectra were collected for each dodecamer as a function of the mixing time in the range from 30 to 150 msec. Inter-proton distances were calculated from cross-relaxation rates derived from the built-up curves of the corresponding NOESY cross-peaks. Preliminary analysis of the data indicate that: (i) the distances from H8 or H6 base protons to sugar H1' protons are consistently larger in the BamHI dodecamer than in the EcoRI dodecamer, and (ii) significant differences between the two dodecamers occur at the base-pairs in position 8 (one of the substitution sites) and in position 9. A detailed comparaison of the conformations in these two dodecamers will be presented. (Supported by a grant from NSF).

**W-Pos273**

NMR STUDIES OF DNA DODECAMERS CONTAINING DEGENERATE RECOGNITION SITES FOR ECORI ENDONUCLEASE.
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EcoRI restriction endonuclease cleaves with largely variable rates sites on the DNA which differ from the canonical recognition site 5'-d(GAATTC)-3' by one or two bases. We have studied the dodecamer 5'-d(CGCGAATTCGCG)-3' and three variants containing: (i) symmetrical AC or GT base-pair mismatches in the first and sixth positions of the EcoRI site and, (ii) a GC-->AT base-pair substitution in the first position of the EcoRI site which results in an EcoRI* site. The enzyme cleaves the EcoRI* dodecamer and, at a slower rate, the dodecamer containing AC mismatches under both canonical and EcoRI* reaction conditions. In contrast,the dodecamer containing GT mismatches is a substrate for the endonuclease only under EcoRI* reaction conditions.

To understand the molecular origin of these changes in specificity, we have characterized the base-pair opening kinetics in the DNA dodecamers of interest by nuclear magnetic resonance (NMR) spectroscopy. Proton resonances of each dodecamer were assigned by established 1- and 2-D nuclear Overhauser effect (NOE) methods. The lifetimes of individual base-pairs in each dodecamer were measured from the dependence of the selective longitudinal relaxation rates and linewidths of the imino proton resonances on the concentration of base catalyst (ammonia). The relationship of these results to the mechanisms involved in DNA recognition by EcoRI endonuclease will be discussed. (Supported by a grant from the NSF).

**W-Pos274**

THE EFFECTS OF CIS-DDP ON THE ANNEALLING OF SYNTHETIC OLIGOS.
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We have previously shown increasing concentrations of cis-DDP had destabilizing effects on the melting of a repeating GC 12-mer. This supports the intrastrand mechanism, involving chelation of 2 adjacent guanines separated by a third base. It is also known that cis-DDP preferentially binds to DNA bases in the following order: G>A>C>>T. We investigated cis-DDP effects on the annealing of 3 different 20-mers (each consisting of 12 alternating GC, GA, GT, flanked by 4 A's and 4 T's, annealed to it's complimentary strand).

Single stranded oligos were synthesized, purified, characterized with PAGE-urea and complimentary strands annealed in 10 mM phosphate buffer, pH 6.8 at 50°C then cooled to 25°C. The annealing process was monitored with a thermoprogrammable spectrophotometer, by heating the oligos to 80°C, then cooling to 25°C, while observing the hypochromic effect. The oligos were redenatured at 80°C and cis-DDP added at a molar ratio of 0.5. The mixture was recooled to 25°C, and hypochromicity monitored at 260nm.

Our data demonstrates a preferential inhibition of annealing in the presence of cis-DDP. The 20-mer with repeating G-C showed the greatest inhibition of reannealing, followed by G-A, then G-T as compared to the control. These trends are supportive of the binding affinity of cis-DDP to the different bases. We additionally report on the effects of cis-DDP on the melting properties of these oligos and a circular dichroic analysis of structural abnormalities induced by cis-DDP at several discrete temperatures. This project was funded by MBRS.

**W-Pos275**

**Influence of Covalent Attachment of Ethidium to DNA on the B-Z Transition.**
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The effects of covalent modification of poly(dGdC)·poly(dGdC) and poly(dGm$^5$dC)·poly(dGm$^5$dC) by ethidium monoazide (a photoreactive analog of ethidium) on the salt induced B to Z transition are used to probe the energetics of the structural transitions between B and Z form DNAs. The degree of inhibition of the B to Z transition is quantitated as a function of the concentration of covalently attached drug. At a concentration of one drug bound per four base pairs, total inhibition of this transition is achieved. Lower concentrations of bound drug were effective in the partial inhibition of this transition. Rates of the B-Z transitions are determined and used to calculate the activation energies for both the B to Z and Z to B transitions. These studies reveal the kinetics of both the B and Z transitions to be dramatically influenced by the amount of ethidium that is covalently attached to the poly(dGdC)·poly(dGdC) and poly(dGm$^5$dC)·poly(dGm$^5$dC) . In contrast, activation energies of the structural transitions are demonstrated to be relatively insensitive to covalent modification by ethidium. Covalent attachment of ethidium results in an inhibition of the B-Z transition by interfering with the propagation step of the mechanism through an irreversible stabilization of the DNA in a right-handed conformation, rendering this region unable to undergo a conversion to the Z conformation. Thus, the propagation of the left-handed conformation along the helix is terminates upon reaching an ethidium-DNA adduct site. (Supported by NIH Grants CA-41474 (D.E.G.) and CA-35635 (J.B.C.)

W-Pos276

## DNASE I HYPERSENSITIVITY AND ASYMMETRIC STRUCTURE TRANSMISSION INDUCED IN A DNA HEXADECAMER BY ACTINOMYCIN D

Karl D. Bishop[1], Philip N. Borer[1], Yao-Qi Huang[2] and Michael J. Lane[2]
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Flanking DNA sequences adjacent to active genes can exhibit unusual increases in sensitivity to nucleases. In some cases, this "hypersensitivity" has been related to local alterations in DNA structure associated with underwinding stress. Although radical changes in structure have been proposed for such sequences it is also possible that less drastic structural alterations can switch a control sequence between active and inactive forms; such alterations might occur as a response to the binding of proteins or other ligands. We have previously shown that actinomycin D binding increases DNase I sensitivity at sites distal to the binding domain of the drug, and that the appearance of enhanced cleavage is a function of sequence. DNase I cleavage kinetics and $^1$H-nmr are used to show that actinomycin D intercalation alters the equilibrium structure of DNA at least five nucleotides from the G-C intercalation site in $[d(AAATATAGCTATATTT)]_2$. The nmr results further indicate that this distal response, while subtle, propagates most strongly from the G-C site toward the 3' ends of the strands. Research supported by NIH grant GM35069 and NCI grant CA45698.

W-Pos277

## STRUCTURE OF A DNA DECAMER AND ITS INTERACTION WITH DNA MINOR GROOVE BINDING DRUGS.

Jordi R. Quintana, Kazimierz Grzeskowiak, Kazunori Yanagi and Richard E. Dickerson. Department of Chemistry & Biochemistry and Molecular Biology Institute, UCLA. 405 Hilgard Ave. Los Angeles, CA 90024, USA

The crystal structure of the self-complementary DNA decamer $d(CGATTAATCG)_2$ has been solved at 1.5Å resolution, in the orthorhombic space group $P2_12_12_1$, with a=38.60Å, b=39.10Å, c=33.07Å.
The DNA helical parameters of the refined structure of this decamer are very similar to those previously found in the crystal structure of the isomorphous decamer $d(CGATCGATCG)_2$ from which only the two central base pairs were changed.[2] There is an alternation in the minor groove width of the two decamers every two base pairs. The pattern of hydration in the minor groove also alternates from a single spine in the narrow regions to a double ribbon of waters in the wider regions.
The relationship between the DNA sequence and the DNA structure analyzed from these B-DNA decamers will be presented.
The decamer $d(CGATTAATCG)_2$ was co-crystallized with the minor groove binding drug SN-7167 in the monoclinic space group $P2_1$ with a=47.79Å, b=19.94Å, c=33.78Å and $\beta$=111°. A 3.0Å X-ray data set collected from these crystals has been used to position an initial model of this drug-DNA complex in its correct position in the unit cell, using the molecular replacement package MERLOT.
Co-crystals of the decamer $d(CGATTAATCG)_2$ with another minor groove binding drug, DISTAMYCIN, belong to the orthorhombic space group C222 or $C222_1$, with a=29.98Å, b=62.54Å, c=67.69Å, as shown by X-ray precession photographs and the progress in the structural analysis of these drug-DNA complexes will be described.
The observation of the same DNA sequence crystallized in several different space groups may lead to a better understanding and to a separation of the effects of crystal packing forces and base sequence on the fine details of the structure of the DNA.

W-Pos278

## NMR STUDIES OF THE BINDING OF CHIRAL METAL COMPLEXES TO OLIGONUCLEOTIDES

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Transition metal complexes have been designed which recognize DNA sites based on *shape-selection*. The focus of this research is the use of NMR spectroscopy to characterize structurally the interactions of these complexes with DNA. Specifically, a new photocleaving rhodium(III) complex {$[Rh(phen)_2phi]^{+3}$ (phen = 1,10-phenanthroline, phi = phenanthrene quinone diimine), Figure 1} has been shown to bind preferentially to B-DNA conformations where the major groove is more open, such as 5'-pyr-pyr-pur-3' steps.[1] Initial NMR experiments have focused on the interaction of $Rh(phen)_2phi^{+3}$ with the structurally well-characterized Dickerson dodecamer $(CGCGAATTCGCG)_2$. Photoinduced cleavage experiments have indicated that the delta isomer binds and cleaves preferentially at the C9-G10 step. The enantioselective recognition of this particular step correlates with the large degree of differential propeller twisting at this step, which results in a chiral "opening" of the major groove.[2] Preliminary NMR studies indicate intermediate to slow exchange of the metal complex with this oligonucleotide. Other interesting oligonucleotides that are being examined include the structurally characterized A form octamer $(CTCTAGAG)_2$ and oligonucleotides containing a 5'-pyr-pyr-pur-3' step. Insights from the NMR studies will be presented. These studies have indicated the usefulness of the combined approach of photocleavage experiments and NMR studies for delineating shape-selective recognition of DNA in solution.

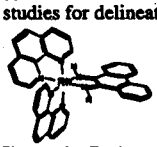


**Figure 1: $Rh(phen)_2phi^{+3}$**

[1]Pyle, A.M., Long, E.C., Barton, J.K., *J. Am. Chem. Soc.* 1989, *111*, 4520-4522.

[2]Pyle, A.M.; Morii, T.; Barton, J.K., *J. Am. Chem. Soc.*, in press.

W-Pos279

## PROTON NMR STUDIES OF A COMPLEX OF [N-MECYS$^3$, N-MECYS$^7$] TANDEM AND $[d(GATATC)]_2$.

Kenneth J. Addess[§], Janet S. Sinsheimer[*], and Juli Feigon[§]. Department of Chemistry and Biochemistry, the Molecular Biology Institute[§], and the Department of Biomathematics[*], School of Medicine, University of California, Los Angeles, CA 90024.

[N-MeCys$^3$, N-MeCys$^7$] TANDEM, an under-methylated analogue of Triostin A, contains two N-methyl groups on the cysteine residues only. Footprinting results from Waring's lab showed that [N-MeCys$^3$, N-MeCys$^7$] TANDEM interacts quite strongly with DNA rich in AT residues. However, it was not known whether [N-MeCys$^3$, N-MeCys$^7$] TANDEM binds to a TpA step or an ApT step. To determine its sequence specificty, we have investigated the binding of [N-MeCys$^3$, N-MeCys$^7$] TANDEM to the hexamer $[d(GATATC)]_2$ using 2D NMR. In a saturated complex with the hexamer $[d(GATATC)]_2$, [N-MeCys$^3$, N-MeCys$^7$] TANDEM binds to the hexamer as a bis-intercalator at the TpA step with the octadepsipeptide ring binding in the minor groove. Analysis of sugar puckers from the phase sensitive COSY data indicates that the T3 sugar from the TpA binding site adopts mainly an N-type sugar conformation, while the remaining sugars on the DNA adopt mainly an S-type conformation, which is observed in Triostin A-DNA and echinomycin-DNA complexes. However, unlike in some CpG specific Triostin A and echinomycin complexes, Hoogsteen base pairing for the [N-MeCys$^3$, N-MeCys$^7$] TANDEM-hexamer complex is not observed on either A-T base pair adjacent to the site of binding. The drug does not bind specifically with the octamer $[d(GGAATTCC)]_2$, which suggests that the drug does not bis-intercalate at an ApT step. Details of the three dimensional structure will be presented.

W-Pos280

THERMODYNAMIC STUDIES OF NETROPSIN BINDING TO DUMBBELL-SHAPED, DOUBLE-HAIRPIN DNA STRUCTURES. D. Szwajkajzer, K. Breslauer, Department of Chemistry, Rutgers University, Piscataway, NJ 08855.

We previously have reported the existence of a new class of double-hairpin, dumbbell-shaped DNA structures. These structures form via intramolecular folding of a single sequence to yield gapped, nicked, and ligated double hairpins with a common 8-mer core duplex $[d(GGAATTCC)]_2$ closed by loops of either 4 or 5 thymine residues at each end [Erie et al. 1987, *Biochemistry*, 26, 7150-7159; Erie et al. 1989, *Biochemistry*, 28, 268-273]. We report here the netropsin binding properties of these DNA structures as determined by both spectroscopic and calorimetric techniques and compare them with the corresponding netropsin binding properties of the isolated core duplex $[d(GGAATTCC)]_2$. This comparison allows us to assess the influence of the adjacent loop domains as well as the influence of backbone discontinuities on netropsin binding thermodynamics. The results of these studies will be presented and discussed in terms of the structural models of netropsin-DNA complexes derived from x-ray and nmr studies. These results set the stage for our subsequent investigations of the binding of EcoRI to the isolated and netropsin-bound forms of the dumbbell-shaped structures.

W-Pos281

AN NMR STUDY OF G•A AND A•A PAIRING IN OLIGONUCLEOTIDE DUPLEXES. Karol Maskos, Darryl A. LeBlanc and Kathleen M. Morden, Dept. of Biochemistry, Louisiana State University, Baton Rouge, LA 70803

The solution structure of the partially complementary oligonucleotide $d(GCGAATAAGCG)_2$ was investigated as a function of temperature and pH by means of NMR spectroscopy and UV absorption. The absorption studies indicate that duplex formation is concentration dependent. The exchangeable and non-exchangeable proton NMR spectra of this oligonucleotide have been completely characterized by NOE experiments in $H_2O$ and $D_2O$ solution at neutral and acidic pH. The NOE results indicate that both the adenine•guanine and adenine•adenine mismatches are stacked into a right-handed helix and that the 3'-guanine exists as a dangling end.

```
      1 2 3 4 5
5'    G C G A A T A A G C G   3'
3' G C G A A T A A G C G     5'
  11 10 9 8 7 6
```

Based on chemical shifts and NOEs, the imino proton of G3 is not involved in a hydrogen bond, however, an amino proton of A8 is involved in the base pairing of the G3•A8 mismatch . Upon lowering the pH we have also observed an unusual exchangeable resonance which can be attributed to the protonation of the N1 position on A4. Structural models of the duplex will be discussed.
Supported by NIH Grant GM38137.

W-Pos282

INFLUENCE OF MISMATCHED BASE PAIRS ON THE ENTHALPIES OF DNA DUPLEXES

Renzhe Jin, Barbara L. Gaffney, Roger A. Jones, and Kenneth J. Breslauer
Department of Chemistry, Rutgers, The State University of New Jersey, Piscataway, NJ 08855

We have used isothermal stopped flow mixing calorimetry to measure duplex formation enthalpies for a family of DNA duplexes both with and without mismatched base pairs. These calorimetric data will be presented and discussed in terms of the influence of base pair mismatches on the enthalpies of duplex formation.

W-Pos283

**SPECTROSCOPIC STUDIES OF POTENTIAL EUKARYOTIC RNA-BINDING SEQUENCES**

**Jose R. Casas-Finet, Nelly Kolodny#, Frank Robey#, Samuel H. Wilson*, and Richard L. Karpel. (Intro. by Robert F. Steiner) Dept. of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21228; #Laboratory of Cellular Development and Oncology, National Institute of Dental Research, and *Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.**

**We have studied the nucleic-acid interactive properties of several oligopeptides with sequences corresponding to either a ribonucleoprotein (RNP) consensus found in nuclear proteins of eukaryotic cells, or a retroviral epitope also identified in the mammalian RNP A1. These peptides are: RNP1 (KSRGYGYUTYAT), RNP1A (KSRGYGAUTYAT), RNP1B (KSRGAGYUTYAT), RNP2 (RKLYUGGLSYE) and A1CT (GGGQYFAKPRNQGGC). At low ionic strength all peptides bind to poly(ethenoadenylic) acid and result in an enhancement of its fluorescence by 260% for A1CT, 76% for RNP1, 40% for RNP1A and RNP1B, and 30% for RNP2. This reflects the unstacking of the polynucleotide bases induced upon binding. At 1 mM $Na^+$ their affinities for poly(εA) range from $6 \times 10^6$ $M^{-1}$ for A1CT to $3 \times 10^5$ $M^{-1}$ for RNP2. Peptide binding to poly(U) or $d(pT)_6$ induces moderate quenching of Tyr emission caused by a reduced contribution of the longest lifetime component of the fluorescence decay. Among the 3 RNP peptides, Y at position 7 has the largest contribution to the fluorescence emission and is perturbed to the greatest extent upon poly(U) binding. UV-crosslinks had been previously shown at this position in an A1-$d(pT)_8$ complex (Merrill *et al., J. Biol. Chem., 263*, 3307 (1988)). The relative affinities of peptide for poly(U) are similar to those for poly(εA), but with magnitudes 2- to 4-fold lower. Increasing the ionic strength results in a significant reduction of affinity ($\delta \log K / \delta \log [Na^+] = -1.9$ for A1CT-poly(εA)). These results indicate that both charged and aromatic residues are involved in the binding process. 500 MHz $^1$H-NMR studies of free and oligonucleotide-bound peptides, monitoring chemical shifts induced upon binding, will be presented. (Supported in part by the American Cancer Society, Grant NP-671, to R. L. K.)**

**W-Pos284**

NMR STUDIES OF LOOP E FROM 5S RIBOSOMAL RNA.
B. Wimberly, G. Varani, and I. Tinoco, Jr. (introduced by L. Packer), Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.

Loop E from the Xenopus laevis 5S ribosomal RNA (5S rRNA) is part of the binding site for the regulatory protein TFIIIA and ribosomal protein L25. As an asymmetric internal loop, it can adopt many different mismatched conformations. The structure of loop E in intact 5S rRNA has been probed using chemical modification and enzymatic digestion, but the base-pairing remains obscure. One- and two-dimensional NMR were used to investigate the solution structure of a 27-nucleotide duplex containing the X. laevis 5S rRNA loop E sequence. A combination of NOESY, correlated experiments, and selective deuteriation were used to assign most nonexchangeable proton resonances. Interproton distances and scalar couplings were used to determine sugar puckers and glycosidic torsion angles. Exchangeable and nonexchangeable proton spectra indicate the formation of two helical stems separated by a closed internal loop containing mismatched base pairs (including A·A and U·U pairs). Base-stacking is conserved in the loop except for one G, which is bulged out. The preliminary structure is generally consistent with the patterns of chemical modification and enzymatic digestion observed for loop E in intact 5S rRNA. Comparison of this structure with that of a very similar internal loop (Varani et al., Biochemistry 28, 7760) shows that the structure of an internal loop can be dramatically changed by the addition of a single loop nucleotide.

**W-Pos285**

**EFFECT OF STEM SEQUENCE ON THERMODYNAMICS AND STRUCTURE OF AN RNA TETRALOOP HAIRPIN**
Marco Molinaro & Ignacio Tinoco, Jr., Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.

Hairpin loops are very common RNA structural motifs. They serve as folding nucleation sites and protein recognition sites for transcriptional and translational control. The sequence and size of these loops is often conserved. We are interested in the very stable and common UUCG 4-nucleotide loop to 1) determine how stability is related to structure in variant hairpins based on this tetraloop , and 2) determine minimum stem size requirements for loop formation.

Current emphasis is on understanding the effect of stability on structure in the two sequences **GC(UUCG)GC** and **CG(UUCG)CG**. The two molecules have been synthesized using RNA phosphoramidite chemistry and purified by anion-exchange HPLC. UV melting studies of the molecules have shown that the sequences form stable hairpins. Thus two base pairs are sufficient to stabilize a hairpin. Thermodynamic parameters are shown below.

| SEQUENCE | $T_m$ | $\Delta H_f^o$ | $\Delta S_f^o$ |
|---|---|---|---|
| | °C | kcal/mol | e.u |
| 5'GC(UUCG)GC3' | 54 | -28 | -84 |
| 5'CG(UUCG)CG3' | 26 | -20 | -67 |

Differences between the two sequences may be largely accounted for by nearest neighbor stacking interactions, but little is known about the effect of the stem sequence on the loop structure. The three-dimensional structure of GGAC(UUCG)GUCC has been determined (C.Cheong,G. Varani & I. Tinoco, Jr., *Nature*, **346**, 680-682 (1990)). We are using similar NMR techniques to determine the structures of the two new hairpins.

**W-Pos286**

## Proton NMR Assignments And Structural Characterization Of An Intramolecular DNA Triplex.

**Román Macaya, Edmond Wang, Vladimír Sklenář, and Juli Feigon**
Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, California 90024.

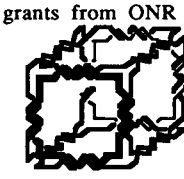
We have synthesized a DNA oligonucleotide designed to form an intramolecular triplex with TAT and C+GC triplets. This molecule exists almost exclusively as a triplex up to 25°C in acidic solution (pH = 5.2). Proton NMR assignments of both exchangeable and non-exchangeable protons have been made using a variety of 2D-NMR techniques and the structural characteristics of the triplex have been investigated. Analysis of the sugar puckers revealed that all purine and most pyrimidine nucleotides in the DNA triplex have a predominantly S-type sugar conformation, with the purine strand sugars showing a higher percent of the S form than those of either of the pyrimidine strands. Only the cytosines involved in Hoogsteen base pairs show a predominantly N-type sugar conformation. There are unusual structural features in the sequential connectivities of some bases; the H2" and H2' protons of certain purine nucleotides are closer to the H8 proton on the 5' neighboring base than to their own H8. A model which is consistent with our data will be presented and discussed.

**W-Pos287**

THE SYNTHESIS FROM DNA OF A MOLECULE WITH THE CONNECTIVITY OF A CUBE, Junghuei Chen and Nadrian C. Seeman, Department of Chemistry, New York University, New York, NY 10003.

A covalently closed molecular complex containing twelve double helical edges arranged about eight vertices has been assembled from DNA. Each edge of this stick-figure consists of 20 nucleotide pairs of DNA. The vertices are the branch points of 3-arm DNA junctions. The construct consists of six single stranded cyclic molecules of DNA, each doubly linked to four other similar molecules. These molecules each define a 'face' of the object. The helix axes of this complex are arranged like the edges of a cube or a rhombohedron; three of them emanate from every vertex, making this a 3-connected object. The molecule has been synthesized by ligating together two synthetic DNA quadrilaterals to form a closed cyclic ribbon. Each of the quadrilaterals contains exocyclic arms whose cohesive ends direct their association to form 4 connecting edges between each quadrilateral. Each edge of the complete object contains a unique restriction site that is used for analytical purposes. This is the first closed 3-connected object formed from double helical DNA.

Supported by grants from ONR and NIH.



W-Pos288

## VIBRATIONAL CIRCULAR DICHROISM STUDY OF THE DNA A-B PHASE TRANSITION

Lijiang Wang, Ligang Yang and Timothy A. Keiderling
Department of Chemistry, University of Illinois at Chicago, Box 4348, Chicago, IL 60680

We and others have previously studied Vibrational Circular Dichroism (VCD) of the B-Z transition of poly(dG-dC) and related oligomers which give rise to distinctly different VCD spectra in the two forms due to the different handedness of their respective polymeric chains. The next problem of interest is determination of the VCD of the A-B transition and its information content. The VCD spectra of A- and B-DNA as prepared from calf thymus DNA are here reported for the C=O and $PO_4^{-2}$ stretching spectral regions. Comparison of the A-form VCD spectra with those of B- and Z-form DNA as well as with that of RNA duplexes and t-RNA will be presented. In the C=O stretching region, the changes between A- and B-forms are small and both spectra are opposite in sense to that of the Z-form. The A-form result is very much like that of duplex poly(rI)·poly(rC).

W-Pos289

## SEQUENCE DEPENDENT STABILITY OF DNA DUMBBELLS

Mitchel J. Doktycz and Albert S. Benight
Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois.

Sixteen DNA dumbbells were constructed that have a sixteen base-pair core duplex sequence linked on the ends by $T_4$ single strand loops. The core duplex contains the sequence: $^{5'}$GTATCC(WXYZ)GGATAC$^{3'}$ and its complement strand, where (WXYZ) represents a unique combination of four base-pairs and consequently includes different combinations of the ten unique nearest-neighbor stacks in DNA. This set of DNA molecules serves as a model system for studying the stability of local sequence dependent interactions in DNA. Melting curves of the DNA dumbbells were collected as a function of NaCl concentration over the range (10 mM $\leq$ [NaCl] $\leq$ 100 mM). In every salt environment, transition temperatures of the dumbbells varied as much as 14°C for different molecules within the set. Results of these experiments were then employed to evaluate the ionic strength dependence of the nearest-neighbor stacking interactions in DNA. These evaluations indicate, within experimental error, (1) nearest-neighbor stacking free-energy is apparently independent of the NaCl concentration over the range examined; (2) the hierarchy from most to least stable stacking interactions is also preserved in different ionic strength environments. The order of stability of nearest-neighbor stacks $^{5'}$MN$^{3'}$ is: GC = CG > CA/TG > AA/TT > AT > TC/GA > AC/GT > TA > CT/AG > GG/CC. We compared our nearest-neighbor values with seven published sets and nearest-neighbor sets evaluated from a re-analysis of published melting data of repeating DNA co-polymers. Consistent analysis of all the available data revealed that nearest-neighbor stabilities evaluated from melting curves of different DNA samples (ie. linear duplex oligomers, repeating co-polymers and restriction fragments) in different salt environments and by molecular orbital theory are in nearly universal agreement with our evaluations. (Supported by NIH)

W-Pos290

## INVESTIGATIONS OF DNA DUMBBELLS WITH A SIXTEEN BASEPAIR DUPLEX STEM AND $T_n$ END-LOOPS (n = 2, 3, 4, 6, 8, 10, 14)

Albert S. Benight, Mohan Amaratunga and Teodoro M. Paner.
Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois.

DNA dumbbell molecules were constructed that contain the 16 base-pair duplex sequence $^{5'}$GCATAGATGAGAATGC$^{3'}$ (set 1) or $^{5'}$GCATCATCGATGATGC$^{3'}$ (set 2). Effects of end-loop size on the gel electrophoretic mobility, circular dichroism (CD) spectra, and restriction enzyme cleavage were investigated. Optical melting curves in 10 mM, 50 mM and 100 mM NaCl were also collected. These investigations revealed as loop size decreases, the electrophoretic mobilities, rates of enzyme cleavage and optical melting temperatures increase. For end-loops with n $\geq$ 3, the observed increases are linear with decreases in loop size. Behavior of the dumbbell with $T_2$ end-loops departs from this linear dependence and is anomalous in every experimental context. For molecules with end-loops n $\geq$ 4, CD spectra were identical but differed considerably from the CD spectrum of the $T_2$-looped molecule. The CD spectra of the dumbbell with $T_3$ end-loops displayed features common to both the dumbbells with larger loops and the $T_2$ loops. Since evidence was obtained that all base-pairs of the duplex stem are intact in the $T_2$-looped dumbbell, these measurements suggest closure of the $T_2$ end-loops induces a structural transition of the stem. The experimental optical melting curves of the dumbbells in set 1 collected over the salt range from (10 mM $\leq$ [NaCl] $\leq$ 100 mM) were analyzed in terms of the statistical thermodynamic theory of DNA dumbbell melting. Exact fits (within experimental error) were obtained between the experimental and calculated melting curves for every molecule in each salt environment. The analysis provides an evaluation of the ionic strength dependence of the free-energy required to form small $T_n$ hairpin loops (n $\leq$ 14) in DNA dumbbells. (Supported by NIH)

W-Pos291

## High resolution NMR studies of monovalent cation-dependent G-quadruplex structures of d($G_2N_5G_2$) in solution

Yong Wang, Carlos de los Santos, Xiaolian Gao, Barbara Gaffney, Roger Jones, Kenneth Breslauer, David Live and Dinshaw Patel

Department of Biochemistry and Molecular Biophysics
Columbia University, New York , NY 10032

We have undertaken two dimensional NMR studies on d($G_2T_5G_2$) and other related sequences, such as d($G_2T_4CG_2$), d($G_2CT_4G_2$) etc., in monovalent cation-containing solution to further charaterize the G-quadruplex alignment established from calorimetric and spectroscopic studies for the structural conformation(s) observed at low temperature. An analysis of the NOE and exchange cross peaks in the NOESY spectrum establishes formation of major and minor structured conformations at low temperature in Na or K cation-containing buffer for both of which G1 and G8 adopt *syn* glycosidic torsion angles while G2 and G9 adopt *anti* glycosidic torsion angles. The conformational equilibrium shifts toward an unfolded conformation upon raising the temperature. These NMR data provides direct evidence that the structured conformations of d($G_2N_5G_2$) in Na or K cation-containing buffer at low temperature involve alternating G(*syn*) and G(*anti*) arrangements around the G-tetrad alignment and consequently adjacent strands are anti-parallel to each other. Moreover, the NMR data establish that at leas: for adjacent G-tetrad in (G-G) segment, the glycosidic torsion angles within each strand alternate between G(*syn*) and G(*anti*) orientations. These NMR derived structural conclusions on G-tetrad alignment in the d($G_2N_5G_2$) sequence complement earlier conclusions deduced from gel mobility studies, chemical protection, interference and cross-linking experiments for repeating G-rich DNA oligomer segments which form G-quadruplexes in the presence of monovalent cations.

**W-Pos292**

**EXPLORATION OF BASE PAIRINGS IN FLEXIBLE RNA CHAINS** Brooke Lustig, Robert L. Jernigan & David G. Covell* NCI/DCBDC/LMMB, Bethesda, MD 20892, *ASCL/PRI/FCRDC, Frederick, MD 21702.

Lattice models of a known tRNA structure are developed and used to enumerate all configurations within a completely filled restricted space. Virtual positions for backbone and base atoms on a lattice are minimized relative to a known RNA crystal structure and show agreement within 2.5 A of the native structure. All possible volume excluded chain configurations can be enumerated on the lattice with an algorithm similar to one applied to small proteins.[1] The optimal base pairing representation for a single configuration cannot be determined unambiguously on the basis of pairing distances alone. We plan to apply and extend existing energy rules to evaluate the various representations for all chain configurations.[2] This approach can lead to methods that generate diverse folded RNA structures and that differentiate among such structures by their approximate energies.

1. Covell, D. G. and Jernigan, R. L. 1990. *Biochemistry 29*, 3287.
2. Turner, D. H., Sugimoto, N. and Freier, S. M. 1988. *Ann. Rev. Biophys. Biophys. Chem. 17*, 167.

**W-Pos293**

INTRAMOLECULAR TRIPLEX FORMATION OF THE PURINE:PURINE:PYRIMIDINE TYPE IN DNA. Fu-Ming Chen, Department of Chemistry, Tennessee State University, Nashville, Tennessee 37209-1561.

Six octadecamers with hairpin motifs are investigated for possible intramolecular triplex formation. Electrophoretic and CD evidence suggest that d(CCCCTTTGGGGTTTGGGG) and d(GGGGTTTGGGGTTTCCCC) form G:G:C intramolecular triplexes via double hairpin formation at pH 8, presumably with the terminal G tract folding back along the major groove of the hairpin duplex. In contrast, d(GGGGTTTCCCCTTTGGGG) and the three corresponding 18-mers containing one G and two C tracts each forms a single hairpin duplex with a dangling single strand. The design of the sequences has led to the conclusion that the two G tracts must be anti-parallel to each other in such a triplex and triplexes having like strands parallel do not appear to form. Titrations with $MgCl_2$ indicate that $Mg^{++}$ is not essential for such an intramolecular triplex formation. The main advantage of our constructs when compared to the intermolecular triplex formation is that shorter triplex stem can be formed in a much lower DNA concentration. The merit of the pu:pu:py triplex, in contrast to that of py:pu:py, lies in the fact that acidic condidtion is not required in its formation and will, thus, greatly expand our repertoire in the triplex strategy for the recognition and cleavage of duplex DNA. Spectral binding studies with actinomycin D (ACTD) and chromomycin $A_3$ (CHR) as well as fluorescence lifetime measurements with ethidium bromide (EB) suggest that although hairpin duplexes bind these drugs quite well, the intramolecular triplexes bind poorly. (Supported by a subproject of MBRS Grant S06RR0892)

**W-Pos294**

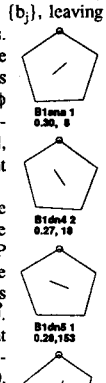
EVIDENCE FOR SPECIFIC MAGNESIUM-DNA INTERACTIONS FROM MAGNESIUM-25 NMR RELAXATION TIMES. Laura Wright and Laura Lerner, Dept. of Chemistry, University of Wisconsin-Madison, Madison WI 53706

Magnesium ions have been shown to dramatically affect the conformation of DNA (1). Earlier $^{25}Mg$ NMR lineshape analysis provided qualitative evidence for site-specific binding of magnesium ions to double-stranded DNA (2,3). To quantify these interactions, and to study them in greater detail, we have measured longitudinal ($T_1$) and transverse ($T_2$) relaxation times of $^{25}Mg$ at 30.6 MHz and 18.4 MHz in the presence of either nucleosomal calf thymus DNA (160 bp) or inorganic polyphosphates (n=24). $^{25}Mg$ relaxation is greatly enhanced by the presence of inorganic polyphosphates, although $T_1 = T_2$ for all ratios of magnesium:phosphate measured thus far. In contrast, $T_2$ is always significantly shorter than $T_1$ in solutions of nucleosomal calf thymus DNA. Thus, magnesium ions bind to both polyelectrolytes, although the nuclei remain in the extreme narrowing region ($w_o t_c << 1$) when bound to polyphosphates whereas they are in the slow motion region ($w_o t_c >> 1$) when bound to DNA. These observations indicate that when magnesium ions bind to DNA the interaction is more complicated than their interactions with simple phosphate groups. (1) Diekmann, S. (1987) *Nucleic Acids Research*, 15, 247-265. (2) Rose, D. M., Bleam, M.L., Record, M.T., Jr. & Bryant, R.G. (1980) *Proc. Natl. Acad. Sci. USA*, 77, 6289-6292. (3) Rose, M.D., Polnaszek, C.F. & Bryant, R.G. (1982) *Biopolymers* , 21, 653-664.

**W-Pos295**

**AN EXACT, FOUR-PARAMETER DESCRIPTION OF THE RIBOSE RING.** C. J. Marzec and L. A. Day, The Public Health Research Institute, 455 First Avenue, New York, New York 10016.

Some studies of nucleic acid structure require an *exact* parametrization of the ribose ring. The 5 ring atoms have 15 degrees of freedom. With 3 each for a center-of-mass translation and an arbitrary solid-body rotation, there remain 9 degrees of freedom which describe the internal ring configuration. Five of these are needed for the bond lengths, $\{b_j\}$, leaving 4 parameters to describe completely all possible configurations. Cremer and Pople showed that for any 5-atom ring, a plane exists such that the displacements of the atoms out of this plane can be *exactly* described by two parameters, an angle $\phi$ (almost identical to P) and a length q (almost linear in pseudorotation amplitude $\tau_{max}$). P and $\tau_{max}$, though widely used, generate *inconsistent* sets of torsion angles, and yield no insight into the two degrees of freedom not covered by q and $\phi$.




The Cremer-Pople formulation *exactly* parametrizes the out-of-plane (z-direction) ring atom coordinates, reducing the problem to parametrizing the projection of the ring in the C-P plane. We introduce two parameters for this, S and $\Gamma$. We start with $\sigma^2(\theta)$, the sum of squares of atom displacements from the projected ring center, measured along a direction $\vec{\theta}$. Its maximum value defines an angle $\Gamma$; the minimum is at $\Gamma \pm 90°$. A distortion or "stretch" of the projected ring is measured by $\sigma^2(\Gamma) - \sigma^2(\Gamma \pm 90°) \equiv S^2$, where S is a length. If S=0, $\sigma$ is independent of angle, which is the condition of maximum symmetry. The transformation from Cartesian coordinates to internal coordinates {q, P, S, $\Gamma$; $\{b_j\}$} is the *exact* inverse of the transformation from the internal coordinates to the Cartesian set. Treating 370 nucleotides in the Brookhaven data bank, we find broad distributions of both q and S, with average values of 0.4 Å for each. At the right are C-P plane projections of six rings from the data bank; under the residue name are q, in Å, and the angle $\phi$; the line inside has length S and angle $\Gamma$.

We have calculated the Jacobian J of the transformation from the 15 Cartesian coordinates to the set of translations, rotations, and {q, P, S, $\Gamma$; $\{b_j\}$}. J gives the phase space volume element used in statistical studies of ring configuration.

W-Pos296

## MOLECULAR MODELING TO PREDICT THE BIOLOGICAL EFFECTS OF MUTATIONS IN A HIGHLY CONSERVED HISTONE mRNA LOOP SEQUENCE.

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The highly conserved 3'-terminal sequence, 5'-GGYYYUUYUNARRRCC-3', is essential for proper 3'-end formation and in regulating the steady-state concentration of histone mRNA (reviewed in Marzluff and Pandey, *Trends Biochem. Sci.* 13:49-52, 1988). This sequence is normally assumed to be a six-base stem with a four-base loop. We have found experimentally that substituting purines for the U's in the loop decreases the processing efficiency 80-90% *in vivo*. Similar effects are seen when the U-A base pair at the top of the stem is changed to a G-C pair. We are attempting to give a structural explanation to these observations using molecular modeling. The wild type sequence has been modeled as a four-membered loop with the sequence, 5'-CCCUUUUUAGGG-3'. All five permutations of a 4-loop were built as described previously (Harvey *et al., Nucleic Acids Res.* 16:11795-11809, 1988) and minimized using the JUMNA algorithm (Lavery, R. Unusual DNA Structures [Eds. Wells R.D. and Harvey S.C., Springer-Verlag, New York, 1988] pp.189-206). We opted to minimize without constraints so that JUMNA was free to break or maintain the A-U base pair to obtain the lowest energy. Consequently, we see a mixed population of four and six-membered loops after energy minimization. The loop regions have been subjected to molecular dynamics in order to extend the range of possible wild type structures. These models and previous experimental results are used as a basis of comparison to predict the effects of mutations which have not yet been tested experimentally.

W-Pos297

## MICROSCOPE MEASUREMENTS OF PERSISTENCE LENGTH AND CHARGE DENSITY ON SINGLE DNA MOLECULES ATTACHED TO MAGNETIC BEADS

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Single DNA molecules (λ phage DNA, 48.5kbp) were biotinylated at both ends. The end labeled DNA was then attached, at one end, to an avidin coated, 2.8μM paramagnetic bead and, at the other end, to an avidin coated glass slide. An external magnet attracted the beads and extended the molecules. The positions of the beads were determined through a microscope to give the full extension of the molecules. The magnetic force on a single bead was inferred from the velocity of free beads moving past the tethered beads and also from the underwater weight of a bead. The force vs. extension data gives a good picture of the contour length, entropic elasticity and tensile strength of single DNA molecules. Comparison with the freely jointed chain model for entropic elasticity gives the persistence length of the molecule. The effects of various salt concentrations and ethidium bromide were investigated. The extension of single anchored DNA molecules in an externally applied electric field is then used to obtain a better estimate of the effective electrophoretic charge density than previously attained. (S. Smith and A. Bendich, Biopolymers 29, 1167-1173)

W-VCR3

## MOLECULAR DYNAMICS ANALYSES OF DUPLEX DODECAMER MODIFIED BY (+) AND (-) ANTI BPDE AND OF MODIFIED CONTROLS.

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In previous work we presented results of molecular dynamics simulations for a DNA duplex heptamer modified by (+) and (-) anti BPDE. These simulations were carried out using AMBER (3.1) with explicit solvent and salt. We have now performed these simulations on a modified duplex dodecamer $d(CG)_6 \cdot d(CG)_6$ and also on the unmodified DNA of the same sequence and on the Dickerson dodecamer as controls. Detailed analyses of the MDS for the three most favored modified conformers, previously obtained by conformational search and build strategies with DUPLEX, and for the unmodified DNAs will be presented.

Supported by NIH, DOE and NSF.

W-VCR4

## AN INFRARED CD INVESTIGATION OF THE THERMAL STABILITY OF DEOXYOLIGONUCLEOTIDES AS A FUNCTION OF IONIC STRENGTH.

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Vibrational circular dichroism (VCD) has recently been used to characterize the conformations of several deoxyoligonucleotides and investigate changes in secondary structure of DNA. We have used VCD to show that the high salt Z-form of poly(dG-dC)poly(dG-dC) in the presence of 3M NaCl is not identical with the Z-form in the presence of 1.4M $MgCl_2$. These data imply that the monovalent and divalent cation effects are different. The B-form of poly(dG-dC)poly(dG-dC) has been shown to be temperature stable whereas the conformation of poly(dG-m$^5$dC)poly(dG-m$^5$dC) is not. The conformation of poly(dA-dT)poly(dA-dT) is also sensitive to temperature changes. In this paper we examine the vibrational secondary structure of poly(dG-dC)poly(dG-dC), poly(dA-dT)poly(dA-dT) and poly(dG-m$^5$dC)poly(dG-m$^5$dC) in the presence of high and low concentrations of NaCl and $MgCl_2$, and as a function of temperature. The spectra are compared with one another and with spectra calculated from our theoretical model.

Grant support: National Science Foundation (NSF 9007807), American Heart Association (AHA-NYC Established Investigatorship and Grant-in-Aid) to D.J.G.; National Institute of Health (GM 28619) to M.D.; and several PSC-CUNY faculty awards to both.

**W-VCR5**

## MODELING MACROMOLECULAR CONFORMATIONAL CHANGES: APPLICATIONS TO THE TRANSITION FROM B-DNA TO Z-DNA

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The development of methods for modeling large, slow conformational changes in macromolecules is one of the most important needs for users of molecular mechanics, molecular dynamics, and Monte Carlo. Broadly speaking, problems of finding transition pathways can be divided into two classes. First are those cases where it is possible to define an appropriate transition coordinate for the conformational change. The availability of a suitable transition coordinate allows one to push the system along the desired pathway using a variety of biasing methods. Second, and much more difficult, are those cases where a transition coordinate cannot be defined *a priori*, but where the initial and final conformations are known. The transition between B-DNA (a right-handed double helix) and Z-DNA (a left-handed one) is an example of the second class.

We have used a variety of methods to investigate the B to Z transition. Systematic searching procedures were designed. Some of these use the ordinary Cartesian coordinates, while others use internal coordinates, either torsion angles (backbone, sugar, and glycosidic), or the set of helicoidal parameters defined by the CURVES algorithm (Lavery and Sklenar, *J. Biomol. Struct. Dyns. 6*, 655 [1989]). We have located trial transition pathways by both constrained energy minimization (adiabatic mapping) and by a minimum biasing molecular dynamics method, CONTRA MD (Harvey, submitted to *J. Phys. Chem.*). Our adiabatic mapping methods are similar to an approach pioneered by Ulitzky and Elber (*J. Chem. Phys. 92*, 1510 [1990]). CONTRA MD is similar in spirit to Elber's constrained MD method (*J. Chem. Phys. 93*, 4312 [1990]), but it does not require the imposition of constraint forces.

**W-Pos298**

The Molecular Origin of the Long Blood Circulation Time of Stealth® Liposomes

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The utility of liposomes for drug delivery after I.V. injection is severely limited by their rapid clearance from the bloodstream. Predominant mechanisms of this fast uptake are 1) opsonization with macromolecules leading to the recognition by body's immune system and uptake by the cells of reticuloendothelial system (RES) and 2) interaction with plasma lipoproteins which results in lipid loss and consequent liposomes disintegration. The half-life of conventional liposomes in blood is, in the absence of the saturation of RES, in the range of minutes to dozens of minutes.

In late 70's and early 80's, it was found that liposomes composed from lipids with a high temperature phase transition gel to liquid crystal and cholesterol are cleared from blood more slowly, with half-lives approaching several hours.

Recently, however, special liposome formulations, containing specific glycolipids, were introduced. After preliminary successes with formulations containing some glycolipids or gangliosides, polymer (PEG) coated liposomes were shown to have blood half-lives approaching one day.

Despite intensive experimental efforts the mechanism of this effect is still unclear. In contrast to the approach which is trying to explain the phenomenon by complex interactions of liposomes with various blood components, we believe, that the effect can be explained, at least in part, by invoking simple considerations from classical colloid stability.

Importantly, steric stabilization provides long circulation times without the requirements of rigid bilayer structure, low surface charge, or presence of cholesterol. It will be shown that attachment of bulky, hydrophilic and water compatible head groups strongly reduces the attractive van der Waals forces and increases repulsive steric, hydration, osmotic and entropic forces.

**W-Pos299**

Phase Diagram of PEG-DSPE (Stealth® Lipid)-Egg Phosphatidylcholine (EPC) Mixtures

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Applications of liposomes for delivery and controlled release of drugs have shown a great deal of promise but are limited by a short blood lifetime due to rapid RES (reticuloendothelial system or macrophage) uptake. Recently, though, advances in lipid compositions of liposomes have resulted in prolonged blood lifetimes and reduced uptake by the liver and spleen (Allen and Chonn, FEBS Lett, 223, 42, 1987; Gabizon and Papahadjopoulos, PNAS 85, 6949, 1988). These Stealth® liposome compositions used natural glycolipids, e.g. $G_{M1}$ and HPI (hydrogenated phosphatidylinositol), in combination with rigid bilayer forming lipids. Further advances in such liposomes resistant to macrophage uptake have been achieved using synthetic lipids with bulky hydrophilic polymers attached to the headgroup. Because of the relatively large polar headgroup, these lipids are not necessarily bilayer forming lipids. In fact, all of these Stealth lipids show an increased water solubility or, in other terms, a higher CMC. Therefore, we have investigated the phase behavior of mixtures of egg lecithin (EPC) and $^{1900}$PEG-distearylphosphatidylethanolamine (PEG-DSPE). In aqueous solutions, PEG-DSPE forms micelles with a hydrodynamic radius less than 10 nm. Examination of PEG-DSPE and EPC mixtures was performed by studies of aqueous entrapment and particle characterizations by light microscopy and dynamic light scattering. At low mole percent EPC, mixed micelles are formed; at high mole percent EPC, liposomes are formed. The CMC and removal of PEG-DSPE during dialysis or gel chromatography also have been investigated. Lipid transfer of PEG-DSPE between EPC liposomes and PEG-DSPE micelles was measured.

**W-Pos300**

## COMPUTER MODELLING OF THE PASSIVE PERMEABILITY OF LIPID-CHOLESTEROL BILAYERS AS A FUNCTION OF ACYL CHAIN LENGTH

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Lipid-cholesterol bilayers are studied by Monte Carlo computer simulations using a microscopic multi-state lattice model. This model is an extension of the Pink model for the main gel-to-liquid crystal phase transition of lipid bilayers in which cholesterol is introduced as a substitutional impurity with no internal degrees of freedom. The model is able to account for the chain melting of lipid molecules at the main phase transition in the absence of cholesterol and is valid at low cholesterol concentrations. The simulation results are used in conjunction with a model due to Cruzeiro-Hansson and Mouritsen to predict the temperature dependence of the passive permeability of lipid-cholesterol bilayers for different cholesterol concentrations and lipid chain lengths corresponding to DMPC, DPPC and DSPC. The model assigns different ionic transfer probabilities to bulk, clusters and interfaces. The basic idea is that defects due to bad packing make the membrane leaky at the interfaces and allow ions to permeate the bilayer with a probability of transfer in the interfacial regions which is much higher than for the bulk or the clusters. A peak in the permeability is observed near the transition temperature in accord with experimental data. The results predict an increase in the membrane permeability for increasing cholesterol concentration for the three different chain lenghts under consideration. Also, an increase in the membrane permeability with decreasing chain length is predicted for the four cholesterol concentrations studied.

**W-Pos301**

EVALUATION OF THE FREE ENERGY OF INTERLIPID HYDROGEN BONDING FROM FLUORESCENCE-PROBE PARTITIONING MEASUREMENTS

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A series of exchangeable fluorescent phospholipid probes has been prepared carrying relatively nonpolar indolyl, carbazole or diphenylhexatriene (DPH-) labels on one acyl chain. Fluorescence intensity measurements can be used to measure the kinetics and the thermodynamics of partitioning of such probes between two different populations of lipid vesicles, one of which contains a non-exchangeable quencher of probe fluorescence while the other does not [Nichols and Pagano, Biochemistry 21 (1982), 1720-1726; Gardam and Silvius, Biochemistry 28 (1989), 884-893]. Using this principle, we have examined the partitioning of a series of neutral phospholipid probes between vesicles rich in phosphatidylcholine, which can serve as a hydrogen-bonding acceptor, and N-palmityl-N-oleyl-N,N-dimethylammonium chloride (PODMA), a bilayer-forming amphiphile that can neither donate nor accept hydrogen bonds. In agreement with previous results, probes with different polar headgroups differ strongly in their rates of desorption from PC-rich bilayers, in a manner that suggests a significant influence of interlipid hydrogen-bonding interactions on the desorption kinetics. In contrast, the rates of desorption of different probes from PODMA vesicles are quite similar, in agreement with the above proposal. Experimentally determined partition coefficients, representing the relative preferences of different probes for PC-rich vesicles over PODMA vesicles, vary with the probe headgroup structure in the order phosphatidylethanolamine (PE) $\approx$ phosphatidyl-2-amino-1-propanol > N-methyl PE > N,N-dimethyl PE > PC. Quantitative analysis of these results suggests that interlipid hydrogen-bonding contributes roughly 300 cal mole$^{-1}$ to the total free energy of interaction of a PE molecule with neighboring phospholipids in a liquid-crystalline lipid bilayer. (Supported by the Medical Research Council of Canada).

**W-Pos302**

COUPLING OF LATERAL POLAR GROUP INTERACTIONS AND HYDROCARBON CHAIN CONFORMATION IN DIDODECANEPHOSPHATE BILAYERS. Y. Fang and R. P. Rand, Biological Sciences, Brock University, St. Catharines, Canada.

DDP bilayers assemble into highly ordered lamellar phases even at large separation. Osmotic stress of this lamellar phase, at 22°C and at various ionic strengths, yields a relation between force and bilayer separation that can be described by electrostatic repulsion from 210 Å to 50 Å. Out to about 100 Å the bilayer is a constant 31 Å thick, but from 100-300 Å separation the bilayers thin as the molecular area decreases. Within this restricted range of osmotic stresses, or water activities, at these large bilayer separations, these changes are accompanied by an unusual reentrant melted-frozen-melted hydrocarbon chain conformation sequence. On either side of the gel state condition, changes in the X-ray high-angle (4.5Å) band characterizing the disordered conformation of the hydrocarbon chain suggest that unusual partial chain ordering results. All this behaviour at large bilayer separations varies with ionic strength and specific counterion. In 2-200 mM tetramethylamonium/Cl solutions, the gel phase exists only over a very narrow range of water contents; a much wider range exists in 6 mM KCl, and even wider in 2 mM NaCl. But DDP becomes completely dehydrated and crystallized at NaCl concentrations greater than 6 mM.

We interpret these structural changes in terms of a sensitive balance in the lateral packing of the polar groups and the hydrocarbon chains whereby only under narrow optimum conditions is the polar group packing consistent with the molecular area of the gel state allowing its formation. Polar group packing depends on the various mutual interactions of water-counterion-polar groups, and small deviations from the optimum conditions results in disordering of the hydrocarbon chains and bilayer thinning.

**W-Pos303**

DYNAMIC COUPLING AND VISCOUS DRAG BETWEEN MEMBRANE LAYERS. Evan Evans*, A. Yeung*, J. Song†, and R. Waugh†. *Pathology and Physics, University of British Columbia, Vancouver, B.C.; †Biophysics, University of Rochester, Rochester, N.Y.

Biological membranes - even lipid bilayers - are weakly-bonded, layered structures. As such, bending will be inhibited dynamically by interlayer viscous drag due to relative displacements between layers. If interlayer coupling (shear stress) is linear in the relative velocity, membrane bending moments relax by a diffusion process; thus, this process will regulate curvature and shape changes at low tension. For two layers, the diffusion coefficient D is given by the geometric mean $\bar{K}$ of the layer area compressibility moduli (dyn/cm) divided by the interlayer drag coefficient b (dyn-sec/cm$^3$), i.e. $D = \bar{K}/b$. Rapid local bending - or membrane flow between regions of low and high curvature - will create transient rigidity. As a result, long wavelength modes of thermally-excited shape fluctuations in vesicles should decay faster than short wavelength excitations as if bending rigidity increases with wavelength. Also, when membrane is rapidly extruded from vesicles or cells to form long-microscopic cylinders (tethers), the rate of tether growth should decay exponentially with time under constant extrusion force. Recent measurements of tether growth rate are consistent with the predictions of the diffusion-like process. Experiments for lipid bilayer vesicles yield values of the effective diffusion coefficient (~7x10$^{-7}$ cm$^2$/sec) that are much larger than lateral diffusion coefficients measured with fluorescent probes. From this transport coefficient, the interlayer drag coefficient is estimated to be ~7x10$^7$ dyn-sec/cm$^3$. This indicates that the interlayer coupling is greater than implied by simple shear of a 3nm thick hydrocarbon liquid (i.e. b >> viscosity/thickness).

**W-Pos304**

BILAYER ASYMMETRY AND PHOSPHOLIPID PACKING IN HIGHLY CURVED PHOSPHATIDYLCHOLINE/LYSOPHOSPHATIDYLCHOLINE SYSTEMS. V.V. Kumar, Barbara Malewicz, and Wolfgang J. Baumann, The Hormel Institute, University of Minnesota, Austin, MN 55912.

Sonication of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC; up to about 30 mol%) produces small unilamellar vesicles (SUV) of 250-265 Å diameter. Phosphorus-31 NMR (32.2 MHz) spectroscopy of the POPC/lysoPC SUV shows four distinct peaks corresponding to POPC and lysoPC in the outer and in the inner bilayer leaflet which can be used to localize and quantify the phospholipids in both vesicle shells. Addition of paramagnetic ions (3 mM $Pr^{3+}$) enhances outside/inside chemical shift differences and allows monitoring of membrane integrity. The data show that lysoPC in highly curved POPC/lysoPC vesicles prefers the outer bilayer leaflet. LysoPC incorporation into POPC SUV also causes a concentration-dependent decrease in spin-spin relaxations ($T_2^s$) of the outside POPC signal from 55 ms for pure POPC vesicles ($\nu_{1/2}$, 5.8 Hz) to 29.5 ms (10.8 Hz) for SUV containing 25 mol% lysoPC. By contrast, $T_2^s$ of the inside POPC phosphorus signal remains constant (44 ms; $\nu_{1/2}$ 7.2 Hz). Because the SUV did not differ in size, line broadening due to differences in the rates of rotational diffusion can be ruled out. Hence, the decrease in $T_2^s$ can be attributed to increased motional constraints on the POPC molecules in the outer shell at higher lysoPC mole percentages. Our findings are consistent with the idea of a cone-shaped lysoPC molecule which, for geometric reasons, is preferentially accommodated in the outer bilayer leaflet. The "wedge"-shaped lysophospholipid does not disrupt the lamellar phospholipid array, as it does in planar bilayers, but actually stabilizes it by filling the "gaps" between the tilted POPC "cylinders" in the highly curved outer bilayer leaflet. Whether the lysoPC "wedge" disrupts or stabilizes a lamellar arrangement is clearly a function of curvature. (Supported by NIH grant HL08214 and the Hormel Foundation)

**W-Pos305**

**ELECTROSTATIC POTENTIALS IN ASYMMETRICAL MEMBRANES : A bilayer and monolayer study.**

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It is generally recognized that lipid asymmetry plays an important role related to a number of membrane functions. Besides the establishment of asymmetric specific interactions between membrane lipids and proteins or between lipids and other constituents in aqueous phases, lipid asymmetry can modulate the activity of membrane proteins by creating various transmembrane gradients such as fluidity or electrical potential gradients. In relation to this, the advent of *in vitro* reconstitution techniques allowing the formation of asymmetrical lipid bilayers of definite composition (namely the Montal-Mueller and "tip-dip" techniques) has led to a better understanding of many biological phenomena such as the gating of ionic channels. In the present work, asymmetrically charged Montal-Mueller phospholipid membranes (DOPE/DOPG and DOPE/DOPS) have been formed under different ionic conditions. Steady-state current-voltage (I-V) curves of these bilayers doped with nonactin yield informations about the electrical profile across the bilayer. The I-V curves are analyzed according to the Nernst-Planck approach assuming trapezoidal barriers. However, a more direct and easier way to get informations on electrostatic potential is provided by monolayer studies. Thus, surface potential measurements ($\Delta(\Delta V)$) of lipid films have been undertaken and the influence of nonactin on the potential was investigated. A comparison will be presented between the differences of $\Delta(\Delta V)$ obtained for monolayers of the constitutive lipid species of a given bilayer and the calculated transmembrane electrical potentials of the corresponding doped bilayer.

W-Pos306

A MICROSCOPIC BASIS FOR THE SURFACE POTENTIAL AT WATER-LIPID INTERFACES. Kamalakar Gulukota and Eric Jakobsson, Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

An amphipathic molecular monolayer consisting of either phospholipid or gramicidin situated at an air-water interface generally results in a potential difference in which the air is a few hundred millivolts positive relative to the water. The magnitude of this voltage varies with the molecular species forming the monolayer. Up to now there has been no consensus on the physical basis for this potential. Lee, McCammon, and Rossky (J. Phys. Chem. 1984. 80:4448) computed by molecular dynamics water orientations near extended hydrophobic surfaces. They reported that in order to maximize hydrogen bonds in bulk, the water molecules had average orientations resulting in "dangling hydrogens" at the hydrophobic surface. We have applied Poisson's equation to Lee et al's published distributions of hydrogen and oxygen to calculate the corresponding surface potential. Across the first water monolayer (3 angstroms from the hydrophobic surface) we find a voltage difference of 1.3 volts with the wall positive relative to the bulk water. Thus the "dangling hydrogen" effect is in the right direction and of sufficient magnitude to account for the measured surface potentials. We are now doing our own water molecular dynamics computations next to a hydrophobic surface to get more precise data on the distributions than is possible from reading the published graphs. We will also do computations including phospholipid head groups at the surface to see how their presence may modulate the measured surface potential.

W-Pos307

OSMOTIC PROPERTIES OF LARGE UNILAMELLAR VESICLES. Barb L-S. Mui, T.D. Madden*, and P.R. Cullis*, Department of Biochemistry, University of British Columbia, Vancouver, B.C. V6T 1W5 and *The Canadian Liposome Co. Ltd., North Vancouver, B.C., Canada, V7M 1A5.

Biological membranes display much higher permeabilities to water in comparison to other molecules and as a result are sensitive to osmotic gradients. Such gradients can have profound influences on cell volume, structure and function and have been implicated in membrane fusion-dependent processes such as exocytosis.

Osmotic properties of lipid membranes have been studied using many different liposomal systems and techniques. Large unilamellar vesicles made by extrusion (LUVETs) were used in the present study as different sized vesicles can be easily made and the method is rapid. When these vesicles are exposed to a hypotonic gradient, they first maximize their volume by assuming a spherical shape before the membrane becomes osmotically stressed. Osmotic lysis has been characterized with respect to the extent of solute release and the residual osmotic gradient determined as a function of the applied osmotic differential. For egg phosphatidylcholine vesicles (100nm in diameter) the osmotic gradient required to produce lysis is similar to the residual osmotic gradient measured after lysis. This suggests that during lysis defects occur in the membrane for solute to be released and then the vesicles rapidly reseal at the maximium osmotic strain it can tolerate. The size of these defects were examined with different sized solutes and were found to be greater than 10nm. Other factors influencing lysis including vesicle size and lipid composition have been similarly examined. Finally, changes in lipid packing resulting from imposed osmotic gradients has been studied using fluorescent membrane probes, diphenylhexatriene and 1-acyl-2[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] dodecanoyl]-phosphatidylcholine. The results indicate, as expected, that when the vesicles are under osmotic strain the membrane is less ordered and the lipids are not as closely packed together.

W-Pos308

MEASUREMENT OF THE NON-LOCAL BENDING MODULUS OF BILAYER MEMBRANES BY TETHER FORMATION FROM LECITHIN VESICLES. Jianben Song[1], Richard E. Waugh[1], Bostjan Žeks[2], and Saša Svetina[2]. (Intro. by Paul LaCelle.) [1] Dept. Biophysics, Univ. of Rochester School of Medicine and Dentistry, Rochester, NY 14642, [2] Institute of Biophysics, Edvard Kardelja University, Ljubljana, Yugoslavia.

Bilayer membranes exhibit an elastic resistance to changes in curvature. This resistance to bending is complex because of the lamellar structure of the membrane. It depends on both the intrinsic stiffness of the constituent monolayers and the expansion or compression of each leaflet relative to the other. Because the molecules of the adjacent monolayers are not connected, the two layers may redistribute over the surface of the membrane capsule to minimize the relative stretching of each leaflet. Both the intrinsic monolayer stiffness ($k_c$) and the modulus for relative stretching ($k_r$) can be measured by tether formation from giant phospholipid vesicles. Previously, we reported measurements of the intrinsic bending stiffness ($k_c$) based on measurements of tether radius as a function of force (Song and Waugh, J. Biomech. Engr. 112:233, 1990). Further analysis has revealed that the contribution from relative stretching can be detected by measuring the change in the aspiration pressure required to establish equilibrium at different tether lengths. Using this approach, we estimate a value for $k_r$ of $3.5 \times 10^{-12}$ ergs. ($k_r = Kh^2/4$, where K is the expansivity modulus and h is the separation distance of the monolayers.) The range of values is broad ($1.0 - 8.0 \times 10^{-12}$ ergs) and may reflect contributions due to dynamic trapping of material in the inner leaflet as the tether is formed. Inclusion of the relative stretching contribution into the calculation of the intrinsic modulus $k_c$ results in a slightly lower value for that modulus ($\sim 1.1 \times 10^{-12}$ ergs), in close agreement with values based on measurements of thermal tensions in membranes.

W-Pos309

**Separation of PE/PC and PC Vesicles based on Hydrophobic Surface Properties.** Chris Kuhn, Kathleen Boesze-Battaglia, Philip Yeagle and Arlene Albert Department of Biochemistry, University at Buffalo School of Medicine (SUNY), Buffalo, N.Y. 14214

Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are two of the most common phospholipids in biological systems. They are also extensively studied in model systems. The ratio of these lipids has been shown to influence the bilayer to hexagonal phase transition in model systems and has been implicated in facilitation of membrane fusion. It has been proposed that surface hydration of the bilayer is central to the observed properties of PE/PC bilayers. Previously the bilayer to hexagonal transition and the aggregation of PE-rich vesicles was shown to be inhibited by chaotropic agents. We now show that PE/PC and PC vesicles can be separated using column chromatography designed to separate hydrophobic proteins. Small unilamellar vesicles composed of PE/PC (4:1) were mixed with an equal amount of PC vesicles. Egg PC and transphosphatidylated egg PE were used to avoid differences in fatty acid distribution between naturally occurring PC and PE. The vesicles were eluted from a phenylsepharose column. The eluate was assayed for total phosphate and for primary amine groups. The elution pattern indicated separation of the two species. Biological implications of these differences in membrane surface properties are presently under investigation.

**W-Pos310**

**BENDING ELASTICITY OF CHARGED BILAYERS: coupled monolayers, neutral surfaces, and balancing stresses,**

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We investigate(*) the contribution of electrostatic double layers to the bending elasticity of charged symmetric bilayers, considering in detail three special problems. First the coupling of the monolayers by the electric field traversing a curved bilayer, second the dependence of the elastic moduli on the position of the monolayer's neutral surface, and third the role of the mechanical stresses balancing the electric ones. According to the results the tendency of charged membranes to spontaneous vesiculation may be stronger than hitherto expected.

(*) J. Phys. Chem. 92, 6865 (1988) and submitted to J. Phys. Chem.

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**W-Pos311**

**MECHANICAL AND INTERACTIVE PROPERTIES OF LIPID MEMBRANES CONTAINING SURFACE-BOUND POLYMER: IMPLICATIONS FOR LIPOSOME DELIVERY SYSTEM DESIGN.** D. Needham and T. J. McIntosh, Departments of Mechanical Engineering and Materials Science, and Cell Biology, Duke University, Durham, N. C. 27706.

The rapid clearance from the blood stream of liposomes used in delivery systems is one of the major obstacles to advancing many applications of liposomes in medical technologies. The incorporation of glycolipids or a lipid that contains a large polymeric polar headgroup into the liposomes greatly enhances the circulation time of the injected dose in the blood. The precise mechanism of action is unclear, but it would appear that the presence of surface bound moeities act to increase repulsive interactions and may also inhibit opsonization of the liposomes, thereby evading identification to the RES. A first step in understanding the mechanism(s) operating in these processes necessarily involves a physical characterization of the mechanical and interactive properties of the liposome systems. We have therefore characterized lipid bilayer systems of typical liposome formulations containing phopholipids, cholesterol, $G_{M1}$ and a polyethylene oxide surfactant (PEOS) in terms of their mechanical and interactive properties. Mechanical stability of inividual lipid vesicles was determined by using a micropipet manipulation method to measure the elastic area expansion modulus K, and several failure parameters. The repulsive, mutual interaction between lipid bilayers was examined by an x-ray method that measured the interbilayer gap separation as a function of applied pressure and also gave an indication of polymer-polymer interactions in the gap from electron density profiles. Compared to the hydrated lipid membrane, the inclusion of $G_{M1}$ and PEOS increased the interbilayer separation distance with the polymer producing the largest effect. In a low pressure regime, the exponential decay constant was ~8 to 10 A for both surface groups, before a stiffer repulsive interaction appeared at high pressure and close separation.

**W-Pos312**

**IMMUNORECOGNITION OF NONBILAYER LIPID PHASES IN VIVO**

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Immunization of mice with phosphatidylethanolamine in the hexagonal (II) phase but not the bilayer phase resulted in the induction of antiphospholipid antibodies. These antibodies which were strongly reactive with phosphatidylethanolamine and cross reactive with cardiolipin were characteristic of a subset of antiphospholipid autoantibodies associated with thrombosis thrombocytopenia and multiple spontaneous abortions common in patients with autoimmune disease. The phase specific immunorecognition of nonbilayer lipid by the afferent limb of the immune system suggests that alterations in lipid polymorphic phase can occur in vivo and lead to the production of autoantibodies with associated pathological sequence.

**W-Pos313**

**X-RAY DIFFRACTION OF FULLY HYDRATED, ORIENTED BILAYERS OF GEL PHASE DPPC.**

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An important datum for the structure of lipid bilayers in gel phases is the tilt angle of the hydrocarbon chains[1]. To measure this angle directly requires oriented samples, which are especially difficult to prepare for the fully hydrated benchmark lipid dipalmitoylphosphatidylcholine (DPPC) in excess water. We have prepared partially oriented samples by evaporation of DPPC from chloroform/methanol on watchglass surfaces, followed by various hydration methods, including incubating the sample at 75 °C in supersaturated humidity for 2 hours. The best samples are judged to be fully hydrated since the d-spacing of 63 Å and the intensities of the lamellar reflections obtained by X-ray diffraction agree with those from fully hydrated unoriented samples for orders 1 through 8. The wide angle ring is attenuated along the equator due to absorption by the sample. However, it exhibits a maximum in intensity off the equator from which the tilt angle can be bounded to less than 32°. A consequence of this value of the tilt angle is that the number of waters of hydration, $n_w$, is less than 12 which is significantly smaller than the values measured by the gravimetric method. This discrepancy supports the model of water-rich defect pools in fully hydrated unoriented multilamellar liposomes, which has important consequences for the methodology for obtaining bilayer structure[1].

This research is funded by NIH grant GM44976-01.

1. Wiener, M., Suter, R. and Nagle, J., Biophys. J. 55, 315 (1989).

**W-Pos314**

THERMOTROPIC STABILITY OF DRY LARGE UNILAMELLAR LIPOSOMES
C. Aurell Wistrom, L.M. Crowe and J.H. Crowe, University of California, Davis, CA 95616.

We have investigated the thermotropic phase transitions in natural eggPE and eggPC and mixtures of these lipids. In freeze-dried eggPE the thermotropic transition from the lamellar to the hexagonal phase is lowered from 30C, the fully hydrated transition temperature, to -5C. We have constructed a hydration dependent phase diagram for natural eggPE spanning from the fully hydrated state to the "complete" dry state. Furthermore, in dry eggPC we have found that the thermotropic transition from gel to liquid crystalline is increased from -8C to 25C measured with Fourier Transform Infrared Spectroscopy. Liposomes made of mixtures of these lipids with an increasing amount of eggPE and extruded through polycarbonate filters with a pore size of 100nm have been freeze-dried in the presence of 5 g of trehalose (a non-reducing disaccharide)/g dry lipid. Retention of entrapped carboxyfluorescein was measured upon rehydration at 25C and 50C. Retention increased in liposomes hydrated at 25C and decreased with liposomes hydrated at 50C suggesting leakage of fluorescent label from the liposomes. In the control liposomes freeze-dried with Tes-buffer the retention of trapped label increased with increasing amounts of eggPE. The liposomes were examined for size distribution and fusion events with Photon Correlation Spectroscopy. In the control all liposome mixtures showed monomodal distribution averaging 122nm before freeze-drying and upon rehydration at 25C bimodal size distributions averaging at 210nm and 1440nm with more than 70% of the vesicles being in the smaller size range and at 50C bimodal distr. at 40nm and 3000nm. In liposomes prepared in presence with trehalose average size was 100nm when freeze-dried and rehydrated at 25C only monomodal size distributions were found averaging 110nm and rehydrated at 50C a bimodal distribution averaging 80nm and 512nm with an increasing amount of the vesicles being in the larger size population with an increasing amount of eggPE.

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**W-Pos315**

EFFECTS OF CHARGED PHOSPHOLIPIDS ON LAMELLAR-HEXAGONAL TRANSITIONS.
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We have studied the effects of charged lipids on the lamellar (L)-hexagonal (H) phase transition in mixtures of dioleoylphophatidyl-ethanolamine (dope) and -serine (dops), using X-ray diffraction and osmotic stress.

From 0 to 12 mole% dops there is a systematic increase in the H lattice spacings, from 64-68Å, that form in excess water. Above 15 mole% dops the lipid forms single L phases at high water contents.

If 20 wt% of the lipid is tetradecane (td), no L phases form to at least 20 mole% dops in excess water, and the H lattice expands systematically to 78Å. The lattice spacing of 10 mole% dops with increasing td shows a broad maximum at about 17% td.

For 10 mole% dops, as the water content is systematically reduced, there is a reentrant H-L-H transition sequence. If 20 wt% of the lipid is td the L phase does not appear.

For 10 mole% dops and no td, the 68Å hexagonal lattice spacing is systematically reduced to 64 Å in NaCl solutions of increasing concentration. Screening of 15 mole% dops, which forms vesicles in excess water, shows coexisting H and L phases in 500 mM and a single H phase in 1M NaCl. At 19 mole% dops, the coexisting L phase does not completely disappear at 1M NaCl.

For 10 mole% dops and with td, the 74Å lattice spacing in excess water is systematically reduced to 54 Å in 1M NaCl, well below the value expected from simple screening.

This experimental evidence shows that the transition between L and H phases is sensitively dependent on electrostatic polar group repulsion tending to drive the system lamellar, and the tendency for dope and dehydration to produce the hexagonal phase. We interpret the H-L-H transition sequence seen on water removal as resulting from first an increase in electrostatic polar group repulsion and expansion, followed by a compressive dehydration of those polar groups. We are analysing the data in an attempt to more quantitatively test this description and measure the charged lipid layer curvature and bending modulus parameters.

**W-Pos316**

**INTERMOLECULAR INTERACTIONS IN THE SUBGEL PHASES OF THE n-SATURATED DIACYL PHOSPHATIDYLCHOLINES. NEW INSIGHTS FROM FT-IR SPECTROSCOPIC STUDIES OF $^{13}C$=O- AND $^{2}H$-LABELED LIPIDS.**
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The subgel phases of unlabeled, *sn*2-$^{13}$C=O and *sn*2-chain perdeuterated samples of DLPC, DMPC and DPPC were studied by Fourier-transform infrared spectroscopy. The use of the specifically labeled lipids simplified the interpretation of the infrared spectra by enabling an unambiguous assignment of the absorption bands arising from the *sn*1 and *sn*2 ester carbonyls, as well as the identification of the components of the $CH_2$ scissoring band arising from interchain vibrational coupling. Consequently, when these lipids form their crystalline or quasi-crystalline subgel phases, it is now feasible to more clearly define aspects of the intermolecular interactions which occur between the hydrocarbon chains, and in the polar/apolar interfacial region of the bilayer. Our data indicate that the structures of all of the various subgel phases formed by the n-saturated diacyl phosphatidylcholines are in many respects incompatible with that deduced from single crystal X-ray studies of DMPC, and we suspect that this is because the lipids have 'crystallized' from water under our experimental conditions, whereas the sample used for the single crystal X-ray study was crystallized from organic solvents.

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

**W-Pos317**

VITAMIN E STABILIZIES NON-BILAYER PHASES IN LIPID MODEL MEMBRANES.
B.A. Cunningham, Bucknell University, Lewisburg, PA; D.H. Wolfe, Lycoming College, Williamsport, PA; O. Kucuk, M.P. Westerman, L.J. Lis, University of Health Sciences/The Chicago Medical School and the Veterans Affairs Medical Center, N. Chicago, IL; P.J. Quinn, Kings College, London, UK; and J.M. Collins, Marquette University, Milwaukee, WI.

Vitamin E has been shown to be a fusogen of erythrocytes as well as an inhibitor of platelet aggregation. In order to probe the interaction of Vitamin E with cell membranes, admixtures of dipalmitoylphosphatidylcholine (DPPC) or 1-palmitoyl, 2-oleoyl phosphatidylethanolamine (POPE) with the tocopherol derivatives Vitamin E, Vitamin E acetate, $\delta$ tocopherol, and $\alpha$ tocopherly acid succinate were studied using real time x-ray diffraction. The $L_\alpha$ - $H_{II}$ phase transition of POPE was observed to decrease by over 20° C in the presence of 5 mole % of any of the tocopherol derivatives studied, with Vitamine E acetate having the largest effect. However, the presence of 5 mole % of the tocopherol derivatives had little effect on the DPPC bilayer phase transition temperatures. We conclude that tocopherols act generally to stabilize lipid non-lamellar phases at the expense of the disordered bilayer phase, but do not disrupt the packing and interactions within the bilayer. The influence of tocopherols thus involve changes at the lipid-water interface, with no evidence of specificity between the lipids and tocopherol derivatives studied.

**W-Pos318**

PRODUCTS OF THE "SPHINGOMYELIN CYCLE" STABILIZE INVERTED PHASES IN DOPE-ME

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Sphingomyelin (SM) undergoes stimulated turnover in vivo, resulting in the successive production of ceramide (CER), and then sphingosine (SP) and fatty acids. SP is a potent inhibitor of protein kinase C. SP production from SM appears to counteract the activation of protein kinase C by diacylglycerol (DAG), which is produced by the phosphatidylinositol (PI) cycle. Previously, we and others (Biochemistry 28:3703 [1989]) found that DAG also stabilized phospholipid inverted phases and induced membrane fusion. CER is similar in structure to DAG and likely has a similar effect. Here, we demonstrate that the lipid mediators produced by the PI & SM cycles, which have antagonistic effects on protein kinase C, both shift the phase behavior of membrane lipids in the same direction. The physiological significance of this is unclear. We examined the effects of SM and it's hydrolysis products on the phase behavior of N-mono-methylated dioleoylphosphatidylethanolamine. We compared the effects of 3 mol % bovine brain SM and it's simulated hydrolysis products (equimolar bovine brain SP and fatty acids with about the same acyl chain distribution as bovine brain SM) on $T_H$, the $L_\alpha/H_{II}$ phase transition temperature. We find that exchanging SM hydrolysis products for SM lowers $T_H$ by ca. 5.5°C/mol % of SM. Experiments with samples containing only SP and only fatty acids at pH 7.4 indicate that, surprisingly, these compounds produce roughly similar and additive effects on $T_H$. In this case the unusually-long ($C_{24}$:1) chains of the acids seem to have little effect on $T_H$ compared to $C_{18}$ chains, contrary to what might be expected (Tate & Gruner, Biochemistry 26:231). In comparison, exchange of DAG for PI lowers $T_H$ by 10-12°/mol %.

**W-Pos319**

## MOLECULAR IMAGING OF PHOSPHOLIPID MEMBRANES

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To interpret our high resolution images of cell membrane surfaces obtained by scanning tunneling microscopy (see related abstract by Tibbitts, *et.al.*) we have begun structural characterization of the molecular components of cell membranes by STM. Our initial work has produced detailed images of phospholipid membranes which were scanned directly without the use of metal coatings. The samples were prepared on freshly cleaved pieces of highly ordered pyrolitic graphite by repeatedly passing the substrate through a lipid monolayer formed at the air-water interface in a Langmuir-Blodgett trough. A tungsten tip was used to scan the membranes at constant current in air at room temperature. In images of dioleoyl phosphatidyl choline (DOPC) layers, ordered arrays of hexagonally packed units with low tunneling conductivity may correspond to lipid polar head groups, since their average center-to-center spacing (9.15 Å) is close to the lateral separation between molecules in lipid crystals. Scans showing less well ordered arrays with 4.5 Å spacings may correspond to the hydrophobic oleic acid chains, since this is a reasonable packing distance for hydrocarbon chains and close to spacings measured from previously published STM images of Cd-arachidate bilayers. Our images of DOPC and structurally related phospholipids will be correlated with analyses of x-ray diffraction data and transmission electron micrographs, by developing a computer-based simulation of STM images from model atomic coodinates.

**W-Pos320**

**FLUORESCENCE STUDIES OF LIPID INTERDIGITATION IN EXTRUDED VESICLES.**

**Hiroaki Komatsu, Peter T. Guy, and Elizabeth S. Rowe. Department of Biochemistry and Molecular Biophysics, University of Kansas Medical Center, and Veterans Administration Medical Center, Kansas City, MO 64111.**

**In recent years we have developed two fluorescence methods for detecting and characterizing the interdigitated phases which occur in phosphatidylcholines (PC's) in the presence of high concentrations of alcohols and other additives. These include DPH (diphenylhexatriene) intensity measurements [Nambi, P., Rowe, E.S., and McIntosh, T. M.,(1988), Biochemistry 27, 9175-9182], and pyrene-PC band intensity ratios [Komatsu, H K., and Rowe, E. S., (1990) Biochemistry, In Press). All of our previous studies have been on hand shaken multilamellar vesicles. Using these two methods we have now investigated the question of whether single lamellar vesicles can also interdigitate. The vesicles studied include sonicated unilamellar vesicles, and vesicles with various diameters produced by extrusion. The vesicles were characterized by freeze fracture electron microscopy and by QELS particle sizer. We have found that vesicles with diameters of 100nm or less do not interdigitate at 2.0M ethanol, but that vesicles with larger diameters can form the interdigitated phase.**

**(Supported by the Research Service of the Department of Veterans Affairs and by the NIAAA.)**

**W-Pos321**

RAMAN SPECTROSCOPIC STUDIES INDICATE TWO TYPES OF BILAYER INTERDIGITATION FOR C(18):C(10)PC IN THE BINARY EUTECTIC SYSTEM WITH C(14):C(14)PC James L. Slater*, Ching-hsien Huang** and Ira W. Levin* *Lab. of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892 and **Dept. of Biochemistry, Univ. of Virginia, Charlottesville, VA 22908

The novel binary mixture of two phosphatidylcholines C(18):C(10)PC and C(14):C(14)PC exhibits a eutectic phase diagram (1). As a consequence of the mixed-interdigitated gel phase packing of the C(18):C(10)PC, the gel phase bilayer thickness for these two components is significantly mismatched, resulting in lateral phase separation with two distinct gel phases coexisting over much of the composition range.

Utilizing Raman spectroscopy, we propose a molecular interpretation of this eutectic phase behavior. Specifically, when C(18):C(10)PC acts as a host gel phase, it retains the mixed-interdigitation (3 acyl chains per headgroup) reminiscent of its single component phase behavior. Therefore, the G(I) gel phase consists of mixed-interdigitated C(18):C(10)PC host accomodating the C(14):C(14)PC guests despite a predicted mismatch in bilayer thickness between these two gel phase components. In contrast, the C(18):C(10)PC molecules exhibit partial interdigitation (2 acyl chains per headgroup) in the G(II) gel phase when they are included as a guests within the C(14):C(14)PC host gel phase matrix. Compliance of C(18):C(10)PC to the requirements of the host gel phase generates a G(II) gel phase without any significant mismatch in bilayer thickness between these two components. One might wonder what prevents a single homogeneous phase with G(II) packing characteristics from forming throughout the entire composition range. The counterforce arises from the loss of the simple bilayer midplane in the G(II) phase, in which the terminal methyl groups of the C(14):C(14)PC experience a reduction in motional freedom.

1)Hai-nan Lin and Ching-hsien Huang (1988) Biochim. Biophys. Acta 946, 178-184

**W-Pos322**

THE DISTRIBUTION OF DECANE WITHIN THE UNIT CELL OF THE INVERTED HEXAGONAL ($H_{II}$) PHASE OF LIPID-WATER-DECANE SYSTEMS USING NEUTRON DIFFRACTION. D.C. Turner and S.M. Gruner, Dept of Physics, Princeton University.

The addition of a free alkane such as decane to lipid-water systems is known to promote the formation of the inverted Hexagonal ($H_{II}$) phase (Kirk and Gruner (1985) *J. Physique* **46**, 761). The decane is thought to promote the $H_{II}$ phase by reducing the hydrocarbon packing stress in the anisotropic unit cell by allowing the lipid chain environment to become more uniform (Kirk, Gruner and Stein (1984) *Biochem* **23**, 1093). Using neutron diffraction data to do a Fourier reconstruction of the $H_{II}$ phase of dioleoylphosphatidylethanolamine (DOPE) + water + deuterated decane it was found that the decane preferentially partitions into the interstitial regions of the $H_{II}$ unit cell where it should be the most effective in alleviating the hydrocarbon chain packing stress, supporting the suggestion of Kirk, *et al.* Using this distribution of decane within the unit cell we have calculated the lipid length distribution for the situations with and without added alkane. With a suitable molecular model this lipid length distribution may eventually be used to calculate the free energy change upon the addition of alkane. Such a measurement is an important clue to a more realistic understanding of the interactions which lead to the formation of the $H_{II}$ phase. Supported in part by NIH (grant GM32614) and DOE (grant DE-FG02-87ER60522-A000), and a Garden State graduate fellowship and NIH traineeship to DCT.

**W-Pos323**

**STRUCTURE AND THERMOTROPIC PROPERTIES OF D-ERYTHRO-N-PALMITOYL-GLYCOSYLSPHINGOSINES (CEREBROSIDES).** Kumkum Saxena, Richard R. Schmidt*, and Graham G. Shipley. Biophysics Department, Boston University School of Medicine, Boston, MA 02118, and *Fakultät Chemie, Universität Konstanz, D-7750 Konstanz, Germany.

A comparative study of the structural and thermal properties of the aqueous dispersions of totally synthetic D-erythro-N-palmitoyl-galactosyl-, glucosyl-, and lactosyl-$C_{18}$-sphingosines (NPGalS, NPGlcS, and NPLacS, respectively) has been done by differential scanning calorimetry (DSC) and X-ray diffraction. On heating, hydrated NPGalS undergoes an endothermic transition centered at 59 °C to produce a stable bilayer crystal form. X-ray diffraction at 70 °C reveals a lamellar bilayer structure (d=55.2 A°) with multiple wide angle reflections characteristic of an ordered hydrocarbon chain packing arrangement. Melting of this crystal structure to the bilayer liquid crystalline state (Lα; d=50.7 A°) occurs at 85 °C with ΔH=17.9 Kcal/mol of NPGalS. Similar behavior is exhibited by hydrated NPGlcS which undergoes the exothermic transition at 49 °C. The endothermic transition leading to the formation of Lα phase occurs at 87 °C. DSC and X-ray diffraction of hydrated NPLacS, show evidence of complex polymorphic behavior. When heated, hydrated NPLacS exhibits four endothermic transitions at 66, 69, 72 and 78 °C. The presence of the 78 °C transition is highly dependent upon the temperature and kinetics of the previous heating run. These studies show that NPGalS and NPGlcS exhibit very similar but complex thermal properties. Increasing the size of the sugar moiety of the cerebroside from a monosaccharide to a disaccharide has a marked influence on its structural properties.

**W-Pos324**

SOLUBILIZATION OF PHOSPHOLIPID LARGE UNILAMELLAR VESICLES BY CONJUGATED BILE SALTS. Charles H. Spink, Stephen Manley and Edwin Mereand, Chemistry Dept., State University of New York, Cortland, N.Y. 13045

Centrifugation experiments show that large unilamellar vesicles (LUV) of dipalmitoylphosphatidylcholine (DPPC) formed by dialysis of mixed micelles above the phase transition temperature fuse to larger vesicles when taurocholate (TC) is added to the phospholipid, and prior to solubilization. Solubilization to mixed micelles begins when the ratio of TC/DPPC is about 1/5. At temperatures below the phase transition the LUV's that form from dialysis of the mixed micelles are already quite large, and there is no evidence from the centrifugation experiments or turbidity increases that the vesicles fuse to larger size. Differential scanning calorimetry of the LUV's formed at low temperature indicates that binding of TC to the vesicles between 0 and 1/10 ratio of bile salt to lipid is simple aggregation of TC with the vesicle. Between ratios of 1/10 to 1/5, a new thermal transition appears, whose enthalpy increases at the expense of the main gel-liquid crystal transition of the DPPC. An analysis of these data indicates a complex between TC and DPPC could be responsible for the thermal changes. The stoichiometry of the complex is approximately 1/3. These results suggest that solubilization and vesicle expansion occurs because of disruption of lipid domains by the 1/3 complex between TC and DPPC. That is, vesicle expansion occurs to provide more area per vesicle for the 1/3 complex, and solubilization occurs when there is little free lipid to maintain the integrity of the vesicle. The fact that in TC/DPPC, as well as other bile salt-lecithin mixtures, the lipid is almost completely mixed micelles by a 1/3 ratio of bile salt to lecithin supports this arguement.

**W-Pos325**

**THE SIZE DEPENDENCE OF INTERDIGITATION**

Boni, L.[1], Minchey, S.[1], Perkins, W.[1], Ahl, P.[1], Tate, M.[2], Gruner, S.[2], and Janoff, A.S.[1].

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Ethanol has been shown to cause a biphasic melting behavior in saturated lecithins, a consequence of the formation of the stable interdigitated phase (Nambi et al., (1988) Biochemistry 27:9175-9182). The membrane systems studied to date have been large vesicle systems in which the membrane surface can be assumed to be planar. An immediate question arises as to whether surfaces of high curvature interdigitate. To address this question we have sought to determine the limiting size at which DPPC liposomes can interdigitate using ethanol as the inducer. We have found that with decreasing vesicle size the degree of interdigitation decreases while the concentration of ethanol required for the onset of interdigitation increases. Furthermore, with decreasing vesicle size, lipid mixing between vesicles increases as the ethanol concentration approaches that necessary for interdigitation. This results in the coalescence of small vesicles into a partially interdigitated matrix.

**W-Pos326**

PRESSURE FACILITATES THE FORMATION OF ALCOHOL-INDUCED INTERDIGITATED STRUCTURES IN DPPC LIPOSOMES. Junwen Zeng and Parkson L.-G. Chong. Dept. of Biochemistry, Meharry Medical College, Nashville, TN 37208.

The effects of pressure on the formation of alcohol-induced interdigitated gel phase in dipalmitoylphosphatidylcholine (DPPC) liposomes have been examined by fluorescence probe techniques. At 20°C and 1 atm, the ratio of fluorescence intensity at 435 nm to that at 510 nm of 6-propionyl-2-(dimethylamino) naphthalene (PRODAN) in DPPC (1:500), F435/F510, exhibits an abrupt decrease at 1.1 M ethanol and at 25 mM benzyl alcohol. This indicates that the polarity around PRODAN increases with alcohol concentration as a result of the formation of alcohol-induced fully interdigitated gel state and that PRODAN is a useful probe for detecting the phase transition from noninterdigitated to fully interdigitated structures. In the presence of 1.2 M ethanol or 35 mM benzyl alcohol and at 20°C, F435/F510 remains virtually unchanged at a level of about 0.3-0.6 in the pressure range of 0.001 - 2kbar. In contrast, at 20°C and in the absence of alcohols, F435/F510 decreases monotonically, with the F435/F510 values in the curve much higher than those obtained with alcohols. At 48-50°C, F435/F510, with or without alcohol, initially increases with pressure up to 0.5 kbar and then decreases with pressure. This abrupt change corresponds to the pressure-induced liquid crystalline-to-noninterdigitated gel phase transition. Above 0.5 kbar, F435/F510 decreases steadily with pressure, in a manner similar to that obtained at 20°C. These results suggest that pressure does not reverse DPPC liposomes back to the noninterdigitated gel state; instead, pressure stabilizes the interdigitated structure previously induced by alcohols, and that pressure and alcohols exert an additive effect on DPPC liposomes. The results obtained with PRODAN have been confirmed by using another probe, namely, 1-palmitoyl-2-(10-pyrenyl)decanoyl)-sn-glycerol-3-phosphatidyl-choline. (supported by NSF-MRCE & ARO).

**W-Pos327**

DETERMINATION OF THE EXCESS WATER POINT IN NON-BILAYER LIPID WATER MEMBRANES USING OPTICAL SCATTERING. P. T. C. So, M. Potters and E. Shyamsunder. Physics Department, Princeton University. – Determination of the excess water point (the maximum amount of water a given system can absorb at full hydration) of biological lipid-water systems is important for an understanding of the thermodynamics of such systems. We report a simple optical method to measure the temperature dependence of the excess water point. The method is based on the following idea: if the temperature of a sample with a single lipid-water phase is changed so that the system crosses the excess water point, bulk water will spontaneously form a coexisting second phase. The formation of the bulk water pools will alter the refractive index mismatch of the lipid-water phase domains with their environment, and can be observed by a simple light scattering technique. By measuring this *excess-water temperature* for several samples with different lipid-water weight ratios, one can can find the excess water point as a function of temperature. To illustrate the utility of the method, we have measured the excess water point of dispersions of dioleoylphosphatidylethanolamine (DOPE) and water, which form an the inverted hexagonal ($H_{II}$) phase. Comparison of the results with X-ray diffraction using both the Luzzatti method and direct Fourier reconstruction of the electron density show that the optical method is in excellent agreement with these more conventional techniques, and has the advantage of being simple to use. The method may be extended also to studies under high-pressure, and even to systems that cannot be studied using X-ray diffraction techniques, such as disordered systems which do not have a periodic lattice.

Support from the ONR, DOE and the NIH is gratefully acknowledged.

**W-Pos328**

**COLD SHOCK DAMAGE IS DUE TO THE LIPID PHASE TRANSITION IN CELL MEMBRANES.** E.Z. Drobnis, L.M. Crowe, P.J. Berger, T.J. Anchordoguy, J.W. Overstreet, J.H. Crowe. Departments of Zoology, Animal Science, Obstetrics & Gynecology, University of California, Davis, CA 95616.

When cells are cooled rapidly above the freezing point of water, they sustain irreversible injury. Reduction of this injury, termed "cold shock" (CS), could increase survival of animals and plants at low environmental temperatures and improve cryopreservation of plant and animal cells. Leakage of solutes across membranes, associated with thermotropic lipid phase transitions (LPT) in membranes, is thought to be responsible for CS, but this hypothesis has not been tested directly. Using FTIR spectroscopy, we measured LPT in intact, living spermatozoa, the animal cell in which CS has been studied most extensively. For sperm of several species, which vary in susceptibility to CS, LPT behavior was consistent with the temperature range over which rapid cooling is damaging as well as the extent of damage produced. Pig sperm, which have the highest known susceptibility to CS had a highly cooperative LPT with loss of motility detected at the LPT mid-point. Leakage of $K^+$ became pronounced at the end of the LPT when phase separation in membranes should be maximal. The pattern of LPT and $K^+$ leakage were similar for sperm of *Sicyonia ingentis*, a shrimp which spawns in 10°C sea water, but these events occured at appropriate lower temperatures. In human sperm, which have membranes relatively high in cholesterol, a definite LPT was difficult to detect and little loss of motility was associated with cooling. Two strains of mouse, which differ by 5°C in the temperature below which CS occurs, had LPT behavior consistent with this difference. This is the first direct evidence that CS results from LPT in cell membranes. Supported by NIH (HD25907-01), NSF (DCB89-18822) and Sea Grant (NA89AA-D-SG138).

**W-Pos329**

**MOLECULAR DYNAMICS OF LIPID NON-BILAYER PHASES**
**III. INTERFACIAL CONFORMATION**

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The lipid/water interfacial conformation of several single, binary and ternary lipid systems containing phosphatidylethanolamine was studied by the use of infrared spectroscopy. The C=O stretching frequency of the lipids has been shown to increase abruptly at the temperature- and composition- driven lamellar liquid crystalline ($L_\alpha$) to inverted hexagonal ($H_{II}$) phase transition in PE and PE/diacylglycerol lipid systems, respectively (Chem. Phys. Lipids, in press). The change in the C=O frequency is attributed to an alteration in the conformation of the lipid/water interfacial region at the $L_\alpha$ to $H_{II}$ transition. Besides the $L_\alpha$-$H_{II}$ transition, we have also studied the $L_\alpha$-I and I-$H_{II}$ transitions of other binary and ternary mixtures. Interestingly, the C=O frequency changes abruptly at the $L_\alpha$-I transition and only slightly at the $L_\alpha$-$H_{II}$ transition. We conclude that the alterations in the interfacial conformation start to occur when the lipids enter the I phase. To delineate the temperature and composition effects on these interfacial changes, 3-dimensional contour maps (temperature vs. composition) were employed to present the dependence of the lipid C=O frequency with temperature and lipid composition for the binary and ternary mixtures. The zone boundaries and transitions of non-bilayer phases were clearly demonstrated by the changes in the contour levels and patterns in these new C=O contour maps.

This work was supported by grants from NIH (CA 47610) and the Robert A. Welch Foundation (D-1158) and the Organized Research Fund of Texas Tech University (0096-44-7346) given to K.H.C.

W-Pos330

## MOLECULAR DYNAMICS OF LIPID NON-BILAYER PHASES II. INTRAMOLECULAR INTERACTIONS

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The intramolecular dynamics of lipids in non-bilayer phases was studied by steady state and time-resolved fluorescence spectroscopy. An intramolecular excimer forming lipid, di-(1'-pyrenemyristoyl) phosphatidylethanolamine (**dipyPE**), was employed to investigate the intra-lipid rotational diffusion and free volume of several single, binary and ternary lipid systems containing **PE**. From the measured fluorescence decays of both the monomer and excimer emissions of **dipyPE**, the intramolecular excimer formation rate constants ($k_E$)of the two pyrene moieties attached to the terminal methyl ends of the two myristic acids of a single **dipyPE** lipid were calculated for different lipid systems. The steady state excimer to monomer fluorescence intensities ratios (**E/M**) were also determined. Both $k_E$ and **E/M** were found to be very sensitive to the temperature- and composition-driven $L_\alpha$-$H_{II}$ transition of the **PE** and **PE**/diacylglycerol systems, respectively (Biophys. Chem., in press). For the binary and ternary lipid mixtures which exhibit intermediate (**I**) phases, these parameters were found to be very sensitive to the onset of the $L_\alpha$-**I** transition. Different strategies of separating the dynamic and geometrical contributions to the measured $k_E$ and **E/M** parameters will be discussed.

This work was supported by grants from NIH (CA 47610) and the Robert A. Welch Foundation (D-1158)given to K.H.C.

W-Pos331

## MOLECULAR DYNAMICS OF LIPID NON-BILAYER PHASES I. INTERMOLECULAR INTERACTIONS

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The molecular dynamics of lipid non-bilayer phases was studied by time-resolved fluorescence anisotropy technique. A fluorescent lipid, diphenylhexatriene-labeled phosphatidylcholine (**DPH-PC**), was used to investigate the intermolecular interactions of lipids in various single, binary and ternary lipid systems containing phosphatidyl ethanolamine. In addition to the lamellar ($L_\alpha$) phase, these lipid systems exhibit various interesting non-bilayer phases, such as, inverted hexagonal ($H_{II}$) and intermediate (**I**). Here **I** is in the form of either amorphous or packing defects depending on the lipid composition and temperature. A first order approximation (Biophys. J. 55:1025-1031) and several newly developed diffusion models (Biophys. J. 58, in press) were applied to obtain the orientational order parameters and diffusion constants (wobbling and hopping) of **DPH-PC** in the above lipid systems. All parameters were found to be very sensitive to the non-bilayer phase transitions ($L_\alpha$-$H_{II}$, $L_\alpha$-**I** and **I**-$H_{II}$), particularly the $L_\alpha$-**I** transition. The interfacial curvatures of the lipid systems were also estimated from the lateral diffusion and hopping diffusion constants as obtained from the pyrene excimer formation and the anisotropy measurements, respectively. The implications of these intermolecular parameters in explaining the molecular features of lipid non-bilayer phases will be discussed.

This work was supported by grants from NIH (CA 47610) and the Robert A. Welch Foundation (D-1158) given to K.H.C.

W-Pos332

## MAGNETIC RESONANCE IMAGING OF DIFFUSION USING PULSED MAGNETIC FIELD GRADIENTS

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Recent advances in quantitative magnetic resonance imaging (**QMRI**) have permitted characterization and evaluation of pathological disorders of tissues at the molecular level. Diffusion imaging represents one of the recently developed **QMRI** techniques. The potential of this new imaging modality in revealing the complex diffusional dynamics of water in tissues has not been fully explored. By incorporating a pair of pulsed magnetic field gradients into a normal spin echo imaging sequence, the effects of the magnitude (0-10 mT/m), directions (slice selection, phase encoding and/or readout axes) and time interval (35-250 ms) of the magnetic field gradients on the image intensity attenuations were quantitated at different echo times (85-500 ms). The pulsed magnetic field gradients were applied from the existing gradient coils of a clinical 1.5-Tesla magnetic resonance imager. Using different diffusion models, molecular dynamics parameters of water, such as lateral diffusion coefficients, diffusion anisotropy and fractal dimension, in various biological tissues and homogeneous polyethylene glycol solutions of different concentrations (controls) were obtained. In addition to the $T_1$ and $T_2$ relaxation times, the above-mentioned parameters allow us to construct a new class of synthetic images based solely on the diffusion properties of water in biological tissues.

This work was supported by grants from NIH (CA 47610), the Robert A. Welch Foundation (D-1158) and the Organized Research Fund of Texas Tech University (0096-44-7346) given to K.H.C.

W-Pos333

## FORMATION OF PROTEIN-VESICLE COMPLEXES: THE ROLE OF VESICLE AGGREGATION

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Membrane proteins have been demonstrated to transfer spontaneously from plasma membranes to dimyristoylphosphatidylcholine (DMPC) vesicles in a number of cell systems. Previous studies have shown this transfer to be insensitive to proteolytic cleavage of exofacial or cytoplasmic domains of the proteins involved. In this work we examined the effect on protein transfer of additional perturbations to the cells and vesicles. Human erythrocytes were incubated with sonicated DMPC vesicles labeled with [14C] dipalmitoylphosphatidylcholine. Protein-vesicle complexes (PVs) were purified by ultracentrifugation. The vesicle yield was determined from the 14C vesicle marker: the protein transferred was examined using SDS gel electrophoresis. In a series of experiments, modifications were made to the cytoskeleton, membrane deformability, surface area, cell age, redox state, cell and vesicle surface charge, and solution ionic strength. No significant difference in PV yield was evident except in those incubations where cell lysis increased or the redox state had been altered. This led us to examine the possibility that PV yield might be sensitive to cell lysate induced aggregation or fusion. Upon resonication of isolated PVs, both a PV and an unaltered vesicle fraction were recovered, suggesting a heterogeneous composition. Positive and negative staining transmission electron microscopy of isolated PVs showed that they were 25 nm vesicles similar in size to the original sonicated vesicles. Aggregation of PVs occured both during the cell-vesicle incubation at 37 degrees and during the low temperature centrifugation. Our results indicate that hemoglobin release, redox state, and centrifugation conditions all have an effect on the recovery of the aggregated PV complex. (Supported by NIH HL 23787 and by a William and Flora Hewlett Grant of Research Corporation. SIW is a GAANN fellow.)

W-Pos334

## INTERACTION OF A PEPTIDE FRAGMENT 828-848 OF THE ENVELOPE GLYCOPROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS TYPE I WITH LIPID BILAYERS

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The nature of the interaction of a peptide fragment corresponding to the 828-848 region of the intraviral cytoplasmic tail of gp 41 with model membrane bilayers was investigated. The peptide sequence is Arg-Val-Ile-Glu-Val-Val-Gln-Gly-Ala-Cys-Arg-Ala-Ile-Arg-His-Ile-Pro-Arg-Arg-Ile-Arg. Electrophoretic mobility studies indicate strong binding of the peptide, which has an excess positive charge of six, to negatively charged liposomes composed of 20 mol.% of DOPG in DOPC. No binding was detected for neutral membranes. Both CD and solution NMR studies show that the peptide adopts a random coil/extended chain conformation in solution which is modified only marginally upon binding to the lipid. Solid state 31P and 2H NMR studies demonstrate that the lipid matrix remains essentially unperturbed upon peptide binding. These results imply a model where there is a strong electrostatic peptide-lipid interaction, but a significant repulsive force prevents deep penetration of the peptide into the lipid matrix. The corresponding segment of the gp 41 could serve to tether the protein to the intracellular face of the viral membrane.

W-Pos335

## RELATIONSHIP BETWEEN AMPHIPATHIC α-HELICAL POTENTIAL IN PEPTIDES AND ACTIVITY AS SYNTHETIC LUNG SURFACTANTS.

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The interactions of a series of amphipathic α-helical peptides containing from 6 to 18 amino acid residues with dipalmitoylphosphatidylcholine (DPPC) were correlated with the activity of the peptides as synthetic lung surfactants when mixed with DPPC. In a lavaged lung model, amphipathic α-helical peptides were more active than other classes of lipid-binding peptides. Consistent with these data, peptides which were most effective as lung surfactants were also optimal in amphipathic α-helical properties. For example, active peptides rapidly decreased the turbidity of DPPC liposomes when mixed at the phase transition temperature. The extent of the clearing depended upon the length of the peptides; the most effective clearing was attained with peptides of 10-12 residues in length. Such peptides also dramatically reduced the enthalpy of the gel to liquid-crystalline phase transition of DPPC. Addition of DPPC liposomes to the peptides increased the α-helicity of the peptides as judged by CD and the single tryptophan was transferred to a less solvent-exposed environment in the presence of lipid, as evidenced by a blue-shift in the fluorescence emission maximum of the peptides. The effectiveness of the peptides in forming clear micellar structures from liposomes was related to the hydrophobic surface area of the peptides. An optimal surface area of 6-7.5 $nm^2$ was found for peptides most effective in liposome clearing. The free energy of transfer of this hydrophobic surface from an aqueous phase to nonpolar phase is just sufficient to exceed the unfavorable increase in free energy associated with restriction of the peptide to 2-dimensions in the peptide-lipid micelle. These data demonstrate that an effective lipid-binding amphipathic α-helix need only be 10 residues in length and that synthetic peptides containing amphipathic α-helical structures, in combination with DPPC, are effective synthetic lung surfactants.

W-Pos336

## CALORIMETRIC AND INFRARED SPECTROSCOPIC STUDIES OF THE INTERACTION OF AN AMPHIPHILIC PEPTIDE AND PHOSPHATIDYLCHOLINE BILAYERS.

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The interaction between the amphiphilic peptide $Lys_2$-Gly-$Leu_{24}$-$Lys_2$-Ala-Amide and a series of n-saturated diacyl-PC's (n=13,14,16,18 & 21) was studied by high-sensitivity DSC and FT-IR spectroscopy. The data indicate that the chain length dependent change in the effect of the peptide on the thermotropic phase properties of the lipids can be related to a mismatch between the bilayer thickness and the hydrophobic length of the peptide. Thus for short acyl chain lengths there is a net ordering of the hydrocarbon chains, whereas the hydrocarbon chains of long chain lipids are disordered. Moreover, there is poor lipid/peptide miscibility in bilayers composed of long chain PCs. FT-IR spectroscopy shows that the peptide remains in a predominantly α-helical conformation irrespective of temperature changes (up to 90°C) or the mismatch between the bilayer thickness and its hydrophobic length. However there is evidence for a small distortion of the α-helix concomitant with a change in the bilayer thickness. These observations are compatible with the so-called Mattress Model proposed by Mouritsen and Bloom (1984).

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**W-Pos337**

CONFORMATIONAL CHANGES THAT CONVERT LYMPHOTOXIN FROM A WATER-SOLUBLE PROTEIN TO A MEMBRANE-EMBEDDED STRUCTURE.

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This study was done to determine whether lymphotoxin (LT), a T-cell derived cytokine with sequence and structural homology to tumor necrosis factor (TNF), has the ability to interact with pure lipid membranes. Like TNF, LT was able to bind to dimyristoylphosphatidylcholine vesicles in a pH-dependent manner. Binding increased with decreasing pH. Intramembranous photolabeling studies revealed that binding was not limited to surface association with lipid head groups but included penetration of the hydrocarbon core of the bilayer. LT's ability to bind and penetrate a lipid bilayer was dependent not only on pH but also on the physical state of the target membrane. Although insertion was enhanced with solid phase lipid targets, binding efficiency showed the opposite behavior. Hence, binding and insertion represent two distinct physical processes. Conformational changes reflecting an increase in hydrophobicity at low pH were detected by intrinsic fluorescence and ANS binding assays. The acid-dependent changes in LT structure were very similar to changes exhibited by TNF, and no doubt serve to facilitate membrane binding and insertion. Interestingly, the trimeric form of LT was maintained after membrane insertion. The acquisition of hydrophobic characteristics through reversible acid-triggered transitions supplies a physical explanation for LT-membrane binding and insertion. [Supported by NIH Grant GM22240; RLB held an NIH Atherosclerosis Training Grant Award]

**W-Pos338**

RECONSTITUTION OF A SYNTHETIC PEPTIDE RESEMBLING PROTEIN SP-B IN PHOSPHOLIPID DISPERSIONS: STRUCTURE-ACTIVITY CORRELATIONS. A. J. Waring[1], R. L. Stevens[2], J.D. Young[2], R. Bruni[1], W. Taeusch[1]. Department of Pediatrics, King/Drew Medical Center, Los Angeles, CA 90059[1] and Peptide Synthesis Facility, Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024[2].

A seventy eight residue synthetic peptide with the same amino acid sequence as human surfactant protein SP-B was synthesized using the method of Merrifield employing Boc strategy. The crude peptide was purified by C4 reverse phase HPLC using a water-acetonitrile gradient containing 0.1% TFA. The expected molecular mass for residues 30-78 was obtained by FAB-Mass spectometry and the amino acid sequence was confirmed by sequencing from residue 1 to the end of the 78mer by an ABI 470A protein sequencer. Purified peptide was cosolvated with surfactant lipids (DPPC:POPG:palmitic acid, 68:22:8, wt:wt) in chloroform:methanol, 2:1, v:v with a molar ratio of peptide to lipid 1:100. The organic solvent was removed by nitrogen evaporation and the lipid-peptide film dispersed in 154 mM NaCl, 5 mM phosphate buffer pH 7.0 by incubation of the dispersion at 45°C under nitrogen with intermittent vortexing for one hour. Circular dichroism measurements of the reduced synthetic SP-B protein in surfactant lipids indicate about 70% alpha helix content. Similar amounts of helix for the reduced SP-B synthetic protein were observed in the structure promoting solvent TFE:phosphate buffer, 1:1, v:v, and 20 mM SDS micelles that mimic a membrane interface environment. Surface activity measurements of the lipid-synthetic SP-B protein dispersion using the Langmuir/Wilhelmy surface balance show rapid decreases in surface tension below 10 mN/m similar to dispersions containing native bovine SP-B protein. These observations suggest that synthetic SP-B with a high helix content in surfactant lipids correlates with high surface activity for the peptide-lipid dispersion.

**W-Pos339**

LIPID-PROTEIN ASSOCIATION AND PROTEIN FOLDING: FLUORESCENTLY LABELED APOLIPOPROTEIN A-II.

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Apolipoprotein A-II (apo A-II) is a major protein component of human plasma high density lipoprotein (HDL). Apo A-II associates with phospholipid to form recombinant HDL of characteristic size and stoichiometry. The lipid association results in an increase of secondary structure in apo A-II that is confirmed by circular dichroism spectroscopy. Apo A-II is a homodimer (MW 17,400) of known sequence (77 residues) linked through a sulfhydryl bridge at cysteine 6. To study lipid-protein association and its related protein folding, reduced apo A-II was labeled at cysteine 6 by the fluorescent probe 6-acryloyl-2-dimethylamino-naphthalene (Acrylodan). In solution, Acrylodan-apo A-II fluorescence occurs to the red (495nm) and is relatively short-lived (2-3nsec) and heterogeneous. Recombinant HDL composed of Acrylodan-apo A-II and dimyristoylphosphatidylcholine (DMPC) exhibits blue fluorescence and multiple decay associated spectra consistent with relaxation phenomena at temperatures above the lipid phase transition. Stopped-flow measurements of the association reaction show that fluorescence intensity changes biphasically in a temperature dependent manner. Frequency domain methods allow for resolution of fluorescence lifetimes (by phase shift and demodulation) during a reaction. Fluorescence lifetime kinetics of the lipid-protein association reaction support the observation of biphasic kinetics. These two phases may represent protein binding and subsequent, slower, conformation change. (Supported by NIH RR03155.)

**W-Pos340**

SEMI-SYNTHETIC PROTEINS AS A MODEL FOR THE STUDY OF PEPTIDE INSERTION INTO LIPID BILAYERS.

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A systematic examination into the mechanism of peptide insertion into bilayers can best be approached by the use of model peptides of graduated length and hydrophobicity. As a reference state, the peptide unit $(Ala)_{20}$ is used, since (1) it is the simplest of all $\alpha$-helix-forming amino acids, and (2) the length would be just long enough to span the bilayer (in an $\alpha$-helical conformation). Variations in length will help distinguish the membrane insertion properties of signal sequences (~12 residues) from those of transmembrane segments (~25 residues). To avoid insolubility problems inherent in small, hydrophobic peptides, they will be coupled to a water-soluble carrier protein, bovine pancreatic trypsin inhibitor (BPTI), which can be readily characterized biochemically and biophysically. By modifying the $\epsilon$-amino groups of the four lysine moieties in BPTI, a heterobifunctional cross-linker can be used that reacts covalently with the unmodified $\alpha$-amino group at the N-terminus (via a N-hydroxysuccinimide ester moiety) and with the free C-terminal cysteine group of the hydrophobic peptide (via a 2-pyridyl disulfide moiety) to form a semi-synthetic protein. Several control peptides (13-15 residues) with a terminal cysteine residue have been selectively attached to the modified BPTI; the extent of peptide conjugation was followed by spectroscopically monitoring the generation of 2-thiopyridone at 343 nm. Peptide-protein conjugates were separated from unreacted species and reagents by size exclusion HPLC (SE-HPLC). Model peptides of the general form $H\text{-}(Ala)_n\text{-}Tyr\text{-}Cys\text{-}CONH_2$ are being synthesized via Fmoc solid-phase synthetic strategies for the membrane interactive studies, and will be conjugated to BPTI in a similar fashion. The family of semi-synthetic proteins thus generated, differing only by the length of hydrophobic tail attached to the N-terminus, will be used in future studies to measure the thermodynamic and kinetic parameters of peptide insertion into lipid bilayers. Supported by NIH grant GM-14628 and a Whitaker Health Sciences Fund.

W-Pos341

ENDONEXIN MEMBRANE-BINDING AFFECTS LIPID DYNAMICS AS DETECTED BY PYRENE FLUORESCENCE. Matthew Junker and Carl E. Creutz, Biophysics Program, Univ. of Virginia, Charlottesville, VA 22908

Endonexin is a member of the annexin family of $Ca^{2+}$-dependent membrane-binding proteins. Endonexin binds to pure phosphatidylcholine (PC) membranes only at very high (>10mM) $Ca^{2+}$ concentrations but binds membranes containing other types of negatively charged phospholipids such as phosphatidylglycerol (PG) at much lower $Ca^{2+}$ concentrations. The interaction of endonexin with PG and PC can be studied using derivatives of these lipids that contain pyrene fluorophores in one acyl chain. The ratio of pyrene excimer to monomer fluorescence intensities (E/M) increases with the collision frequency of pyrene molecules, and so provides a sensitive measure of the mobility and local concentration of pyrene labelled lipids. Endonexin binding to pure PG (97% PG/3% pyrene-PG) vesicles at 2mM $Ca^{2+}$ caused a significant increase in monomer and decrease in excimer pyrene fluorescence suggestive of reduced PG mobility upon endonexin-membrane binding. This decrease in E/M was $Ca^{2+}$ dependent, and could be titrated with protein to a maximum change of 31%. The protein titration was similar to protein titrations of endonexin-membrane binding detected by an energy transfer assay. At 10mM and less $Ca^{2+}$, endonexin did not affect the E/M of pyrene-PC in pure PC (95%PC/5% pyrene-PC) vesicles. Increasing the mole % PG in PC/3% pyrene-PG vesicles from 10% to 97% resulted in progressively larger maximum changes in E/M, with a 3-4 fold decrease between 10% and 50% PG and only a 15-20% further decrease between 50% and 97% PG. Small increases in E/M were observed with 90% PC/10% pyrene-PG vesicles, suggesting that endonexin-membrane binding can cause some lipid segregation as well as reduced lipid mobility.

W-Pos342

ORIENTATION OF MELITTIN IN PHOSPHOLIPID BILAYERS, A POLARIZED ATTENUATED TOTAL REFLECTION INFRARED STUDY.

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The orientation of the 26-residue amphiphilic bee venom peptide melittin was measured in dry phospholipid multibilayers (MBLs) and in fully hydrated single supported planar bilayers (SPBs) by polarized attenuated total reflection infrared spectroscopy (ATR-IR). Melittin adopted an α-helical conformation in MBLs of dipalmitoyl-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), a 4:1 mixture of POPC and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), and in SPBs of POPC:POPG (4:1). The orientation of the α-helix in the bilayers depended on the hydration, but not on the lipid composition of the bilayers. In dry MBLs the α-helix long axis of melittin was oriented parallel to the bilayer normal. In fully hydrated SPBs, however, the α-helix of melittin was oriented parallel to the plane of the membrane. This study represents the first IR spectroscopic investigation of the orientation of melittin under conditions of full hydration (i.e. in $D_2O$) and opens new possibilities for studying the structure of other proteins under native conditions.

W-Pos343

MELITTIN INTERACTION WITH LIPID BILAYER.
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The binding of bee venom melittin to DMPC (dimyristoylphosphatidylcholine) unilamellar vesicles was studied with fluorescence spectroscopy. The fluorescence of the tryptophan and its quenching by acrylamide and iodide were used as indicators of protein penetration in lipid bilayers. The influence of temperature on melittin binding to lipid bilayer was measured. The blue shift of the tryptophan emission maximum increases at the DMPC phase transition at 22° C and does not change further at higher temperatures. The efficiency of quenching tryptophan fluorescence by acrylamide decreases slightly with increased temperature. However, the quenching efficiency of tryptophan fluorescence with KI shows five fold increase in the quenching at the lipid phase transition and falls again to pretransition level at temperatures above the phase transition. The possibility of iodide penetration into the hydrocarbon part of the membrane was determined by using pyrene-phosphatidylcholine (pyrene was attached to the 10th carbon of the sn-2 chain) labelled DMPC vesicles. The quenching efficiency of pyrene by iodide, again, shows a maximum at the lipid phase transition. Therefore, we conclude that the iodide penetrates deep into the membrane at phase transition through an increased number of defects. The data shows, in the presence of lipid vesicles, that the tryptophan residue of melittin is inaccessible to the bulk water. The magnitude of observed changes in quenching efficiency upon bilayer phase transition (5-6 times) provides a sensitive technique to probe the lipid organization in membranes.

W-Pos344

SPECTROSCOPIC INVESTIGATION OF THE INTERACTIONS OF NEUTRAL AND ACIDIC PHOSPHOGLYCERIDES WITH THE CATIONIC ANTIMICROBIAL PEPTIDES, POLYMYXIN B AND MAGAININ 2

Melody Ferguson, Fazale Rana, Kelly Schumacher and Jack Blazyk (intro. by Paul Sullivan), Chemistry Department, Molecular and Cellular Biology Program, and College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701.

The ability of two small cationic peptides, polymyxin B and magainin 2, both of which possess potent antimicrobial activity, to bind to and alter the fluidity of phosphoglyceride bilayers was studied by FT-IR spectroscopy. A major feature of the structure of biological membranes is the presence of a lipid bilayer, in which phosphoglycerides are usually a major component. The center of the bilayer is composed of a hydrophobic core formed by the fatty acyl chains of the lipid molecules. Controlling the fluidity of this hydrocarbon region, which is determined by the nature of the fatty acids and polar headgroups and influenced by external factors such as temperature and the binding of exogenous molecules, is critical to the survival of the organism. Two members of the phosphoglyceride family are phosphatidylcholine (PC), which possesses a neutral zwitterionic polar group, and phosphatidylglycerol (PG), which is negatively charged at neutral pH. Both polymyxin B and magainin 2 are believed to exert their antimicrobial activity by disrupting the native membrane structure. Using FT-IR spectroscopy, the effect of the peptides on the thermotropic phase behavior of lipid bilayers containing PC, PG, and mixtures of the two lipids (in which the fatty acyl chains of one of the lipids were perdeuterated) was determined by monitoring changes in the peak position and bandwidth of the symmetric methylene C–H stretching band as a function of temperature. Polymyxin B disorders PG bilayers while having little or no effect on PC. Magainin 2 increases the phase transition temperature of both PC and PG, but at high peptide levels, the fatty acyl chains of PG are highly mobile at all temperatures. Amide I bands were analyzed to detect changes in the secondary structure of the peptides as they interact with these lipids. The mechanism of interaction between the lipids and peptides is discussed in light of these data.

**W-Pos345**

SHORT-CHAIN PYRENE-PC AS A PROBE OF PHOSPHOLIPASE ACTIVITY. Jinru Bian & Mary F. Roberts. Department of Chemistry, Boston College, Chestnut Hill MA 02167

A new lipid analogue, 1-hexanoyl-2-[1-pyrenebutyroyl]-phosphatidylcholine (1-$C_6$-2-$C_4$Pyr-PC) has been synthesized as a probe of water soluble phospholipase activity. This molecule forms micelles in aqueous solution with a critical micelle concentration of 0.009 mM. Like other short-chain PC molecules it can induce formation of small bilayer particles with gel state long-chain PC's such as dipalmitoyl-PC. Either as a pure micelle or in combination with dipalmitoyl-PC in the small bilayer aggregates it serves as an excellent substrate for phospholipase $A_2$. Both products, the fluorescent fatty acid (1-pyrenebutyric acid) and the lyso-PC, have cmc's higher than the parent lipid and will partition as monomers into the aqueous phase. Activity can then be monitored by following the increase in monomer fluorescence of 1-pyrenebutyrate as a function of time. One of the problems in interpreting phospholipase kinetics is distinguishing between how a molecule as a monomer or the same species solubilized in a micelle, particularly a species that could be inhibitory, interacts with the enzyme. This can be determined with the 1-$C_6$-2-$C_4$Pyr-PC assay system. For example, we have examined how other short-chain lipid substrates and lyso-PC species which are monomeric around 0.1 mM affect the activity of the enzyme. Monomeric lysolecithins and the asymmetric species 1-lauroyl-2-acetyl-PC are good inhibitors of the enzyme-catalyzed hydrolysis of 1-$C_6$-2-$C_4$Pyr-PC. The effect of the other enzyme product, free fatty acid, on 1-$C_6$-2-$C_4$Pyr-PC hydrolysis can also be be compared. In general monomeric amphiphiles with two chains (e.g., di$C_7$PC) are not good inhibitors under these conditions while single chain amphiphiles are quite potent. This reflects a difference in binding affinity for two-chain versus single chain amphiphiles. The results of these fluorescence assays at low PC concentrations are compared to results from pH-stat assays at high PC levels where micellar lyso species and free fatty acids are found to be only slightly inhibitory. This information is then used to generate a picture of relative affinities of lipids for the phospholipase $A_2$ molecule.

**W-Pos346**

EFFECT OF SATURATED DIACYLGLYCEROL ON THE ACTIVATION OF PHOSPHOLIPASE $A_2$ ON THE SURFACE OF PHOSPHATIDYLCHOLINE BILAYERS. J.D. BELL, S.M. BAY, S.D. BROWN, K.P. COLLINS, B.A. PADGETT, and R.P. WARD. Department of Zoology, Brigham Young University, Provo, Utah 84602.

Diacylglycerol has been suggested as a possible intracellular mediator of hormone stimulation of phospholipase $A_2$. The effect of dicapryl glycerol on the time course of hydrolysis of multilamellar vesicles of dipalmitoylphosphatidylcholine by the basic aspartate-49 phospholipase $A_2$ from *Agkistrodon piscivorus piscivorus* venom was examined to determine possible mechanisms of such regulation. The hydrolysis time course in the absence of diacylglycerol is characterized by an initial slow rate of hydrolysis followed by a sudden and rapid increase in enzymatic activity. The initial time dependence of the reaction has been interpreted as slow activation of the enzyme upon interaction with the vesicles. The sudden increase in activity presumably results from structural perturbation of the lipid bilayer by the hydrolysis products (fatty acid and lysolecithin). Maximum activity is achieved most quickly when the vesicles are at the temperature of the gel-liquid crystalline phase transition (41.5 °C). At 37 or 39 °C, dicapryl glycerol decreases the length of time before the sudden increase in enzyme activity and increases the maximum rate of hydrolysis. These effects are maximal at approximately 10 mole percent diacylglycerol. Higher concentrations are inhibitory. Similar effects are found at 45 °C, although the dependence on diacylglycerol concentration is quantitatively different; 1 mole percent is optimal for activation and higher concentrations are inhibitory. These effects can be explained partly by the effect of diacylglycerol on the lipid phase transition temperature. However, additional effects on the structure and dynamics of the bilayer also appear to be important. Fluorescence spectroscopy and differential scanning calorimetry were used in attempt to study the effects of diacylglycerol, temperature, and the hydrolysis products and correlate them with the kinetics of phospholipase $A_2$ activation.

**W-Pos347**

EVIDENCE FOR COMPOSITIONAL PHASE SEPARATION IN LIPID VESICLES DURING PHOSPHOLIPASE A2 CATALYSED HYDROLYSIS
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Previous work has suggested that the reaction products of phospholipase A2 hydrolysis induce a cooperative structral change in lipid vesicles which promotes a rapid increase in phospholipase A2 activity. In order to explore the details of this putative lipid structural change, high sensitivity differential scanning calorimetry has been employed to characterize the phase behavior of dipalmitoylphosphatidylcholine in the presence of various amounts of palmitic acid and lysophosphatidylcholine. The ternary lipid phase diagram exhibits complex behavior. At about 7% of reaction products an abrupt broadening of the heat capacity function is observed, a result consistent with phase separation of the components of the vesicles. Fluorescence has been employed to further characterize the system using a pyrene labelled fatty acid probe (1-pyrenyldecanoic acid). The excimer/monomer ratio (E/M) increases abruptly during the phospholipase A2 hydrolysis process, suggesting preferential partitioning of pyrene fatty acid into palmitic acid rich domains. This increase in E/M is temporally correlated with the onset of the rapid increase in hydrolytic activity. These results are consistent with our previous proposition that the temporal sequence of events in the induction of rapid PLA2 hydrolysis in lipid vesicles involves a cooperative structural change in the vesicle, which, in turn induces activation of the surface-bound enzyme. Furthermore, this change in the vesicle is a compositional and structral phase separation process.(Supported by the NIH Grant GM 5-35583)

**W-Pos348**

BINDING OF PHOSPHOLIPASE C-$\delta$ TO PHOSPHOLIPID BILAYERS. Arnold A. Peterson, Mario J. Rebecchi, and Stuart McLaughlin. Dept. of Physiology & Biophysics, SUNY, Stony Brook, NY 11794-8661.

Phosphoinositide-specific phospholipase C, PLC, a key enzyme in many signal transducing pathways, is found in both the cytoplasmic and plasma membrane fractions of cells. We wished to determine if the $\delta$ isozyme of PLC binds to the lipid component of membranes, and if so to identify those lipids important for binding. We mixed PLC-$\delta$ with multilamellar vesicles, sedimented the vesicles, and assayed the residual PLC activity in the supernatant. PLC-$\delta$ did not bind measurably to vesicles composed of the zwitterionic lipid PC, but bound increasingly to PC:PS or PC:PI vesicles as the percent anionic lipid increased. We define the affinity constant for PLC-$\delta$ binding to a vesicle surface, $K_a$ = (PLC)/[PLC], where (PLC) = number of bound PLC/area of membrane and [PLC] = free PLC concentration in the bulk aqueous phase. The $K_a$ was approximately 0.1 $\mu$m for vesicles composed of a 3:1 ratio of PC:PS or PC:PI, and 1 $\mu$m for vesicles of pure PS or PI. PLC-$\delta$ bound more strongly to PC:$PIP_2$ than to PC:PS or PC:PI vesicles. We also measured the binding of basic peptides, including one corresponding to a conserved portion of the sequence of PLC-$\delta$ containing 5 basic residues, to phospholipids. The pentavalent peptides did not bind to PC vesicles, bound in increasing amounts to vesicles containing increasing amounts of anionic lipids, and bound more strongly to PC:$PIP_2$ than to PC:PS vesicles. These results are consistent with the hypothesis that a cluster of positive charges on PLC-$\delta$ may bind negative lipids. Supported by NIH grant GM-24971 and NSF grant DMB9044656 to SM, and NIH Grant BM-43422 to MJR.

**W-Pos349**

## MODULATION OF THE STRUCTURE AND STABILITY OF FERRICYTOCHROME C BY ACIDIC PHOSPHOLIPIDS.

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The effect of membrane binding on the structure and stability of ferricytochrome c was studied by Fourier-transform infrared spectroscopy, circular dichroism and differential scanning calorimetry. Association of cytochrome c with phospholipid membranes containing phosphatidylglycerol or cardiolipin results in only slight, if any, perturbation of the protein secondary structure. However, upon membrane binding there is a pronounced increase in the accessibility of protein backbone amide groups to hydrogen-deuterium exchange, which suggests a lipid-mediated "loosening" and/or destabilization of the protein tertiary structure. A lipid-induced conformational perturbation of ferricytochrome c is also indicated by a marked decrease in the thermodynamic stability of the membrane-bound protein. The temperature of a major denaturation step decreases from 82°C for ferricytochrome c in solution to 52-55°C for the protein bound to membranes containing phosphatidylglycerol as a single lipid component. For ferricytochrome c bound to membranes containing the mixture of acidic and zwitterionic phospholipids, the extent of structural perturbation depends on the surface density of negatively charged lipid head groups. The observed destabilization of protein structure mediated by acidic phospholipids (and possibly formation of folding intermediates at the membrane surface) may represent a general property of a larger class of water soluble proteins for which the membrane binding is governed by electrostatic forces.

**W-Pos350**

## Cytochrome $b_5$/DMPC Bilayer Structure and Physical Dynamics.

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Cytochrome $b_5$ is a microsomal membrane bound protein which, through it's interaction with $b_5$ reductase, provides reducing potential to the Δ5-, Δ6- and Δ9-fatty acid desaturases. Cytochrome $b_5$ membrane binding is accomplished via the NonPolar Peptide (NPP) portion of the molecule at the -COOH terminus. This protein must interact with a variety of other membrane bound proteins which differ in bilayer mass distribution. We have been attempting to evaluate the structural interaction between DMPC and $b_5$ to discern protein mass distribution about the bilayer normal and the effects of reconstitution on bilayer physical dynamics. We will present x-ray diffraction data further refined to ~6 Å resolution and discuss its correlation with acyl chain packing, fluorescence anisotropy and calorimetry results. Our data indicate that the NPP does not penetrate beyond the center of the bilayer. This result is consistent with data previously obtained in this laboratory and others. Additionally, equitorial scatter experiments yield no evidence of a 1/10 Å reflection indicative of α-helical structures paralell to the bilayer normal. The data also suggest that asymmetric $b_5$ reconstitution increases acyl chain order. This effect was observed both above and below the DMPC thermal phase transition. We are currently attempting to place our membrane profile structures on an absolute electron density scale by using specifically iodinated $b_5$ derivatives which will, hopefully, allow us to obtain difference profile constructs and location of the -COOH terminus. These studies were supported by NIH GM-15924.

**W-Pos351**

## INCORPORATION OF CYTOCHROME b5 INTO INTERDIGITATED C(18):C(10)PHOSPHATIDYLCHOLINE.

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The incorporation of the membrane bound protein cytochrome b5, isolated from rat liver, into interdigitated C(18):C(10)phosphatidylcholine [C(18):C(10)PC] was investigated using gel chromatography. C(18):C(10)PC is an asymmetric mixed-chain phospholipid, known to form highly ordered mixed-interdigitated bilayers below the transition temperature (Tm) and partially interdigitated bilayers above the Tm. Known amounts of purified cytochrome b5 were incubated with multilamellar C(18):C(10)PC vesicles at temperatures above (37°C) and below (4°C) the Tm (18.4°C). The unincorporated cytochrome b5 was then separated out from the vesicles on a 1 x 20 cm Sepharose CL-2B column. The partition coefficient of cytochrome b5 into C(18):C(10)PC was determined from the absorbance of cytochrome b5 and Bartlett's phosphate assay. The results showed that cytochrome b5 can insert itself into highly ordered interdigitated lipid structures. However, slightly more cytochrome b5 partitions into C(18):C(10)PC above the Tm (1:62) than below the Tm (1:42). This result is expected since the ability of any foreign molecule to insert itself spontaneously into the highly ordered mixed-interdigitated bilayers would be expected to be more difficult. This implies the existence of an inhomogeneous protein distribution in a biological membrane which contains local domains enriched with interdigitated lipids.
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**W-Pos352**

## SPONTANEOUS INCORPORATION OF SALT-EXTRACTED INTEGRAL MEMBRANE PROTEINS INTO PREFORMED VESICLES

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The spontaneous reconstitution of phosphatidylinositol (PtdIns) synthase (E.C. 2.7.8.11) and other membrane proteins extracted from rat pituitary $GH_3$ cell membranes into preformed unilamellar vesicles (SUVs) of dimyristoylphosphatidylcholine and cholesterol was examined. The $GH_3$ cell membrane proteins were extracted with 3 M KCl without detergent. In the absence of detergent, they spontaneously inserted into gel phase SUVs and remained stably incorporated, as demonstrated by equilibrium density gradient centrifugation. This direct reconstitution of a salt-extracted PtdIns synthase into preformed SUVs has shown that the enzyme is an integral membrane protein and that the protein retains its hydrophobic character after salt extraction. The reconstitution of PtdIns synthase and other integral membrane proteins in the salt extracts of GH3 membranes is consistent with the idea that the ability of hydrophobic membrane proteins to insert into preformed bilayers is related, in part, to a general, intrinsic property of the proteins. (Supported by NIH grants GM-36651 and DK-33468).

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**W-Pos353**

## INTERACTION OF THE CATIONIC EOSINOPHIL MAJOR BASIC PROTEIN (MBP) WITH LIPOSOMES.

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MBP, one of the eosinophil cationic granule protein (pI> 11), is toxic to a number of mammalian cells and helminths, the mechanism of its toxicity is not known. We hypothesized that MBP mediates its toxicity by interacting with and disrupting the lipid bilayer of its targets. Liposomes prepared from from synthetic phospholipids [labeled with the fluorescent probe diphenyl hexatriene (DPH)] were used as targets for MBP. The interaction of MBP with the liposomes was studied utilizing anisotropy and polarization measurements, electron microscopy (EM), and radioimmunoassy (RIA). MBP caused a change in the temperature transition profile of liposomes made from the negatively charged palmitoyl oleoyl phoshatidyl serine (POPS), as well as liposomes made from an equimolar mixture of dimyristoyl phaspatidyl choline (DMPC, neutral) and dimyristoyl phosphatidic acid (DMPA, negatively charged). A significant dose-dependent reduction in the polarization measurements of those lipids [at temperatures below the transition temperature ($T_c$)], is seen with MBP concentrations from 1 to 10 $\mu$M, at a constant lipid concentration of 2 mM. Above the $T_c$, at 1:200 MBP:lipid molar ratio, the MBP/POPS, and MBP/DMPC/DMPA mixtures precipitate. This precipitate is composed of both MBP, as measured by RIA, and lipids, as measured by the intensity of emission of DPH. The measured levels of MBP in those mixtures, however, is lower than the added MBP, in control experiments the addition of lipids has no effect the MBP RIA. Negative staining EM studies show that MBP causes aggregation of the liposomes. In contrast, MBP did not affect the tepmperature transition profile of liposomes made of the neutrally charged DMPC or palmitoyl oleoyl phosphatidyl choline; nor did it affect micelles made from monooleoyl phosphatidyl choline. The results indicate that MBP strongly interacts with negatively charged lipid bilayers in the absence of other membrane proteins.