Tu-Pos236

^{1}H NMR STUDIES OF THE FOLATE AND METHOTREXATE COMPLEXES OF DHFR FROM *E. COLI*. Christopher J. Falzone‡, Peter E. Wright†, Marlon Cowart‡, and Stephen J. Benkovic‡. (Intro. by William D. Taylor). ‡Department of Chemistry, The Pennsylvania State University, University Park, PA 16802. †Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Sequence-specific assignments have been made for about 30% of the amino acid side chains in the folate[1] and methotrexate complexes using a combination of site-directed mutagenesis and a knowledge of the X-ray crystal structure[2]. As a consequence of these assignments, the orientation of both the pterin ring of folate and the pteridine ring of methotrexate could be determined based on NOEs to the protein. The H-7 proton on the pterin ring of folate showed NOEs to Ile-5 and Ile-94 which indicated an orientation similar to a productive dihydrofolate complex. Methotrexate binds in an 'upside-down' orientation and showed NOEs to Leu-28 in the active site. In the folate complex, only one bound form of the pterin ring could be detected. This was confirmed in heteronuclear experiments using ^{13}C-7/^{13}C-9 labeled folate. In contrast to the folate complex, the binary methotrexate complex presented two bound forms. In addition, many of the amino acid side chains revealed doubled resonances with these residues located in the β-sheet and the active site. NOESY experiments at 323 K showed that these two forms are interconverting slowly on the NMR time scale. Preliminary studies on the apoprotein also revealed doubled resonances in the absence of inhibitor indicating the existence of two protein isomers. Apparently, methotrexate has an affinity for both protein isomers whereas folate can bind only one form. (Supported by NIH grants GM 36643 (PEW) and GM 24129 (SJB). CJF is supported by a fellowship from Merck, Sharp & Dohme.)

1. Falzone, C. J., Benkovic, S. J., & Wright, P. E. *Biochemistry*, in press.
2. Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., & Kraut, J. (1982) *J. Mol. Biol. 257*, 13650-13662.

Tu-Pos237

PRELIMINARY ASSIGNMENTS OF THE ^{1}H-RESONANCES OF RESIDUES AT THE METAL BINDING SITE IN CHEY

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CheY is a 14 kD cytoplasmic protein that is activated by the transfer of a phosphoryl moiety to Asp57 from phospho-CheA during signal transduction in bacterial chemotaxis. It has been established that metal ions play an important role in the phosphorylation and dephosphorylation of CheY. We have used two-dimensional NMR methods to assign the aromatic resonances of CheY in the metal-free and metal-bound conformations. In addition, sequential assignment has been obtained for an octapeptide segment including the phosphorylation site, Asp57. A T87I mutant has been used to assign Thr87. It is shown that these preliminary assignments are sufficient to probe the conformational change that occurs at the phosphorylation site on metal binding. Comparison of chemical shifts show that in addition to Trp58 (next to Asp57), Thr87 and Tyr106 are significantly affected by the metal binding. Comparison of NOESY spectra for the metal-free and metal-bound forms show that the conformational change is limited to the residues close to the metal binding site. A hydrophobic cluster of phenylalanines (8, 30, 53 and 124), which give rise to a network of long range NOESY cross-peaks, remain unaffected by the conformational change. These results are compared with the the high resolution X-ray structure of metal-free CheY (Volz and Matsumura, manuscript in preparation).

Tu-Pos238

SEQUENCE-SPECIFIC ^{1}H-NMR ASSIGNMENTS, SECONDARY STRUCTURE, AND LOCATION OF THE CALCIUM BINDING SITE IN THE FIRST EGF-LIKE DOMAIN OF FACTOR IX. L. Huang, H. Cheng, W. Sweeney, A. Pardi*, J. Tam#. Dept. Chemistry, Hunter College, N.Y., NY 10021; *Dept. Chemistry, Univ. Colorado, Boulder, CO 80309. Dept. Biochemistry, #The Rockefeller Univ., N.Y., NY 10021;

A wide range of proteins have been found to contain EGF-like domains, including blood clotting factors VII, XI, X, and XII. While the function of these domains is largely unknown, the first EGF-like domain of factor IX (human factor IX (45-87)) has been shown to bind calcium. Two-dimensional NMR techniques have been used to determine the sequence-specific assignments and the secondary structure of this domain. Two antiparallel β-sheet structures are found: a major sheet, involving residues 16-20 and 25-29, and a shorter β-sheet at the N-terminal end. Neither β-turn appears to be a classical type I or II turn. Preliminary distance geometry studies indicate that its tertiary structure is very similar to that of EGF. Studies of the peptide in the presence of 20mM calcium find that only 12 of 43 residues exhibit resonances which are shifted by more than 0.02 ppm. This demonstrates that the protein does not undergo any major changes in conformation as a result of calcium binding. Preliminary distance geometry calculations, in conjunction with a consideration of the specific amino acid residues which exhibit calcium-dependent chemical shift changes, yield a model for the calcium binding site (supported by NIH HL41935).

Tu-Pos239

CONSTRUCTION AND APPLICATION OF A SECONDARY STRUCTURAL "MAP" CORRELATING ^{1}H NMR CHEMICAL SHIFTS TO SECONDARY STRUCTURAL ELEMENTS. Lisa M. Smith and Joyce E. Jentoft. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

A secondary structural "map" of the ^{1}H NMR spectrum was constructed by rigorous statistical analysis of a database of 3280 assigned proton resonances from 11 proteins for which complete NMR assignments and structures have been obtained. This analysis involved the division of the spectrum into two regions, one which includes primarily C^α proton resonances (3.70 - 6.13 ppm), and one which includes primarily side chain carbon proton resonances (-3.75 - 3.70 ppm). The distribution of secondary structural elements in each of these two regions of the spectrum was analyzed statistically by the use of a Runs Test Analysis and the Conservative Chi-Squared Analysis for Sparse Data to identify small windows of the spectrum having a distribution of secondary structural elements that is statistically significantly different from that of the entire region. The "map" of these significant windows in the ^{1}H NMR spectrum that was derived from this analysis was then applied to ^{1}H NMR data obtained from the nucleocapsid protein (NC) from avian myeloblastosis virus (AMV). NC is the protein responsible for the recognition and packaging of the viral genomic RNA molecules into the budding AMV virions. All known retroviruses, including HIV, have NC proteins that carry out these functions. Although AMV NC is the best characterized of all the retroviral NC proteins, little is known about its structure in solution. Circular dichroism experiments have shown AMV NC to have no helical structures, and to be 30 - 50 % beta sheet and 50 - 70 % random coil and turn structures (Jentoft, et al., (1988) PNAS 85, 7094 - 7098 and Gelfand and Jentoft, personal communication). thus, further characterization of AMV NC by ^{1}H NMR has been undertaken. (Supported by GM 36948.)

Tu-Pos240

NMR STUDIES OF SINGLE-SITE MUTANTS OF HUMAN EPIDERMAL GROWTH FACTOR (hEGF).

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Site directed mutagenesis and NMR spectroscopy have been combined to examine the roles of several residues of hEGF in determining its structure and biological activity. These studies provide structural and functional data which are being used to map out the receptor recognition site of hEGF. The mutant proteins have been expressed in E. coli and purified as described previously[1]. Biological activities have been measured in both receptor binding and receptor kinase assays[1]. NMR data have been obtained in D_2O at both pH 7, to examine the effects of the mutations at physiological pH, and at pH 3 to examine the effects of mutations on the rates of exchange of amide protons involved in hydrogen bonds of β-sheets. Although several mutations at positions Arg-41 and Leu-47 result in greatly diminished binding activities, the mutated proteins have NMR spectra and rates of hydrogen-bonded amide proton exchange which are nearly indistinguishable from the wild-type. These data indicate that the reduced biological activities of these mutants are due to their essential role in the function of hEGF, rather than to significant changes in the overall structure of the protein. Mutations at positions Tyr-13 , Ile-23, Leu-26, and Tyr-37 which also result in diminished biological activities exhibit some differences in the NMR spectra and / or increased rates of amide proton exchange compared to wild-type hEGF. These studies of the biological activities and NMR spectra of single-site mutants combined with comparisons of the solution structures of human and murine EGF provide information about the spatial distribution of atoms involved in recognizing the EGF receptor. Our progress in completing more detailed structural studies of wild-type and mutant hEGF molecules will be presented.

1. Engler, D. A.; Matsunami, R. K.; Campion, S. R.; Stringer, C. D.; Stevens, A.; Niyogi, S. K. **1988** J. Biol. Chem. 263, 12384-10390.

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Tu-Pos241

DELETION OF THE OMEGA LOOP AT THE ACTIVE SITE OF STAPHYLOCOCCAL NUCLEASE AND ITS EFFECT ON PROTEIN STRUCTURE AND DYNAMICS BY HETERONUCLEAR, 2D AND 3D NMR SPECTROSCOPY.

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We have used multidimensional, heteronuclear NMR techniques to probe the structure and dynamics of the wild-type enzyme, Staphylococcal nuclease (E43 SNase), and three mutants of the protein. These mutants are : a) D43 SNase, made by a Glu - Asp substitution at position #43, b) E43 ΔSNase, resulting from a deletion of residues 44-49 from the flexible, active site Ω loop of the wild type enzyme and c) D43 ΔSNase, resulting from a deletion of residues 44-49 from the E43D point mutant . All four enzymes are complexed to an irreversible inhibitor, 3',5' - thymidine bisphophate, and a calcium ion. Deletion of residues 44-49 from the sequence of E43 SNase results in an enzyme (E43 ΔSNase) that is significantly more active than D43 SNase which differs from the wild-type enzyme by deletion of a single methylene group. In addition, both E43 ΔSNase and D43 ΔSNase are significantly more stable than their respective parent enzymes.

We have used high resolution 2D and 3D NMR spectroscopy to characterize the solution conformations of the four enzymes in order to better understand their variations in stability and activity. The backbone assignments of E43 SNase were extended to the three mutant proteins (uniformly ^{15}N enriched) using 2D HSQC, 3D HOHAHA-HMQC and 3D NOESY-HMQC spectra. All available data obtained from the NOESY spectra indicate that the solution structures of E43 and D43 SNase are essentially the same as the crystal structures. The NOESY data also show that the intact and deleted proteins have essentially the same structures except that the disordered Ω loops in the intact proteins are replaced by tight type II' turns, formed by residues 43-50-51-52, in the deleted proteins. Except for regions neighboring the Ω loops the intact and deleted proteins show nearly identical ^{15}N and ^{1}H chemical shifts. In contrast, there are widespread, small and similar chemical shift differences between E43 SNase and D43 SNase and between E43 ΔSNase and D43 ΔSNase. This observation indicates that deletion of a single methylene group causes small, widespread and similar changes in the structures of E43 SNase and E43 ΔSNase.

Tu-Pos242

THE STRUCTURE OF THE SOYBEAN TRYPSIN/CHYMOTRYPSIN BOWMAN-BIRK INHIBITOR DETERMINED BY TWO-DIMENSIONAL NMR

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The trypsin/chymotrypsin Bowman-Birk inhibitor (BBI-I) from soybeans is a small protein (M_r=7975 based on amino-acid sequence), isoforms of which are found in a variety of leguminous plants. It is a potent inhibitor of serine protease (K_I=10^{-8}) with the unique property of simultaneous inhibition of both trypsin and chymotrypsin at kinetically independent binding sites. In an effort to understand the inhibitory properties of this protein, we present the ^{1}H-resonance assignments and structure of BBI-I, determined by NOE constraints as input to a distance geometry algorithm. Comparison to other Bowman-Birk inhibitors and other serine protease inhibitor families is discussed.

Tu-Pos243

DIFFERENTIAL BROADENING OF PROTON COUPLED ^{13}C DOUBLETS OF [2 - ^{13}C] ATP BOUND TO VARIOUS ATP-UTILIZING ENZYMES.

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The ^{13}C NMR spectrum of [2 - ^{13}C] ATP free in solution is a doublet of equal intensities and separated by 205 Hz due to spin-spin coupling with the attached proton. However, the two lines are differentially broadened when ATP is bound to various ATP utilizing enzymes (four kinases and one amino acyl tRNA synthetase). The primary cause for this effect is an 'interference' between the two mechanisms of ^{13}C relaxation viz., ^{13}C chemical shift anisotropy (CSA) and ^{13}C-^{1}H dipolar interaction both of which are tensors of the second rank. Linewidth measurements were made at three different ^{13}C frequencies. In order to quantitatively analyze the observed effects, the ^{13}C CSA-tensor has been measured. Although the differential broadening is observed in all the enzyme-bound ATP complexes, the effect is not observed when ATP is dissolved in highly viscous sucrose solutions that are expected to have isotropic rotational correlation times similar to the enzyme complexes. This contrasting behavior suggests the presence of an internal mobility in free ATP which persists in highly viscous solvents and is arrested in the enzyme complexes. The glycosidic rotation of the adenine base is most likely to be this internal motion.

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Tu-Pos244

NMR AND FLUORESCENCE INVESTIGATIONS OF TRYPTOPHAN DYNAMICS IN THIOREDOXIN.

Kenneth E. Nollet†, Marvin D. Kemple‡, Bruce D. Ray‡, James A. Fuchs§, and Franklyn G. Prendergast†, †Dept. of Biochem. and Mol. Biol., Mayo Fdn., Rochester, MN 55905, ‡Dept. of Physics, IUPUI, Indianapolis, IN 46205-2810, §Dept. of Biochem., Univ. of Minn., St. Paul, MN 55108.

E. coli thioredoxin is an intracellular oxidoreductase of low molecular weight (11,700) containing two tryptophans (W28 and W31). Thioredoxin was expressed in a tryptophan auxotroph of *E. coli* in order to incorporate two different specifically labeled tryptophans, *viz.* $^{13}C\varepsilon_3$-trp and $^{13}C\delta_1$-trp, into the protein. The internal motion of the two tryptophan residues in the oxidized and reduced forms of thioredoxin in liquid solution is being examined through high resolution ^{13}C-NMR relaxation and fluorescence anisotropy decay measurements. The ^{13}C-NMR signals of W28 and W31 are sufficiently separated (*e.g.*, 1.2 ppm for $^{13}C\varepsilon_3$-trp and 2.8 ppm for $^{13}C\delta_1$-trp in the oxidized form) to allow unambiguous determination of T_1, T_2, and steady state NOE values for each. The NMR relaxation data are analyzed using the formalism of Lipari & Szabo (*J. Amer. Chem. Soc.* **104**, 4546 (1982)) which yields three motion-dependent parameters including an order parameter pertaining to the amplitude of the local motion of the particular tryptophan residue. The results are considered in light of the expectation that $^{13}C\varepsilon_3$-trp and $^{13}C\delta_1$-trp will have different order parameters in the protein. In addition, motional information will be compared for W28 and W31 in the reduced and oxidized forms and related to the environment of those residues. This work is supported in part by NIH grant GM34847.

Tu-Pos245

STRUCTURAL AND DYNAMIC STUDIES OF SILK FIBROIN USING SOLID-STATE ^{15}N NMR.

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The structure and dynamics of *Bombyx mori* silk fibroin have been investigated using solid-state ^{15}N NMR techniques. Isotopic labels were incorporated biosynthetically by rearing silkworms on an artificial diet enriched with ^{15}N-labeled glycine, alanine or tyrosine. Spectra have been obtained from various preparations of randomly dispersed silk fibroin to yield the principal values of the ^{15}N chemical shift tensor for representative glycine, alanine and tyrosine sites in the silk I and silk II structures. ^{15}N chemical shift and ^{15}N-^1H dipolar spectra have been obtained from uniformly aligned fiber samples containing [^{15}N]-glycine or [^{15}N]-alanine to yield high resolution structural information on the silk II conformation. This structural information is compared to various models for the silk II conformation reported in the literature. The results of attempts to orient the silk I conformation by elongation of the sample or by application of a high magnetic field will be presented. The results of hole-burning (selective saturation) and T_1 experiments will be presented and interpreted in terms of dynamics in the protein backbone.

Tu-Pos246

^{19}F-NMR STUDIES OF THE MEMBRANE-BINDING REGION OF D-LACTATE DEHYDROGENASE OF *ESCHERICHIA COLI*.

Hoai-Thu N. Truong, E. A. Pratt, Susan R. Dowd and Chien Ho. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

D-Lactate dehydrogenase (D-LDH, 65,000 Da) is a membrane-bound respiratory enzyme from *E. coli*, which contains 571 amino acid residues with FAD as a cofactor and requires lipid or detergent for full activity. We are using ^{19}F-NMR to investigate the structure of D-LDH by labeling with fluoro-amino acids and studying its interaction with membranes. Our studies on the effect of a spin-labeled fatty acid on the spectrum of D-LDH labeled with 5-fluorotryptophan have shown that residues 243, 340, and 361 are located in or close to the lipid phase [Peersen et al., Biochemistry 29, 3256 (1990)]. Further studies with D-LDH mutants generated by site-specific mutagenesis show that the spectrum of residue 309 is perturbed by the lipophilic spin label, while those of residues 300 and 315 are not. The depth of the penetration of the spin-label sensitive residues into the bilayer is currently being studied using phospholipid vesicles prepared from acyl chain spin-labeled dimyristoylphosphatidylcholines. The extent of the membrane-binding region is being investigated by observing the number of fluorinated aromatic residues in the ^{19}F-NMR spectra perturbed by a lipophilic spin label. [This work was supported by a research grant from the NIH (GM-26874) and a postdoctoral fellowship from the American Cancer Society to HTT (PF-2877)].

Tu-Pos247

NMR AND BIOCHEMICAL STUDIES OF PARTIALLY ACTIVE MUTANTS OF THE MEMBRANE-BOUND D-LACTATE DEHYDROGENASE OF *ESCHERICHIA COLI*.

E. A. Pratt, Hoai-Thu N. Truong, Gordon S. Rule, Priscilla Y. Hsue and Chien Ho. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

D-Lactate dehydrogenase (D-LDH) is a membrane-bound respiratory enzyme from *E. coli* (65,000 Da). We have been using a combination of site-specific mutagenesis and ^{19}F-NMR to study the structure of D-LDH labeled with 5-fluorotryptophan. Trp has been substituted for Phe, Tyr, Ile, and Leu at various positions throughout the enzyme, to use as a probe of the local environment. All 24 mutants thus generated are expressed in *E. coli*. Ten are fully active and purifiable, while fourteen are inactive or partially active. All of the expressed protein in the inactive mutants is Triton-insoluble, and is not purifiable. Most of the expressed D-LDH from the partially active mutants, which are temperature-sensitive, is also Triton-insoluble; however, a small fraction is soluble in Triton and can be purified. All purified partially active mutant D-LDHs have a normal K_m, while several have a V_{max} lower than normal. The ^{19}F-NMR spectra of many of these partially active mutants show abnormalities compared to the spectra of wild-type or fully active mutant D-LDHs. The structure-function relationship of these mutant D-LDHs will be discussed. [This work was supported by a research grant from the NIH (GM-26874) and a postdoctoral fellowship from the American Cancer Society to HTT (PF-2877)].

Tu-Pos248

X-RAY CRYSTAL STRUCTURE OF THE PROTEASE INHIBITOR DOMAIN OF ALZHEIMER'S AMYLOID β-PROTEIN PRECURSOR BOUND TO TRYPSIN.

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Alzheimer's amyloid β-protein precursor contains a Kunitz protease inhibitor domain (APPI) potentially involved in proteolytic events leading to cerebral amyloid deposition. To aid the identification of physiological targets of the inhibitor, the crystal structure of the complex of APPI with trypsin has been determined and refined to 1.8 Å resolution. The backbone conformation of APPI was found to be very similar to the homologous inhibitor BPTI, indicating that altered specificity results from the five inhibitor-protease interface residues which differ between APPI and BPTI. Arg-15 of APPI forms a direct salt bridge with Asp-189 of trypsin as compared to the water bridged interaction of Lys-15 in BPTI. APPI side chains Met-17 and Phe-34 are involved in hydrophobic interactions with Tyr-151 of trypsin in contrast to the hydrogen bond between Arg-17 of BPTI and the His-40 backbone oxygen of trypsin. This suggests that protease targets will have hydrophobic residues facing this region of the inhibitor. Arg-39 in BPTI hydrogen bonds to trypsin. Gly-39 of APPI does not interact with trypsin and could accommodate a larger protease loop or side chain in this region. Together these observations provide an outline of the structural and chemical nature of *in vivo* protease targets.

Tu-Pos249

STRUCTURE DETERMINATION OF NUCLEOSIDE DIPHOSPHATE KINASE FROM *MYXOCOCCUS XANTHUS* AND MOLECULAR MODELING OF NUCLEOTIDE SOLVATION.

Williams, R.L.(1), Munoz-Dorado, J.(2), Grant, A.G.(4), Jacobo-Molina, A.(1), Inouye, M.(2), Inouye, S.(2), Arnold, E.(1,3) (Intro. by W. K. Olson)

(1) Center for Advanced Biotechnology and Medicine, (2) Dept. of Biochem., Univ. of Medicine and Dentistry, (3) Dept. of Chemistry, Rutgers Univ., Piscataway, NJ
(4) Dept. of Chemistry, Cornell Univ., Ithaca, NY.

Preliminary X-ray diffraction analysis of the enzyme nucleoside diphosphate (NDP) kinase from *M. xanthus* indicates that high resolution structure determination is feasible. Two crystal forms have been obtained under the same conditions and diffract to at least 2.2Å and 1.9Å, respectively. One form has symmetry I222 with cell dimensions a=53.5, b=74.1, c=75.1Å. The second crystal form also grows as space group I222 but with cell edges a=266.0, b=74.1, c=75.1Å. Examination of the diffraction intensities indicates that the second space group is related to the first with pseudo crystallographic translational symmetry. Phase determination with heavy atom derivatives is in progress. Although NDP kinase is a key enzyme in nucleotide metabolism, no three dimensional structure is yet available for any NDP kinase.

Because *in vivo* phosphorylation is required for the pharmacologic activity of many anti-viral and anti-neoplastic nucleoside analogs, structural studies of NDP kinase/nucleotide complexes should provide a basis for improved design of such drugs. During the process of phosphate transfer, the drug must form more stable interactions with the enzyme than with the solvent. As a foundation for computational modeling of these interactions, methods have been developed for calculating solvation energy and forces for a free nucleotide. Reaction field calculations have been used to calculate the electrostatic solvation energies and forces. Estimation of the non-electrostatic forces and energies has been carried out with a solvent accessible surface area model using analytical calculation of surface areas and their derivatives with respect to atomic coordinates.

Tu-Pos250

USE OF MAD PHASING FOR STRUCTURE DETERMINATION OF *B. STEAROTHERMOPHILUS* TRYPTOPHANYL-TRNA SYNTHETASE.

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Tryptophanyl-tRNA synthetase (TrpRS) from *Bacillus stearothermophilus* is a dimeric enzyme composed of identical subunits of molecular weight 37,000 Daltons. The enzyme displays unusual crystal polymorphism when crystallized with different low molecular substrates but under otherwise identical conditions. Underlying factors responsible for this crystal polymorphism appear to contribute to loss of isomorphism during attempts to prepare heavy atom derivatives and this has lead us to use alternative phase determination methods. Direct methods were used to determine the molecular envelope (Carter, et al., *Acta Cryst.*, A46:5768, 1990). We chose the Multiwavelength Anomalous Dispersion (MAD) method for high resolution studies because measurements contributing to a single phase can be independent of the isomorphism of different crystals. The TrpRS monomer contains of 327 residues, 10 of which are methionines, making it a very good candidate for seleno-methionine substitution. Diffraction ratios for the selenium atoms in the modified protein are quite favorable. The Bijvoet ratio <|ΔFanom|>/<Fnat> = 2.6% and the partial structure ratio <|FSe|>/<Fnat> = 22.8% at the selenium absorption edge.

The *E.coli* strain containing the plasmid producing TrpRS was converted to methionine auxotrophy by P1 transduction. The resulting strain grew fairly well on selenomethionine and allowed us to undertake a large scale fermentation. The purification protocol was slightly modified to avoid oxidation. Selenomethionyl TrpRS crystallizes under conditions similar to those used with native protein. Three dimensional 2.8 Å data from tetragonal crystals of selenomethionyl enzyme complexed with tryptophanyl-2'3'ATP were collected at Brookhaven at three wavelengths, two close to the absorption edge of selenium (0.9809 Å at the inflection point, 0.9795 Å at the peak value), and one away from it at 0.91Å. Fluorescence measurements were used to reposition the monochromator before each cycle of wavelength changes. Crystals were pre-aligned so that mirror-related Bijvoet mates would be recorded simultaneously on the FAST detector. Because of radiation damage, 15 crystals were used to get a complete set of multiwavelength data. Diffraction data are currently being processed. Supported by NIGMS RO1-26203

Tu-Pos251

THE NEUTRON STRUCTURE OF SUBTILISIN BPN': EFFECTS OF CHEMICAL ENVIRONMENT ON H-BONDING GEOMETRIES AND THE PATTERN OF HYDROGEN-DEUTERIUM EXCHANGE IN SECONDARY STRUCTURE ELEMENTS.

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The neutron structure of subtilisin BPN' has been refined and analyzed at 2.0 Å resolution. The structure studied was a mutant variant of subtilisin, Met222→Gln, and was used because large, uninhibited crystals could be grown, which was not the case for the native molecule. Comparison of the structure with that of the native molecule indicated that the two structures are essentially the same. Using the capability of the neutron method to locate hydrogen and deuterium atoms, the protonation states of the six histidine residues were assigned. The active site histidine, His64, was found to be neutral at the pH of the analysis (pH 6.1). This group had an unexpectedly low pKa compared to assignments made by other techniques. The altered pKa of the group could result from electrostatic effects of other molecules in the crystal lattice. The dihedral conformations of a majority of the hydroxyl rotors were assigned. The preferred orientation was trans (180°) with the other two low energy conformers (60°, 300°) about equally populated. For the serines, about 21% of the hydroxyls act exclusively as H-bond acceptors, 37% as H-bond donors, and in 42% the group functions as both. The experimentally observed dihedral conformations were compared to predicted conformations based on calculated energy criteria and showed a strong correspondence. Deviation from low energy states could usually be explained by local electrostatic effects. The hydrogen exchange pattern of subtilisin identified the β-sheet and α-helix secondary structure elements to be the most resistant to exchange. 55% of the peptide amide hydrogens were fully exchanged, 15% unexchanged and 30% partially exchanged. The largest concentration of unexchanged sites was in the 7-stranded parallel β-sheet, in which there were 12 protected groups. Little correlation was found between H-bond length and angle and a peptide group's susceptibility toward exchange. Of the 5 α-helices the most protected from exchange is the one defined by residues 224-236. The pattern of exchange identifies regions in this helix where the H-bonding regularity is disrupted.

Tu-Pos252

CRYSTALLIZATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-CoA) REDUCTASE FROM *PSEUDOMONAS MEVALONII*. C. Martin Lawrence [1], Young-In Chi[1], Richard J. Lewis[3], Victor W. Rodwell[2], and Cynthia V. Stauffacher[1]. Departments of [1]Biological Sciences and [2]Biochemistry, Purdue University, West Lafayette, IN 47907, [3]Hereford College, Oxford University, Oxford, England

HMG-CoA reductase (HMGR) is a four electron oxidoreductase that catalyzes the interconversion of HMG-CoA and mevalonate, the rate-limiting step in isoprene biosynthesis. The mevalonate produced from this reaction eventually is used in the synthesis of cholesterol and steroid hormones, as well as other isoprenoid products. Mammalian HMGR is a large enzyme (M_r~100,000) with a C-terminal catalytic domain and an N-terminal membrane attachment domain. In *P. mevalonii* the enzyme is a cytosolic tetramer of identical subunits with M_r= 43,000. It also utilizes NAD(H) as its oxidoreductant which is consistent with its ability to serve a catabolic role when mevalonate is the sole carbon source for growth of *P. mevalonii*. Substantial sequence homology between this enzyme and the catalytic domain of the mammalian HMGR indicates a high probability of similarity in their three-dimensional structures, particularly around the active site. The gene for *P. mevalonii* HMGR has been cloned and overexpressed in *E. coli* and large quantities of purified enzyme are available for mechanistic and structural studies.

We have produced well ordered crystals of the *P. mevalonii* HMGR in a high symmetry cubic space group ($I4_132$, a=229Å). A full native data set has recently been collected at CHESS from these crystals, which diffract to 2.4Å in the high intensity X-ray beam of a synchrotron source. Lower resolution (3.5Å) data from native HMGR has been collected on an area detector (Rsym=7.9%) and this data has been used to identify two mercurial derivatives of the enzyme. The Patterson maps for these potential derivatives show consistent sets of peaks and are now being solved to provide isomorphous replacement phases for the structure. We have recently shown that substrates can also be accommodated in the HMGR crystals and have produced both soaked and cocrystallized complexes of the enzyme with the CoA substrate. Further progress in these areas toward the structure determination of HMGR will be reported.

Tu-Pos253

CRYSTALLOGRAPHIC IDENTIFICATION OF WATER MOLECULES REQUIRED FOR PENICILLINASE FOLDING AND CATALYSIS. **JAMES R. KNOX AND PAUL C. MOEWS, DEPARTMENT OF MOLECULAR AND CELL BIOLOGY, THE UNIVERSITY OF CONNECTICUT, STORRS, CT 06269-3125.**

In the crystal structure of the penicillinase (beta-lactamase) of *Bacillus licheniformis* (Moews et al., Proteins 7, 156-171(1990)), 484 water molecules are associated with two independent copies of the 29,500-Da enzyme. Refinement of the hydrated model has produced an R factor of 0.15 for 27,700 data with F≥3σ(F). Not only do the two protein molecules have very similar folding (0.17Å RMS difference in positions of alpha-carbon atoms), but they also have many tightly-bound water molecules which coincide when the two protein molecules are superposed. Twenty-six water molecules are found to fall within 0.25Å of matching water molecules in the second protein molecule; 69 fall within 0.50Å of matching waters. Because two independent views of the hydrated protein are available, each at the same pH, ionic strength, etc., one can analyse the role of these conserved water molecules with a good degree of confidence. For example, six water molecules in the protein are buried. Three of these are helix N-cap water molecules which promote alpha-helix stability by H-bonding to mainchain NH groups at the beginning of the helices. One of these waters also serves an important role in linking two helices in the penicillin binding site by bridging between mainchain groups.

The penicillin-binding site contains seven water molecules. Upon penicillin entry, a water molecule in the oxyanion hole, hydrogen-bonded between the N-terminus of helix (70-83) and ß-strand (230-238), would be displaced by the oxygen atom of the penicillin's carbonyl group. An unexpelled molecule of water is proposed to be the catalytic water required for penicillin hydrolysis. It is strongly hydrogen-bonded to Glu166, a conserved residue in all penicillinases, and it lies 3Å from the α-face of a previously-modelled penicillin. The position of the water-Glu166 pair is stabilized in the active site by a *cis*-peptide bond at Pro167.

Tu-Pos254

THE MOLECULAR PACKING IN ARTICULAR CARTILAGE COLLAGEN FIBRILS. M. Volpi, J.-H. Zhang, E.P. Katz, Department of BioStructure & Function, University of Connecticut Health Center, Farmington, CT 06032.

In our past studies of bovine femoral articular cartilage collagen by x-ray diffraction, we have shown that: 1) the collagen fibrils are osmotically compressed by vicinal proteoglycans, and 2) the fibril structure changes with age. These changes, possibly an adaptive response to changing mechanical needs, are due to variations of the molecular packing in the collagen fibrils as well as in changes in the amount of interfibrillar associated water. In order to characterize the molecular packing we obtained the x-ray diffractograms of the fibrils as a function of water content and osmotic pressure. To this end we conducted the following experiments. The proteoglycans were enzymatically removed from samples of bovine femoral articular cartilage, from animals of different age, and then, a) equilibrated with different concentrations of the osmotically active molecule polyethylene glycol, or alternatively, b) progressively dehydrated by air drying. From the equatorial distribution of intensities of the diffractograms we obtained the most frequent intermolecular spacing (MFIS), a statistical measure of the lateral organization of the collagen fibrils by means of a Patterson analysis. This analysis revealed that the molecular packing did not have any long range lateral order and that, even in the short range, the packing was essentially random. Water contents calculated assuming a liquid-like lateral packing, was in very good agreement with the experimental data. The MFIS as a function of the water/collagen ratio, changed in an analogous way with dehydration in samples from animals of different age. Furthermore, the fibrillar water content was age-dependent.

Supported by NIH grant AR 37604.

Tu-Pos255

CRYSTALLOGRAPHIC AND BINDING STUDIES ON A CYTOKININ - PEANUT AGGLUTININ COMPLEX, K. W. Olsen, E. J. Zaluzec, M. M. Zaluzec, E. J. Fernandez and S. F. Pavkovic, Dept. of Chemistry, Loyola University, 6525 N. Sheridan Rd., Chicago, IL 60626

The binding of hydrophobic ligands to peanut agglutinin (PNA) was followed by fluorescence titrations. A single binding site per tetramer was found for 2,6-toluidinyl-naphthalenesulfonate (TNS), with an affinity constant of $9.1 \pm 0.2 \times 10^3\ M^{-1}$. Binding of the non-fluorescent ligand N^6-benzylaminopurine (BAP) was followed by the ability of BAP to quench the fluorescence of PNA-TNS, yielding an affinity constant of $2.1 \pm 0.4 \times 10^2\ M^{-1}$. 1,8-Anilino-naphthalene sulfonate (ANS) had a K_a of $24.2 \times 10^3\ M^{-1}$. Titrations of the fluorescent sugar, N-dansyl-galacto-samine (Dns-GalN) with PNA in the presence of various amounts of BAP gave K_a of 6.9 ± 0.3, 9.4 ± 0.4, 10.9 ± 0.2, and $12.4 \pm 0.5 \times 10^3 M^{-1}$ in the presence of 0.0, 0.1, 0.5, and 1.0mM BAP, respectively. BAP, a naturally occurring phytohormone, slightly enhances carbohydrate binding. Crystals of PNA have been grown in the presence of BAP, TNS, ANS and indole acetic acid, an auxin. Preliminary diffraction data collected on PNA-BAP crystals indicate a monoclinic cell (P2) with a=67.0 Å, b=35.2 Å, c=65.8 Å, and β=68.6°. This is the first example of a legume lectin crystallized with a bound phytohormone. Crystals of PNA grown in the presence of lactose have an orthorhombic space group ($P2_12_12$) with a=128.8 Å, b=126.0 Å and c=76.1 Å and one tetramer per asymmetric unit. The V_m for the PNA-BAP crystals is 2.62 $Å^3$/dalton assuming one monomer of PNA per asymmetric unit. Thus while the PNA-lactose complex crystallized as tetramers, the PNA-BAP complex is, at most, dimers in the crystal. These results indicate that BAP can modify the quaternary structure of PNA by dissociation and change its carbohydrate valence. (Supported in part by a Biomedical Research Support Grant from NIH.)

Tu-Pos256

INVESTIGATIONS OF THE SOLUTION BEHAVIOR OF THE NATIVE ADHESIVE POLYPHENOLIC PROTEIN FROM THE COMMON BLUE MUSSEL M. edulis.

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M. edulis is a common intertidal bivalve which attaches itself to substrates via a mechanism of byssal threads and plaques. The plaques contain an adhesive of exceptional properties. The adhesive is able to maintain high strength bonds to a wide variety of substrates in aqueous environments. In previous work, we determined that 10 amino acid peptide analogs of the adhesive polyphenolic protein have the ability to stably aggregate in solution to form two-dimensional extended arrays. This aggregation correlated with the ability of the peptides to form stable 3_{10}-helices. We postulated that this aggregation was important for proper adhesive function. We here report studies on the native adhesive protein in solution by means of x-ray solution scattering. These studies indicated that the adhesive protein has a radius of gyration (Rg) of ~ 78Å. Analysis of the solution scattering data indicate, that in contrast to the peptide analogs, the native protein is either a monomer or dimer in solution with a non-spherical shape and size consistent with an extended structure. This work was supported by the State of Connecticut Department of Higher Education Cooperative High Technology Program with BioPolymers Inc. of Farmington, Connecticut.

Tu-Pos257

DISTANCE BETWEEN THE ANTIGEN BINDING SITES OF DIFFERENT SUBCLASSES OF IgG MEASURED USING NEUTRON SCATTERING

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The most common antibody class is IgG, which is a 150 kD symmetrical Y or T shaped molecule with two identical antigen binding sites at the ends of the two arms. We have measured the distances between the antigen binding sites for 3 different IgG subclasses using neutron scattering from deuterated antigen complexed with proteated IgG. Mouse monoclonal antibodies (subclasses IgG1, IgG2a and IgG2b) specific to nuclease (a 17 kD protein) were used. Neutron scattering data were measured for each IgG-antigen complex in a 41% D_2O solvent. The 41% D_2O solvent has the same mean neutron scattering density as the proteated antibody molecule, and hence the antibody contributes negligibly to the scattering signal. (IgG complexed with proteated antigen also has very low contrast, and is used as a blank.) The deuterated antigens, however, are strongly contrasted against the 41% D_2O solvent and give rise to a scattering profile that contains an interference term related to the distance between the deuterated antigens. The scattering profile has been modeled to give the distance between the antigens and the variance of this distance. The variance gives measure of the relative flexibility between the subclasses. We have quantified the mean distance and its variance for each subclass and found differences that may relate to their function in the immune response.

Tu-Pos258

NEUTRON AND X-RAY SCATTERING STUDIES OF CALMODULIN AND TROPONIN C IN SOLUTION

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We have extended our studies of calcium-binding protein/peptide interactions (1,2) to troponin C/peptide complexes. Small-angle X-ray scattering data have been measured from solutions of troponin C complexed with the venom peptides mellitin and mastoparan as well as synthetic peptides based on regions of the Troponin I sequence (including the Troponin I peptide 104-115). These data will hopefully contribute to further understanding of the conformational flexibility exhibited by calcium-binding proteins when they interact with their respective partners.

We have also continued our studies of the structure of calmodulin as a free monomer in solution using neutron resonance scattering from ^{240}Pu•calmodulin to measure distances between the ion-binding sites (more normally occupied by Ca^{2+}) (3). The resonance scattering experiment was repeated during September 1990 with significantly improved instrument conditions resulting in much reduced background scattering. The new data show stronger resonance scattering (signal-to-noise is improved by a factor of approx. 10), and we are in the process of reducing the data to the form of scattered intensity versus scattering vector, Q, which is predicted to show an interference term that can be interpreted in terms of specific distances between the ion-binding sites in the protein.

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Tu-Pos259

MONOCLONAL ANTIBODIES TO BACTERIAL PHOSPHATIDYL-INOSITOL-SPECIFIC PHOSPHOLIPASE C.

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Bacterial phosphatidylinositol-specific phospholipases C (PI-PLCs) display similar substrate specificity as their eukaryotic counterpart involved in signal transduction of insulin and Ca^{2+}-mobilizing hormones, and are used in the study of the novel glycosyl-phosphatidylinositol-protein anchors. Structure-function relationships of the PI-PLC from Bacillus cereus were investigated with monoclonal antibodies raised against the purified enzyme. Two of the monoclonals inhibited reactions catalyzed by the bacterial enzyme: cleavage of phosphatidylinositol, release of acetylcholine-esterase from its membrane anchor, and conversion of inositol 1,2-cyclic phosphate into inositol-1-phosphate. At saturating concentrations of inhibitory antibody only a few percent of the enzyme activity remained. The epitope recognized by one of the inhibitory antibodies, A72-24, was mapped by proteolytic digestion, protein sequencing, and Western blotting of the generated fragments. The data indicate that at least part of the epitope resides within an 8 kDa stretch of the bacterial polypeptide which has also been found to display significant primary sequence similarity to several of the eukaryotic PI-PLCs (Kuppe et al. (1989) J. Bact. 171, 6077). These results suggest that the conserved peptide (peptide X) may contain functionally important residues.

Tu-Pos260

LIGAND BINDING FEATURES OF THE Apo[a] PROTEIN OF HUMAN LIPOPROTEIN [a]

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Lp[a] is a lipoprotein whose plasma concentration in man is highly correlated with cardiovascular and cerebrovascular disease. Interdigitated in the Lp[a] surface is one molecule of apoprotein (apo) B-100 disulfide-linked to a molecule of apo[a]. Apo[a] is highly glycosylated, exists in more than 12 polymorphic forms (M_r = 419-838 Kd), and possesses kringle and protease domains similar to those of plasminogen (PG). The capacity of lysine-Sepharose to bind certain Lp[a] subfractions but not others has stimulated us to compare each apo[a] kringle (AK) with kringles from other proteins which have been shown to bind lysine, ϵ-amino caproic acid (EACA), and/or fibrin. PGK1, PGK2, PGK5, and tPA-K2 exhibit binding affinity for lysine and/or fibrin. We have identified at least 32 residues in these kringles that may be essential for intra-kringle interactions necessary for binding lysine, EACA and/or fibrin. Among the 11 kringle types present in apo[a], AK10 has the highest probability for binding these ligands (comparable to PGK1). AK11 has a lower probability for binding lysine (similar to PGK5). AK8, AK6, AK7, and AK5 have decreasing probabilities for binding lysine (comparable to PGK2 and PGK3). AK1, AK2, AK3, AK4, and AK9 are not predicted to bind lysine. Hence, apo[a] contains a number of ligand binding sites within its kringles which can impart significant affinity for lysine side-chains of fibrin, thereby providing a rational basis for the observed binding of Lp[a] to fibrin clots.

(Supported by NIH, HL32971)

Tu-Pos261

TIME-RESOLVED FLUORESCENCE STUDIES OF α-NEUROTOXINS BINDING TO PEPTIDES DERIVED FROM THE α-SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR.
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The complex formed between the dodecamer α185-196 (KHWVYYTCCPDT) and α-bungarotoxin (BGTX) results in an enhancement of the steady-state intrinsic fluorescence while peptide binding to α-cobratoxin (CBTX) produces a quenching of the net fluorescence. The fluorescence lifetimes were analysed to investigate the interactions and orientations of tryptophan (Trp) in the peptide and in the two neurotoxin complexes. The dodecamer Trp, excited at 295 nm, shows three resolvable lifetimes at 3.8, 1.7 and 0.6 nsec with amplitude ratios of 17:46:28% respectively suggesting that the Trp indole ring has more than one rotameric conformation. The more rapid decays are favored in amplitude ratio, suggesting that the Trp fluorescence is normally somewhat quenched. Both BGTX and CBTX have Trp lifetimes of 3.4 nsec (3-6%) of the total amplitude, 1.1 nsec (40-60%) and 300 psec (20-40%). The very rapid fluorescence lifetime components may reflect quenching of the Trp fluorescence due to proximity of the toxin disulphides.

In the complex between the dodecamer and BGTX a larger percentage of the total amplitude is attributable to the slower component, which now has a decay time of 5.5 nsec. In contrast, the lifetimes in the complex formed between the dodecamer and CBTX are indistinguishable from CBTX alone although the amplitudes vary slightly. These lifetime studies suggests that the decreased fluorescence observed in the steady-state experiments with CBTX may be due to static quenching.

Experiments were carried out at the Regional Laser Biotechnology Laboratories, University of Pennsylvania, Philadelphia, Pa. Supported by NIH grant GM32629 and training grant CA-09085 and the AHA.

Tu-Pos262

Secondary structure and orientation of the amphipathic peptide GALA in lipid structures : an infrared spectroscopy approach

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A synthetic, amphipathic 30-amino acid peptide based upon a Glu-Ala-Leu-Ala motive was designed to mimic the behavior of viral fusion proteins. GALA is a water soluble peptide with an aperiodic conformation at neutral pH and becomes an amphipathic α helix as the pH is lowered to pH 5 where it interacts with phospholipid bilayers. Attenuated total reflection infrared spectroscopy using polarized light provides information on the structure and orientation of the peptide and the lipids which is not subject to artifacts due to light scattering on large particles. H/D exchange rate of the amide N-H group and analysis of the shape of the amide I' by Fourier self deconvolution and curve fitting indicate that the α helix content increases from 19% to 69% upon pH lowering. A further increase to 100% α-helix is observed after interaction with palmitoyloleoylphosphatidylcholine (POPC) vesicles. Dichroism data obtained on oriented bilayers of the POPC-GALA complex demonstrate that POPC hydrocarbon chains and the peptide helical axis are essentially perpendicular (± 15°) to the membrane plane. At neutral pH, in the presence of dimyristoylphosophatidylcholine (DMPC), GALA is known to form discoïdal structures similar to those formed under the same conditions by apolipoproteins A1 and A2. In these discoïdal complexes, the α helical content was estimated to be 65% with the rest of the structure being essentially unordered. No significant modification of the all-trans conformation of the hydrocarbon chain of dimyristoylphosphatidylcholine (DMPC) is detected upon disc formation. Dichroism measurements show that the helical axis is essentially parallel to the hydrocarbon chains. The infrared spectrum of GALA in this complex was found to be very similar to those of apolipoprotein A1 and A2 which form discoïdal complexes with DMPC but the spectrum is quite different from that of apolipoprotein B100 in low density lipoproteins which does not form discoïdal complexes.

Tu-Pos263

CONFORMATIONAL SENSITIVITY OF AMIDE III INFRARED VIBRATIONAL CD OF PEPTIDES

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Infrared or Vibrational CD (VCD) has previously been observed in the amide I vibration of peptides in a number of studies [1]. Here, we report our efforts to utilize the amide III VCD signals as a conformational probe.

Amide III VCD for a number of small peptide models (L-Ala-L-Ala, L-Ala-L-Ala-L-Ala, N-acetyl- L-Ala-N'-methyl amide), as well as a number of large homo-oligopeptides (poly-L-lysine, poly-L-tyrosine) in water and organic liquids, has been studied. We find that left-handed structures, such as the poly-L-tyrosine helix in acidified DMSO, gives rise to large and characteristic amide III VCD signals. The α-helical form, on the other hand, shows much less VCD. This is also true for amide I VCD.

The interpretation of the amide I VCD in terms of dipole-dipole coupling has been proposed previously [2]. However, an interpretation of the amide III region is much more difficult, since the amide III vibration is a complicated coupled vibration of adjacent C-H and N-H deformation coordinates. We propose an interpretation of the amide III VCD signal, based on detailed normal coordinate calculations, and the extended coupled oscillator model.

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Tu-Pos264

CONFORMATIONAL ORIGIN OF DIFFERENTIAL IMMUNE RESPONSES IN TRANSMEMBRANE PROTEINS OF HIV-1, HTLV-1 AND MuLV

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Solution secondary structures of peptides corresponding to phylogenetically conserved regions in the transmembrane envelope glycoproteins of HIV-1, HTLV-1, and MuLV are determined by NMR and CD in combination with restrained molecular dynamics computations. For HIV-1, this region encompasses residues 576-592, and represents the most conserved region of gp41. All of the peptide fragments studied were found to be quite similar and to have appreciable α-helical content. A point mutation from Ala to Thr at position 582 in both the peptide fragment and the intact virus significantly decreases the ability of HIV-1 antibodies both to recognize the peptide fragment and to neutralize the mutant virus. Furthermore, the difference in energies between the Ala and Thr peptide analogs is very small. Analogous mutations to Gly or to Ser do not result in diminished antibody recognition for either the peptide analog or the mutated virus. One possible explanation for the observed differences in the immunologic responses between the two peptides as well as between the wild type and mutant viruses involves the structures of Ala or Thr side chains and their orientation with respect to Arg 579 and Tyr 586.

Tu-Pos265

MAGAININ 2, A SMALL NONHEMOLYTIC CATIONIC ANTIMICROBIAL PEPTIDE: DETERMINATION OF ITS STRUCTURE AND INTERACTIONS WITH LIPIDS BY ROTATIONAL-ECHO DOUBLE-RESONANCE (REDOR) NMR

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Magainin 2, a naturally occurring 23-amino acid cationic peptide isolated from the skin of the African clawed frog, is lethal to a wide variety of microorganisms and enveloped viruses. The bactericidal activity of the peptide is believed to be associated with the disruption of the integrity of the membrane system of the target organism. Magainins have the potential to adopt a highly amphiphilic α-helical secondary structure, which is typical for many membrane-active peptides. Unlike other cationic peptides, such as melittin, which are cytotoxic, magainins are nonhemolytic at antimicrobial concentrations. Thus, magainins and related peptides may have therapeutic potential as antibiotics. The molecular nature of the interaction between magainin 2 and the cell surface has yet to be determined. One proposal suggests that an oligomeric association of the peptide (6 to 8 monomers) forms a channel across the membrane through which anions can flow. Another suggestion is that magainins bind primarily to the membrane surface and destroy the integrity of the lipid bilayer. We have applied a new solid-state NMR technique, REDOR (rotational-echo double-resonance), which measures dipolar coupling, to accurately determine distances between labeled nuclei. We have synthesized magainin 2 containing ^{15}N-Ala at position 9 and 2-^{13}C-Gly at position 13. When the peptide adopts an α-helical structure, the distance between the labeled atoms will be about 7 Å. Determination of this interatomic distance is useful in verifying α-helical content under different conditions. In addition, we have synthesized two phosphoglycerides, DPPC and DPPG, containing ^{13}C at the 12 position of the palmitoyl chains. Measurements of dipolar coupling between ^{15}N in the peptide and ^{13}C in the membrane lipids are used to reveal the penetration of the peptide into the lipid bilayer. These data will provide insight into the mechanism by which magainins kill microorganisms.

Tu-Pos266

Short range distance determination in integral membrane proteins by magic angle spinning NMR.

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Rotational resonance magnetization transfer, a new technique for selectively measuring homonuclear dipolar coupling in magic angle spinning NMR, is being used to investigate internuclear distances in membrane proteins.

The current study is concerned with comparing the structures of a hydrophobic undecapeptide in crystals and in lipid bilayers. The peptide has been used to model the N-terminal half of alamethicin, a fungal peptide that forms voltage gated pores in membranes, and it has the sequence given below (Aib = amino isobutyric acid). In addition, the X-ray crystal structure of the peptide has been solved to 0.9 Å resolution, showing an α-helical motif.

Boc-L-Ala-{Aib-Ala}$_2$-Glu(OBzl)-Ala-{Aib-Ala}$_2$-OMe

Five different ^{13}C labeled peptides have been synthesized which are labeled at pairs of carbonyl carbons and alanine methyl carbons. Based on the crystal structure of the peptide, these pairs are 3.5, 4.3, 4.8, 5.1, and 6.6 Å apart.

The first set of experiments consist of evaluating the range and accuracy of magnetization transfer as a technique for determining inter-nuclear distances. Using crystal of the peptide, where the relative geometry of the labeled sites is known, significant transfer has been observed between ^{13}C nuclei as far as 6.6Å apart, and the rate of transfer does correlate with the internuclear distance.

The second set of experiments involve placing the same labeled peptides in DPPC bilayers and determining the internuclear distances in a hydrophobic environment. A comparison of the distance data from the peptide in crystals and in a bilayer provides a means for determining if and how the structure changes upon incorporation into a lipid bilayer.

Tu-Pos267

GEOMETRY OF THE HELIX→COIL TRANSITION IN SHORT HELICAL PEPTIDES AS OBSERVED BY ELECTRON SPIN RESONANCE

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Recently, short alanine based peptides have been synthesized that have a high α-helical content of up to 80% at 1.0°C (Marqusee and Baldwin,1987; Marqusee et al,1989). Spin labeled analogs of these peptides enable a unique view of peptide conformation and dynamics throughout the helix-coil transition on a subnanosecond timescale. Each analog contains a single cysteine substituted for an alanine at one position and to which a methanethiosulfonate spin label (MTSSL) is specifically attached by a disulfide linkage. The sequences of the three analogs used in this study are:

i+4c AcAEAAAKEACAKEAAAKANH$_2$
3Kc AcAAAAKAACAKAAAAKANH$_2$
3Kt AcAAACKAAAAKAAAAKANH$_2$

Fractional helicity (θ), as determined by CD, decreases with increasing temperature for all three analogs. At each temperature, θ for each analog is approximately the same for the unsubstituted and unlabeled peptides, indicating that the cysteine+MTSSL is not perturbing. From the motionally narrowed ESR spectra and the Stokes-Einstein relation, one can calculate the local tumbling volume, V_L, which is the volume that reorients with the nitroxide. The behavior of V_L as a function of temperature provides a new way of probing local dynamics in peptides through the helix-coil transition. V_L is highest at low temperatures (high θ), consistent with a helical structure tumbling as a rigid unit. V_L, declines at higher temperatures, indicating the onset of segmental motion. V_L(T) for 3Kc and 3Kt are superimposable except near the middle of the transition, indicating a cooperative helix-coil transition and a somewhat compact 'coil' state. V_L(T) for the i+4c deviates from the trend of the other analogs at low temperature, which apparently reflects the formation of salt bridges. Our data also indicate that the helical ends are more flexible than the central region at intermediate helicities.

Tu-Pos268

INTERPRETING THE HELIX-COIL TRANSITION OF SHORT PEPTIDES OF KNOWN SEQUENCE.

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In the past several years large amounts of experimental data have become available on the thermal transitions of helices of short polypeptides (Marqusee & Baldwin (1987), PNAS, 84, 8898). It is desirable that a systematic approach be developed to analyze these results to obtain physical parameters which characterize the helix-forming tendencies of individual amino acid residues.

The helix-coil transition of sequenced polypeptides can be introduced into matrix theories by utilizing sequenced matrix products. We have incorporated the necessary algebra into a computer program which will generate helix-coil statistics and thermal transition curves for any sequence with known statistical weights.

Analysis of experimental curves to obtain the physical parameters, i.e., nucleation and elongation factors σ and s, of individual amino acids is the main challenge and is considerably more difficult because normally only two macroscopic parameters, the apparent ΔH and ΔS, can be determined from a single experimental curve. Synthetic strategies which vary the position of key amino acids and the length of the chain are helpful since ΔH and ΔS depend on them. In confronting this problem we have made comparisons between the Zimm-Bragg, Lifson-Roig, single-sequence, and two-state models for the experimental data. We thank R.L. Baldwin and M. Scholtz for providing data before publication, this work is supported by NIH grant GM20195.

Tu-Pos269

TRIPLET STATE SPECTROSCOPIC STUDIES OF ALPHA COBRATOXIN BINDING TO ACETYLCHOLINE RECEPTOR ALPHA SUBUNIT PEPTIDES.
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The photoexcited triplet states of the tryptophan residues in the neurotoxin cobratoxin (CBTX) and in peptides derived from the alpha subunit of the nicotinic acetylcholine receptor have been used as a probe of their local environment, and of the binding interactions of the peptides and toxin. The observed low phosphorescence yield of CBTX is attributed to the proximity of a disulfide group, which also gives rise to short components (1 sec and less) in the phosphorescence decay. Multiexponential Trp phosphorescence decay in this single-tryptophan-containing toxin is consistent with conformational heterogeneity in solution, as are the comparatively large linewidths for phosphorescence and optically detected magnetic resonance (ODMR) spectra. The ODMR of Trp in CBTX is also anomalous in that the usually strong D - E (1.7 GHz) transition is absent under broadband optical excitation. By contrast, the dodecamer peptide α185-196 and the 18-mer α181-198 show strong phosphorescence and D - E and 2E ODMR transitions. The 18-mer phosphorescence and ODMR frequencies are shifted relative to those of the dodecamer in directions indicating a more hydrophobic environment for the emitting Trp residues, consistent with recent fluorescence work. Binding of the dodecamer to CBTX produces shifts in spectral parameters indicative of an increase in local hydrophobicity of the tryptophan residues contributing to the ODMR. Work supported by the NYU Research Challenge Fund, NIH grant GM32629 and training grant CA-09085.

Tu-Pos270

DETERMINATION OF INTRAMOLECULAR DISTANCES AND FLEXIBILITY IN ZINC FINGER PEPTIDES USING FREQUENCY-DOMAIN FLUORESCENCE ENERGY TRANSFER MEASUREMENTS.
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The "zinc finger" motif consists of a protein domain which tetrahedrally coordinates a zinc ion via cysteine (sulfhydryl) and histidine (imadazole nitrogen) sidechain atoms (for a recent review see Berg, *JBC* 265:6513-16, 1990). Three distinct classes of zinc finger proteins have been identified. Differences between the classes include the size of the domain (ranging ~12-33 residues), the number of coordinating cysteine (C) and histidine (H) residues (CCHH, CCHC, CCCC), and the number of metal-binding domains per protein molecule (1-37, possibly more). Zinc finger proteins are nucleic acid binding molecules. The CCHH and CCCC classes consist of eukaryotic proteins that bind double-stranded DNA, whereas the CCHC class consists primarily of retroviral nucleocapsid proteins and are believed to interact with single-stranded RNA although no specific nucleic acid interaction has been found yet.

Zinc finger peptides containing a single metal-binding domain of the type CCHH and CCHC were synthesized for fluorescence energy transfer measurements. An intrinsic conserved tryptophan or tyrosine residue serves as the energy donor to various acceptors in different regions of the peptide. Distance distributions between the donor and acceptor, in the absence and presence of metal ion, were determined for these peptides using time-resolved frequency-domain fluorometry. The distances recovered from the metal-bound peptides were shorter and had narrower distributions than those recovered from peptides without metal, which is consistent with previous experimental evidence (CD, NMR) indicating that metal ion is required to form a defined structure. The mean distances calculated from these measurements will be compared to existing theoretical and NMR-determined structures.

Tu-Pos271

STRUCTURAL STUDIES OF A THYROID HORMONE RECEPTOR SINGLE "ZINC FINGER" DOMAIN

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Thyroid hormones can both induce and repress the expression of specific genes via a cascade of events beginning with recognition by a nuclear thyroid hormone receptor (T3R). The T3Rs belong to a superfamily of ligand-modulated transcription factors that contain two "zinc finger" motifs within their DNA binding domains. A 26 amino acid peptide has been synthesized that comprises the first "zinc finger" of the DNA binding domain of r-erbA-β-1, a T3R. The peptide sequence contains the Cys-X_2-Cys-X_{13}-Cys-X_2-Cys motif. The reduced peptide has been studied using UV-VIS, CD and NMR spectroscopies and is found to bind to Zn^{2+} and Co^{2+} with high affinity at pH values greater than 5.0. In contrast to the recently published solution structure of the glucocorticoid receptor DNA binding domain (Science 1990, 249, 157), NMR studies indicate that the His residue situated in the X_{13} loop plays a role in metal binding of the T3R peptide. CD studies indicate a small but reproducible decrease in the magnitude of (negative) ellipticity around 200 nm upon addition of $ZnCl_2$. This suggests either an increase in ordered secondary structure or a change in the aromatic contribution to far UV CD upon zinc binding. Solution conditions under which predominantly a single conformation of Zn-bound peptide exist have been identified by ^1H NMR, and 2-D NMR experiments are underway to define further the secondary structure and metrical parameters of metal ligation.

Tu-Pos272

RECOGNITION OF DIFFERENT STRUCTURAL MOTIFS BY H-2^b AND H-2^d CLASS II MOLECULES IN MURINE EXPERIMENTAL MYASTENIA GRAVIS.
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Experimental Autoimmune Myastenia Gravis (EAMG) is induced in mice strains of H-2^b haplotype (C57BL/6 and BALB/b) by immunization with Torpedo nicotinic acetylcholine receptor (nAChR). Mice of H-2^d haplotype (CB17 and BALB/c) are less or not susceptible to EAMG, although they develop anti-nAChR antibodies and anti-nAChR T-helper (Th) response.

Using panels of overlapping synthetic peptides corresponding to the complete sequence of Torpedo nAChR α subunit, we have identified the sequence segments containing epitopes recognized by nAChR-specific Th cell of mice strains of different H-2 haplotype. H-2^b strains recognized primarily the aminoacid sequences 149-169 and 360-378, and to a lesser extent peptide 181-200. H-2^d strains recognized the sequence segments 1-20 and 304-322.

Analysis of the α subunit sequence for propensity to form amphipatic helices, obtained with the AMPHI program, indicated that the segments 6-27 and 306-322, which contain Th epitopes for the H-2^d strains, have the highest amphipatic scores (56.1 and 34.2 respectively). The sequences recognized by H-2^d haplotype were not predicted to form α-helix. Secondary structure preditions using the Chou & Fasmann and Garnier-Osguthorpe-Robson algorithms, support the possibility of formation of helical structure within the region 1-27.

Circular dichroism spectra of synthetic peptides corresponding to the sequences 1-20 and 304-322 in solution confirmed the presence of a high content of α helical structure, in the absence and/or in the presence of the helix inducing detergent SDS. Of the three sequences recognized by the H-2^d strains, two did not have any measurable α helical component, but had a strong β-pleated component (79% and 32% respectively), and the third peptide contained both β-pleated (50%) and α-helical (18%) structures, which were insensitive to SDS (2-3%).

Different class II restriction elements, in spite of the permissive nature of their recognition, may preferentially bind peptide antigens with different structural motifs.

Tu-Pos273

SOLVENT EFFECTS ON CONFORMATIONAL CHANGES OF BIOMOLECULES IN WATER.

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SOLVENT EFFECTS ON THE THERMODYNAMICS AND STRUCTURE OF BIOLOGICALLY INTERESTING MOLECULES, SUCH AS ORGANIC DRUG MOLECULES AND PEPTIDES, WERE STUDIED BY ANALYTICAL METHODS BASED ON INTEGRAL EQUATION THEORIES. USING JORGENSEN'S THREE-SITE TIPS MODEL FOR WATER AND ALL-ATOM MOLECULAR MECHANICS NONBONDED-ENERGY PARAMETERS FOR THE SOLUTE MOLECULE, DETAILED SOLUTE-SOLVENT SITE-SITE DISTRIBUTION FUNCTIONS WERE CALCULATED. FROM THESE DISTRIBUTION FUNCTIONS, THE INTRA-MOLECULAR POTENTIAL OF MEAN FORCE FOR EACH PAIR OF SOLUTE ATOMS IN WATER WAS CALCULATED TO DETERMINE THE FREE ENERGY OF SOLVATION OF THE MOLECULE AS A FUNCTION OF CONFORMATION. THE CONFORMATIONAL FREE ENERGIES OF THESE MOLECULES WILL BE DISCUSSED IN TERMS OF THE RELATIVE STABILITY OF IMPORTANT CONFORMERS, AND COMPARED TO APPROPRIATE SIMULATION DATA WHERE APPLICABLE.

Tu-Pos274

OLIGONUCLEOTIDE DYNAMICS - A SOLID STATE ^2H NMR STUDY

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The internal dynamics of deuterium labeled nucleosides and the corresponding synthetic dodecamer [d(CGCGAATTCGCG)]$_2$ have been investigated using solid state NMR. Synthetic deuteration of the nucleosides [*methyl* ^2H]-2′-deoxythymidine, [6 ^2H]-2′-deoxythymidine, [5′5″-$^2H_{1,1}$]-2′-deoxythymidine [2′,2″ ^2H]-2′-deoxycytidine, and [2″ ^2H]-2′-deoxyadenosine and subsequent incorporation into oligonucleotides have allowed the investigation of methyl, backbone, sugar and base motions within DNA. Analysis of the quadrupolar echo line shape, plus T_1 and T_{2e} relaxation allowed the variation in the dynamics as a function of hydration levels (W H_2O/nucleotide) ranging from dry (W$\sim$ 0) to a liquid crystal phase (W$\sim$ 30) to be determined. A variety of discrete and continous motional models have been investigated in the simulation of the experimental line shapes, including inversion recovery behavior and pulse spacing dependence. The amplitude of the internal motions were found to increase slightly with hydration levels, with only minor variation for different labels. At high hydration levels a liquid crystal phase was observed with the helix axis aligning perpendicular to the magnetic field. The description of internal dynamics for furanose rings in DNA is still unresolved. This has prompted investigations utilizing the nucleosides [2″ ^2H]-2′-deoxyadenosine and [2′,2″ ^2H]-2′-deoxycytidine. The energy of activation for the sugar ring motion has been determined from T_1 measurments, while the quadrupolar echo line shape has been simulated with a variety of motional models which include unequal site probabilities.

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Tu-Pos275

ROTATIONAL AND INTERNAL DYNAMICS OF SHORT DNA FRAGMENTS. Bryant S. Fujimoto, N. Susan Ribeiro and J. Michael Schurr, Department of Chemistry, University of Washington, Seattle, WA 98195

A significant discrepancy exists between the hydrodynamic radii of DNA determined by fluorescence polarization anisotropy (FPA) (Wu et al., Biopolymers 26, 1463 (1987)) for DNAs containing 43 and 69 bp (R = (12.0 ± 0.6 Å) and by depolarized dynamic light scattering (DDL) (Eimer et al., Biochemistry 29, 799 (1990)) for DNAs containing 8, 12 and 20 bp (R = 10.2 ± 0.5 Å). The possible origins of this discrepancy are addressed here. The rotational dynamics of a 12 bp GC-rich DNA are measured by time-resolved FPA and the hydrodynamic radius of the equivalent cylinder is determined using the theory of Tirado and Garcia de la Torre, as in the previous two works. We obtain a value R = (9.8 ± 0.2) Å, in good agreement with the measurements of Eimer et al. on short DNAs. It is conceivable that this low value is associated with the short length of the DNAs studied. For example, the radius obtained from modelling the real non-cylindrical DNA as a cylinder would be expected to decrease somewhat with decreasing DNA length in any case. Studies on longer fragments are currently in progress and will hopefully be completed by the time of the meeting.

We present a theoretical analysis of the NMR relaxation data of Eimer et al. to obtain the root-mean-squared amplitudes of local angular motion of the DNA bases in a two-dimensional harmonic potential. Using the neutron scattering value for the C5H - C6H distance (2.52 Å), and a hydrdynamic radius of 10 Å, we obtain an rms amplitude of angular motion in any one-dimension of less than 9 degrees. This value is much less than that reported by Eimer et al..

Tu-Pos276

DYNAMICS OF SHORT DUPLEX DNAs MONITORED BY A SITE SPECIFIC SPIN PROBE Eric J. Hustedt, Andreas Spaltenstein, James E. Kirchner, Colin Mailer, Paul B. Hopkins, Bruce H. Robinson,* Dept. of Chemistry, University of Washington, Seattle, WA 98195.

Recent work has suggested that there are differences in the dynamics of duplex DNA as a function of base sequence or composition. The resolution of this issue has been confounded by the problem of determining to what extent the dynamics are due to local independent motions of the base and those which are due to the collective modes. We have prepared an EPR active spin-labeled analogue of thymidine site specifically incorporated into duplex DNA of 12, 24, 48 and 96 bases.

The EPR data have been analyzed in terms of a model in which 1) the overall motions (or uniform modes) are determined by the hydrodynamic theory for a right circular cylinder and 2) all internal motions are considered to be sufficiently rapid that the net effect of such motions on the spectra is to reduce both the chemical shift (or g tensor) anisotropy and the splitting (or A tensor) anisotropy. The internal motion is parameterized by a single adjustable parameter as a rotational or librational motion of defined amplitude about either 1 or 2 axes for either a square well or a harmonic well potential. A consequence of the above model is that no estimate of the time scale of the internal dynamics is possible.

With this model all of the EPR data is simulated with a single set of tensors and a single oscillation amplitude. The optimum tilt angle of the probe from the helix axis is 20°, making this a probe that is more sensitive to flexural motions than to twisting motions of the DNA at X-band. An analysis of EPR spectra at Q-band will be done to identify twisting motion as well as flexural motion.

The motion of the probe on the DNA comes from three sources: 1) overall rotation of the duplex DNA, 2) the collective internal (length dependent) motions of the base pairs, and 3) the (length) independent motion of the probe-base entity. The conclusions of this study are: 1) The overall rotation is excellently described by the hydrodynamic theory for a right circular cylinder [of Tirado and de la Torre]. 2) The internal oscillation amplitude is only weakly dependent upon viscosity, verifying that the local probe mobility is not determined by direct solvent interactions. The length independent motion is characterized by an amplitude of oscillation of around 9° at 20°C and has a strong temperature dependence indicating that the local structure of the DNA changes with temperature. 3) The length dependence of the internal dynamics, using the continuous filament model for DNA dynamics, leads to an estimate of the flexure component of the dynamic persistence length of around 1900 ± 450 Å. at 20°C, with a temperature dependence that suggests that the restoring potential for the collective flexural modes of a weakly bending rod are well described as harmonic.

Tu-Pos277

DYNAMIC VS. STATIC BENDING RIGIDITIES FOR DNA AND M13 VIRUS. J. Michael Schurr, Lu Song, and Ug-Sung Kim, Department of Chemistry, University of Washington, Seattle, WA 98195

The bending rigidities of filamentous biopolymers have generally been inferred from their equilibrium or static persistence lengths (P_s). However, if the bending potential is biased and/or exhibits multiple minima (e.g. if the filament exhibits slowly interconverting microstates not all of which are straight), then the dynamic bending rigidity measured at short times may significantly exceed that inferred from P_s. A normal mode theory for the Brownian dynamics of weakly bending rods with preset hydrodynamic interactions has been developed and tested against Brownian dynamics simulations. This theory is used to analyze literature data pertaining to the off-field decay of the transient electric dichroism and birefringence of DNA restriction fragments containing N+1 = 95 to 250 base-pairs. The relaxation times (τ) of the longest bending normal modes for all fragments lie on the same theoretical curve of τ vs. $(N+1)^4$, which is calculated for a dynamic persistence length P_d = 2100 angstroms. This is about three times the consensus static value P_s = 700 angstroms under the prevailing millimolar salt conditions.

This normal mode treatment for weakly bending rods is incorporated into a theory of the dynamic structure factor, which is valid up to several relaxation times at large K^2. This theory differs from previous attempts to treat the effect of flexure on dynamic light scattering in that stretching along the axis is not allowed, as motions are constrained to be perpendicular to the end-to-end vector. Comparison of the experimental D_{app} vs. K^2 data with theoretical curves computed for various values of P_d permit estimation P_d. Unlike the case of DNA, the best-fit value of P_d for M13 virus is close to the static value P_s (which is estimated for the essentially identical fd virus by Maeda and Fujime).

The possibility that DNA is a linear mosaic comprising extended domains of different secondary structure that interconvert only slowly is consistent with the observed large ratio of P_d/P_s, and also with the recent finding of a very long range (100 base-pairs or more) effect of a $(GC)_8$ insert on the secondary structure of its flanking DNA sequences.

Tu-Pos278

A UNIVERSAL CURVE FOR POLYMER BIREFRINGENCE DECAY?. M.Hong, R. Austin, Dept. of Physics, Princeton Univ.- One of the unsolved problems in polymer dynamics is the functional form of the bending decay function for a long polymer of many persistence lengths. A recent paper (Degiorgio et al. ,Phys. Rev. Lett. 64, 1043-1046, 1990) has done a calculation using gaussian coils of self-avoiding chains to show that in the limit of a static configuration of chains that the birefringence decay function should go as $\exp[-(t/\tau)^{\beta}]$, that is a stretched exponential. The predicted value for β was 0.44.

We will present evidence using restriction fragment DNA's showing that (1) In the limit of infinitely short pulses the analysis is correct and the birefringence decay is a stretched exponential with $\beta = 0.44$. (2) For finite pulses the curves move from stretched exponentials to power laws, that is $(1 + t/\tau)^{\alpha}$. Experimentally, we measure $\alpha = -1.5$. We will discuss the physical implications of this change from a stretched exponential to power law. (3) The functional form of the change in β for polymers much less than one persistence length ($\beta = 1$) to infinite length polymers ($\beta = 0.44$) should provide a hydrodynamic-free way to find the actual persistence length of DNA.

Tu-Pos279

MULTIVALENT CATION BINDING ENVIRONMENTS ON DNA. W. H. Braunlin, Q. Xu, and T. M. Garver, Department of Chemistry, University of Nebraska, Lincoln, NE 68588-0304

As monitored by cation NMR, the mobility of multivalent cations trapped near the surface of double-helical DNA is remarkably high - comparable to that previously noted for univalent cations such as $^{23}Na^{+}$. Despite this apparently diffuse mode of binding, the observed NMR parameters of bound multivalent cations show dramatic dependences on the local structure of the DNA. The resolution of this apparent contradiction can be found in the interplay between local and global effects. Thus, the long-range electrostatic attraction of the highly charged DNA molecule for simple cations results in a very high local concentration of cations near the surface of the DNA. This high local ion concentration in turn favors otherwise intrinsically weak interactions of simple cations with specific structural features on the DNA. Such specific interactions in turn provide a driving force for large-scale structural transitions of DNA.

These conclusions follow from multinuclear NMR studies of $Co(NH_3)_6^{3+}$ binding to natural DNA's of variable %GC. The structural specificity of $Co(NH_3)_6^{3+}$ binding to DNA has been further defined through NMR studies of $Co(NH_3)_6^{3+}$ binding to several different oligomeric DNA's. Finally, additional studies utilizing stereoisomers of C^{13} and N^{15} labelled $Co(en)_3^{3+}$ will be reported.

Tu-Pos280

HYDRATION AND MELTING BEHAVIOR OF DNA HAIRPINS. Dionisios Rentzeperis, Dimitry P. Kharakoz and Luis A. Marky. Department of Chemistry, New York University, 4 Washington Place, Room 514, New York, NY 10003.

Hairpin structures have been implicated as binding sites and/or control regions in DNA duplexes. We studied the effects of temperature and salt concentration on the helix-coil transition of DNA hairpins with the following sequences: $d(GCGCT_5GCGC)$, $d(GTACT_5GTAC)$ and $d(GCGCT_5GCGCGTACT_5GTAC)$. We used a combination of UV melting and differential scanning calorimetric techniques. Over a tenfold range in salt concentration, the two single hairpins melt in a monomolecular monophasic two state transitions with a difference in transition temperatures, ΔT_m, between them of 42°C. On the other hand the double hairpin melts in a monomolecular biphasic transition with a ΔT_m equal to 27°C. The differential stabilization with both hairpins in a single molecule corresponds to a $\Delta\Delta G°$ = -0.9 kcal/mol., $\Delta\Delta H°$ = -2.4 kcal/mol. and $\Delta\Delta S°$ = -5 e.u.. The overall counterion uptake of the double hairpin is substantially higher than the sum of the individual hairpins. Parallel ultrasound velocities and density measurements indicate that the double hairpin is qualitatively more hydrated than any of the single hairpins. These results indicate that the increase in base stacking interactions at the junction of the two hairpin molecules is accompanied by an uptake of counterions and water molecules.

This work was supported by NIH Grant GM-42223.

Tu-Pos281

HYDRATION EFFECTS OF OLIGO-DNA DUPLEXES CONTAINING DEOXY-ADENYLATE and DEOXY-THYMIDYLATE BULGES.

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The role of water in the formation of stable duplexes of nucleic acids is being studied by determining the concurrent volume, heat and counterion uptake changes that accompany the duplexation process. The variability of the volume contractions that we have observed in the formation of a variety of homoduplexes suggest that sequence and conformation acutely affect the degree of hydration. We have used a combination of magnetic density and calorimetric techniques to measure the change in volume and enthalpy resulting from the mixing of two complementary strands to form: a) Fully paired duplexes with 10 or 11 base pairs, and b) bulged decameric duplexes with an extra dA or dT unmatched residue. We also obtained absorbance versus temperature profiles as a function of strand and salt concentration. Relative to the fully-paired duplexes, both bulged duplexes are 10-12°C less stable. Both the counterion uptake and enthalpies are very similar. Our densimetric results indicate that an unmatched residue increases the amount of bound water molecules. The combined results strongly suggest that the destabilizing forces in bulged duplexes are entropic in nature and due to an increase in hydration levels.

This work was supported by NIH Grants GM-42223 (L.A.M.) and GM-34938 (D.W.K.).

Tu-Pos282

RNA-DRUG INTERACTIONS; IN VITRO ANTIVIRAL ACTIVITY OF RNA-INTERCALATING AGENT COMBINATIONS.

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Twenty intercalating agents were tested to examine the effects of intercalating agent-induced perturbations upon the antiviral activity of poly (A-U). Neither poly (A-U) alone nor each intercalating agent shown effective antiviral activity. When poly (A-U) was combined with major groove intercalating agents (acridine orange or proflavine), no synergism was observed. When poly (A-U) was combined with minor groove intercalating agents (erhidium (EB), propidium (PI), adriamycin (ADR) or daunomycin (DMN)) or minor/major groove intercalating agents (9-aminoacridine (9-AA), N^2-methyl-9-hydroxy-ellipticine (NMHE) or N^2,N^6-dimethyl-9-hydroxy-ellipticine (DMHE)), the 50% effective doses (ED_{50}) of the poly (A-U), 9-AA, ADR, DMHE, DMN, EB, NMHE and PI decreased 18-, 22-, 60-, 274-, 61-, 154-, 113- and 299-fold, respectively. When poly (A-U) was combined individually with eleven dyes whose mode of intercalation was not known, the ED_{50} of ametantrone (HAQ), chloroquine (CHL), mitoxantrone (DHAQ) and quinine (QUI) decreased 125-, 65-, 251- and 32-fold, respectively. These results suggest the four dyes may intercalate into poly (A-U) from the minor groove. Ten (ADR, CHL, DMN, DMHE, EB, HAQ, NMHE, PI, QUI) of the twenty agents evaluated exhibited significant synergism with poly (A-U), as quantitated by the fractional inhibitory concentration index. Interferon neutralization assays demonstrate that the interferon-inducing capability of the dye/poly (A-U) combinations approximates the sum of the capabilities of the poly (A-U) and the dyes employed. These results suggest that the majority of the dyes tested potentiate the antiviral activity of poly (A-U) without affecting the amount of interferon induced.

Tu-Pos283

THERMODYNAMICS OF DOUBLE-HELIX FORMATION OF AN OLIGONUCLEOTIDE COVALENTLY MODIFIED BY (+) AND (-) *anti*-BENZO[*a*]PYRENE DIOL EPOXIDE

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The thermodynamic parameters for helix formation and the temperature dependent absorption spectra of adducts derived from the covalent binding of the (+) and (-) enantiomers of BPDE (*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetra hydrobenzo[*a*]pyrene) at the N^2-dG position of the duplex [d(CACATG*TACAC):d(GTGTACATGTG)] were investigated. Both (+)- and (-)-BPDE *trans* double-stranded adducts give rise to conformations in which the pyrene chromophore is located at the exterior of the double-helix, while the *cis* adducts appear to be intercalative in nature. Modification with BPDE results in a lowering of the melting temperature T_m, relative to the T_m (41° C) of the unmodified double-stranded oligonucleotide. The T_m values depend on the stereochemical characteristics of the adducts as follows: (+)-*trans:* 30°, (-)-*trans*: 25°, (+)-*cis:* 34°, and (-)-*cis:* 21°. Relative to the native duplex, $\Delta H°$ is more favorable (6-11% more negative) for the two (+)-adducts, whereas in the two minus adducts $\Delta H°$ is less favorable (17% and 50% more positive for the (-)-*trans* and (-)-*cis*-adducts, respectively). The $\Delta S°$ values on the other hand, are 10-15% more negative for the two (+)-adducts, and 15% ((-)-*trans*) and 50% ((-)-*cis*) more positive for the two (-)-adducts. These differences can be attributed to the differences in the conformations of the adducts which in turn, may account for the differences in the biological activities of the (+) and (-)-enantiomers of *anti*-BPDE.

Tu-Pos284

SEQUENCE SPECIFIC BINDING MODES OF DAPI TO DNA

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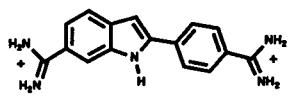
Unfused aromatic cations have a well characterized groove-binding mode at AT sequences, but we have found that some of these molecules bind to both AT and GC DNA sequences by intercalation. Spectroscopic, kinetic and hydrodynamic analyses of the interaction of an unfused aromatic cation, 4',6-diamidino-2-phenylindole (DAPI), with DNA polymers, revealed that it binds in the minor groove of AT sites as expected, however, it intercalates at GC and mixed sequence sites in DNA (1). To obtain more molecular detail on the intercalation of DAPI, we have conducted NMR experiments and molecular modeling studies on oligonucleotide-DAPI complexes. The following observations define the intercalation binding mode for DAPI: 1) intercalation with the amidine groups in the minor groove has severe steric clash with no low energy complex possible, 2) threaded intercalated models in which one amidine is in each groove are possible but are rejected based on biophysical observations from kinetics and NMR experiments, and 3) intercalation with the amidines in the major groove results in a low energy complex with favorable interactions. This latter model is supported by the NMR experiments. In this low energy complex, the phenyl-indole aromatic system is essentially planar and stacks well with the base pairs at the intercalation site. The amidine groups have electrostatic and hydrogen-bonding interactions with phosphate groups connecting the base pairs that form the intercalation site.

(1) Wilson et al., Biochemistry, 29 (1990) 8452-8461.

DAPI



Tu-Pos285

Interaction of a Macrocyclic Bisacridine with DNA

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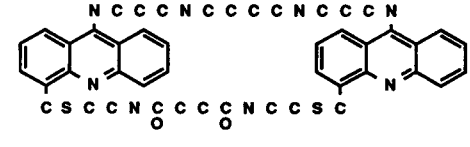
The macrocycle SDM binds to DNA by bisintercalation (1), and because of its structure there are three possible binding modes: (i) bisintercalation with the two sidechains in the same groove; (ii) bisintercalation with the two sidechains in opposite grooves according to the neighbor exclusion principle; and (iii) bisintercalation with the two sidechains in opposite grooves in violation of the neighbor exclusion principle. Studies employing visible spectroscopy, stopped-flow kinetics, and NMR spectroscopy are inconsistent with model (i), do not eliminate model (ii), and best support model (iii). A large hypochromicity upon binding to polymer DNA, and an apparent binding site size of three base pairs are observed from visible spectroscopy studies. Dissociation kinetics are slow, and the dissociation rate constant from poly $d(AT)_2$ is similar in magnitude to that observed for nogalamycin. 2D NMR studies with oligonucleotides support full intercalation of the acridine ring systems with the side chains residing in opposite grooves. Molecular modeling is completely consistent with experimental results, and further supports the conclusion that binding of SDM to DNA requires temporary disruption of Watson-Crick hydrogen bonds (models (ii) or (iii)). Molecular modeling also indicates that bisintercalative binding in violation of the neighbor exclusion principle (iii) is as energetically favorable as model (ii).

(1) Zimmerman et al., J. Am. Chem. Soc. 111 (1989), 6805-6809.



Tu-Pos286

THE NON-COVALENT DNA BINDING OF BIS-(1,10-PHENANTHROLINE) COPPER(I) AND RELATED COMPOUNDS.

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The bis-(1,10-phenanthroline) Cu(I) complex, $(Phen)_2Cu(I)$, causes spontaneous cleavage of double stranded DNA in the presence of molecular oxygen and a reducing agent. Cleavages occur preferentially at T-3',5'-A steps, particularly in TAT triplets. We have examined the non-covalent DNA binding of $(Phen)_2Cu(I)$ under anaerobic conditions by absorption and circular dichroism spectroscopy, and viscometry, as a function of phenanthroline concentration. Analyses according to the McGhee-von Hippel method indicated that binding exhibited both neighbor exclusion and positive cooperativity effects, with a neighbor exclusion parameter n = 2 and a cooperativity parameter ≈ 4. The maximal association constant for DNA binding of $(Phen)_2Cu(I)$ extrapolated to zero concentration of excess phenanthroline, was $4.7 \bullet 10^4$ M-1 (base pairs) in 0.2 M NaCl and 9.8% ethanol. The association constant decreased with increasing concentration of excess phenanthroline. The magnitude of the neighbor exclusion parameter, changes in spectral properties of $(Phen)_2Cu(I)$ induced by DNA binding, and the increase in DNA solution viscosity upon $(Phen)_2Cu(I)$ addition are consistent with a model for DNA binding by $(Phen)_2Cu(I)$ involving partial intercalation of one phenanthroline ring of the complex between DNA base pairs in the minor groove as suggested previously (Veal & Rill, *Biochemistry* 28, 3243-3250 (1989). Viscosity measurements indicated that the monophenanthroline copper(I) complex also binds to DNA by intercalation, but no evidence was found for intercalation of the bis-(2,9-dimethyl-1,10-phenanthroline)Cu(I) complex. The bis-(1,10-phenanthroline)Cu(I) complex is formed when ascorbic acid is used to reduce Cu(II), but not when thiols such as mercaptopropionic acid are used as the reducing agent. Substitution of ascorbate for mercaptopionic acid increases the rate but does not alter the sequence selectivity of DNA cleavage.

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Tu-Pos287

COMPARISON OF THERMODYNAMIC BINDING PROFILES DETERMINED FOR DAUNOMYCIN-DEOXYPOLYNUCLEOTIDE COMPLEXES AS A FUNCTION OF IONIC STRENGTH.

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In an effort to evaluate the influence of electrostatics on the daunomycin-DNA interaction, we have studied the binding of this anthracycline antibiotic to a series of synthetic deoxypolynucleotide host duplexes as a function of ionic strength. The DNA sequences examined to date include the alternating copolymers poly d(AT) • poly d(AT) and poly d(GC) • poly d(GC), the homopolymers poly d(A) • poly d(T) and poly d(G) • poly d(C), and the mixed sequences poly d(AC) • poly d(GT) and poly d(AG) • poly d(CT). Previous studies employing stopped-flow microcalorimetry have demonstrated the sensitivity and utility of this technique for measuring daunomycin binding enthalpies at *monomeric* drug concentrations [53 (1988) p. 480a], effectively precluding the need to correct for aggregation effects. In conjunction with our microcalorimetric measurements, UV/Vis and fluorescence spectroscopy facilitate determination of daunomycin binding affinities for these deoxypolynucleotide host duplexes at 25.0 °C (pH = 7.0) in sodium phosphate buffers of varying ionic strength. The resultant thermodynamic binding profiles will be presented and discussed in terms of the influence of electrostatics on the putative mode of drug binding as deduced from structural studies of daunomycin-DNA complexes.

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Tu-Pos288

INTERACTION OF ETHIDIUM WITH AN OLIGONUCLEOTIDE CONTAINING A B-Z JUNCTION.

Dongchul Suh and Jonathan B. Chaires*, Dept. of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216 and Richard D. Sheardy, Dept. of Chemistry, Seton Hall University, South Orange, NJ 07079.

Junction regions between DNA segments of different conformation have been suggested to be possible high affinity antibiotic binding sites. We have tested this possibility by studying the interaction of the simple intercalator ethidium with the oligonucleotide

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      * * * *
5'CGCGCGCGACTGACTG
3'GCGCGCGCTGACTGAC
      * * * *
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(where $\overset{*}{C}$ = 5-methylcytosine), which adopts a hybrid B-Z form in high NaCl solutions. Circular dichroism (CD) spectra show that the duplex is fully right-handed under low salt conditions. In high salt, however, the oligonucleotide possesses both left and right-handed regions and therefore contains a B-Z junction. Ethidium binding to the oligonucleotide was measured using a steady state fluorescence titration method. Binding isotherms shows positive cooperativity over the range of 0 - 4.5 M NaCl concentrations. Upon the addition of saturating amounts of ethidium bromide to the oligonucleotide in high salt, the CD spectrum of the B-Z form is switched to that of an intercalated right-handed form. The positive cooperativity can be quantitatively accounted for by application of Crothers' allosteric model, in which drug binding is postulated to be coupled to a conformational transition in the oligonucleotide. Our quantitative analysis shows that ethidium bromide binds more tightly to the oligonucleotide containing a B-Z junction than to the same oligonucleotide when it is entirely in the right-handed conformation. It is therefore possible that ethidium preferentially interacts with the B-Z junction. (Supported by N.I.H. Grant CA 35635(JBC) & NSF Grant DMB 8996232(RDS).)

Tu-Pos289

THERMAL STABILITY AND DNase I DIGESTION PROPERTIES OF TWO DECAMERS, 5'd(ATCGATCGAT) AND 5'd(CGGAATTCCG).

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Circular dichroism, thermal denaturation, and the rate of DNaseI digestion of two decamers, $5'd(ATCGATCGAT)_2$(E10) and $5'd(CGGAATTCCGG)_2$(P10), have been studied. These studies were undertaken to define the conditions necessary for proper "single site" drug-oligonucleotide footprinting studies. The thermal stability of the decamers under the footprinting conditions (50mM tris-HCl pH 7.6, 0.1M NaCl, 10mM $MgCl_2$) were studied using circular dichroism and UV spectroscopy. The E10 decamer exhibited biphasic melts and post-melting changes in its circular dichroic spectra. Sodium chloride and oligonucleotide concentration dependencies of the melts are suggestive of hairpin formation. The thermal denaturation of the P10 decamer, in contrast, exhibited behavior consistent with a two state helix-coil transition. Under the conditions of "single hit" kinetics, the most prominent DNase I cleavage site for both decamers was 7 base pairs from the 5' end. A secondary cleavage site shared by both decamer was 8 base pairs from the 5'end. The E10 decamer showed a third prominent cleavage site 6 base pairs from the 5' end. Complete kinetic analysis of the DNase I digestion of the two decamers were done at three temperatures. A parallel reaction mechanism is the most appropriate kinetic model to describe our data. For such a mechanism, the rate of disappearance of the decamer is a composite rate constant comprised of the sum of the individual rates of product formation. It was necessary, in addition, to modify the model to account for loss of DNase I activity during the course of the assay. Individual rate constants for the formation of each product oligonucleotide , as well as the overall composite rate of disappearance of the decamer have been estimated by non-linear least squares analysis.

(Supported by N.I.H.Grant CA 35635)

Tu-Pos290

DOUBLE- AND TRIPLE-STRANDED HYBRIDS FORMED BY HOMOPURINE AND HOMOPYRIMIDINE OLIGOMERS WITH PHOSPHODIESTER AND METHYLPHOSPHONATE BACKBONE.

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The 16-mers, d(AG)8 and d(CT)8, having either negatively charged phosphodiester backbone or nonionic methylphosphonate, (NpN), backbone, were investigated for their duplex and triplex formation in 0.1 M NaCl solution. UV mixing curves at neutral pH indicate the formation of 1:1 complexes for (AG):(CT), (ApG):(CT) and (AG):(CpT). Melting profiles of these duplexes give Tm's of 50, 46 and 40 °C, respectively. CD spectra indicate that the conformation of (ApG):(CT) is very similar to that of (AG):(CT) and very different from that of (AG):(CpT). Hydrogen-bonded imido proton resonances are observed for the (AG):(CT) and (AG):(CpT) duplexes. For both duplexes the 12.6 ppm resonance may be assigned to Watson-Crick A T base pairs. The G C imido resonance occurs at 13.7 and 13.9 ppm for the all phos-phodiester and hybrid duplex, respectively. At acidic pH (< 5.5), the CD spectra indicate the formation of triple-stranded complexes of similar conformation for phosphodiester and hybrid complexes at 1:2 pur:pyr strand stoichiometry. The imido proton region of the NMR spectra of hybrid complexes at acidic pH show similar resonance patterns which, when compared to the triplex having only phosphodiester strands, can be attributed to Watson-Crick and third-strand hydrogen bonds of T•A•T and C+•G•C base triads. The temperature dependence of resonances in this region indicates the presence of triplexes up to 60, 70 and 80 °C for the (C+pT)•(AG)•(CpT), (C+T)•(AG)•(CT) and (C+T)•(ApG)•(CT) complexes, respectively. This NMR data also indicates that these triple-stranded complexes melt directly to single strands without going through a double-stranded intermediate. These studies show that hybrid complexes containing phosphodiester and methylphosphonate strands can be studied in terms of structure and stability by physical chemical methods in spite of the presence of multiple stereoisomers introduced at the chiral methylphosphonate linkage. (Supported in part by DOE and NIH).

Tu-Pos291

THERMODYNAMICS OF ANTIPARALLEL HAIRPIN-DOUBLE HELIX EQUILIBRIA IN DNA OLIGONUCLEOTIDES FROM EQUILIBRIUM ULTRACENTRIFUGATION.

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Five highly palindromic DNA dodecamers, four of which may form G-A or I-A purine-purine mispairs at either the 5,8 or 6,7 positions, have been studied at sedimentation equilibrium in the analytical ultracentrifuge. Each DNA oligonucleotide forms an equilibrium mixture of ordered antiparallel-hairpin and double-stranded helical structures in buffered solutions of 0.1 M or 0.5 M NaCl between 5° and 40°. The dimeric form is favored by high salt and low temperature. The values of K_{12}, the monomer–dimer association constant, vary from 5×10^6 to 5×10^3 and are unique for each DNA dodecamer. Analysis of the temperature dependence of the values of K_{12} shows that the conversion of double-helix to hairpin is driven by a positive entropy change and is associated with an endothermic enthalpy change. The mispair substitutions at the 5,8 positions and the IA(6,7) mispair have the lowest values for K_{12} and exhibit significantly larger entropy changes than the non-mispaired d-GGTACGCGTACC parent sequence and the thermodynamically similar GA(6,7) DNA. The consequences of such hairpin-double helix equilibria must be considered in the interpretation of other kinds of experiments carried out on oligonucleotides at different concentrations such as ultraviolet spectophotometry at ~10^{-5} M and calorimetric, NMR and infrared studies at ~10^{-4} M. The state of association must be considered in order to avoid attributing apparent thermodynamic parameters characteristic of monomeric hairpins or of hairpin-helix mixtures to structural features associated with a predominantly double helical conformation.

Tu-Pos292

DNA LOOP FORMATION BETWEEN *LAC* OPERATORS ALLOWS DETERMINATION OF PHYSICAL CHARACTERISTICS OF DNA *IN VIVO*

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Transcriptional repression of a lac promoter controlled by an isolated weak binding lac operator, O^c, is increased 5-50 fold with the addition of a strong binding lac primary operator, O^+, 100-200 base pairs upstream of the O^c operator on a multicopy plasmid in E. coli.[1] Incremental variations of the inter-operator distance yield an oscillatory pattern of modulation of repression with three characteristics: periodicity, amplitude, and an envelope defining its overall shape.[2,3]

The periodicity, analyzed by Fourier methods or visual fitting, yields an estimate of the helical repeat of 11.3 - 11.6 base pairs/turn.[1] In a similar study, Lee and Schleif determined a helical repeat of 11.1 base pairs/turn.[5] Amplitude analysis yields a torsional rigidity value of $1.1 \pm 0.3 \times 10^{-19}$ erg cm.[1] The apparent persistance length estimated by application of (SY)[3] cyclization analysis of the envelope is approximately 30 base pairs.[1] For comparison, in vitro values of these quantities obtained from cyclization analysis are 10.5 base pairs/turn, 2.4×10^{-19} erg cm and approximately 150 base pairs, respectively.[2-4]

The difference between in vivo and in vitro values for the helical repeat has been interpreted in terms of global and or local effects of supercoiling.[1] The relatively small apparent persistance length may indicate the inapplicability of the SY model because of CAP induced bending of the inter-operator region and/or intermolecular "sandwich" structure formation.[1] Experiments are in progress which examine the role of bending and intermolecular vs. intramolecular effects on the length dependence of looping in vivo.

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Tu-Pos293

ACIDIC DOMAINS AROUND NUCLEIC ACIDS.

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The hydrogen ion concentration in the vicinity of DNA was mapped out within the Poisson-Boltzmann approximation. Experimental conditions were modeled by assuming Na-DNA to be solvated in a buffer solution containing 45 mM Tris and 3 mM Mg cations at pH 7.5. Three regions of high H^+ concentration (> 10^{-5} M) are predicted: one throughout the minor groove and two localized symmetrically in the major groove near N^7 of guanine and C^5 of cytosine for a G-C base pair. These acidic domains correlate well with the observed covalent binding sites of benzo(a)pyrene epoxide (N^2 of guanine) and of aflatoxin B_1 epoxide (N^7 of guanine), chemical carcinogens which presumably undergo acid catalysis to form highly reactive carbocations that ultimately bind to DNA. It is suggested that these regions of high H^+ concentration may also be of concern in understanding interactions involving proteins and noncarcinogenic molecules with or near nucleic acids.

Tu-Pos294

A COMPARISON OF POISSON-BOLTZMANN THEORY WITH MONTE CARLO CALCULATIONS OF THE DISTRIBUTION OF COUNTERIONS AROUND ALL-ATOM MODELS OF DNA. George R. Pack, Linda Wong, and Gene Lamm, Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford, 1601 Parkview Ave., Rockford, IL 61107.

A comparison of the results of Metropolis Monte Carlo (MC) calculations with those obtained from the Poisson-Boltzmann (PB) equation is presented. The system considered is an all-atom cell model for the polyanion DNA and the quantity calculated is the three-dimensional distribution of counterion charge around the central polyion. The two types of calculations were set up to be identical with the single difference being that the PB approach uses the mean field approximation whereas the Monte Carlo method explicitly includes interionic correlation. The effect of including electrostatic hard sphere and cavity potential corrections in the PB approximation is also investigated. The results indicate that at the surface of the macromolecule there are regions in which the PB calculation predicts greater concentrations that does the MC method while in other regions the reverse is true. These regions are also slightly dependent upon ion size. Although local difference in counterion density are found for these two methods, average ionic distributions over slightly more global regions are quite similar.

Tu-Pos295

DETECTION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN RAT HEPATOMA CELLS WITH GYLCOLYTIC ENZYME cDNA PROBES. C. Wigley, D. W. Mittanck, and R. A. Nakashima, Dept. of Chemistry & Biochemistry, Texas Tech University, P. O. Box 4260, Lubbock, TX 79409

The technique of restriction fragment length polymorphism has been used for the detection of several genetically linked diseases, including sickle cell anemia and Huntington's chorea. More recently, RFLP analysis has been applied to human colon carcinomas by Vogelstein's laboratory (Science 244:207, 1989). The goal of the present study is to determine whether or not a generalized RFLP test can be developed for transformed cells, using glycolytic enzyme cDNA probes. An increased rate of aerobic glycolysis is one of the earliest and most consistent biochemical markers for cell transformation. Increased glycolysis is accompanied by a ten-fold increase in specific activities for the key glycolytic enzymes hexokinase, phosphofructokinase, and pyruvate kinase. Slot blot analysis using glycolytic enzyme cDNA probes shows that increased activities of these enzymes result at least partially from an increase in specific messenger RNA levels. When restriction digests of AS-30D hepatoma and normal rat liver DNA were probed with a cDNA specific for hexokinase (courtesy of Dr. John Wilson), several apparent fragment length polymorphisms were detected. Analysis of DNA from a series of Morris hepatomas and the AS-30D hepatoma suggest that a band of apparent size 12,300 base pairs may provide a progression-linked marker for tumors. (Supported by Grant #003644-010 from the State of Texas Advanced Research Program.)

Tu-Pos296

FRACTAL DYNAMICS OF DNA'S COUNTERIONS. T. Gregory Dewey, Department of Chemistry, University of Denver, Denver CO 80208.

The diffusional dynamics of polyelectrolyte counterions was investigated using a quantum simulation. In this approach the Langevin equation governing the counterion diffusion is transformed into a "Schroedinger-like" equation. Thus, a "wavefunction" simulating the motion of the counterion can be determined. This theoretical development has two advantages. First, for simple, electrostatic potentials, it provides an exactly solvable problem and the parameters of the system are readily calculated using standard formulae from quantum mechanics. The second advantage is that more sophisticated, Poisson-Boltzman potentials may be treated using perturbation theory calculations. For an unscreened, line potential the probability distribution function, P(r,t), of counterions can be determined exactly. It has a functional form at short times which is identical to that obtained for a particle diffusing on a fractal lattice. The long time limit shows normal diffusional behavior. Additionally, the average mean squared displacement, $\langle r(t)r \rangle$, has a normal diffusional time dependence. A similar approach can be used to calculate the dynamics of the coions. The extension of these results to more realistic potentials is possible using the quantum mechanical perturbation theory and the basis functions from the simple case. This extension will also be discussed.

Tu-VCR4

UNWINDING DYNAMICS OF U-SHAPED DNA MOLECULE DURING GEL ELECTROPHORESIS. *Lu Song, and Marcos F. Maestre*, Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, CA 94720*

Motions of T4 DNA molecule undergoing gel electrophoresis were studied by using fluorescence microscope. When DNA molecules move in an electrophoresis gel, they would periodically change their configurations from extended to contracted forms. Very often it would be caught by gel fiber somewhere along the chain with two arms fully streched by the electric field and exhibit U-configuration. The longer arm would always move forward pulling the shorter arm around the obstacle until the whole molecule is released. The projections of the lengths of both arms on the confocal plane were measured at 0.4 second time intervals. The length decrease of the shorter arm always appeared to be exponential in time. The length increase of the longer arm could be either exponential or linear. The time constants of the two processes are different. The results clearly show that the characteristic time constant of the lengthening arm is about one half of that of the shortening arm. This might indicate that the friction force at the hooking point is comparable to the tensions of DNA chain near the obstacle. Theoretical calculations of the unwinding dynamics will also be shown.

Tu-Poa297

TRANSCRIPTIONAL SUPERCOILING OF DNA

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We have constructed a model system for the study of driven rotation of the DNA template by RNA polymerase. Our plasmid constructs utilize strong promoters (based on the P_{A1} promoter from phage T7) under the control of the *lac* repressor and long transcribed regions (the *trp* and *lac* operons) to efficiently produce positive supercoils upon inhibition of DNA gyrase *in vivo*. Accumulation of positive supercoils occurs independent of gene orientation in these constructs, suggesting that an interaction with the DNA, in addition to the transcription complex, may form a topological anchor in cells. The production of the overwound species is rapid, and reaches its fullest extent within 4 min of addition of novobiocin, a gyrase inhibitor, to a culture. Treatment with novobiocin alone does not result in the production of overwound DNA. Activation of transcription by the addition of IPTG to the culture is obligatory for the production of this species. A mechanism for the production of supercoiled domains by transcription is discussed in light of these results.

Tu-Pos298

INTERACTION OF CHOLESTEROL WITH SATURATED PHOSPHOLIPIDS: ROLE OF THE C(17) SIDE CHAIN. Michael A. Singer* and Leonard Finegold**, * Department of Medicine, Queen's University, Kingston, Ontario K7L 3N6 (Canada) and ** Department of Physics, Drexel University, Philadelphia, PA 19104 (U.S.A.).

Cholesterol and 5-androsten-3β-ol differ structurally only in the presence of an eight carbon side chain at the C(17) position in the former sterol. Both molecules decrease the main transition enthalpy change (ΔH) in a series of phosphatidylcholines and phosphatidylethanolamines, of acyl chain length n, with the reduction being a linear function of sterol concentration (c). The sterol concentrations at which ΔH = 0 bear a straight line relationship to n and are equivalent for both cholesterol and 5-androsten-3β-ol. In addition, both sterols give identical ΔH versus c slopes. These results underscore the importance of acyl chain length in the cholesterol/phospholipid interaction and also indicate that the cholesterol C(17) side group is not an essential requirement for the capacity of the sterol to decrease the enthalpy change of the main transition.

Tu-Pos299

CHOLESTEROL'S INTERACTION WITH MULTI-LIPIDS IN MULTI-LAMELLAR BILAYERS

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To extend our knowledge of model membrane systems based upon one lipid component, multi-lamellar bilayers were made of cholesterol with two phospholipids in equimolar ratio, and the enthalpy change ΔH of the main phase transition of the ternary mixture was measured by differential scanning calorimetry (d.s.c.) as a function of increasing cholesterol concentration c. The lipids were saturated phosphatidylcholines CnPC of acyl chain length n, and as the n of the two lipids became more different (from C13PC/C14PC to C14PC/C15PC to C14PC/C18PC to C14PC/C19PC) distinct breaks in the ΔH versus c plots were observed. These mixtures displayed only one broad d.s.c. endotherm. Mixtures of an unsaturated lipid C18:1PC (dioleoyl) with C16PC or with C18PC showed two peaks, with each peak being associated with its parent lipid. However, the ΔH versus c plots for each of these peaks showed an initial independence of cholesterol concentration followed by a dependence on cholesterol concentration. These results indicate that, in lipid mixtures, the type of interaction of cholesterol with each lipid component depends on the concentration of cholesterol present.

Tu-Pos300

NEW LIQUID CRYSTAL PHASE IN DILAUROYLPHOSPHATIDYLCHOLINE (C12PC) BILAYERS, BY SYNCHROCYCLOTRON X-RAY DIFFRACTION

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Whereas phosphatidylcholines CnPC of saturated acyl chain length $n \geq 13$ carbons show only one sharp peak ($P_{\beta'}$ to L_α) in differential scanning calorimetry d.s.c., C12PC shows one sharp peak at -1.8°C and one additional broad peak at 5°C. Extensive purity and other studies [1] show that the broad peak is real, and not an impurity, and it was suggested [1] that there exists a partially ordered liquid crystalline phase "L_χ" between -1.8 and 5°C. To elucidate the nature of the L_χ phase, we have used synchrocyclotron x-ray diffraction on identical (to those of [1]) samples of C12PC, and at corresponding scan rates to the d.s.c. work of [1]. Small-angle diffraction results show that below -1.8°C a ripple peak indicating a $P_{\beta'}$ phase is seen; then with increasing temperature the lamellar spacing increases suddenly at the $P_{\beta'}$ to L_χ transition, to decrease in the L_χ phase, to show gradual inflection at the L_χ to L_α transition, and then at the highest temperature to show the usual liquid crystal L_α behaviour. Recent wide-angle diffraction shows two peaks at 4.26 Å (stronger in the L_χ phase) and at 4.49 Å (stronger in the L_α phase) which coexist and which together are consistent with the broad d.s.c. peak at 5°C. Hence it is established that there is a new liquid crystal phase in C12PC.

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Tu-Pos301

PLASMA MEMBRANE VISCOSITY MEASUREMENTS ON HUMAN LIVING KERATINOCYTES BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY.

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Rapidly proliferating adult human keratinocytes grown in the absence of polyunsaturated (essential) fatty acids have been postulated by us to have abnormal membrane viscosities. An innovation of the standard technique of spin-labelling was developed to measure the viscosity of the plasma membrane of living keratinocytes (epidermal skin cells) using an electron paramagnetic resonance (epr) spectrometer. This technology uses exacting delivery systems defining spin probe [16-doxylstearic acid methyl ester] and carrier concentrations and ratios, and incubation temperature and label loading times. Precise epr running conditions controlling temperature, field modulation amplitude, microwave power, sample and, microwave cavity geometry were used to measure the viscosity in the plasma membranes of living keratinocyte monolayers. The epr data were analyzed by our newly developed spectral simulation method based on a previously written iterative non-linear minimization algorithm. The output of this algorithm (five parameters) was converted to viscosity by comparing these parameters to an emperically determined library of values. These were obtained by multi-temperature epr experiments on an oil of known viscosities, thus avoiding the usual ambiguities in analyzing the data from spin-label experiments. Reproducible measurements show that the viscosity of keratinocyte plasma membranes varies five-fold or greater, depending on the saturated, mono- and polyunsaturated fatty acid composition of the phospholipids in the membrane. The fatty acid content of the cells varies, depending on the cell strain, passage number and growth medium formulation. Supported by NIH grants GM 32785 and AR 26009.

Tu-Pos302

CHARACTERIZATION OF MEMBRANE COMPRESSIBILITY USING FLUORESCENCE TECHNIQUES S. Scarlata* and M. Sassaroli*, Cornell Univ. Med. College, New York, NY 10021* and Mt. Sinai School of Medicine, New York, NY 10029

We have measured the hydrostatic pressure dependence of the free volume of several membrane systems by fluorescence polarization and pyrene excimer formation techniques. Fluorescence polarization studies were done using a series of anthroyl fatty acids in which the probe is located at varying membrane depths. These experiments allow for extraction of the microscopic compressibility. Within error, the compressibility is constant with membrane depth. By monitoring in-plane and out-of-plane probe rotations we can assess the compressibility in different membrane planes. Our data show a large decrease in free volume perpendicular to the lipid chains with much smaller changes in the planes more parallel to the chains consistent with an increase in bilayer thickness. Unsaturated systems are more compressible than saturated and the values are close to those of organic solids. Our data also indicate membrane expansion with temperature. Studies utilizing dipyrenyl phospholipid probes show that the volume of activation for the intramolecular excimer formation process decreases as a function of increasing temperature. This can be attributed to a temperature dependent expansion of the lipid matrix consistent with the polarization results. Supported by N.I.H. GM39924 and R24-RR05272.

Tu-Pos303

THERMAL STABILITY OF LIPOSOMES FORMED FROM TETRAETHER ARCHAEBACTERIAL LIPIDS. Barbara Herendeen[1,2], Rick Light[1] and E.L. Chang[1,3], [1]Center for Bio/Molecular Science and Engineering, NRL, Washington DC 20375-5000; [2]Geo-Centers, Newton Upper Falls, MA 02164; Dept. of Protein & Molecular Biology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF.

Sulfolobus acidocaldarius, a thermoacidophilic archaebacterium lives optimally at Ph 2-3 and 65-75°C. The majority of lipids from this archaebacterium are bipolar, bidiphytanyl, tetraether lipids that have the potential to span the biomembrane. However, since these lipids make up only 25-40%, by mass, of the native membrane, it is not obvious that liposomes formed from these lipids (and without the proteins) can possess the same remarkable thermostability as demonstrated by the native membrane.

We report here the preliminary results of a study on the thermostability of liposomes made from linear saturated and unsaturated lipids, as well as monopolar and bipolar branched lipids. For the experiments, stability is taken to mean membrane integrity, as quantified by the leakage of carboxyfluorescein, a polar solute, across the lipid membrane.

The aims of the study are to determine the "normal" thermal stability of lipid bilayers, the effect of the state of the lipid, and the role of methyl branching and bipolarity on lipid membrane stability. Results of the study will be presented and discussed in relation to the various unique structural features of the tetraether lipids. A possible thermodynamic model for the stability of lipid membranes will be examined.

Tu-Pos304

THERMODYNAMIC STABILITY OF PHOSPHATIDYLCHOLINE VESICLES. Dov Lichtenberg, Gabrielle Bains, Sarna Valliappan and Ernesto Freire. Departments of Physiology and Pharmacology, Tel Aviv University, Tel Aviv, Israel (D-L) and Biocalorimetry Center, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Isothermal titration calorimetry has been used to determine the stability of phosphatidylcholine unilamellar vesicles of different sizes, below and above the gel-liquid crystalline phase transition temperature. These experiments have been performed by measuring the amount of heat released or absorbed upon injecting the phospholipid vesicles into a detergent solution containing excess Triton X-100. In doing so, the vesicles are brought from the conditions under consideration to a common reference state. Analysis of the enthalpy of solubilization allows determination of the enthalpy differences between the various vesicle preparations. These experiments indicate that in the gel phase, the enthalpies of vesicle solubilization are highly endothermic (8.9 kcal/mole for large unilamellar extruded DPPC vesicles of 1000 Å diameter and 6.0 kcal/mole for DPPC sonicated vesicles of 400Å diameter). Similar experiments performed with POPC (fluid phase at 25° C) indicate that in the fluid phase the enthalpy of solubilization is very small (330 cal/mole)and almost independent of the vesicle size. The larger enthalpy of solubilization in the gel phase accounts for the smaller transition enthalpy observed for small sonicated vesicles and supports the idea that in small vesicles which have a high curvature the gel phase is more disordered than in large vesicles which have a lower curvature. These results also suggest that vesicle fusion in the gel phase has a strong exothermic enthalpic component whereas in the fluid phase this component is negligible, and therefore any fluid phase fusion must be primarily entropic. (Supported by NIH Grant RR-04328)

Tu-Pos305

AMPHOTERICIN B-PHOSPHOLIPID INTERACTIONS RESPONSIBLE FOR REDUCED TOXICITY.

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When interacting with DMPC/DMPG (7:3) in an aqueous environment amphotericin B forms non-liposomal ribbon-like structures of markedly reduced toxicity [Janoff et al.(1988) *Proc. Nat'l. Acad. Sci. USA 85*, 6122-6126]. At amphotericin B to lipid ratios of 1:3 to 1:1 these structures are found exclusively, while at lower mole percentages of drug these structures occur in combination with liposomes. Circular dichroism (CD) spectroscopy revealed that the ribbon-like structures are comprised of a lipid-polyene complex possessing a distinct and reproducible signature. Liposomal amphotericin B on the other hand exhibited a monomeric CD spectrum and was dramatically more toxic to RBCs. Minimal inhibitory concentrations for yeast of liposomal and complexed species were comparable and could be attributed to lipid breakdown within the complexed material by an extracellular yeast product (lipase). Examination of nystatin-DMPC/DMPG mixtures indicated that, unlike amphotericin B, nystatin does not form a stable 'ribbon' phase and as a consequence remains significantly toxic. The formation of stable non-liposomal amphotericin B-lipid structures such as those described here indicates a possible new role for lipid as a stabilizing matrix for drug delivery of lipophilic substances, specifically where a highly ordered packing arrangement between lipid and compound can be achieved.

Tu-Pos306

NORMAL AND ABNORMAL ADSORPTION ISOTHERMS OF DPPC MEMBRANES AND NEGATIVELY CHARGED LIPOPHILIC IONS

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We studied the adsorption of pentachlorophenolate, PCP^-, dipicrylamine, DPA^- and tetraphenylborate, $TPhB^-$ by measuring the ζ-potential of DPPC vesicles: (1) ζ-potential isotherms, and (2) ζ-potential vs temperature. Our objective was to determine how the physical state of lipids affects the adsorption of lipophilic ions to the membrane. The ζ-potential isotherm of membranes in both the gel and fluid state is well described by a Langmuir-Stern-Grahame model from which the association constant, K, the area of the adsorption site, P_s, and the linear partition coefficient β were determined. Results: K,($\times 10^4$ M^{-1}): K(gel):PCP^-(0.49±0.28), DPA^-(25±10), $TPhB^-$(31±10); K(fluid): PCP^-(4.5±0.9), DPA^-(74±21), $TPhB^-$ (59±14); P_s,(nm^2): P_s(gel): PCP^-(5.4±2.3), DPA^-(5.9±2), $TPhB^-$(5.0±1.7); P_s(fluid): PCP^-(4.5±0.4), DPA^-(5.2±0.4), $TPhB^-$ (4.1±0.2); β,($\times 10^{-5}$ m): β(gel): PCP^-(0.15±0.09), DPA^- (7.1±0.3), $TPhB^-$(10±7); β(fluid): PCP^-(1.7±0.3), DPA^- (24±7), $TPhB^-$(24±6). Conclusions: the adsorption site area for PCP^-, DPA^-, and $TPhB^-$ were similar for both the gel and fluid membranes and were independent of the size and molecular structure of the adsorbing species. Using a simple discrete charge model, the adsorption site areas for all species were consistent with a dielectric constant of 8-10 and an ion adsorption depth of 0.4-0.6 nm below the water/dielectric interface. At certain temperatures (37 °C for PCP^- and 25 °C for DPA^- and $TPhB^-$), we observed anomalous isotherms that are inconsistent with the one-component adsorption model. The anomalous isotherms correspond to an isothermally driven gel-to-fluid phase transition by the lipophilic ions. Supported by NIH ES-05202.

Tu-Pos307

INTERACTION BETWEEN LONG-CHAIN AMINO-BASES AND DIPALMITOYLPHOSPHATIDYLSERINE

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We have studied the interaction of the long chain amino-bases stearylamine and sphingosine with dipalmitoylphosphatidylserine (DPPS) vesicles, in the presence and in the absence of Ca^{2+}. At neutral pH, stearylamine and sphingosine have a net positive charged, so that electrostatic interactions between these compounds and DPPS are possible.

Differential scanning calorimetry measurements were done at different concentrations of the bases and phase diagrams were elaborated. In the absence of Ca^{2+} and at low concentrations of bases, Tc of DPPS was increased indicating an stabilization of the system. On the other hand, whereas the addition of 1.5 mM Ca^{2+} to pure DPPS totally suppresses the phase transition of the phospholipid, this transition is still present in the presence of 20 mol% of stearylamine. A very broad transition is also detected in the presence of 20 mol% of sphingosine.

Fourier transform infrared spectroscopy experiments were also carried out. The C=O stretching band of DPPS was broadened in a significant extent in the presence of stearylamine, indicating a conformational change in the interfacial region of the phospholipid. Apart from that, the symmetric stretching band of the phosphate group indicates a dehydration of the phosphate moiety in the presence of stearylamine, but not in the presence of sphingosine.

This work was supported by grant no. PA86-0211 from CAICYT (Spain).

Tu-Pos308

THE INFLUENCE OF OXIDIZED STEROL COMPOUNDS ON PHASE TRANSITIONS IN LIPID MODEL MEMBRANES. J.M. Collins, Marquette University, Milwaukee, WI; O. Kucuk, M.P. Westerman and L.J. Lis, University of Health Sciences/The Chicago Medical School and The Veterans Affairs Medical Center, N. Chicago, IL; B.A. Cunningham, Bucknell University Lewisberg, PA; P.J. Quinn, Kings College, London, UK; and D.H. Wolfe, Lycoming College, Williamsport, PA.

Preliminary TLC and GC/MS results have clearly indicated the presence of oxidized sterol compounds in both normal and sickle red blood cell membrane total lipid extracts. In order to probe the possible correlation between oxidized sterol compounds and biological processes, admixtures of various oxidized sterol compounds and 1-palmitoyl,2-oleoylphosphatidylethanolamine (POPE) in buffer, or 1,2 dihexadecylphosphatidylcholine (DHPC) in water were examined using real time x-ray diffraction. The formation of nonlamellar POPE phases was enhanced by the presence of cholesterol and a variety of oxidized sterols, but not by the presence of 5 -cholestane-3 ,5,6 -triol. Structural parameters could be correlated to the theoretical spontaneous radius of curvature which describes the preference for lipid systems to form nonlamellar phases. The presence of cholesterol, or any of the oxidized sterol compounds studied was sufficient to eliminate the DHPC interdigitated gel bilayer phase over its usual temperature range, and to replace it with a rippled bilayer phase. Moreover, the presence of 5 ,6 -epoxycholesterol or 5 cholestane-3 ,5,6 -triol induces the formation of rippled phases with disordered DHPC acyl chains. It can be inferred that the presence of oxidized sterol compounds prevents the dissipation of thermomechanical stresses within the bilayer as a function of temperature during the transformation from the crystalline to the gel state acyl chain packing. These results can be related to differences in function and in morphology between normal and sickle red blood cells.

Tu-Pos309

GLYCOLIPID CONFORMATIONAL ANALYSIS BY ^{13}C AND ^{2}H NMR OF ORIENTED SYSTEMS.

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Previous work has used ^{2}H nuclear magnetic resonance (NMR) to determine the membrane conformational properties of the glucolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL). β-DTGL was selectively labelled with deuterium on the pyranose ring at C3 of glycerol. The results established the orientation of the director of motional averaging and the segmental order parameters for various regions of β-DTGL in the membrane. In addition, an estimate of the ^{1}H-^{2}H dipolar coupling between the hydrogen at C2 and deuterons at C3 of glycerol was obtained. This allowed calculation of the dihedral angle about the C2-C3 bond and the determination of the average conformation of this part of the backbone.

Such studies required specific assumptions to be made during data analysis. In order to test their validity, the ^{13}C NMR spectroscopy has been used to obtain additional information on the β-DTGL system labelled with ^{13}C on the pyranose ring. The systems studied include oriented planar multibilayers and oriented liquid crystals. The useful parameters for such determinations include the orientation of the bilayer normal with respect to the applied magnetic field, the associated ^{13}C-^{13}C dipolar couplings of the carbon atoms in the pyranose ring, and ^{13}C-^{1}H dipolar couplings. The results are used to calculate the average β-DTGL headgroup orientation, independent of any assumptions made in the deuterium studies.

Finally, molecular mechanics procedures are used to identify possible minimum-energy conformations about the glycosidic bond. This approach is an attempt to relate the ^{13}C and ^{2}H results to a cohesive description of the carbohydrate headgroup motions. Such motions were proposed in a recent dynamic ^{2}H NMR study, but are inconclusive without the information made available from the ^{13}C NMR results and the above calculations.

Tu-Pos310

GANGLIOSIDE G_{D1a} INDUCES AN ISOTROPIC PHASE IN PHOSPHATIDYLETHANOLAMINES AS MEASURED BY ^{31}P NMR

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We have studied the effect of G_{D1a} on the polymorphic phase behaviour of Egg phosphatidylethanolamine and dielaidoylphosphatidylethanolamine using ^{31}P NMR and differential scanning calorimetry. G_{D1a} facilitates the formation of large unilamellar liposomes from Egg PE and also induces the formation of an isotropic phase in PE bilayers. Calorimetry seems to indicate a biphasic effect. At low mol fractions of GD1a the temperature of the $L_\alpha \rightarrow H_{II}$ phase transition is shifted downward, while at higher mol fractions this transition temperature is raised. The Egg PE/G_{D1a} complex has a stoichiometry of 23 ± 3 Egg PE lipids per G_{D1a} as derived from the increase in intensity of the isotropic ^{31}P NMR signal with increasing ganglioside concentration. In the gel-phase, none or only small amounts of isotropic phase were observed. Above the gel-to-liquid crystalline phase transition temperature, the intensity of the istropic ^{31}P NMR signal increased rapidly to a plateau. The isotropic phase co-exists with the liquid-crystalline Egg PE bilayer, indicating that the exchange rate of Egg PE lipids between both phases is slow on the ^{31}P-CSA averaging timescale (slower 0.2 ms). At higher temperatures (> 40 °C), a second isotropic phase is observed which does not correlate with the concentration of GD1a in the Egg PE bilayers. The neutral pH fusion of Sendai virus to Egg PE/G_{D1a} liposomes has a maximum fusion rate constant (150% increase) at 4-5 mol% G_{D1a} in Egg PE liposomes corresponding to liposomes yielding completely isotropic ^{31}P NMR powder patterns. Supported by MRC grant MT-7654 (RME) and a NSERC pre-doctoral fellowship (JJC).

Tu-Pos311

DIVALENT CATION INTERACTIONS WITH BILAYERS COMPOSED OF 1,2-DIACYL-3-SUCCINYLGLYCEROL. A. Tari[1], N. Fuller[2], P. Rand[2] and L. Huang[1], [1]Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840 and [2]Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada.

Interactions of divalent cations (Ca^{2+} and Mg^{2+}) with bilayers prepared from 1,2-diacyl-3-succinylglycerol (DASG) were studied. Under identical conditions where divalent cations were limited, Mg^{2+} was found to be more effective than Ca^{2+} in inducing the aggregation and lipid mixing of liposomes composed of 1,2-dioleoyl-3-succinylglycerol (DOSG). Differential scanning calorimetric studies on 1,2-dipalmitoyl-3-succinyl-glycerol (DPSG) also showed that in the presence of limiting divalent cations, Mg^{2+} was more effective than Ca^{2+} in driving up the chain-melting temperature (Tm) of DPSG. X-ray diffraction studies on the hydrated DOSG and DPSG were also done. With limiting divalent cations, heterogenous mixtures of dehydrated and hydrated lipid structures were observed. But in the excess of divalent cations, both Mg^{2+} and Ca^{2+} could induce the lipids to undergo a complete lamellar to hexagonal phase transition. These studies suggest that with limiting divalent cations, Mg^{2+} is more effective than Ca^{2+} in destabilizing the DASG bilayers. Implications of these results in the drug delivery activity of liposomes composed of DASG will be discussed. Supported by NIH grants CA 24553 and AI 25834 and NSERC of Canada.

Tu-Pos312

THE COVALENT ATTACHMENT OF CRYOPROTECTIVE SUGARS TO MEMBRANE PHOSPHOLIPIDS.

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A number of sugars have been shown to have the ability to preserve biological and synthetic membranes under periods of water stress such as freezing and desiccation. This action may be related to the ability of the sugar to form hydrogen bonds to the interfacial region of the bilayer altering membrane phase properties and preventing membrane fusion and leakage during stress. Recently, synthetic modification of cholesterol derivatives containing galactose or maltose have been shown to have cryoprotectant ability (Goodrich, R.P. et al., Biochem. Biophys. Acta, 938, 143-154, 1988). We have extended these studies to study the ability of these molecules to alter the phase properties of dry DPPC. Calorimetric and spectroscopic studies of the phase behavior of dry films of DPPC co-dried with 3,6,9-trioxaoctan-1-B-D-galactosylcholesteryl-3B-ol (TEC-Gal) and 3,6,9-trioxaoctan-1-B-D-maltosylcholesteryl-3B-ol (TEC-Mal) will be presented. In addition, we have synthesized derivatives of two lecithins to contain these same sugars with similar linker and sugar groups. The synthetic schemes for two glycolipids, one a derivative of DPPC and the other a derivative of DC8,9PC (1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine), a polymerizable diacetylenic phosphatidylcholine. Modification of the lipid headgroups results in the covalent attachment of the sugars by a triethoxy linkage similar to the linkage used in the cholesterol synthesis. The effect of the sugar moiety on the ability of this lipid to form cylindrical microstructures and the stability of these microstructures to freezing and dehydration will be presented.

Tu-Pos313

THE INFLUENCE OF FIBRINOGEN ON THE PHASE TRANSITIONS AND STRUCTURES OF DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS. O. Kucuk and L.J. Lis, University of Health Sciences/The Chicago Medical School and the Veterans Affairs Medical Center, North Chicago, IL; H.C. Kwaan, Northwestern University Medical School, Chicago, IL; B.A. Cunningham, Bucknell University, Lewisburg, PA; and P.J. Quinn, Kings College, London, UK.

The presence of an acquired fibrinogen abnormality which causes red blood cell (RBC) aggregation and ischemia in distal extremities has been recently described. Raman spectroscopic examination of the abnormal fibrinogen suggests an increase in the alpha helical content when compared to normal fibrinogen. In order to determine if differences exist in the interactions between normal or abnormal fibrinogen with RBC membranes, model membrane systems were examined. Specifically, dipalmitoylphosphatidylcholine (DPPC) bilayers were hydrated in the presence of phosphate buffered saline (PBS) solutions containing 4mg/ml normal or abnormal fibrinogen, and examined using differential scanning calorimetry (DSC) and real time x-ray diffraction. DSC scans reveal that the DPPC pre-transition is eliminated but the main transition remains essentially the same for bilayers hydrated in PBS solutions containing normal or abnormal fibrinogen. Real time x-ray diffraction indicates that the main transition observed calorimetrically involves the transformation from the gel state bilayer ($L_{\beta'}$) into a bilayer containing disordered acyl chains (L_α). These observations can be interpreted to indicate that fibrinogen, in general, interacts with the lipid bilayer interface, and that differences in cell aggregation in the presence of an abnormal fibrinogen is not dependent on changes in the fibrinogen-membrane interaction but is dependent on changes in the interactions within the intercellular fibrinogen network.

Tu-Pos314

DOES THE PREFERENTIAL INTERACTION HYPOTHESIS APPLY TO LIPID BILAYERS? Lois M. Crowe and John H. Crowe, Dept. of Zoology, University of California, Davis CA 95616

We have investigated the effect of two monosaccharides, glucose and fructose, and two disaccharides, sucrose and trehalose, on the thermotropic phase transition of unilamellar extruded vesicles of DPPC. All the sugars investigated raise the main transition temperature (T_m) of some fraction of the lipid, but there are differences between the effect of glucose and the other three sugars. At low [glucose], T_m is lowered. At high [glucose] there are two transitions, one with a low T_m and one with a high T_m. The data suggest that at low concentrations, all of the glucose present may bind to the bilayer and increase headgroup spacing by physical intercalation or increased hydration. The other three sugars increase T_m, but at very high concentrations a second peak occurs at a low T_m. The appearance of a T_m above that of pure hydrated DPPC at high [glucose] suggests the possibility of the dehydration of some other population of phospholipid molecules. Sucrose, trehalose and fructose appear to dehydrate the bilayer at low concentrations, but may show some binding or increase hydration at very high concentrations. The sugar effects on unilamellar vesicles are strikingly different from the effects of these sugars on multilamellar vesicles.

All of the sugars decrease the cooperativity and calorimetric enthalpy of the transition.

Measurements of osmolality and surface tension of sucrose and fructose solutions show that the increase in T_m of DPPC in solutions of these sugars is correlated with surface tension but not osmotic pressure of the solution.

The answer to the question posed awaits the results of direct studies of the interaction of sugars with bilayers.

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Tu-Pos315

MIXED-CHAIN PHOSPHOLIPIDS: THERMOTROPIC AND MIXING BEHAVIOR. E. Brumbaugh, T. Bultmann, C. Huang, A. Klinger, H-n. Lin, Cui-jing Liu, R. B. Sisk, & Z-q. Wang. Dept. of Biochemistry, Health Sciences Center, Univ. of Virginia, Charlottesville, VA 22908.

The thermotropic phase behavior of three homologous series of mixed-chain phosphatidylcholines with molecular weights identical with C(16):C(16)PC, C(15):C(15)PC and C(14):(14)PC, respectively, have been examined by high-resolution differential scanning calorimetry. The thermodynamic parameters (T_m, ΔH, ΔS) associated with the main phase transition for dispersions of these mixed-chain phosphatidylcholines in each series are found to be inversely related to the corresponding values of the normalized chain-length difference ($\Delta C/CL$), when the values of $\Delta C/CL$ for these lipids are within the range of 0.09-0.40. However, when the value of $\Delta C/CL$ reaches the range of 0.42-0.57, the observed thermodynamic properties deviate markedly from the linear function. These observations suggest that phospholipids with $\Delta C/CL$ values in the range of 0.09-0.40 are packed, at $T<T_m$, into the partially interdigitated bilayer, whereas those with $\Delta C/CL$ values of 0.42-0.57 are parked into the mixed interdigitated bilayer, at $T<T_m$, in excess water as hypothesized by Lin et al. [Biochemistry (1990) 29, 7063]. The phase diagrams of binary mixtures of C(16):C(16)PC/C(10):C(22)PC, C(16):C(16)PC/C(12):C(20)PC and C(20):C(12)PC/C(10):C(22)PC are constructed, and the results support the view that mixed-chain phosphatidylcholines in the gel-phase bilayer pack differently when the values of $\Delta C/CL$ are greater than 0.42. In addition, the effects of changing the headgroup from PC to dimethyl PE, monomethyl PE, and PE on the thermotropic phase behavior of bilayers containing a single component of mixed-chain phospholipids and on the mixing behavior of two-component systems for mixed-chain phospholipids will also be presented. Finally, the mixing behavior of C(18):C(10)PC/C(18)lysoPC at temperatures above and below the fully interdigitated bilayer→micelle transition temperature of C(18)lysoPC will be addressed. Supported by NIH grant GM-17452.

Tu-Pos316

RAMAN SPECTROSCOPIC STUDY OF THE DOMAIN ARCHITECTURE WITHIN MULTILAMELLAR VESICLES COMPOSED OF HIGHLY UNSATURATED PHOSPHATIDYLCHOLINES AND CHOLESTEROL.

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Although virtually all naturally occurring lipids contain highly unsaturated sn-2 chains, physical studies, to date, have largely focused on systems comprised of either symmetric saturated chains or those with sn-2 chains containing only a single double bond. This study uses data derived from dispersive and Fourier-Transform Raman spectroscopy made on multilamellar vesicles to monitor the packing and dynamic properties of a series of phosphatidylcholines containing a saturated, perdeuterated sn-1 chain and varying sn-2 chains with either 1, 4 or 6 double bonds. Complete deuteration of the sn-1 chain enables separate observations to be made of the packing characteristics of either the sn-2 (C-H stretching mode region 2800-3100 cm^{-1}) or sn-1 (C-D stretching mode region 2000-2300 cm^{-1}) chains unoccluded by spectral contributions from the opposing chain. Phase transition data and order parameters derived from these Raman spectroscopic measurements, strongly suggest the formation of microdomains within the plane of the bilayer in which the sn-1 chain interactions are specifically promoted. This phenomenon is more pronounced in the more unsaturated phosphatidylcholine vesicles. The effect of cholesterol on these packing properties was determined by making similar measurements on vesicles containing 30 mole% cholesterol. These results demonstrate that varying levels of sn-2 unsaturation and cholesterol content modulate bilayer physical properties by influencing the level of domain formation. The implications of these studies relative to the effect of membrane lipid composition on integral membrane protein function will be discussed.

Tu-Pos317

PHOSPHATIDYLETHANOLAMINE ENHANCES THE CONCENTRATION-DEPENDENT EXCHANGE OF LIPIDS BETWEEN BILAYERS. William C. Wimley and T.E. Thompson. Department of Biochemistry, University of Virginia, Charlottesville, Va. 22908.

It has previously been demonstrated that lipid exchange between phosphatidylcholine vesicles, at higher total lipid concentrations, is characterized by a second-order, concentration-dependent exchange process in addition to the usual first-order process measured at lower concentrations (Jones and Thompson, *Biochemistry* (1989) **28**,129-134). Furthermore it was demonstrated that the second order process occurs as a result of an enhancement of the first-order desorption process, probably resulting from attractive interactions between potentially desorbing lipid molecules and transiently apposed bilayers (Jones and Thompson,*Biochemistry* (1990) **29**,1593-1600). Based on this model it is expected that bilayer surface properties such as hydration or charge will have a strong affect on the second order exchange process by altering the equilibrium distance between vesicles in the transient collisional complexes as well as the average interaction lifetime. In this work we have tested these ideas by studying the exchange of ^{3}H-dimyristoylphosphatidylcholine (DMPC) between large vesicles of three compositions: 100% DMPC, 70/30(mol/mol)DMPC/dimyristoylphosphatidylethanolamine(DMPE)and 68.25/30/1.75 DMPC/DMPE/DMPG. The second order exchange process is greatly enhanced (by 50-fold or more) in vesicles containing 30 mol % DMPE relative to 100% DMPC and is eliminated by the addition of 1.75% of the anionic lipid DMPG. This effect of PE is consistent with the decreased hydration and stronger interactions known to occur between PE-containing bilayers compared to PC bilayers. These results are in accord with the model of Jones and Thompson and indicate that relatively low concentrations of PE in a PC bilayer can have significant effects on bilayer surface properties and bilayer-bilayer interactions. Supported by PHS NIH grant GM14628.

Tu-Pos318

CALORIMETRY OF THE CRITICAL UNILAMELLAR LIPID STATE.

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Equilibrium conditions for the assembly of isolated phospholipid bilayers have been reported (1). The unilamellar structure appears simultaneously in the dispersion and in the equilibrium surface film, but only at a critical temperature T* that is characteristic of the lipid. Thus, for DMPC dispersions T* is 29°C. These conditions were obtained from studies in which the thermodynamic properties of phospholipid dispersions were monitored by the equilibrium lipid films that form at the air-water surface. In principle, the bulk dispersion should also exhibit properties characteristic of the critical bilayer at T*. Using a heat conduction calorimeter that has been modified for heat capacity measurements, we have been able to measure directly the critical unilamellar state properties in DMPC dispersions. Predictably, DMPC dispersions exhibit a heat capacity anomoly at 29°C, the identical temperature T* reported for this lipid by surface film measurements. The magnitude of the heat capacity change is consistent with the formation of the unilamellar state.

(1) N.L. Gershfeld, Biochim. Biophys. Acta, 988 (1989) 335-350; J. Phys. Chem., 93 (1989) 5256-5261.

Tu-Pos319

MELTING BEHAVIOR OF $^{13}C{=}O$ LABELED DIPALMITOYLPHOSPHATIDYLCHOLINE: RAMAN SPECTROSCOPIC DETERMINATION OF THE LIPID BILAYER INTERFACE REGION

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Conformational and packing properties of the phospholipid bilayer interface region can be determined from the Raman spectra of the carbonyl stretching modes. Interpretation of the spectral features is complicated by the overlap of bands attributed to the sn-1 and sn-2 acyl chains. Band overlap becomes more acute during melting and under hydrated conditions. In order to separate the spectral bands and to monitor the changes in the interface region, we have synthesized dipalmitoylphosphatidylcholine (DPPC) with the sn-2 chain C=O moiety substituted with ^{13}C. Isotopic substitution decreases the sn-2 chain carbonyl stretching mode by 40 cm^{-1}. Changes relevant to both acyl chain carbonyl groups that occur during melting and with varying degrees of hydration can be monitored independently of each other. In this study, the temperature dependence of anyhydrous, polycrystalline 2-[1-^{13}C]DPPC is investigated from 20-120°. A cooperative phase transition occurs at 108°. At temperatures below the phase transition (20-100°) and under extremely dry conditions, as indicated spectrally by the C-N symmetric stretching mode at 710 cm^{-1}, each acyl chain carbonyl band displays two distinct components. Individual doublets are attributable to various conformational arrangements for the sn-1 and sn-2 carbonyl groups. At 108° and above, the splitting patterns merge into broad single peaks. Results from temperature studies of hydrated 2-[1-C^{13}]DPPC also indicate the presence of multiple conformational arrangements. Experimental results will be presented in detail.

Tu-Pos320

TITRATION CALORIMETRY AND DIFFERENTIAL SCANNING CALORIMETRY STUDIES THE INTERACTIONS OF n-BUTANOL AND DIPALMITOYLPHOSPHATIDYLCHOLINE

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The saturated like-chain phosphatidylcholines (PC's) exist in a fully interdigitated gel phase in the presence of various ligands. The alcohol induced interdigitated gel phase of dipalmitoyl-phosphatidylcholine (DPPC) and distearoyl-phosphatidylcholine (DSPC) have been extensively studied in our lab. In the present investigation, the interactions of n-butanol with DPPC were studied using differential titration calorimetry (DTC) and differential scanning calorimetry (DSC). The DSC results indicated that n-butanol induces the interdigitated phase in DPPC at higher concentration. Both the main transition temperature and the pre- or interdigitated transition temperature decrease with increasing n-butanol concentration. A new method was developed to determine the partition coefficient of n-butanol between lipid and water by using DTC. The partition coefficients and the heat of binding of n-butanol to DPPC were measured for each of the various phases. The results indicated that the partition coefficient of n-butanol between DPPC and water for the interdigitated gel phase is smaller than that for the liquid crystal phase, but much larger than that for the non-interdigitated gel phase.

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Tu-Pos321

NON-IDEAL MIXING OF PHOSPHATIDYLSERINE AND PHOSPHATIDYLCHOLINE IN MODEL MEMBRANES.

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Our method to measure the activity coefficients of lipids mixed in a bilayer utilizes the constraint on the variance of the mixture that occurs when the heterogeneous equilibrium is established, $Ca^{2+} + 2PS^{-} \rightleftharpoons Ca(PS)_2$. In our two earlier papers that introduce this procedure, we did not take into account the surface potential of the multilamellar PS/PC vesicles, hence we did not correctly account for the surface concentration of free Ca^{2+}. In the new experiments described here, we have calculated the surface concentration of Ca^{2+}, based upon the concentration of PS^{-} surface charges, the weak binding of Ca^{2+} and K^{+}, and the ionic strength, following the procedure of Mclaughlin, et al (J. Gen. Physiol 77:445 [1981]) and of Nir, et al (Bioelect. & Bioenerg. 5:116 [1978]). We examined the non-ideality of mixing of C16:0,C18:1-PS with either C16:0,C18:1-PC or with C14:1,C14:1-PC, at ionic strengths of 55, 110 and 330 mM. We measured the high-affinity binding of Ca^{2+} to these multilamellar vesicles by means of the Ca^{2+} chelator/indicators BAPTA and BrBAPTA. These experiments yield two kinds of information:(i) The free energy to transfer PS that is completely surrounded by one type of PC, into another type of PC. Here we find a two-fold increase in the activity coefficient of C16:0,C18:1-PS for transfer from C16:0,C18:1-PC into C14:1,C14:1-PC, corresponding to a molar free energy increase of about 0.4 kcal; (ii) In all six cases examined so far, the excess molar free energy to transfer C16:0,C18:1-PS from pure PS to pure PC is positive, and corresponds to an activity coefficient *increase* of 5 to 10-fold, or an excess molar free energy increase of 1 - 1.5 kcal. These data imply that the electrostatic repulsion energy of PS^{-} - PS^{-} is far exceeded by the unfavorable interaction of phosphoserine headgroups with phosphocholine headgroups.

Tu-Pos322

MOLECULAR MODELING OF DRUG/LIPID BILAYER INTERACTIONS

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We have extended our AMBER-based molecular modeling calculations to examine the interactions of amphiphilic drug molecules with lipid bilayers. We have studied the energetics of a drug/bilayer interaction by creating a lipid bilayer environment, positioning the drug in the bilayer, and equilibrating the system. A bilayer consisting of 20x2 DMPC molecules was used with periodic boundary conditions to create the lipid environment for the drug. This system was heated to 310° allowed to equilibrate for several psec and then hydrated and reequilibrated. Several features of the model bilayer were analyzed and compared to experimental data to validate the model. For example, the electron density profile calculated from the model matched well an experimentally measured profile at the same temperature. There was significant disordering of the lipid matrix from the original hexagonal packing and extensive disordering of the lipid acyl chains. In the next phase, a central lipid molecule was removed and replaced by one drug molecule. The drug-containing bilayer was minimized and then reheated to 310° over 1 psec, hydrated, and run for several psec. The structure of the drug and the membrane were monitored, as well as the composition of the total energy related to the drug. This analysis was repeated with the drug positioned in different orientations at locations determined by X-ray and neutron diffraction studies. The structure of the drug in the bilayer matrix was compared to the crystallographic coordinates, the computed structure using MMP2, and typical structures computed using AMBER for water or in vacuo environments. We feel that this approach will prove a valuable tool for confirming experimental data for drug/membrane structures (drug location), and can supplement such data, suggesting orientations and conformations which are are beyond the resolution of the experimental approach (~5-10 Å).

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Tu-Pos323

AN INVESTIGATION OF PLASMALOGEN CONFORMATION BY NMR

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Plasmalogens are analogues of commonly studied phospholipids and occur widely in nature. While plasmalogen glycerophosphoethanolamines (PE) occur in several human tissues, the heart is unique in that about 40% of its glycerophosphocholines (PC) are plasmalogens[1]. Plasmalogens are known to be partly resistant to various phospholipase A_2's. In plasmalogens the linkage between glycerol and the hydrocarbon chain at the sn-1 position is a vinyl ether rather than an ester. The physical behavior of hydrated plasmalogens has been the subject of relatively few studies. We have initiated studies of plasmalogen conformation by ^{13}C and ^{31}P NMR methods. Both the PE and PC fractions of bovine heart containing diacyl and plasmalogen phospholipids were isolated. ^{13}C NMR spectra were obtained for each fraction in solvents of differing polarity and hydrogen bonding capacity. The chemical shift of the plasmalogen carbonyl was assigned by using purified plasmalogen PC or PE. The carbonyl group of plasmalogens and diacyl phospholipid in both fractions showed a significant chemical shift change as the solvent hydrogen bonding capacity increased. In addition one of the vinyl ether carbon resonances of both PC and PE showed a significant shift in solvents of increased H-bonding capacity. Small unilamellar vesicles of purified plasmalogen PC were prepared to study the exposure of the carbonyl and vinyl groups to the aqueous milieu. The carbonyl chemical shift relative to nonpolar solvent was interpreted in terms of degree of hydration[2]. There was little or no effect of the sn-1 linkage in plasmalogens on the sn-2 carbonyl hydration for plasmalogen PC. In addition the vinyl chemical shift indicated a less polar environment for the ether.

[1]Diagne, A. et al. Biochim. Biophys. Acta (1984) 793, 221.
[2]Schmidt, C.F. et al. Biochemistry (1977) 16, 3948.

Tu-Pos324

CHOLESTEROL AND LANOSTEROL IN PHOSPHOCHOLINE MEMBRANES: A ^2H NMR AND DSC STUDY.

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Cholesterol, with its smooth, rigid sterol moiety, has been shown to induce the formation of a 'liquid ordered' phase at concentrations above about 20 mol % in phosphocholine (PC) membranes. This liquid ordered phase is characterized by high acyl chain order and high lateral mobility of the lipids. Lanosterol's sterol ring system, with three 'bumpy' methyl groups and differing double bond position, has been postulated to be less effective than cholesterol in inducing the liquid ordered phase.

Partial phase diagrams for cholesterol/PC and lanosterol/PC are presented as determined from ^2H NMR and DSC experiments. The phospholipid employed in this study is 1-[$^2H_{31}$]palmitoyl-2-petroselenoyl-*sn*-glycero- 3-phosphocholine (PPetPC-d_{31}) which is analogous to the common natural lipid 1-palmitoyl-2-oleoyl-PC except for the position of the double bond (*cis*Δ^{6-7} in petroselenic acid) and hence the transition temperature (T_m = 15° C in PPetPC-d_{31}).

Results from the two sterols are compared in the context of the evolutionary development of the plasma membrane of eucaryotic cells.

Tu-Pos325

STUDIES OF PHOSPHOLIPID BILAYERS CONTAINING ω3 AND ω6 POLYUNSATURATED FATTY ACIDS BY ^2H NMR, X-RAY DIFFRACTION, AND MOLECULAR MODELING.

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Studies of the structural properties of polyunsaturated phospholipids containing arachidonic (20:4ω6) and docosahexaenoic (22:6ω3) acids are essential to understanding their different roles in biological membranes. Therefore, comparative studies of bilayers of (per-^2H-16:0)(20:4)PC and (per-^2H-16:0)(22:6)PC in the liquid-crystalline state have been carried out by deuterium (^2H) NMR, x-ray diffraction, and molecular modeling methods. The order profiles, obtained from ^2H NMR studies, are higher for the (per-^2H-16:0)(22:6)PC bilayer than for those of (per-^2H-16:0)(20:4)PC at the same absolute temperature. The pronounced low-density trough in the center of the electron density profile of (16:0)(22:6)PC also suggests that its acyl chains are more ordered than those of (16:0)(20:4)PC, for which there is only a broad minimum. Given an energy minimized "angle-iron" conformation of the polyvinylic segments, the (16:0)(22:6)PC molecule shows closer inter-chain contact and a more extended structure than does (16:0)(20:4)PC. The difference may reflect the bent average conformation near the beginning of the *sn*-2 arachidonyl (20:4) acyl chain, together with the more flexible pentyl group at its terminus. The ^2H NMR order profiles, in conjunction with the x-ray diffraction and molecular modeling results, suggest that bilayers of phospholipids containing ω3 fatty acids are somewhat more ordered than those containing ω6 acids at the same absolute temperature.

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Tu-Pos326

A DSC STUDY OF THE EFFECTS OF STEROL SIDE-CHAIN STRUCTURE ON THE PHASE TRANSITION OF DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS.

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In order to compare the role of sterol side-chain structure on sterol-phospholipid interaction, the variation of the main transition enthalpy of dipalmitoylphosphatidylcholine (DPPC) was monitored by differential scanning calorimetry (DSC) using multilamellar vesicles containing 1 to 15 mol % of the following Δ^5-sterols: cholesterol (chol), sitosterol (sito), campesterol (camp), 20-isocholesterol (20-iso), androst-5-en-3β-ol (andr), *cis*-22-dehydrocholesterol (Δ^{22}), 20(*R*)-*n*-nonylpregn-5-en-3β-ol (C30), and cholesta-5,22*E*,24-trien-3β-ol ($\Delta^{22,24}$). Plots of ΔH (obtained from cooling scans) vs. mol % sterol were linear. A comparison of the slopes of these plots provides an estimate of the sterol's ability to pack with phospholipid acyl chains. The DSC data show that sterols with very long or with very bulky side chains (C30 and $\Delta^{22,24}$) do not pack optimally with the acyl chains of DPPC; the nine-carbon substituent of C30 may interdigitate into the adjacent leaflet of the bilayer. 24-Alkylsterols (sito = 24-ethylchol and camp = 24-methylchol) interact with the fatty acyl chains of DPPC as effectively as chol. Sterols having a kinked side-chain conformation (as in Δ^{22}) and even no side chain at all (andr) also reduced the transition enthalpy of DPPC about as effectively as chol. Inversion of the configuration of chol at C-20 resulted in a derivative (20-iso = 20(*S*)-stereoisomer of chol) that interacts with DPPC somewhat better than chol. [Supported by NIH HL-16660]

Tu-Pos327

STUDY OF THE STRUCTURE AND EFFECT OF CALCIUM IONS ON N-ACYL DIPALMITOYLPHOSPHATIDYL-ETHANOLAMINES DISPERSIONS BY VIBRATIONAL SPECTROSCOPIES

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The effect of the headgroup chain length on the structure and on the thermotropic behavior of N-acyl dipalmitoylphosphatidylethanolamines (N-acyl DPPE) has been studied by infrared and Raman spectroscopies. The results show that the N-acyl DPPEs can be divided in two classes depending on the N-acyl chain length. When the N-acyl chain contains 10 carbon atoms or more, it penetrates into the bilayer while it remains at the level of the glycerol backbone for shorter N-acyl chains. For both classes of N-acyl DPPE's, the rotation of the lipid chains in the liquid crystalline phase is hindered by the presence of the N-acyl group. In addition, the disruption of the hydrogen bonds between the amino and phosphate groups by the N-acylation of the amino group results in an increase of the hydration of the phosphate group compared to DPPE. For N-acyl DPPEs with short N-acyl chains, the frequency of the amide I vibration appears at 1625 cm^{-1}. This indicates that, for these lipids, the amide group is exposed to the solvent and forms strong hydrogen bonds with water molecules. In the case of N-acyl DPPEs with longer N-acyl chains, the amide I band is centered at 1640 cm^{-1} which is very close to that of self-associated N-methyl acetamide where both NH and CO groups formed intermolecular hydrogen bonds. The existence of such intermolecular hydrogen bonds in N-C16 DPPE has been confirmed by the isotopic dilution of this lipid with DPPC-d_{62}. Finally, the influence of calcium ions on the N-C16 DPPE dispersions has been investigated.

Tu-Pos328

AGGREGATIVE STABILITY IN LECITHIN LIPOSOMES: A STATISTICAL CRYSTALLOGRAPHIC APPROACH

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The evaluation of bilayer stability has been a formidable undertaking. Here, the powerful techniques of statistical crystallography are applied to extract stability information from random networks of two-dimensional bilayer liposomes. Such an investigation gives rise to structural equations of state governing stability of the phase transition. Analysis was carried out on 350 angles and sides taken from digitized images of lecithin liposomes. Both long- and short-term behavior shows good agreement with Aboav's law and a perimeter relation. In contrast to previous reports of long-term order, an increase in the second moment of the distribution function indicates a trend towards disorder and not asymptotic behavior. Considerable anisotropy is found, with vertex switching as the most favored mechanism of instability. This instability, in turn, leads to a high fraction of vesicles with small area (e.g. defect sites). The statistical result, likewise, points toward maximum energy carried by the interface between vesicles and dominant dipolar forces of a long-range nature. Defect generation and disorder in liposomes are found to be more strictly constrained by the mathematical requirements of space-filling than previous considerations of short-range ordering forces alone would justify. The statistical equations of state (Aboav's, perimeter law) give a measure of this constraint In three-dimensions, these crystallographic techniques should apply equally to studying the biomorphology of dense proteinoid microspheres and hence bear on theories of abiogenesis.

Tu-Pos329

A POTENTIAL PACKING MOTIF FOR GRAMICIDIN/LIPID CO-CRYSTALS

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Single crystals of a complex of gramicidin A, a transmembrane channel-forming polypeptide, and dipalmitoyl phosphatidylcholine, a phospholipid, have been prepared and characterized by X-ray diffraction. They belong to space group $P222_1$, with unit cell dimensions a=26.8A, b=27.5A, c=32.8A. The assymetric unit appears to be a complex of one gramicidin monomer and two phospholipid molecules. Molecular graphics techniques have been used to model the possible packing arrangements of the gramicidin and lipid molecules in these crystals. The unit cell dimensions, space group, and chemical composition are compatible with lipids packing in a bilayer-like motif, and an end-to-end association of gramicidin monomers to form a functional dimeric unit. This packing model is being used as the starting model for minimization by molecular mechanics.

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Tu-Pos330

LOCATION OF ION BINDING SITES IN THE GRAMICIDIN CHANNEL BY X-RAY DIFFRACTION

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X-ray diffraction of gramicidin in its membrane-active form was measured by using uniformly aligned multilayer samples of membranes containing gramicidin and various ions. From the difference electron density profiles, we found a pair of symmetrically located ion binding sites for Tl^+ at 9.6±0.3Å and for Ba^{++} at 13.0±0.2Å from the midpoint of the gramicidin channel. The location of Ba^{++} binding sites is near the ends of the channel, consistent with the experimental observation that divalent cations do not permeate but block the channel. The location of Tl^+ binding sites is somewhat a surprise. It was generally thought that monovalent cations bind to the first turn of the helix from the mouth of the channel. But our experiment shows that the Tl^+ binding site is either near the bottom of or below the first turn of the helix. Results on other ions, including distributions of anions will be presented.

Tu-Pos331

THE GRAMICIDIN CHANNEL BACKBONE FOLDING MOTIF BY SOLID STATE NMR

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Solid state ^{15}N NMR spectra of single site isotopically labeled gramicidins have been obtained from oriented lipid bilayers containing gramicidin in a 1:8 molar ratio with dimyristoylphos- phatidylcholine (DMPC). Quantitative interpretation of ^{15}N chemical shifts have been made possible by recent determinations of the orientation of the chemical shift tensors with respect to the molecular frame (Teng and Cross, J. Mag. Res. 85,439,1989). The chemical shifts of the first seven residues in gramicidin have recently been reported along with predicted chemical shifts based on several model structures (Chiu et al., Biophys. J. in press). Here, the ambiguities raised in the amino terminal residues are resolved with the determination of the tensor orientations for Val-1 and Gly-2. We also report on the chemical shifts for Val-8 through Trp-15 and make comparisons with the latest computed structures from molecular dynamics studies. While it is not possible to determine the backbone conformation from the chemical shift interaction alone, it does provide a very substatial orientational constraint which when obtained for numerous consecutive sites dramatically reduces the conformational space available to the backbone.

Tu-Pos332

ISOLATION AND SOLUTION NMR STRUCTURE DETERMINATION OF A GRAMICIDIN A DIMER.

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Gramicidin A forms a mixture of intertwined double-helices in non-polar solvents. Four different species have been resolved by thin layer chromatography (Veatch et al., Biochemistry, 13, 5249, 1974). One of these conformations, species 3, was preparatively isolated and then characterized by 2D NMR (Arseneiv et al., FEBS Lett.,165, 51,1984). Bystrov et al. (Bull. Mag. Res, 8, 84,1987) have published folding motifs for the other three species based on NOE pattern recognition, and use of gramicidin analogs. We have utilized a normal phase silicon HPLC column with a 100/1 dioxane/water mobile phase to separate preparative amounts of a previously unisolated species. NMR spectra show the conformation remains stable in pure dioxane for several weeks. Spin assignments were made via DQCOSY, NOESY and HOHAHA spectra. The CD spectra and backbone NOEs are consistent with those of species 4. We are using distance geometry and standard computational techniques to generate and refine a molecular model of both the backbone and sidechain conformation.

Tu-Pos333

NMR STUDIES OF ACYLATED GRAMICIDIN IN d_6DMSO SOLUTION AND d_{25}SDS MICELLES.

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Gramicidin K collectively is a set of acylated linear gramicidins in which the ethanolamine hydroxyl of either gramicidin A, B, or C is esterified to one of a heterogeneous population of fatty acids. Previous GC-MS studies have identified the fatty acids as primarily octadecanoic, hexadecanoic, pentadecanoic, and 12-methyl-tetradecanoic acids. We have further characterized the ester linkage and the fatty acyl chains by ^{13}C and ^{1}H NMR spectroscopy of acyl gramicidin A. In d_6DMSO, a new ester carbonyl resonance appears at 172.8 ppm in the ^{13}C spectrum of acyl gramicidin; the reference compound myristic acid ethyl ester shows a similar resonance. The Trp^{15} carbonyl resonance is shifted from 171.44 to 171.73 ppm upon acylation, whereas the formyl, glycyl and other carbonyl resonances are unchanged, suggesting that acylation affects only the C-terminal of the molecule. In the aliphatic carbon region, a new ^{13}C resonance at 19 ppm appears in the midst of the Val-$C_{\gamma 1}$ resonances at 18.9, 19.1, 19.15 and 19.2 ppm, thus confirming the presence of a major branched fatty acid component. We have also incorporated acyl gramicidin A into d_{25}SDS micelles for two-dimensional ^{1}H NMR spectroscopy. The molecule incorporates into the micelles in similar fashion to gramicidin A, whereas several sequence-substituted analogues do not incorporate. Resonances are being assigned through analysis of DQCOSY and NOESY spectra. NOESY spectra with mix times of 30 to 100 ms are being used to construct a set of distance restraints.

Tu-Pos334

THE EFFECT OF LIPID PACKING ON THE ROTATIONAL MOTION OF GRAMICIDIN TRYPTOPHANS S. F. Scarlata, Cornell Univ. Med. College, 1300 York Ave., F-231 New York, NY 10021

We have studied the influence of lipid packing on the rotational motion of gramicidin tryptophans. Previous temperature studies (Scarlata, Biophys.J. 54, 1149) have shown that in fluid phase DMPC or in octylglucoside micelles, the rotational motion of gramicidin tryptophan increase more than permitted by the solvent and it was postulated that this was due to the disruption of aromatic ring stacking with temperature. To determine whether this is the case, and to isolate changes in lipid packing from those due to heat content we subjected the protein to high hydrostatic pressure. Under pressure ring stacking should be stabilized. In micelles no changes in tryptophan rotational motion was observed from 0.001-1kbar which most likely reflects the incompressibility of the protein as compared to the micelle. In DMPC bilayers, the tryptophans showed a consistent and reproducible increase in rotational mobility from 0.001 to 2kb without evidence of protein elimination or reorientation from the membrane. The magnitude of this increase was temperature dependent and much steeper in the gel phase. Based on these results and studies by others (O'Connell et al., Science in press), we postulate that increased pressure results in a breaking of hydrogen bonds between the indole rings and the lipid surface which due to the large, pressure-induced change in bilayer dimension relative to the protein. Thus, disruption of hydrogen bonding must also contribute to the increase mobility observed with temperature. Supported by N.I.H. GM 39924.

Tu-Pos335

TAUR16-GRAMICIDIN A FORMS HYBRID CHANNELS WITH GRAMICIDIN A. Xian-Zheng Jin and David Busath, Section of Physiology, Brown University, Providence, RI 02912

The handedness of the helix assumed by gramicidin analogs in lipid bilayers can be assessed by measuring whether the analog forms hybrid channels with gramicidin A (GA) or with gramicidin A- (GA-) (Andersen et al., BJ 57: 100a,1990). The structure of the former is thought to be right handed, the later, which is an analog of gramicidin A with D amino acids replaced by L and *vise versa*, left handed (Andersen et al., 1989). Hybrids are readily detected because they ususally have a conductance which is between the conductance of the two "pure" channels. Taur16-gramicidin A (TGA) is an analog having a compact negative charge at the C-termini which are located at the entry and exit of the dimeric channel. When both TGA and GA are present in a GMO/hexadecane (50 mg/ml) bilayer which is bathed in 0.1 M NaCl with 100 mV applied, 25 - 26°C, conductance peaks were observed at 0.7 pA (GA-GA), 1.3pA (TGA-GA) and at 1.5 pA (TGA-TGA). There was no evidence of splitting in the hybrid peak. Gramicidin A-, synthesized using standard methods, was also tested. When mixed with TGA, two prominent conductance levels were observed, 0.7 and 1.5 pA. There were some channels at 1.3 pA, but it is impossible to distinguish whether these were hybrids, or low-conductance variants of TGA-TGA channels. The observation that TGA form hybrids with GA but not GA- indicates that TGA is right-handed, or at least has the same handedness as GA. Supported by NIH grant GM33361.

Tu-Pos336

[L-Leu^9-D-Trp^{10}-L-Leu^{11}-D-Trp^{12}-L-Leu^{13}-D-Trp^{14}-L-Leu^{15}]-GRAMICIDIN FORMS BOTH SINGLE- AND DOUBLE-HELICAL CHANNELS.

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Gramicidin A (gA) forms $\beta^{6.3}$-helical channels in membranes, as first proposed by Urry in 1971. In organic solvents, gA assumes a variety of conformations, most of which are double-helical, as first observed by Veatch and Blout in 1974. It has remained a puzzle why no double-helical gA channels were observed in membranes. Cation-conducting intertwined channels were first suggested to explain the long-lived hybrid channels that were observed to form between a chain-shortened analogue (g14C) and an optically reversed analogue, gM$^-$ (Durkin et al., Biophys. J. 51, 451a). We have synthesized [L-$Leu^{9,11,13,15}$-D-$Trp^{10,12,14}$]gramicidin (gLW) and have found that this analogue forms two types of channels in DPhPC membranes: "Urry-type" single-helical channels that exhibit a 20 pS conductance and a 100 ms average duration (1 M CsCl, 20 mV) and intertwined "Veatch-type" channels that exhibit a 22 pS conductance and a 25,000 ms average duration. Hybrid channel experiments show that gA channels and gLW single-helical channels have the same helix sense. The rearrangement of Trp and Leu residues in gLW has therefore not altered the helix sense of the monomers that constitute $\beta^{6.3}$-helical channels, but has increased the stability of a conducting double-stranded conformer with respect to $\beta^{6.3}$-helical monomers and dimers.

Tu-Pos337

COMPUTATIONAL STUDIES ON THE SIDE CHAIN CONFORMATIONS OF GRAMICIDIN A.

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We present here results from both high temperature molecular dynamics (HTMD) and Biased Monte-Carlo (BMC) on the side chain conformations of gramicidin A. In biased Monte Carlo the local interactions are calculated using adiabatic mapping to obtain an approximate potential energy map for each side chain. This information is then used to increase the effiency of our simulation by adding a step prior to normal Metropolis Monte-Carlo which "biases" the sampling to those regions of space which are predicted to be most energetically favorable. The results will be useful in investigating whether the side chains have a number of rotameric states and what their effect on gramicidin's activity is.

Our HTMD work indicates that the side chain conformation proposed by Urry and Venkatachalam (J. Comp. Chem., vol. 4, pg. 461, 1983) is not the most stable. HTMD at 400K produced large scale rotations in tryptophans 9 and 15 from the Urry-Venkatachalam structure to a conformation suggested by Koeppe and Kimura (Koeppe, personal communication) in which the direction of their dipole moments are reversed. Energy minimization of the two conformers reveals the Urry-Venkatachalam conformation to be of significantly higher energy.

At this writing we have completed BMC calculations on a four-peptide fragment that forms part of the gramicidin sequence and verified for this system that the computational efficiency is much better than ordinary unbiased Monte Carlo. We are now doing BMC calculations for the entire gramicidin molecule and will present our preliminary results.

Tu-Pos338

BROWNIAN DYNAMICS COMPUTATION OF ACCESS RESISTANCE TO THE MOUTH OF A GRAMICIDIN CHANNEL.

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We have set up a Brownian dynamics computation to calculate access resistance for ions to the mouth of the gramicidin channel, using the lipid conformation around the channel we previously calculated by liquid crystal theory. (Helfrich and Jakobsson, 1990, Biophys. J.) The computation generates Brownian trajectories in a region outside the channel mouth. Periodic boundary conditions are imposed in the direction parallel to the planar membrane surface. To reduce spurious anisotropy the cell for periodic boundaries has a hexagonal rather than a square cross section. The outer face of the region in which the Brownian trajectories are calculated is joined to bulk solution by an extension into three dimensions of the one-dimensional "entrance tube" introduced for the boundary between bathing solution and channel mouth by Cooper, Jakobsson and Wolynes. (1985, Prog. in Biophys. and Mol. Biol.) The concentration in the computational cell equilibrates with the bulk solution, indicating the boundary conditions are properly constructed. At this writing we are putting into the program the electrostatics associated with charges in the membrane head groups, using linearized Poisson-Boltzmann theory. (Klapper, Hagstrom, Fine, Sharp, and Honig. 1986. Proteins 1:47) We will report on access resistance computations including these effects.

Tu-Pos339

ION, WATER, AND D_2O MOTION IN THE GRAMICIDIN CHANNEL: FLUCTUATION ANALYSIS OF MOLECULAR DYNAMICS SIMULATIONS.

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We probe the physics of permeation of the gramicidin channel by doing molecular dynamics simulations with univalent cations of varying size in the channel lumen and doing fluctuation analysis of the trajectories. We also vary the water properties by using D_2O instead of water in some simulations. The fluctuation analysis for ordinary water in the channel was reported at a previous Society meeting. In more extensive water simulations, we have seen more examples of water conformational state transitions, of which we previously reported one. Another recent computation for which our analysis is relatively complete is that of sodium ion in the channel. For this case we find the local friction similar to that in bulk solutions, but the effective diffusion coefficient is reduced by a series of potential barriers between 5 and 6 kT in height. The barrier crossing process is neither high-friction (Brownian) nor low-friction (Eyring Transition-state) in nature, but rather an intermediate-friction case requiring the full Langevin equation for its analysis. For larger ions the barriers are lower than for sodium.

We have done computations with D_2O rather than H_2O and are analyzing the trajectories. Since the velocity of sound in D_2O is different from that in H_2O, we intend to use this analysis to test the hypothesis that collective periodic water motions in the channel are sonic in nature.

Tu-Pos340

EFFECTS OF CATION VARIATION ON ION-WATER-POLYPEPTIDE INTERACTIONS IN A GRAMICIDINLIKE CHANNEL.

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Ionic variation greatly influences the transport properties of gramicidin. I present preliminary results of molecular dynamics studies on a series of alkali metal ions interacting with a gramicidinlike channel (the polyglycine analogue). Thermodynamic cycle perturbation methods and umbrella sampling techniques are used to construct and correlate portions of the free energy profiles of the various ions in the channel and to investigate details of ion-channel correlation. The free energy profile exhibits more pronounced structure as the cation radius decreases and ion-polypeptide interaction becomes stronger. Ionic solvation in the model channel is notably dependent upon ionic size and upon where in the channel the ion is located. The nature of ion-water correlations change markedly as ionic size decreases. Cesium and potassium interact with the model channel in basically quite similar fashions; sodium differs notably from its larger cognates. Within the channel, sodium binds and polarizes water more strongly than do the other two ions; it induces markedly stronger water-water interactions. The differences between cations are most noticeable with sodium near the exit from the single-file; an additional water is present in the channel and the waters exhibit greater orientational ordering. The possible relationship of these observations to gramicidin's multiple occupancy properties is considered.

Tu-Pos341

HOW DOES CONFORMATION AFFECT CHANNEL CONDUCTANCE? A MOLECULAR DYNAMICS STUDY ON GRAMICIDIN A.

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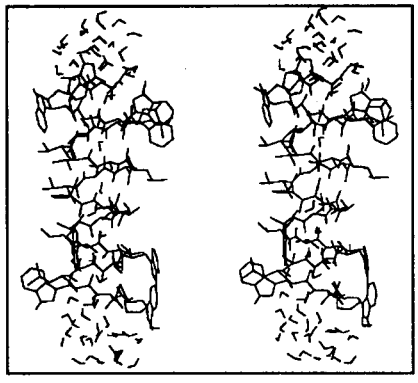
Stankovic, et al. (*Science* **244**: 813, 1989) showed gramicidin dimers have different conductances depending on the chirality of the covalent link between the monomers. To understand the interactions that result in an apparent change in the central barrier height, we are computing the free-energy profile of K^+ in the water-filled channel with the X-PLOR program. The models consist of right-handed beta helical dimers with SS- and RR- dioxolane linking rings. The simulations are based on those of B. Roux (thesis, Harvard, 1990), including his improved ion-peptide potentials, water caps at the channel ends, and a hydrophobic region surrounding the channel. Figure: a stereo view of SS-gramicidin and water molecules.



Tu-Pos342

MONTE CARLO STUDIES OF GRAMICIDIN A IN MODEL MEMBRANES: . J. Xing and H.L. Scott, Department of Physics, Oklahoma State University, Stillwater, Ok. 74078.

We present current results of ongoing Monte Carlo calculations of the equilibrium properties of lipid chains in interaction with a Gramicidin A peptide in a model membrane environment. In the present work, our calculations are extended to allow simulation of full bimolecular layers of lipid chains interacting with Gramicidin A dimers. As in our earlier work, interactions are optimized 6-12 potentials between all CH_2 subunits of all chains and between all chain and Gramicidin helix and residue molecules within a cutoff distance. The standard Metropolis Monte Carlo method is utilized, with the rotational isomeric model for chain conformational states. We use calculated $C-C$ bond order parameter profiles as measures of the manner in which the lipid chains are affected by the peptide, by comparing the profiles for chains which are near neighbors to the Gramicidin to those which are in the pure lipid portion of the simulation cell. In the simulations, all chains are allowed to undergo whole-chain lateral translation and axial rotation in the bilayer plane, *trans- gauche* isomerization, and chain motion in a direction *perpendicular* to the bilayer plane. Since hydrophobic interactions are not explicitly considered in the calculations, their effects are modeled by an elastic restraining interaction which inhibits large extrusions of chains out of the bilayer volume. Inclusion of the additional perpendicular degree of freedom allows us to examine the mismatch between the hydrophobic lengths of the chains and the Gramicidin dimer. The effect of the mismatch on both the order parameter profiles and on the shape of the surface of the bilayer will be discussed, and will be compared with existing experimental data. Results will be provided for a comprehensive study of C-14 chains in a bilayer in interaction with a Gramicidin A dimer, and preliminary results will be shown for simulations involving C-16 and C-18 chains. (Supported by NSF Grant No. DMB-8703644)

Tu-Pos343

DIRECT DETERMINATION OF THE EFFECT OF GRAMICIDIN ON DPPC CONFORMATIONAL DISORDER BY INFRARED SPECTROSCOPY Mark A. Davies and Richard Mendelsohn, Department of Chemistry, Rutgers University, 73 Warren Street, Newark, NJ 07102, USA

A direct determination of the percentages and classes of trans and gauche conformers present in the acyl chains of 6,6,6',6' DPPC (6-d_4 DPPC) in the presence of the peptide Gramicidin D has been made using FT-IR. Intensities and frequencies of conformationally sensitive CD_2 rocking modes were monitored in 10:1 and 30:1 (mol:mol) DPPC:Gramicidin mixtures and in pure DPPC vesicles. At 44°C, the gauche percentages in the 10:1 and 30:1 mixtures were 15% and 17%, respectively. At 34°C, the corresponding values were 9.8% and 2.6%. The percentage of (single gauche bend + kink) conformers, relative to multiple gauche forms, decreases from 78% in the 30:1 mixture to 15% in the 10:1 mixture at 44°C. These data provide the quantitative measure of the extent to which a membrane - spanning peptide disorders phospholipid gel phases and orders liquid crystal phases at a particular lipid acyl chain position. Gauche state populations averaged over the entire acyl chain are obtained using CH_2 wagging modes. The application of these modes to Gramicidin/DPPC and other systems will be discussed.

Tu-Pos344

GRAMICIDIN CHANNEL FUNCTION SHOWS LITTLE DEPENDENCE ON PHOSPHOLIPID CHIRALITY. L. L. Providence, O. S. Andersen, R. Bittman*, and R. E. Koeppe II‡. Dept. Physiol. Biophys., Cornell Univ. Med. Coll., New York, NY, *Dept. Chem., Queens College of CUNY, Flushing, NY, and ‡Dept. Chem. Biochem., Univ. Arkansas, Fayettevile, AR.

In order to examine how the function of gramicidin channels depends on the properties of the host bilayer, we compared the single-channel conductances, and average durations of channels formed by [Val[1]]gramicidin A (gA^+) and its enantiomer (gA^-) in bilayers formed by the enantiomeric ether phospholipids (*R*)- and (*S*)-dioleylglycerophosphocholine (di-18:1-PC). In 1.0 M NaCl (200 mV, 25 °C), there was little difference between the functional characteristics of the channels observed under the four different experimental conditions: gA^+/(*R*)-di-18:1-PC; gA^+/(*S*)-di-18:1-PC; gA^-/(*R*)-di-18:1-PC; gA^-/(*S*)-di-18:1-PC. The single-channel conductances varied between 16 and 18 pS; and the average durations varied between 900 and 1200 ms; likewise, the channel-forming potency did not vary significantly among the combinations tested. These results suggest that the gramicidin channel structure is invariant among the four combinations tested, which indicates that the tendency of gramicidin A to form $\beta^{6.3}$-helical channels does not depend on chiral interactions with polar groups in the glycerolipid backbone but rather on the anisotropic nature of the host bilayer per se. The results also show that chiral interactions between the channels and the glycerolipid backbone cannot be critical for channel function, but do not exclude the possibility that such specific interactions may alter subtle aspects of channel function.

Tu-Pos345

STRUCTURE AND DYNAMICS OF VAL-1 (d_8) DEUTERATED GRAMICIDIN IN RELATION TO H_{II} PHASE FORMATION

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To obtain insight into the role of peptide conformation and aggregation in gramicidin-induced hexagonal H_{II} phase formation we used ^2H NMR to study the structure and dynamics of Val-1 (d_8) deuterated gramicidin in different lipid systems.
^2H NMR spectra of gramicidin incorporated into DMPC bilayers and oriented between glass plates showed four distinct quadupolar splittings, which could be assigned to the $C_\alpha D$, $C_\beta D$ and two types of $C_\gamma D_3$ deuterons. In randomly dispersed bilayers of gramicidin and DMPC, a two-component ^2H NMR spectrum was observed, originating mainly from the two CD_3 groups. The spectral properties appeared virtually independent of the gramicidin/lipid molar ratio in the range of 1/5 to 1/25. Cooling below the phase transition temperature resulted in a strong line broadening, consistent with a reduced rate of axial reorientation of the helix. In bilayers of gramicidin with DOPC at a molar ratio of 1/25 and at 40 °C identical spectra were observed as in DMPC under the same conditions. Upon increasing the gramicidin/lipid ratio to 1/5 the lipid organization changed from a pure bilayer to a H_{II} phase, but the only spectral change observed for deuterated gramicidin was a slight broadening, suggesting that no major conformational change of the peptide occurs and that in the H_{II} phase the peptide is present in its channel conformation. Lowering the temperature resulted in a line broadening, that was much more pronounced at high gramicidin/lipid ratios, suggesting a reduced rate of axial rotation, most likely due to intermolecular interactions between gramicidin molecules.
The results are consistent with the notion that the channel conformation of gramicidin and self-association of the peptide both play a role in H_{II} phase induction in DOPC, but they do not show evidence for extensive peptide aggregation in the gramicidin-induced H_{II} phase.

Tu-Pos346

GRAMICIDIN CHANNEL CONDUCTION BLOCK BY POLYVALENT CATIONS AND TL(I) STUDIED WITH POTENTIAL RAMPS.

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Conduction in gramicidin channels is reduced by two distinct mechanisms for bathing solutions containing polyvalent ions and low mole fractions of Tl(I) ion, respectively. Some polyvalent ions do not obstruct the channel but produce a surface charge barrier to univalent ion entry. This barrier is maintained by the applied potential. The nature of this block is established from current-voltage curves generated using ramp potentials and asymmetric bathing solutions. Driving potentials from the bath containing the polyvalent ion give the current saturation typical of blocking ions. The currents as the ramp potential drives the opposite polyvalent-free bath are linear indicating complete removal of this block. Polyvalent ions are driven to the surface where they create an electrostatic barrier which is dispersed when the polarity of the applied potential is reversed. By contrast, ramp potential studies of Tl(I)-Na(I) solutions of constant total concentration and variable mole fraction, which give anomalously low currents for small Tl(I) mole fractions, produce two current regimes for current voltage curves generated with ramp potentials. These data are consistent with a displacement model where only Tl(I) ion can displace a Tl(I) ion in the channel. The reduced conduction at lower potentials utilizes only Tl(I)-free channels. The break point between the two modes is displaced to lower potentials as the Tl(I) mole fraction increases.

Supported by the Office of Naval Research.

Tu-Pos347

KINETICS OF ION MOVEMENT THROUGH GRAMICIDIN CHANNELS WITH SINGLE TRP-->PHE REPLACEMENTS.

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The four Trp at positions 9, 11, 13 and 15 in Gramicidin A influence the single channel conductance. In order to quantify this effect, we fit a 3-barrier-2-site-1-ion model with aqueous diffusion limitation (3B2S1I/D) to the current-(voltage,$[Na^+]$) relation of channels formed by Gramicidin A and four single Trp-->Phe substituted gramicidins. The 3B2S1I/D model has 7 adjustable parameters, including the diffusion limited access permeability and a central (translocation) barrier "shape" factor. Parameter error estimates were provided by Monte Carlo simulations. For Gramicidin A channels, the intrinsic entry and exit rate constants were $1.3\pm0.1\cdot10^8$ $M^{-1}s^{-1}$ and $3.9\pm0.2\cdot10^7$ s^{-1} (K_d = 0.15±0.01 M) and the translocation rate constant is $7.1\pm0.3\cdot10^6$ s^{-1}. In channels formed by Gramicidin A and by the Trp-->Phe substituted gramicidins, the access permeability clusters around $1.5\cdot10^8$ $M^{-1}s^{-1}$. Single Trp-->Phe substitutions at position 9 or 15 decrease the ion affinity (K_D = 0.26±0.01 and 0.27±0.01 M, respectively) suggesting that Trp at these positions stabilizes ion binding to the channel. Single Trp-->Phe substitutions decrease the translocation rate constant to approximately $3.9\cdot10^6$ s^{-1} for Phe-15, Phe-13, and Phe-11 and to $0.25\pm0.01\cdot10^6$ s^{-1} for Phe-9. This suggest that favorable interactions between the permeating ions and the Trp dipole decrease the central (electrostatic) barrier for ion movement through gramicidin channels, consistent with the notion that the orientation of the Trp indole NH's is determined by hydrogen bonding to the polar groups at the membrane/solution interface.

Tu-Pos348

ELECTROCHEMICAL KINETICS AND NONEQUILIBRIUM EFFECTS OF GRAMICIDIN

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The applicability of electrochemical kinetics and nonequilibrium mass transport has been evaluated in gramicidin in lipid bilayers. Kinetic effects are described using the electrochemical current voltage relation, and the Henderson equation for nonequilibrium potentials.

The current voltage relation relates the current magnitude to the applied potential, and defines a potential dependent kinetic barrier at the charge transport limit. The Henderson equation describes the contribution of mass transport to the experimentally determined potential formed at an interface between electrolyte phases.

Gramicidin will be tested with a series of inorganic and and organic electrolytes in aqueous solution.

Tu-Pos349

PERMEABILITY TO ORGANIC CATIONS OF COLICIN E1 CHANNELS IN PLANAR LIPID BILAYERS. J.O. Bullock, J.L. Shear, and E.R. Kolen. *Department of Physiology, University of Missouri-Columbia, Columbia, MO 65212*

Although the channels formed by the bactericidal protein colicin E1 conduct small ions at comparatively slow rates, large ions are known to permeate the channel, and no ions have yet been reported as impermeant. We have investigated the permeability properties of this channel by measuring the zero current potentials (ZCP) of planar lipid bilayers containing open colicin E1 channels exposed to ten fold gradients of the chloride salts of a series of organic cations. In gradients of NaCl, colicin E1 exhibits a strong selectivity for Cl^- at pH<3 and a moderate preference for Na^+ at pH>7. Our measurements were made in Asolectin membranes at pH 5.0; conditions which, because of the anionic lipids in Asolectin, favor Na^+ over Cl^- (ZCP≈+25 mV). The measured chloride equilibrium potentials (E_{Cl}) of the gradients fell into the range from −40 to −46 mV. All of the eight monovalent amines investigated were significantly permeable, producing ZCP values in the range from −22 to −30 mV. There was no correlation of the ZCP with the molecular weight of the ions, which ranged from 113 to 218 Daltons, or with the backbone structure or nature and position of polar substituent groups of the ions. Seven polyvalent cations, ranging in molecular weights from 172 to 284 and in valency from 2 to 4, appeared to be relatively impermeant, since the ZCP values for these compounds fell within a narrow range (−34 to −39), quite close to their corresponding E_{Cl}'s. The ZCP of the divalent quaternary ammonium ion, hexamethonium, was found to be independent of *cis* pH in the range from 3.5 to 6, whereas the ZCP in NaCl solutions varied by 55 mV in similar experiments. When these measurements were repeated in membranes composed of the neutral lipid, bacterial phosphatidal ethanolamine, the ZCP for hexamethonium was not only independent of pH, but was equal to E_{Cl}. These results suggest that, at least for hexamethonium ion, the slight deviation of ZCP from E_{Cl} observed in Asolectin membranes is the result of an experimental artifact rather than significant permeation of the colicin E1 channel by the ion under study. From these of experiments, we infer that the major barrier to the permeation of colicin E1 channels by polyvalent cations is electrostatic rather than steric in nature. If this barrier were located within the lumen of the channel, we would expect the presence of such ions to interfere with the movement of Na^+. While we as yet have no data bearing on this question, we do note that, since large currents were observed in all these experiments, none of the cations had any marked effect on the movement of Cl^-. (Supported by GM37396)

Tu-Pos350

ION SELECTIVITY OF AMPHOTERICIN B CHANNELS IN SUV. Sandra K. Benz, W. Ayenew, T. Stephan and S.C. Hartsel. *Intro. by* W.H. Gallagher. University of Wisconsin-Eau Claire, Eau Claire WI 54702.

It has been shown that Amphotericin B (AmB) may induce ion permeability in sterol-free small unilamellar membrane vesicles (SUV). As a first step in the characterization of these sterol-free channels, we have measured the monovalent cation selectivity sequence of these structures in lipid membrane vesicles using the pH sensitive probe pyranine. We have also studied divalent cation selectivity properties. In these experiments H^+ was exchanged for the transported cation by making the membrane highly permeable to protons with the protonophore FCCP. A stopped-flow system was used to introduce the antibiotic and/or create a salt gradient. When different cationic species were present on either side of the membrane, a bi-ionic potential developed. The direction and magnitude of the resulting pH change indicated which, if any, cation was more permeant in the AmB channel. Sulfate salts of Li^+, Na^+, Cs^+ and Rb^+ were all tested versus K^+ in this system. The sequence determined for sterol-free vesicles, $Na^+ > K^+ \geq Rb^+ > Cs^+ > Li^+$, corresponds closely to Eisenman selectivity sequence number VII. This sequence deviates from the sequence previously determined for AmB channels in cholesterol-containing planar bilayer studies. This suggests a different channel structure. AmB channels in sterol-free or sterol-containing vesicles showed almost no preference for Mg^{2+} vs. Ca^{2+}. In fact, sterol-free vesicles showed almost no permeability enhancement for either of these ions. This indicates that sterol-free channels may pass monovalent but not divalent cations. In contrast, when $CaCl_2$ gradients were imposed on AmB treated vesicles containing 10 mole percent cholesterol, a modest Ca^{2+} permeability enhancement was observed and vesicles with ergosterol (the normal fungal sterol) showed a much higher Ca^{2+} permeability. This difference in selectivity may have great importance concerning the mechanism of selective toxicity of AmB toward fungal cells and on the nature of AmB channels.

Tu-Pos351

CONFORMATIONAL CHANGES IN SENSORY RHODOPSIN I: SIMILARITIES AND DIFFERENCES WITH BACTERIORHODOPSIN, HALORHODOPSIN AND RHODOPSIN.

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FTIR difference spectra have been obtained for the $sR_{587} \rightarrow S_{373}$ phototransition of sensory rhodopsin I (sR-I), a signal transducing protein of *Halobacterium halobium*. The vibrational modes of the sR_{587} chromophore have frequencies close to those of the bacteriorhodopsin bR_{568} chromophore, confirming that the two chromophores have very similar structures and environments. However, the sR-I Schiff base C=N stretch frequency is downshifted relative to bR, consistent with weaker hydrogen bonding with its counterion(s). The carboxyl (COOH) stretch modes of sR-I and halorhodopsin (hR) are at the same frequencies. Based on sequence homologies, these bands can be assigned to Asp-106 on helix D and/or Asp-201 in helix G. In contrast, no band was found which could be assigned to the protonation of Asp-76. In bR, the homologous residue, Asp-85, serves as the acceptor group for the Schiff base proton. Bands appear in the amide I region at similar frequencies in sR-I, hR and bR, indicating that despite their different functions they all undergo closely related structural changes. Bands are detected in the C-H stretch region, possibly due to alterations in the membrane lipids. Similar spectral features are also observed due to the lipids of rhodopsin containing photoreceptor membrane upon light activation. It is hypothesized that the retinal isomerization in sR-I leads to alterations in surface domains which interact with subsequent signal transducing components.

This work has been supported by grants from the NSF (DMB-8509587), NIH (EY05499) and ONR (N00014-88-K-0464) to KJR and NIH (GM27750) and ONR (N00014-89-J-1629) to JLS

Tu-Pos352

PURIFICATION AND CHARACTERIZATION OF WILD-TYPE AND MUTANT FORMS OF BACTERIORHODOPSIN EXPRESSED IN *E. COLI.*

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Bacteriorhodoposin, expressed at high levels as a fusion of bacterio-opsin with thirteen additional residues at the amino terminal end, has been purified to homogeneity using detergents and size exclusion and dye ligand chromatography. Purified bacteriorhodopsin and bacteriorhodopsins containing single amino acid substitutions contain no bacterio-opsin and have an absorbance ratio ($A280nm/A\lambda max$) of 1.5. This provides a scheme for preparative scale production of pure bacteriorhodopsin in a single detergent system. Expressed bacteriorhodopsin has identical refolding rates, absorbance spectra, and light-induced proton pumping activities as wild-type bacteriorhodopsin purified from *H. halobium*. Proton pumping rates of bacteriorhodopsins with the wild-type sequence and variants D85N, R82Q, and D96N were determined in phosholipid vesicles as a function of pH. D85N was inactive at all pH values. D96N was inactive from pH 7.0 to pH 8.0, where wild-type is most active, but had some activity at low pH. R82Q showed diminished proton pumping with the same pH-dependence activity profile as for wild-type.

Tu-Pos353

ION TRANSLOCATION BY BACTERIORHODOPSIN AT LOW pH. A. Dér+, S. Száraz+,*, L. Keszthelyi+, W. Stoeckenius*. +Inst. of Biophys.,Biol.Res.Ctr., Szeged, PO Box 521, Hungary H-6701; *CVRI, Univ. CA, San Francisco, CA 94143.

The bacteriorhodopsin(bR) is a light-driven proton-pump in the membrane of halobacteria, where it can form crystalline domains, the purple membranes(pm). If pm is oriented by electric field and immobilized in gel, charge transport through the membrane elicited by short light flashes gives rise to transient currents, which are recorded with external electrodes. Acidification of pm gels to pH 0.5 reversibly red-shifts the absorption maximum from 568 to 605 nm. In this "blue membrane", illumination still elicits charge shifts but the net current flow due to the ion transport is abolished. Addition of Cl^- ions at this pH restores the purple absorption band (maximally to within 1 or 2 nm of the original value) and the ion current in the same proportion. Continuous illumination up to 4s produces continuous current. Replacement of H_2O to ^2H_2O in pm at neutral pH has a pronounced effect on the charge transport kinetics. We found no isotopic effect on the rate-limiting step at acid pH using ^2HCl and ^2H_2O. Br^- can replace Cl^- in restoring the absorption maximum and charge transport at low pH and in the case of the rate of the slower phase(s) of the photocurrent is reduced by a factor of 2. No Cl^- or Br^- effects have been observed at neutral pH. These observations strongly support the assumption that at low pH the halide anions are the transported species. Halorhodopsin is a closely related protein occurring in the same bacteria. It is a light-driven Cl^- pump at neutral pH. Since the amino acid sequence and to a good approximation the structure for both proteins is known, a comparison suggests a molecular model for the transport mechanism.

Tu-Pos354

A Method for Quantum Efficiency Measurement and Its Application to the Bacteriorhodopsin Photoreactions

Aihua Xie, Roberto A. Bogomolni, and Walther Stoeckenius

For a photoreaction system A $\underset{\phi_2}{\overset{\phi_1}{\rightleftharpoons}}$ B involving linear transition dipoles like retinals the extent of photosaturation after a linearly polarized actinic flash is fully determined by the resulting dichroic ratio. Consequently, the quantity $\varepsilon_A\phi_1+\varepsilon_B\phi_2$ can be measured in terms of the dichroic ratio and the absolute actinic flash intensity. In the case that the extinctions of the A state ε_A are known, while those of the B state ε_B are unknown, the sum of the quantum efficiencies, $\phi_1+\phi_2$, can be measured if the actinic wavelength is set to the isosbestic point of the A and B states so that $\varepsilon_B = \varepsilon_A$. This method is sensitive at low saturation so that any complications associated with high saturation such as partial bleaching and possible cooperativity can be avoided. We applied this method to the photoreactions between the bR and the K state, and determined that $\phi_1+\phi_2$ is 1.52±0.11, i.e. larger than 1.0. Therefore, the bR and the K state can not share a common excited state. With the ratio ϕ_1/ϕ_2=0.55±0.02 obtained in (1), we calculate ϕ_1=0.54±0.07 and ϕ_2=0.98±0.07.

1. Aihua Xie. Quantum Efficiency of Bacteriorhodopsin Photochemical Reactions. 1990 *Biophys. J.* 58:(November issue).

Tu-Pos355

A RAMAN SPECTROSCOPIC STUDY OF OCTOPUS BATHORHODOPSIN WITH DEUTERIUM LABELED RETINAL CHROMOPHORES. G. Weng, D. Manor, H. Deng , P. Rath, R. Gebhart., J. Lugtenberg, Y. Koutalos, T. Ebrey, M. Tsuda, and R. Callender. Physics Department(H. D., D. M., G. W., P. R.,and R. C.), City College of City University of New York , New York New York 10031. Chemistry Department (R. V., and J. L), Leiden University , 2300 RA Leiden, The Netherlands. Department of Physiology and Biophysics (Y. K., and T. E.), University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois 61801. Department of Physics (M. T.), Sapporo Medical College, Sapporo, Japan.

The resonance Raman spectrum of octopus bathorhodopsin in the fingerprint region and in the ethylenic-Schiff base region have been obtained at 80°K using the 'pump-probe' technique as have its deuterated chromophore analogues at the C_7D, C_8D, C_7,C_8D_2, $C_{10}D$, $C_{11}D$, C_{11}, $C_{12}D_2$, $C_{14}D$, $C_{15}D$, C_{14}, $C_{15}D_2$ and $N_{16}D$ positions. The essential configuration of the chromophore is *trans*, like that in bovine bathorhodopsin. Analysis of the C=N stretching and the NH bending modes, and their shifts upon isotope labelling reveal differences from bovine bathorhodopsin, and suggest a weaker hydrogen bonding pattern with the protein. The spectra will be presented and the significance of the difference between bovine and octopus bathorhodopsin will be discussed.

Tu-Pos356

RESONANCE RAMAN SPECTRA OF BACTERIORHODOPSIN MUTANTS WITH SUBSTITUTIONS AT ASP-85, ASP-96, AND ARG-82. Steven W. Lin, Stephen P. A. Fodor, and R. A. Mathies Chem. Dept., Univ. of Calif., Berkeley, CA 94720 and Larry J. W. Miercke, Richard F. Shand, Mary C. Betlach, and Robert M. Stroud Dept. of Biochemistry and Biophysics, Univ. of Calif., San Francisco, CA 94143.

Detergent solubilized bacteriorhodopsin (BR) proteins which contain alterations made by site-directed mutagenesis (Asp-96→Asn, D96N; Asp-85 →Asn, D85N; Arg-82→Gln; R82Q) have been studied with time-resolved resonance Raman (RR) spectroscopy at room temperature. The mutant proteins and native BR were reconstituted into CHAPSO micelles in delipidated acetate buffer at pH 5. Resonance Raman spectra of the light-adapted (BR_{LA}) and M species in D96N are identical to those of native solubilized BR, indicating that this residue is not located near the chromophore. The BR_{LA} states of D85N and especially R82Q contain more of the 13-*cis*, C=N *syn* (BR_{555}) specie under ambient illumination compared to native BR. This may account for part of the decrease in the proton-pumping activity of R82Q. Replacement of Asp-85 with Asn also causes a 25 nm red-shift of the λ_{max} and a frequency decrease in both the ethylenic and $C=NH^+$ stretching modes of BR_{LA}. These changes indicate that Asp-85 is located close to the protonated retinal Schiff base. The BR_{LA} spectrum of R82Q exhibits slight perturbation of the $C=NH^+$ band, suggesting weaker interaction with the Schiff base. These data support the involvement of Asp-85 and Arg-82 in the counterion environment of BR_{LA} and are consistent with a complex counterion model of mutually interacting $Asp\text{-}85^-$, $Arg\text{-}82^+$ and $Asp\text{-}212^-$ residues. The protein-chromophore interaction and hence the λ_{max} are sensitive to the overall charge of the environment around the protonated Schiff base. These studies demonstrate that RR spectroscopy on BR mutants can be used to obtain spectroscopic evidence of interactions between specific protein residues and the retinal in the photocycle intermediates of BR.

Tu-Pos357

PICOSECOND INFRARED PROBE OF BACTERIORHODOPSIN DYNAMICS
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We have developed an infrared spectroscopic method to study the Bacteriorhodopsin dynamics on the picosecond timescale after photoexcitation. The time resolution is thereby improved by six orders of magnitude compared to fast Fourier transform infrared methods and allows probing of the early intermediates of the photocycle at ambient temperature.
We present spectral and kinetic data monitored by both protein and chromophoric infrared absorbance changes in the region above 1530 cm^{-1}.

They show the bleach of BR570 (e. g. negative signal at 1641 cm^{-1} , due to the depletion of the C=NH stretch absorption) and the appearance of picosecond intermediates (e.g. positive signals at 1622 and 1610 cm^{-1}). The rise time of both the negative and the positive signals are smaller than the time resolution of the apparatus (10 ps), indicating the fast photoisomerisation process. Differences and similarities between our data and those obtained by FTIR techniques at low temperature are evident.
Photoselection experiments underway using this method permit the study of ultrafast molecular geometrical changes of both the protein and the chromophore at ambient temperature and are being extended to the subpicosecond timescale.
R. D. is a fellow of the Deutsche Forschungs-gemeinschaft. Supported by NIH and the NIH Laser Resource at PENN.

Tu-Pos358

FTIR STUDY OF THE M TO BR BACKPHOTOREACTION OF BACTERIORHODOPSIN. Hiroyuki Takei† and Aaron Lewis*, †Dept. of Applied Physics, Cornell University, Ithaca, NY 14853 *Dept. of Applied Physics, The Hebrew University of Jerusalem, Israel.

We have applied low temperature FTIR difference spectroscopy to investigate intermediates produced from the M intermediate upon blue light excitation (< 480 nm). In agreement with an earlier report by Balashov and Litvin, who studied these intermediates with visible absorption spectrophotometry, we have observed at least three intermediates. The initial photoproduct is stable at 100 K, and two subsequent products of thermal reactions are observed upon raising the temperature to 130 K and 160 K respectively.

As is expected from a small shift (~2nm) in the maximum peak in the visible absorption spectrum during the initial photoreaction at 100 K, a corresponding FTIR difference spectrum shows unusually small features in the C=C and C-C regions. Only following thermal reactions, peaks presumably due to these modes appear. We have used ^{15}N isotopic labeling and deuteration to look for the C=N peak as well as the step of the Schiff base reprotonation. Features in the carboxyl group region, 1720-1770 cm^{-1}, show de/protonation of aspartic acids, in accord with the observation that the direction of photo-induced signal during the backphotoreaction is opposite from that observed during the usual pumping; during the backphotoreaction the deprotonated Schiff base in the M intermediate is probably reprotonated from an proton donor other than Asp 96.

Tu-Pos359

THE EFFECT OF REDUCING THE RETINAL IN BACTERIORHODOPSIN ON ITS CD SPECTRUM: ANOTHER EVIDENCE AGAINST EXCITON COUPLING

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(Introduced by David S. Eisenberg)

The question whether or not the photosynthetic system of bacteriorhodopsin (bR) has an antenna system resulting from exciton coupling was recently discussed (1) (M. A. El-Sayed *et al, Proc. Natl. Acad. Sci.* USA 86(1989), 5376-5379). Evidence for the exciton coupling was the observed biphasic nature of its CD spectrum and against it was the observed polarized fluorescence, the polarized retinal absorption and the CD spectra of the daughter molecules in the bR photocycle. It was then proposed (1) that the biphasic nature might result from the combination of the CD of distorted retinal molecules with opposite sense of distortion and thus with opposite signs for their CD spectra, i.e. the biphasic band shape results from heterogeneity. Reduced bR (RbR) has a planar retinal electronic system and its trimer structure is found to have a hexagonal structure similar to bR (2) (Oesterhelt *et al, Biochem.* 17(1978), 5353-5359). In the present study (*Biophys. J.*), the CD spectrum of RbR was examined and compared with that simulated by using the exciton model and assuming that the CD spectrum of bR is a result of exciton coupling. The observed monophasic band shape as well as the small observed value of rotational strength of RbR do not agree with predictions made from the exciton model. These results are better understood in terms of the planar retinal system in RbR. This eliminates the cause of the retinal heterogeneity within the protein and thus the presence of retinals with opposite signs for their CD. Furthermore, it eliminates the contribution of retinal distortion to the rotational strength of the individual retinals in RbR, thus explains its reduced value.

Tu-Pos360

THE SECONDARY β-DEUTERIUM ISOTOPE EFFECT IN DARK ADAPTATION OF BACTERIORHODOPSIN CONTAINING RETINAL-20,20,20-d_3[#]. Dov Birnbaum[+] and Stanley Seltzer Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973

The secondary β-deuterium isotope effect on the rate of dark adaptation of bacteriorhodopsin, reconstituted with retinal-20,20,20-d_3, has been found to be $k_H/k_D = 0.87 \pm 0.02$. The isotope effect reflects the change in C_{20}-H(D) hyperconjugative stabilization of positive charge density at C_{13} in going from the reactant to the transition state of the rate controlling step of the dark adaptation process. Two mechanisms for catalyzed bicycle-pedal double cis-trans isomerization, which occurs upon dark adaptation, are considered: 1) reversible addition of a nucleophile and 2) electrostatic stabilization of positive charge development at C_{13} [J.A.C.S. **109** 1627 (1987)]. The results are rationalized with the aid of MNDO semiempirical quantum calculations as being due to a mechanism whereby a nucleophile, presumably the carboxylate of aspartate-212, adds to retinal's C_{13} to catalyze the double cis-trans isomerization.

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+ On sabbatical leave from A. D. A. Israel.

Tu-Pos361

FURTHER EVIDENCE FOR STRUCTURAL ACTIVITY NEAR TRY-185/PRO-186 IN HELIX F OF BACTERIORHODOPSIN

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The mechanism by which light-driven retinal isomerization in bacteriorhodopsin (bR) couples to vectorial proton transport has been investigated by FTIR difference spectroscopy. It has previously been shown by incorporating ^{15}N-prolines into bR that a structural change occurs in one or more Xaa-Pro C-N bonds during the bR photocycle (Rothschild et al., *Proc. Natl. Acad. Sci. USA 86*, 9832-9835 (1989)). In order to localize the region(s) in the primary sequence where these changes occur, we have measured the bR→K and bR→M difference spectra for bR molecules labelled with various 1-^{13}C-amino acids. Bands in the amide I region are found which have contributions from both tyrosine and proline amide carbonyl stretching vibrations. These results, along with earlier FTIR studies on isotopically labelled tyrosines and site-directed mutants of bR suggest that Tyr-185 and Pro-186 form part of a hinge in helix F of bR which allows conformational changes of this helix to occur during proton pumping.

This work was supported by grants from National Science Foundation (DMB-8806007) to K.J.R and from the National Institutes of Health (GM36810) to J.H..

Tu-Pos362

SOLID STATE ^{15}N NMR STUDIES OF ^{15}N-PRO- AND ^{15}N-TRP-BACTERIORHODOPSIN

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Spectroscopic studies of bacteriorhodopsin (bR) and bR mutants suggest that tryptophan and proline residues are involved in wavelength regulation and in the photocycle. These inferences are consistent with recent structural information. We have undertaken solid state NMR studies of ^{15}N-Pro-bR and indole-^{15}N-Trp-bR in order to characterize these residues in the dark-adapted state and to identify changes that may occur during the photocycle. ^{15}N-Pro-bR was prepared using the JW-3 strain of *Halobacterium halobium* by substituting L-^{15}N-Pro for L-Pro in a defined growth medium. Indole-^{15}N-Trp-bR was similarly prepared by adding ^{15}N-anthranilic acid to a defined medium that contains no tryptophan. Any scrambling of the labels that may occur is expected to be spread broadly in the nitrogen pool (enhancing the natural abundance signal), since normal biodegradation pathways would transfer the ^{15}N on proline directly to glutamate and the ^{15}N on anthranilic acid or indole directly to ammonia. The ^{15}N cross polarization, magic angle spinning (CP-MAS) spectrum of ^{15}N-Pro-bR in the dark adapted state shows three resolved resonances, spread over 12 ppm, downfield of the amide backbone signal. The intensities of the lines suggest that one of the 11 proline residues is particularly distinctive and the others belong to two approximately equally populated groups with chemical shifts differing by 3 ppm. Preliminary results reveal no change in the spectrum upon light adaptation. In contrast, the CP-MAS spectrum of indole-^{15}N-Trp-bR in the dark adapted state shows an unresolved peak with two shoulders. Preliminary results suggest a small shift of one shoulder on light adaptation.

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Tu-Pos363

SOLID STATE NMR STUDIES OF [δ-15N] ARGININE LABELED BACTERIORHODOPSIN

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Bacteriorhodopsin was 15N labeled at the δ-position of the arginine residues by replacing L-arginine in a defined *halobacterium halobium* medium with δ-15N labeled L-ornithine. The 15N solid state NMR spectra, in the dark and light adapted states, revealed a 5ppm splitting in the arginine peak indicating that there are two classes of arginine residues. 48 hour deuterium exchange studies at a neutral pH using delay without decoupling pulse sequence show that all the arginine residues have exchangeable protons at the δ - nitrogen. Experiments that monitor the kinetic transfer of magnetization from bulk water protons to the 15N nuclei (Harbison et al.,JACS,110,7221,1988) show that neither the peptide nitrogens nor the δ-arginine nitrogens exchange protons rapidly with bulk water at neutral pH. At higher pH values, some rapid proton exchange from bulk water is seen at the δ-arg nitrogens, although there is still no exchange at the peptide nitrogens. Studies on the M- photocycle intermediate and the low pH forms of bacteriorhodopsin are in progress.

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Tu-Pos364

Simultaneous Replacement of Asp85 and Asp96 in bacteriorhodopsin leads to halorhodopsin-like properties

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Homologous expression of double mutated bacteriorhodopsin Asp85,96 ->Asn in Halobacterium halobium L33 leads to formation of membrane fragments with the same buoyant density as that of purple membrane. Absorption maxium of the mutated protein is at 608 nm (10 mM Tris, pH 7.0). The pK of the Schiffbase in the dark is lowered from >13 in BR to 7.2 in 4 M NaCl and 8.6 in H_2O. This behavior is known from halorhodopsin. Flash-induced absorbance changes show striking similarities with the halorhodopsin photocycle, i.e. no appearance of the M-intermediate The intermediate with the most blue shifted λ_{max} has a difference maximum at 490 nm and repopulates the initial state with a time constant of 5 ms. Attachment of membranes to black lipid membranes reveals anion dependent peak currents. Large peak currents are obtained with blue light after preillumination with green light. This effect again is known from halorhodopsin. Furthermore deprotonation of the Schiff base can be catalyzed by azide as in HR. Incorporation of mutated BR in azolectine vesicles and subsequent attachement to BLM allows detection of anion dependent photostationary currents. Taken together these results show that the two aspartic acids in position 85 and 96 are crucial for ion specifity in the retinal protein ion pumps.

Tu-Pos365

THE EFFECTS OF GENETIC SUBSTITUTIONS OF INDIVIDUAL ARGININE RESIDUES ON THE DEPROTONATION AND REPROTONATION KINETICS OF THE SCHIFF BASE DURING THE BACTERIORHODOPSIN PHOTOCYCLE

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(Introduced by Juli Feigon)

There are a total of seven arginine residues in the bacteriorhodopsin (bR) molecule. By mutagenesis, each of the arginine (Arg) residues can be replaced by the neutral glutamine (Gln) or cysteine (Cys), one at a time [1]. We have studied the kinetics of photocycles of such arginine mutants of bR by monitoring the M intermediate at 405 nm and the transient at 296 nm using transient absorption spectroscopy. The results show that while the decay rate of the M intermediate is greatly enhanced for the Arg-164-->Gln mutant, it is dramatically decreased in the case of Arg-227-->Gln mutant. The observation that the Arg-227-->Gln mutant shows a decreased rate for reprotonation of the Schiff base (SB) is in agreement with the previous finding by Stern *et al.* [1]. The decay of the 296 nm transient also exhibited similar trend, although not as dramatic. Furthermore, in terms of the absorption of the 296 nm transient formed versus that of the slow rising M intermediate, the arginine mutants showed no significant difference from that of ebR (bR express in *E. Coli*) and native bR, except for the Arg-227-->Gln mutant which is reduced by ~70%. Based on temperature dependence study of the slow-decaying component of the M intermediate, we show that while there is no significant difference between native bR and ebR in terms of activation energy and entropy, the corresponding activation energy and especially entropy are drastically decreased in the case of the Arg-227-->Gln mutant. These results are discussed in terms of the structural model of bR by Henderson *et al.* [2].

[1] Stern, L. J. and H. G. Khorana. 1989. *J. Biol. Chem.* 264:14202-14208.
[2] Henderson, R., J. M. Baldwin, T. A. Ceska, F. Zemlin, E. Beckmann, and K. H. Downing. 1990. *J. Mol. Biol.* 213:899-929.

Tu-Pos366

REPLACEMENT OF ASP-85 BY GLU IN BACTERIORHODOPSIN KINETICALLY DECOUPLES SCHIFF'S BASE DEPROTONATION AND H^+-EJECTION

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Point-mutated bacteriorhodopsin (BR) residing in the purple membrane was investigated by time-resolved flash-spectroscopy in combination with optical pH-indicators. When Asp-96 is replaced by Asn, the decay of M is extremely slowed down whereas its rise is less affected. This separates H^+ release and uptake by 5 orders of magnitude and allows to clearly distinguish the three proton transfer steps monitored by the pH-indicators fluorescein or coumarin covalently attached to BR, i.e., H^+ appearance at the surface, surface/bulk transfer, H^+ reuptake by BR. The pumped H^+ appears at the extracellular surface during the L - M transition. However, in contrast to the wildtype, where the proton is taken up after the reprotonation of the Schiff's base (i.e., after the 410 nm absorbance has nearly completely decayed), in the mutant H^+-uptake by BR occurs concurrently with the absorbance decay at 410 nm. Replacement of Asp-85 by Glu leads to an approx. 20 times faster formation of M (τ_1=1 μs, τ_2=10 μs), but instead of following this acceleration, the appearance of H^+ at the surface is strongly delayed (τ=500 μs as compared to 60 μs in the wildtype). The presence of Glu induces a faster deprotonation of the Schiff's base, thereafter, the transferred H^+ is retained at this amino acid for a long time period. The proton is not ejected from bacteriorhodopsin until the beginning of the M decay and taken up again after M had decayed completely.

Tu-Pos367

CRYSTALLIZATION OF AN *E.COLI* EXPRESSED BACTERIOPSIN FUSION PROTEIN. A.K. Mitra, L.J.W. Miercke, M.C. Betlach, R.F. Shand, and R.M. Stroud. Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448.

Bacteriorhodopsin (BR) is a light-driven proton pump found in the purple membranes (PM) of *Halobacterium halobium* where it is organized in a highly ordered 2-dimensional p3 lattice. We are pursuing site-directed mutagenesis on an *E.coli* expressed bacteriopsin fusion protein (e-BO) to determine which residues are functionally critical for proton pumping in BR. In order to help establish the role of single amino acid substitution which lead to altered activity, it is necessary to characterize any changes in function to changes in structure. For this purpose we are using electron diffraction on 2-d crystalline sheets as a sensitive high-resolution assay (Mitra and Stroud.1990.Biophys. J. 57:301-311). We have grown 2-d lattices of e-BR after retinylation of e-BO with all-*trans* retinal followed by reconstitution with *Halobacterium halobium* lipids. Two types of crystals were seen. First is a previously undescribed tubular crystal in which BR is organized in an orthorhombic lattice (a=127Å, b=67Å) with pgg symmetry and a novel packing scheme. Identical tubular lattices were also observed for wild type BR from PM. Second is the hexagonal lattice that is identical to the well-characterized p3 lattice in PM. Electron diffraction from glucose embedded crystals of these e-BR p3 lattices show reflection out to <3.9Å and are ideal for high-resolution structure-function studies on BR mutants. Three mutants D85N, D96N and R82Q are under investigation and crystallization and data collection are in progress.

Tu-Pos368

MEMBRANE LOCATION OF THE PALMITOYLATION SITE OF RHODOPSIN. Julie Kiss and T. Gregory Dewey, Department of Chemistry, University of Denver, Denver, CO 80208.

Bovine rhodopsin in rod outer segments can be specifically modified with pyrene-dodecanoic acid. This in situ modification is ATP and CoA dependent and corresponds to labelling the palmitoylation sites on rhodopsin. Palmitoylation of two of the cysteins in the carboxyl tail are thought to "anchor" this region to the membrane. The membrane location of modifying fatty acids was established by collisional quenching using doxyl fatty acids. Quenching experiments were performed on rhodopsin reconstituted into phospholipid vesicles. Stern-Volmer behavior was seen for all of the doxyl-stearic acid quenchers studied and the slopes of these plots indicated that the pyrene was located deep in the bilayer (16 doxyl > 12 doxyl > 6 doxyl). A comparison was made of the quenching of the protein-labelled pyrene-dodecanoic acid and of free pyrene-dodecanoic acid. The free label did not show as large a difference in quenching by the doxyl probes at different membrane locations. This indicates that the rhodopsin-acylating fatty acid is located within a more restricted region of the lipid bilayer than the free fatty acid. These results suggest that acylation of the carboxyl tail does indeed "anchor" it to the membrane in a well-defined structure. Circular dichroism experiments were also performed on palmitoylated and depalmitoylated rhodopsin. These spectra indicated a slight decrease in alpha helical content upon palmitoylation.

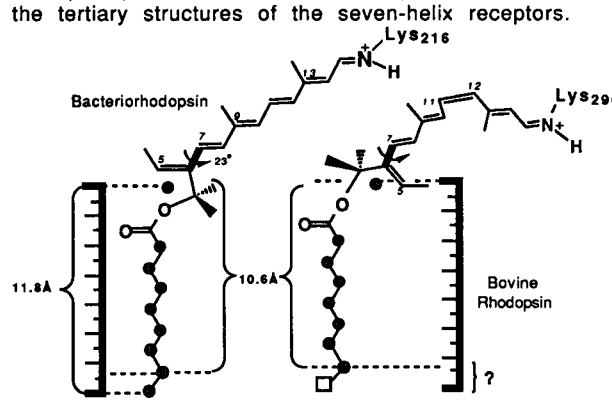
Tu-Pos369

LIGHT-DEPENDENT REACTIONS OF RHODOPSIN IN DETERGENT-LIPID MIXTURES. B. E. Knox and H. G. Khorana, M.I.T., Cambridge, MA 02139. Rhodopsin reactions are usually carried out in hydrophobic media, which influence the stability of the reactive intermediates produced upon illumination. We have now studied the effects of the detergents, cholate-related CHAPS and lauryl maltoside (LM) with and without phospholipids on the light-dependent phosphorylation of rhodopsin and activation of transducin. Rhodopsin used was purified using immunoaffinity chromotography in 0.1% LM or 1% CHAPS. Rhodopsin Phosphorylation. This was essentially absent in 0.6% (CMC concentration) to 1% CHAPS. Addition of LM (0.01-0.6%) to CHAPS (0.6%) also did not allow rhodopsin phosphorylation. In LM alone, phosphorylation was observed, with maximal activity (1.2 mole ^{32}P/mole rhodopsin/60') being near the CMC (0.01%). Asolectin increased phosphorylation at detergent concentrations above CMC. For LM the extent of ^{32}P incorporation depended on both the LM and asolectin concentrations. An optimum ratio of LM to asolectin of approximately equal weight gave maximal activity (approximately 3.0 mole ^{32}P/mole rhodopsin/60'). Phosphorylation of rhodopsin in CHAPS occurred with asoletin, but peak activites were only 60% of comparable LM samples. Transducin Activation. Rhodopsin in LM activated transducin, with the activation showing a strong dependence on the LM concentration. Optimal exchange activities (75 mole GTPγS^{35}/mole rhodopsin) were found at LM concentrations near the CMC (0.01%), and showed a four fold higher sensitivity (2.1 nM vs. 8.8 nM) compared to rhodopsin in washed rod outer segment membranes. Asolectin also increased transducin activations at LM concentrations above the CMC, similarly to the phosphorylation results. The results have described conditions for investigating both rhodopsin phosphorylation and transducin activation in rhodopsin detergent-lipid mixtures. Supported by NIH grants GM28289 and AI11479 and ONR grant N00014-82-K-0668.

Tu-Pos370

SPACER-ARMED RETINALS AS PROBES FOR INTER-HELICAL SPACES IN RHODOPSINS. Valeria Balogh-Nair,* Liang Chen and Wei-Xing Li, Department of Chemistry, City College of CUNY, New York, N.Y. 10031.

All-trans and 11-cis retinals with spacer arms of varying lengths attached to seco-rings were synthesized and bound to bacterio- and bovine opsins to yield rhodopsin analogs with absorption maxima and properties similar to the natural pigments. Length of the spacer arms was used to determine the maximum distance from the ring to the membrane external surface which still allows binding. The remarkable similarity of these distances found in bacteriorhodopsin and bovine rhodopsin points to an identical design in this domain of the tertiary structures of the seven-helix receptors.



Thanks to: NSF BNS 8812506 & The ACS Petroleum Fund.

Tu-Pos371

CHLAMYDOMONAS RETINAL-DEPENDENT PHOTORECEPTION: EVIDENCE THE NATIVE CHROMOPHORE POLYENE CONFIGURATION FOR THE STOP RESPONSE IS ALL-TRANS. D. Zacks[1], M. Lawson[1], F. Derguini[2], K. Nakanishi[2], & J.L. Spudich[1]. [1]Dept. of Anat. & Struct. Biol., A. Einstein Coll. of Med., Bronx, N.Y., [2]Dept. of Chem., Columbia Univ., New York, N.Y.

Wild type gametic cells of the unicellular, eukaryotic alga *Chlamydomonas reinhardii* exhibit two behavioral responses to 500nm light: orientation with respect to the direction of the beam (phototaxis), and brief "photophobic" or "stop" responses to millisecond flashes (Boscov & Feinleib, Photobiochem. Photobiol. 30:499, 1979; Hegemann & Bruck, Cell Motil. 14:501, 1989). Foster et al. (Nature 311:756, 1984) reported dependence of phototaxis on exogenously added retinal using a pigment-deficient mutant (FN68) and a population migration assay. By computerized cell tracking and motion analysis of individual cell behavior (~1000 cells/assay), we find all-trans retinal reconstitutes flash-induced photophobic responses in a phenotypically similar pigment-deficient mutant cc-2359 (Iroshnikova et al, Genetika 22:761, 1986). Response saturation occurs at 0.1 nM ($1.5x10^8$ retinal molecules added/cell), at which all cells in the population exhibit a stop (peak response within 67 msec). We compared the rate of restoration of the response by the retinal isomers 9-cis, 11-cis, and 13-cis to that of all-trans. Isomers were purified by HPLC and added from a methanolic solution (maximum 20 μl to 20 ml cell suspensions) at $7.5x10^8$ retinal molecules/cell (all-trans, 11-cis, 9-cis) or $1.5x10^8$ molecules/cell (all-trans, 13-cis). All-trans was most effective, fully restoring responses within 60 min and 15 min at the lower and higher retinal concentrations, respectively. Within 60 min, 13-cis, 11-cis, and 9-cis restored responses to 50%, 30% and 0% of the all-trans value at the same concentration, respectively. The data suggests all-trans as the native polyene configuration of the chromophore for the photophobic response. After overnight incubation at 1 μM, full restoration occurs with 6-s-trans-locked retinal, 10% with a 6-s-cis-locked, and 0% with 13-trans-locked, 13-cis-locked, and phenyl retinal analogs. The 13-cis and 13-trans-locked analogs competitively inhibit all-trans restoration of the stop response (Lawson et al, Abstr. IV Inter. Conf. Cell Mol. Biol. of *Chlamydomonas*. Wisconsin, 1990).

Tu-Pos372

IRBP LIGANDS: IS THERE COMPETITION BETWEEN ALL-TRANS RETINOL AND PALMITIC ACID?

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IRBP reportedly binds one or two moles of all-trans retinol (ATR) with an affinity of about 1.0μM and up to six moles of fatty acids (FA), two moles of which are covalently attached. We report here experiments designed to evaluate the possible competition for common binding sites between ATR and palmitic acid in vitro.

IRBP (3.0μM, in 0.1M TRIS-HCl) in the presence and absence of palmitic acid (120μM) was titrated with ATR (0-27μM) and the fluorescence monitered (350nm excitation, 400-600nm emission). The binding parameters were analyzed in a standard manner (Cogan et al., 1976, Eur.J.Biochem. 65, 71). In the presence of FA the molar ratio of IRBP:ATR was 2.1; in the absence of FA the molar ratio was 3.0. A formal analysis of palmitic acid as a competitor for ATR binding is underway. These preliminary results suggest the possibility of three ATR binding sites, one of which is absent in the presence of FA. This study is part of a series designed to localize and characterize the ATR binding sites on IRBP. Our results should help further illuminate the functional role of the IRBP molecule. (Supported by NIH grants EY04939 and HL07260).

Tu-Pos373

IRBP AND VITAMIN E PROVIDE PROTECTION FOR RETINOL IN AQUEOUS MEDIA

Rosalie K. Crouch[1], Starr Hazard[1], Gerald Chader[2], Barbara Wiggert[2] and D. Wesley Corson[1]. [1]Medical University of South Carolina, Charleston, SC 29425 and [2]National Eye Institute 20892

The mechanism of transport of retinol and retinal between photopigments and ocular storage pools is being studied extensively by various means including recording from isolated photoreceptors. As large excesses of retinoids are routinely delivered to isolated rods by liposomes in physiological experiments, maintenance of the isomeric and oxidative integrity of the retinoid is essential. We have reported previously that retinol can autooxidize to retinal in liposomes. We therefore have examined methods of protecting retinoids from oxidation and/or isomerization.

Two methods have been developed to protect retinoids in experiments involving exposure of the retinoids to aqueous media over several hours. Prior incubation of the retinoid with IRPB (3 μM) protects retinol and retinal from oxidation and isomerization for at least 48 hours. Vitamin E (25 μM) added to liposomes containing retinol or retinal likewise prevents decomposition of retinol or retinal for up to 17 hours. Neither of these agents interferes with the successful regeneration of pigment in vitro or the restoration of sensitivity to isolated bleached photoreceptors. (Supported by NIH grants EY04939 and EY07543.)

Tu-Pos374

THERMAL RELAXATION EFFECTS IN BACTERIORHODOPSIN

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Thermally induced relaxation effects were observed in aqueous suspensions of bacteriorhodopsin using modulation technique. Conductivity changes in the suspensions due to modulated near infrared radiation were monitored at various modulation frequencies and the lifetimes of the effects measured. The response of the apparent lifetimes with temperature showed a maximum at approximately 302 K and addition of D_2O slowed the lifetimes by approximately 4 ms. The observed relaxation processes may reflect heat-induced conformations or transformations of bacteriorhodopsin in purple membrane. Cooperativity mechanism involving bacteriorhodopsin trimers may also be involved in the processes.

Tu-Pos375

REGULATION OF MAGNESIUM TRANSPORT ACROSS CARDIAC AND LIVER CELLS. A. Romani, E. Dowell, M. Fatholahi and A. Scarpa. Dept Physiol. & Biophys. CWRU, Cleveland, OH 44106.

The adrenergic stimulation of perfused rat hearts and livers as well as the stimulation of isolated rat myocytes and hepatocytes resulted in a considerable Mg^{2+} efflux across the plasma membrane. The Mg^{2+} efflux was measured by atomic absorbance spectrophotometry or by the fluorescence and absorbance of specific Mg^{2+} indicators. The Mg^{2+} movement resulted being regulated via a modulation of intracellular cAMP level. In fact, the additions of permeant cyclic AMP analogs (dBu-cAMP, 8-Cl-cAMP, 8-Br-cAMP) or forskolin mimicked the adrenergic stimulation in both cell types whereas the addition of carbachol and vasopressin (inhibiting the cAMP production via muscarinic and vasopressin receptors, respectively) stimulated a consistent Mg^{2+} uptake. Large Mg^{2+} efflux from either type of cell could also be stimulated by uncouplers, vanadate and ouabain. The presence of Ca^{2+} and Na^{+} in the extra-cellular medium was necessary for the NE induced Mg^{2+} efflux. As the concentration of each ion was decreased a progressive decrease of Mg^{2+} efflux was observed. By contrast, changes in extracellular Mg^{2+} (from 0 mM up to 1.2 mM) were not affecting the amplitude of Mg^{2+} release. These results indicate that: i) a mechanism(s) of Mg^{2+} transport is present in the plasma membrane of rat cardiac and liver cells; ii) this transport is regulated by b-adrenergic receptors through a modulation of cellular cAMP level; iii) it requires physiological levels of extracellular Ca^{2+} and Na^{+}; iv) this mechanism is operating also in presence of a broad concentration of extracellular Mg^{2+}. (Supported by NIH 18708).

Tu-Pos376

CYTOSOLIC CONTROL OF MITOCHONDRIAL RESPIRATION IN THE INTACT HEART. Joseph W. Starnes and Douglas K. Bowles, Department of Kinesiology, University of Texas, Austin, TX 78712.

Adenine nucleotide metabolism in rat hearts was altered for the purpose of identifying the cytosolic signal that links myocardial energy demand to mitochondrial energy production. In the first experiment, rats were fed ß-guanidinopropionic acid (ßGPA) for 10-12 weeks which decreased myocardial total creatine 78%. No hemodynamic differences were observed between control (C) and creatine-depleted (CD) hearts when perfused by the working heart mode. In retrogradely perfused hearts utilizing glucose and acetate as substrates energy demand was set at approximately 6 or 17 µmoles O_2/min/g by adjusting perfusion pressure. After 30 min of perfusion, hearts were freeze clamped and appropriate compounds measured to calculate free cytosolic concentrations of ADP, ATP/ADP, and ATP/ADPxPi. At the lower energy demand, CD hearts had a two-fold higher ADP and two-fold lower ATP/ADP and ATP/ADPxPi. When energy demand was increased three-fold, neither ADP or ATP/ADP changed in C or CD, but ATP/ADPxPi decreased by 30-35%. In another experiment oxygen consumption was held constant while intracellular Pi was elevated by increasing the perfusate Pi concentration from 0 to 2.5 mM. The increase in tissue Pi was countered by a comparable decrease in ADP such that ATP/ADPxPi did not change. We conclude from these experiments that the cytosolic signal that controls mitochondrial respiration in the intact heart is ATP/ADPxPi.

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Tu-Pos377

MOLECULAR SIZE OF A NA^{+}-DEPENDENT AMINO ACID TRANSPORTER IN EHRLICH ASCITES CELL PLASMA MEMBRANES DETERMINED BY RADIATION INACTIVATION. *John I. McCormick, Marc Jetté, Michel Potier, Richard Béliveau and *Rose M. Johnstone. *Department of Biochemistry, McGill University, Montreal and Laboratoire de Membranologie Moléculaire, Département de Chimie, Université du Québec à Montréal, Canada.

Radiation inactivation was used to estimate the molecular size of a Na^{+}-dependent amino acid transport system in Ehrlich ascites cell plasma membrane vesicles. Na^{+}-dependent α-aminoisobutyric acid uptake was measured after membranes were irradiated at -78.5° C in a cryoprotective medium. Twenty-five percent of transport activity was lost at low radiation doses (<0.5Mrad) suggesting the presence of a high molecular weight transport complex. The remaining activity decreased exponentially with increasing radiation dose and a molecular size of 466 kDa was calculated for this carrier system. Vesicle permeability and intravesicular volume were measured to ensure that loss of Na^{+}-dependent transport activity was due to a direct effect of radiation on the transporter and not through indirect effects on the structural integrity of membrane vesicles. Intravesicular volume decreased during irradiation but losses became significant only at radiation doses 2-3 fold higher than those required to inactivate transport. In addition, vesicle permeability was unaffected by irradiation. A 120-130 kDa peptide has previously been identified as a possible component of a Na^{+}-dependent amino acid transport system in Ehrlich cells. The relationship between the fragmentation of the 120-130 kDa peptide and loss of transport in irradiated membranes was also examined. Peptide loss was quantitated by Western blot analysis. Estimation of the rate of disappearance of the 120-130 kDa peptide and the rate of loss of transport activity from irradiated membranes gave similar values of 13.4%/Mrad and 10.9%/Mrad respectively, when measured as % decrease per unit amount of radiation. The data suggest that fragmentation of the 120-130 kDa peptide is related to loss of amino acid transport in irradiated Ehrlich cell plasma membranes.

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Tu-Pos378

EXPRESSION STUDIES OF HUMAN UREA TRANSPORTER cDNA. J.J. Gargus, E. Steele, Y. Feng, W.L. Whaley and L. Malone. Department of Physiology and Section of Medical Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Rare individuals have been found to be homozygous null for the Kidd blood group antigen [Jk(a-b-)]. Such individuals have been demonstrated to be null for mediated urea transport in red cells, by direct measurement (Fröhlich et al., AJP, in press); and in kidney, by extrapolation from a renal concentrating defect (Sands et al., J. ASN, in press). Genetic arguments have been used to suggest that the antigen and transport activity are manifestations of the same protein affected by the null mutation (Gargus & Mitas, AJP 255:F1047). Kidd isoantibody has been used to isolate a 2.3 Kb candidate cDNA clone for the antigen/transporter from a reticulocyte library, the sqeuence of which was compatible with the protein's transmembrane orientation (Allen et al., Am. J. Hum. Gen. 43:A1). In the absence of protein sequence information, proof that the candidate sequence in fact encodes the transporter depends on its functional analysis. Towards this end, eukaryotic expression constructs of the cloned cDNA were prepared to allow the isolation of stable transformants of mouse L cells and transient high-copy transformants of temperature-sensitive Cos cells. As the 5' terminus is not contained in the cDNA clone, forced initiation is driven off of an ATG cassette. Constructs were prepared to contain the full 2.3 Kb cDNA, the open reading frame, and just the 3' half of the ORF which contains all of the putative transmembrane segments. Transfection was carried out with $CaPO_4$ and selection imposed for the G418-resistance marker carried on the vector. Northern blot, antibody binding, and unidirectional urea flux are used to assign functional phenotype to the transformants. (Supported by NIH grants GM34436 and GM34939 and carried out during the tenure of an AHA Established Investigatorship to JJG.)

Tu-Pos379

^{31}P NMR EVIDENCE OF SARCOLEMMAL PHOSPHATE INFLUX IN ISOLATED PERFUSED RAT HEART. J.A Hoerter. (intro. by G. Vassort). U241 INSERM, Physiologie Cellulaire Cardiaque, Bat 443, Université Paris Sud, 91405 Orsay, France.

In a normoxic perfused heart the sum of intracellular phosphorus compounds (ΣP) is constant: sarcolemmal inorganic phosphate (Pi) influx should equal its efflux. To study Pi membrane transport in the perfused heart ΣP was followed by ^{31}P NMR together with contractile function in presence or in absence of external Pi. Experimental conditions were designed to achieve Vmax of Pi influx. Saturating concentration of Pi_o was chosen: 5mM. Low content of Pi_i was maintained by unrestricted substrate availibility to the mitochondria and by 2 deoxyglucose perfusion. Phosphorylation into 2DG6P traps cellular phosphate moieties, without major alteration of heart function (Hoerter et al., Amer.J.Physiol. 1988, 255, C192). In absence of Pi_o, ΣP remains constant over 30min (98±3 natoms P/mg prot). In most cases, in the presence of Pi_o, ΣP increased but the end diastolic pressure (EDP) rose preventing functionnal stability. However, in low Ca medium (Ca/4) and in presence of Pi_o, EDP did not rise. ΣP steadily increased to 135±5 natoms P/mg prot (n=5) in 60 min while Pi_i content remained low. The Pi influx was slow: 0.6 natom/min/mg prot, a velocity similar to Vmax of the Na-Pi cotransport measured by radioactivity in sarcolemmal vesicles (Jack et al., J. Biol. Chem, 1989, 264, 3904). We postulate that a rise in Ca_i via Na-Ca exchange is the result of a Na-Pi influx because of the rise in EDP observed in normal Ca medium. Thus in the perfused heart Pi influx could partly be due to a Na-Pi cotransport and could thus interfer with other sarcolemmal Na transporters. (NMR performed in Groupe de Biophysique, Dir. M.Guéron)

Tu-Pos380

EFFECTS OF OKADAIC ACID AND STAUROSPORINE ON Na,K,Cl COTRANSPORT IN SQUID GIANT AXON. A.A. Altamirano, G.E. Breitwieser*, and John M. Russell. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550; *Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Using the internally dialyzed squid giant axon we have previously demonstrated that vanadate and fluoride can significantly decrease the rate of inactivation of the Na^+,K^+,Cl^- cotransporter caused by ATP depletion (AJP 254:C582, 1988). We have postulated that phosphorylation of some protein(s) activates the cotransporter whereas dephosphorylation causes inactivation of the cotransporter. To further test this hypothesis we examined the effect of both okadaic acid (OKA), an inhibitor of types 1 and 2A protein phosphatases and, of staurosporine (STAURO), an inhibitor of protein kinase C. OKA was tested using two different protocols. First, we examined its effect on the rate of inactivation of bumetanide-sensitive (B-S) ^{36}Cl influx caused by dialyzing out cellular ATP. The half-time for inactivation in the absence of OKA was 11.9 ± 1.3 min (n=7) whereas in the presence of 100 nM OKA, the half-time for inactivation was 22.6 ± 1 min (n=9). A second approach was to dialyze the axon with a low [ATP] (50 μM) which is very near the $K_{0.5}$ for the ATP activation of the cotransporter. If the phosphorylation/dephosphorylation model is correct, under this condition the flux will be determined by a dynamic steady-state between these two processes. After application of 200 nM OKA + 100 μM vanadate, B-S ^{36}Cl influx increased from 33.5 ± 3.8 to 45.7 ± 3.7 pmol/cm²·s (n=4). The results of both types of experiments are consistent with an inactivating role for protein phosphatases. STAURO (2 μM) had no effect on 3 axons dialyzed with 50 μM ATP suggesting that protein kinase C is not involved in cotransporter activation. (Supported by DHHS NS11946).

Tu-Pos381

PROLINE PORTER II: GENE ISOLATION, SEQUENCE DETERMINATION AND EXPRESSION IN E. COLI. D.E. Culham, H. Goulding, B. Lasby, A.G. Marangoni, J.L. Milner, B.A. Steer, R. Van Nues and J.M. Wood.

Proline porter II (PPII) permits *Escherichia coli* to utilize proline as a protein constituent or an osmoprotectant. Transporter activity is enhanced in bacteria subjected to hyperosmotic stress or to amino acid limited growth and it is eliminated by mutations at the *proP* locus. DNA fragments containing that locus, mobilized by bacteriophage mini-Mu, were selected through their ability to restore osmoprotection by proline to a *proP* mutant. A 5 kb DNA fragment common to the resulting molecular clones was incorporated into plasmid vector pGEM4Z, deletion derivatives were prepared and the nucleotide base sequence of the insert was determined. Both the original plasmid (pDC1) and a deletion derivative retaining only 2.5 kb of the original insert (pDC15) complemented the ability of strains containing *proP* deletions to utilize exogenous proline as a protein constituent or an osmoprotectant. Unlike bacteria which contained only a chromosomal copy of gene *proP* and those containing plasmid pDC15, those containing plasmid pDC1 could also utilize exogenous proline as a carbon or nitrogen source for growth. The rate of proline uptake by bacteria containing plasmids pDC1 and pDC15 was elevated when they were cultivated in hyperosmotic media or subjected to amino acid limitation; for pDC1, the proline uptake activity was up to 9-fold higher than that observed in bacteria containing a single, chromosomal copy of *proP*. The transport activity of bacteria containing plasmid pDC15 was lower under all conditions than that of strains containing plasmid pDC1 and the proline transport activity of strains containing no plasmid or vector pGEM4Z was negligible. The sequence of the chromosomal DNA insert common to plasmids pDC1 and pDC15 includes two open reading frames. ORF1 corresponds to a 17,964 kd polypeptide with 45.4% hydrophobic amino acids and ORF2 corresponds to a 24,594 kd polypeptide with 44.4% hydrophobic amino acids. The chromosomal DNA insert in plasmid pDC15 ends immediately downstream from ORF2. These data suggest that expression of ORF2 is required for PPII activity. Further deletion analysis is being conducted to determine whether both ORF1 and ORF2 are required to complement *proP* defects and the maxicell technique is being used to identify the polypeptides encoded in pDC1 and pDC15.

Tu-Pos382

EVIDENCE FOR TWO GALACTOSIDE BINDING SITES IN *ESCHERICHIA COLI LAC* PERMEASE. Bengt Persson and H. Ronald Kaback, Howard Hughes Medical Research Institute, Molecular Biology Institute, University of California, Los Angeles, CA 90024-1570.

The *lac* permease, the product of the *lacY* gene in *E. coli*, is a hydrophobic polytopic transmembrane protein that catalyzes the coupled translocation of a single galactoside molecule with a single H^+. Previous reports from this laboratory (Lolkema, J.S. & Walz, D. (1990) *Biochemistry*, in press; Lolkema, J.S., Carrasco, N. & Kaback, H.R. (1990) *Biochemistry*, in press) are consistent with the possibility that *lac* permease contains more than one binding site per monomer. This idea is supported by the present studies which demonstrate that the rate of photoaffinity labeling of purified reconstituted *lac* permease by *p*-nitrophenyl-α,D-galactopyranoside exhibits two distinct pH optima, one at pH 6.4 and another at pH 8.3. The properties of the photolabeling reaction at pH 8.3 are consistent with a high-affinity site as judged by protection experiments with lactose and other galactosides. On the other hand, the properties of the photolabeling reaction at pH 6.4 are indicative of a lower affinity site. Moreover, photoaffinity labeling studies with several site-directed mutants (e.g. K319L, H322R and E325A) reveal an altered pH dependence, and only a single pH optimum is observed. In addition to the mechanistic implications of two sites, the usefulness of the photoaffinity labeling technique for probing pK_a changes resulting from various mutations in the permease will be discussed.

Tu-Pos383

HISTIDINE TRANSPORT BY PERIPLASMIC PERMEASE RECONSTITUTED INTO PROTEOLIPOSOMES

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The periplasmic histidine transport system of *Salmonella typhimurium* has been partially purified and reconstituted into proteoliposomes. The reconstituted system has been previously shown to be completely dependent on the external periplasmic protein (HisJ), on the membrane bound complex (HisQMP) and on internal ATP (Bishop *et al.* 1989, PNAS 86, 6953-57). The initial rate of histidine transport depends on the external concentration of liganded HisJ and follows Michaelis-Menten kinetic with an apparent K_m value of about 3 μM and a V_{max} value of about 4 nmol/min/mg. The total amount of transported histidine at the attainement of a plateau level is independent of liganded HisJ concentration. Both the inside accumulated histidine and the ADP formed during the transport-coupled ATP hydrolysis are shown to be inhibitory. Proteoliposomes were non-leaky as shown both by the maintenance of the histidine gradient when ATP hydrolysis concomitant with histidine uptake ended and by the lack of the exit of accumulated labeled histidine as the proteoliposomes were exposed to a very large excess of external unlabeled histidine. The data demonstrate the unidirectionality of the histidine flux through the permease as reconstituted in proteoliposomes.

Tu-Pos384

KINETICS OF 0-TRANS L-GLUTAMATE INFLUX IN BARNACLE MUSCLE.

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L-Glutamate (Glu) influx was studied in internally dialyzed barnacle muscle fibers at 10°C and constant membrane potential. Internal Glu and Na concentrations were kept constant at zero. Influx-substrate activation curves were measured for Glu at five different Na concentrations and, independently, activation curves were measured for Na at three different Glu concentrations. The influx appears to be tightly Na-coupled since the apparent Glu influx "leak" permeability is unaffected by external Na or by the presence of a 10-fold excess of a competitive inhibitor of the mediated flux (L-Aspartate). Analysis of all results in terms of specific rapid equilibrium and steady state binding kinetic models leads to the following conclusions:

1. The Na:Glu coupling stoichiometry is 1:1. This is supported by a Hill analysis of Na-activation data.
2. The principle (though not necessarily the sole) substrate binding sequence at the extracellular membrane interface is Glu-first.

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Tu-Pos385

INORGANIC ION TRANSPORT CHARACTERISTICS OF DICTYOSTELIUM DISCOIDEUM.

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Myxamoeba of Dictyostelium discoideum (strain Ax-3) were grown in axenic media at room temperature. Ion composition was calculated from measurements of trapped space with C-14-PEG, water content by drying the constant weight, and atomic absorption and chloridometry of acid extracts of packed cells. When the growth medium contained (mM): Na 18, K 27, and Cl 45, the cells contained (mM/kg cell water): Na 30, K 65, Cl 6, and 3.1 kg cell H_2O/kg dry cell solids. Tracer influxes (M) were measured by centrifugation and washing cell samples taken at known times after addition of isotope. M(Na) = 500 μmoles/kg protein • min; M(K) = 320; M(Cl) = 710; in growth medium at 26°C. All three fluxes are linear in extracellular concentration. ^{86}Rb and ^{42}K gave the same flux values for K. Ouabain (10^{-4} M) inhibited cation fluxes less than 20%. The apparent activation energies (5-27°C) for cation fluxes were negligible but 11-15 kcal/mole for chloride fluxes. In comparison with human red cells, the cation fluxes in Dictyostelium discoideum are comparable to ouabain-insensitive fluxes but the chloride fluxes are 65,000 times smaller at 25°C. Supported in part by NIH grant HL28674.

Tu-Pos386

PASSIVE TRANSPORT STUDIES OF NUCLEOSIDE ANALOGS IN A TWO-PHASE MEMBRANE MODEL

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To understand the molecular properties that allow certain nucleoside analogs to permeate passively cell membranes, 3'-azido-3'-deoxythymidine (3'-N3dThd), 5'-azido-5'-deoxythymidine (5'-N3dThd), 2',3'-dideoxythymidine (3'-ddThd), and 2',5'-dideoxythymidine (5'-ddThd) were studied in a stacked, two-phase water:chloroform model of the interfacial barrier encountered at the surface of a membrane. The rate constants for crossing from the aqueous to organic phase, k_{in}, and from the organic to aqueous phase, k_{out}, and the equilibrium distribution coefficients, K_p, for each compound were determined by monitoring the disappearance of the compound from the buffered aqueous phase. The value of k_{in} determined for each compound was .0047 s^{-1} and was independent of the initial concentration in the aqueous phase. The values of k_{out}, which reflects the energy of desolvation for the chloroform phase, were .016, .029, .036, and .044 s^{-1} for 3'-N3dThd, 5'-N3dThd, 3'-ddThd, and 5'-ddThd, respectively. The rates of influx for these nucleoside analogs into human erythrocytes (Zimmerman et al., J. Biol. Chem., 262, 5748-54 (1987); Domin et al., BBRC 154, 825-31 (1988); and Domin et al., in prep.) are inversely proportional to k_{out}. These results suggest that the rate-determining step in the membrane permeation process may be the rate of crossing from the membrane interior to the intracellular aqueous compartment. The role of molecular conformation in the permeation process will be discussed. This simple model of the membrane interface can be used to establish the relative permeability of a series of compounds and thereby serve as a useful technique for directing drug design.

Tu-Pos387

SODIUM DEPENDENT GLUCOSE TRANSPORTERS CONTAIN A TRANSMEMBRANE AQUEOUS WATER CHANNEL.

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We reported previously (PNAS 86:8397, 1989; PNAS 87:3244, 1990) that facilitative glucose transporters (GTs) also facilitate the osmotically driven transmembrane passage of water. These findings, and others, suggested that the facilitative glucose transporters form an aqueous pore that spans the membrane. To determine whether the Na^+-dependent GTs found in epithelia also form transmembrane aqueous pores, we obtained the cDNA clone from Dr. E. Wright (cf. Hediger et al., Nature 330:379, 1987). Following in-vitro transcription of this clone with T3 polymerase, we injected the mRNA encoding this transporter into Xenopus Laevis oocytes (11 ng/oocyte). We then measured GT activity and osmotic water permeability of control and mRNA injected oocytes. The mRNA-induced expression of Na^+-dependent GTs increased oocyte uptake of ^{14}C-α-methyl glucoside by 95 fold, and increased their osmotic permeability by about two-fold with respect to water-injected control oocytes. In the absence of Na^+, or in the presence of 100 μM phlorizin, glucose uptake of mRNA-injected oocytes was negligible, and their osmotic water permeability was equal to that of water injected controls. These results suggest that: 1) Na^+-dependent GTs contain an aqueous pore that spans the membrane; 2) both glucose and water traverse the membrane via this pore; and 3) extracellular sodium acts as an allosteric regulator of the patency of this pore.

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Tu-Pos388

TREHALOSE TRANSPORT BY YEAST MEMBRANES. J.H. Crowe[1,2], P.S. Araujo[2,3], L.M. Crowe[1,2], A.C. Panek[2,3], and A.D. Panek[2], [1]Univ. California, Davis; [2]Federal University, Rio de Janeiro, [3]University of Sao Paulo

In previous studies we have established that trehalose stabilizes membranes, liposomes, and proteins in the absence of water. In an effort to find means for introducing this molecule into cells, we have begun a study of a transport system for trehalose known to be present in baker's yeast, Saccharomyces cerevisiae. Several strains were found to possess a specific transporter for trehalose distinct from the maltose transporter also found in yeasts. Kinetic analysis shows that the transporter follows sigmoidal kinetics, with separate low and high affinity binding sites. Transport activity is low in cells in log phase growing on glucose, but it increases after glucose is exhausted in early stationary phase. Addition of glucose to stationary phase cells results in rapid loss of transport activity, an event that is reversible when the glucose is removed, but requiring protein synthesis. We have isolated membranes from these cells, with the following properties: (1) Transport activity was seen in the isolated membranes only when it was present in the intact cells. (2) Two strains were chosen for study, one of which transported trehalose three times as fast as the other. When membrane vesicles were made from these two strains, they transported trehalose at about the same relative rates as the intact cells. (3) The kinetics of the transport system were very similar to those seen in the intact cells. (4) The influx of trehalose into the cells depends on a transmembrane pH gradient. If the gradient is collapsed, trehalose accumulated inside the vesicles is leaked into the medium. (5) After aging for several days the ability of the vesicles to transport was lost. However, transport was partially restored by elevating internal pH in the vesicles.

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Tu-Pos389

A MODEL RELATING THE POTENTIAL DEPENDENCE OF K_m VALUES FOR NA^+ AND SUGAR OF THE NA^+/GLUCOSE COTRANSPORTER. Eric Bennett and George Kimmich, Dept. of Biophysics, Univ. of Rochester, Rochester, NY 14642

Kinetics of the Na^+/glucose cotransporter in LLC-PK_1 epithelial cells were studied using whole cell recording techniques. Alpha-methyl-glucoside (AMG) induced currents (I_{AMG}) were normalized by cell capacitance. Kinetic parameters were determined by measuring I_{AMG} versus one of the transporter solute concentrations (the other solute was at a high concentration: AMG=20mM, Na^+=155mM). The maximal current (I_{MAX}) in both sets of experiments showed a marked potential dependence (doubling over the range of -13mV to -113mV). The concentration of solute at half I_{MAX} (K_{ms} for AMG and K_{mn} for Na^+) for both solutes decreased with hyperpolarization (K_{ms}: 2.02-.95mM; K_{mn}: 50-27mM over the range -13mV to -113mV). If the K_m for one solute is potential dependent, then, by simple Hill equation analysis, the other K_m must also depend on the membrane potential (V). Describing the effect of the membrane potential on K_m as $K_m(V)=K_{mo}*f(V)$, where K_{mo} is the K_m at zero membrane potential and f(V) is a potential dependent function, an equation relating f(V) for Na^+ to its counterpart for AMG can be determined. The Hill equation can be used to characterize I_{AMG} in two equivalent manners using coefficients of one to describe AMG dependence and two to describe Na^+ dependence. Since kinetic parameters calculated through these equations are affected by nonsaturating levels of solute, only the I_{AMG} under conditions where both solute concentrations are high (AMG=20mM, Na^+=155mM) can be used to compare the two Hill equations. Using values of 2 and 48.5 mM for K_{mos} and K_{mon}, respectively, $f(V)_n=1.01*(f(V)_s)^{1/2}$ (eqn.1) was calculated for a 2:1 stoichiometry. Comparing the measured values of K_{mn} with the values calculated from eqn.1 showed good agreement (errors were 0 to 18% over the full range of potentials). This relationship links the kinetic analysis of the two methods used to study Na^+/glucose cotransport (one describing AMG flux, the other describing Na^+ flux).

Tu-Pos390

PARTIAL PURIFICATION OF RECONSTITUTIVELY ACTIVE Na^+/Ca^{2+} EXCHANGER FROM BEEF HEART MITOCHONDRIA. W. Li and K.D. Garlid. Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

The mitochondrial Na^+/Ca^{2+} antiporter catalyzes electroneutral exchange of 2 Na^+ for 1 Ca^{2+} and was first demonstrated by Crompton et al (Eur. J. Biochem. 79, 549-558, 1977). Emerging concepts on the role of Ca^{2+} in regulating key matrix enzymes require Na^+/Ca^{2+} exchange to regulate mitochondrial Ca^{2+} (Denton and McCormack, Am. J. Physiol. 249, E543-E554, 1985). Structure-function analysis of this important carrier requires its purification and reconstitution, and, since no tightly bound ligand is known for the Na^+/Ca^{2+} antiporter, purification must be followed by reconstitutive activity. Mitochondrial proteins were extracted from beef heart SMPs, using Triton X-100, and fractionated on DEAE cellulose. Fractions were reconstituted into proteoliposomes containing the Na^+-selective fluorescent probe SBFI, and Na^+ uptake or efflux was measured with a spectrofluorometer. We have succeeded in purifying reconstitutively active Na^+/Ca^{2+} antiport with a purification factor of about 1,200. Net Na^+ transport is blocked by EGTA and specifically requires trans Ca^{2+} for activation. The K_m for Ca^+ stimulation of Na^+ efflux was found to be 20 nM, lower than that reported in intact mitochondria (Crompton, et al, ibid). Diltiazem, a relatively specific inhibitor of Na^+/Ca^{2+} antiport (Vaghy, et al, J. Biol. Chem. 257, 6000-6002, 1982), inhibited Ca^{2+}-dependent Na^+ efflux with a K_i of 10 μM, similar to the value obtained in intact mitochondria. Ba^{2+} was also found to inhibit this process in the reconstituted system. In the presence of EGTA, Li^+ was found to stimulate rapid, diltiazem-sensitive Na^+ efflux, with a K_m of 30 mM. This is the first indication that the Na^+/Ca^{2+} antiporter can catalyze Na^+/Li^+ exchange. We conclude that we have partially purified the beef heart mitochondrial Na^+/Ca^{2+} antiporter and that it is reconstitutively active. This research was supported by NIH grants HL 31573 and HL 43814.

Tu-Pos391

THE NA-CA EXCHANGER PRODUCES A "GATING" CURRENT WHEN ACTIVATED BY PHOTORELEASE OF CAGED Ca^{2+}.

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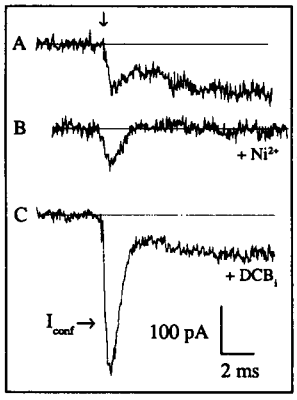
Whole-cell patch clamp currents were recorded in isolated guinea pig cardiac myocytes while intracellular Ca^{2+} jumps ($\tau \approx 200$ μs) were produced by flash-photolysis of caged Ca^{2+} (DM-Nitrophen). These experiments show that two components of current (Fig. A) are activated by the photorelease of Ca^{2+}: (1) a transient current lasting ≈ 2 ms is followed by (2) a "maintained" current (that decays slowly as $[Ca^{2+}]_i$ falls). We identify the maintained current as the usual Na-Ca exchange current by its voltage-dependence and its known sensitivity to inhibitors (e.g. zero $[Na^+]_o$, Ni^{2+} (see Fig. B), La^{3+}, etc.). The transient current component is not significantly affected by voltage, zero $[Na^+]_o$ and extracellular inhibitors. However, intracellular application of the inhibitor dichlorobenzamil (DCB) dramatically ***increases*** the size of the transient current component (Fig. C). This effect is consistent with model predictions of intracellular DCB-binding to the Na-Ca exchanger. It suggests that the transient component may represent a conformational transition current (here labeled as I_{conf}) and may result from a charge movement associated with a partial reaction step of the exchange cycle. The apparent charge on the Na-Ca exchanger, the charge moved during the transient, and the peak-current observed experimentally indicate a lower limit of 250/μm² for the site-density of the exchanger and an upper limit of 2500s^{-1} for the turn-over rate.



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