T-Pos86 DOES THE FLUORESCENCE QUENCHER ACRYLAMIDE BIND TO PROTEINS? Camillo A. Ghiron,[1] Samira Barghouthi,[2] and Maurice R. Eftink,[2] [1]Department of Biochemistry, University of Missouri, Columbia, MO 65211, and [2]Department of Chemistry, University of Mississippi, University, MS 38677.

Blatt *etal* (BBA 871 6, 1986) have recently reported that the magnitude of the Stern-Volmer constant, K_{sv}, for the acrylamide quenching of the tryptophanyl fluorescence (steady state) of human serum albumin (HSA), monellin and ovalabumin depends on the concentration of protein employed. They argued that this is due to the fact that acrylamide interacts with these proteins and they analyzed their quenching data to obtain partition coefficients of the order of 30-300 for the association process (with as many as 350 acrylamide molecules being bound to a single protein molecule). They further argued that the quenching mechanism involves diffusion of these bound quencher molecules through the protein to strike the tryptophanyl residues. We have repeated these studies using both steady state and lifetime (phase/modulation) measurements for a protein concentration range of 0.5 to 10 mg/ml. For HSA and monellin we find the acrylamide K_{sv} to be independent of protein concentration and similar to our previously reported values. Furthermore, we have performed equilibrium dialysis studies that show no significant association of acrylamide with HSA. Thus we find no evidence that acrylamide associates with these proteins as strongly as suggested by Blatt *etal* and we are unable to explain the difference between our results and theirs. This work was supported by NSF grant DMB-8511569.

T-Pos87 A 2 GHz FREQUENCY-DOMAIN FLUOROMETER-PICOSECOND RESOLUTION OF PROTEIN FLUORESCENCE INTENSITY AND ANISOTROPY DECAYS. Joseph R. Lakowicz, Gabor Laczko, I. Gryczynski and H. Cherek, University of Maryland, Department of Biological Chemistry, Baltimore, Maryland U.S.A

We developed a frequency-domain fluorometer which operates from 4 to 2000 MHz. The modulated excitation is provided by the harmonic content of a laser pulse train (7.52 MHz, 5 ps) from a synchronously pumped and cavity dumped dye laser. The phase angle and modulation of the emission are measured with a microchannel-plate photomultiplier. The precision of the measurements is adequate to detect differences of 20 ps for decay times of 500 ps. This instrument was used to examine tyrosine intensity and anisotropy decays from peptides and proteins. The data demonstrate that triply-exponential tyrosine intensity decays are easily recoverable, even if the mean decay time is less than 1 nsec. Importantly, the extended frequency range provides good resolution of rapid and/or multi-exponential anisotropy decays. Correlation times as short as 15 psec have been recovered for indole, with an uncertainty of ± 3 psec. We recovered a doubly exponential anisotropy decay of oxytoxin (29 and 454 psec), which probably reflects torsional motions of the phenol ring and overall rotational diffusion, respectively. Also, a 40 psec component was found in the anisotropy decay of bovine pancreatic trypsin inhibitor, which may be due to rapid torsional motions of the tyrosine residues and/or energy transfer among these residues. The availability of 2 GHz frequency-domain data extends the measurable time scale for fluorescence to overlap with that of molecular dynamics calculations.

T-Pos88 FLUORESCENCE DECAY STUDIES OF THIOREDOXIN: QUENCHING, OXIDATION AND REDUCTION

M. vandeVen, M. Han, D. Walbridge, J. Knutson, D. Shin, C.B. Anfinsen and L. Brand. Department of Biology, The Johns Hopkins University, Baltimore, Md. 21218 and The Laboratory of Technical Development, NHLBI, NIH, Bethesda, Md. 20205. Supported by NIH grant No. GM11632.

Thioredoxin from *E coli* contains two tryptophan residues, Trp 28 and Trp 31, close to two cysteine residues, Cys 32 and Cys 35, which form a disulfide bond. Time-resolved fluorescence decay of tryptophan was monitored with emission at 340 nm. and excitation at 295 nm. The experiments were carried out in 100 mM potassium phosphate, pH 5.7, 1 mM EDTA at 5 °C. Oxidized thioredoxin exhibits a triple fluorescence decay with decay times of 0.3(60%), 1.4(39%) and 5.9(1%) ns. Upon reduction of the disulfide bond by dithiothreitol, the steady-state fluorescence intensity increases and decay times of 0.4(43%), 2.4(12%) and 5(45%) ns. are found. The fluorescence enhancement is thus mainly due to the increased amplitude of the long lifetime component. The kinetics of reduction were also studied by nanosecond time-resolved measurements. Thirty sequential decay curves with a two minute collection time were obtained over a period of one hour. Global analysis of the data with linkage between the decay times indicated that the amplitude associated with the longest decay time increased while the amplitude of the shorter decay time decreased. External quenching of the tryptophan fluorescence was studied with the reduced protein in 100 mM potassium phosphate at pH 7.1 with 1 mM EDTA at 23 °C. Iodide was used as a quencher. Fluorescence intensity and decay time Stern-Volmer plots were linear up to 0.6 M KI and showed virtually the same slopes, suggesting dynamic quenching. The longer decay time was quenched while the medium decay showed little sensitivity to iodide. The origin of the complex decay will be discussed.

T-Pos89 INTRAMOLECULAR EXCIMER FLUORESCENCE OF PYRENE MALEIMIDE-LABELED DITHIOTHREITOL (P-DTT).
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The fluorescence properties of type I and type II P-DTT, with intact and cleaved succinimido-rings, respectively, were studied as a model system for intramolecular excimer (E) formation in pyrene-labeled proteins. In water, the fluorescence properties of the two P-DTT types were similar to those of P-tropomyosin with a low and high degree of E formation for type I and type II, respectively (Betcher-Lange and Lehrer, J.B.C. 1978). Excitation spectra of both P-DTT types indicated that the E originated from a subset of ground-state interacting pyrenes. For P(II)-DTT, as the labeling ratio increased, the M and E yields decreased and increased, respectively, reaching E/M (peak value) = 25. For P(I)-DTT, the M yield decreased without much increase in E (E/M = 1) indicating that the pyrenes interact in a configuration which quenches M wihout forming E. Lifetime studies confirmed the independent emission of E and M since there was little time delay between the E and M peak intensities (<3ns). In organic solvents, both P-DTT types formed some E. For type I, E/M only changed slightly with the polarity of the solvent (E/M=0.45, 0.59, 0.75, for dioxane, 2-propanol and methanol, respectively). Excitation spectra indicated that M and E originate from pyrenes in a similar environment and the E decay showed a ~15 nsec time delay (in propanol) due to formation by rotational diffusion, consistent with viscosity effects on E formation in glycerol/ethanol mixtures. The relatively long M lifetime (~90 nsec) therefore allows enough time for E formation by rotational diffusion. P(II)-DTT gave E/M ratios of 0.35, 0.45 & 1.65 in the same organic solvents, respectively, which increased with solvent polarity. In glycerol the E/M ratio was consistent with solvent polarity rather than viscosity. The much shorter M lifetime of the type II adducts (~10 nsec) decreases the possibility of E formation by rotational diffusion. The greater flexibility resulting from the ring cleavage,however, facilitates optimum hydrophobic pyrene interactions in more polar solvents. Thus, in type II-DTT, the E/M ratio appears to be determined by the polarity whereas in type I-DTT it is determined primarily by the viscosity of the solvent. Supported by NSF, NIH and the MDA.

T-Pos90 NANOSECOND TIME-RESOLVED FLUORESCENCE STUDIES OF PYRENE MALEIMIDE LABELED ENZYME I OF THE PTS. M. Han, D. Walbridge, J. Knutson, S. Hong, S. Roseman, and L. Brand. The Johns Hopkins University, Dept. of Biology, Baltimore, MD 21218 and The Laboratory of Technical Development, NHLBI, NIH, Bethesda, MD 20205.

Enzyme I, one of the protein components of the phosphoenolpyruvate:glycose phosphotransferase system (PTS), contains two tryptophan and four cysteine residues per monomer. All the sulfhydryl groups of Enzyme I react with 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), and the enzyme loses activity upon DTNB modification. The kinetics of the DTNB reaction with Enzyme I were analyzed with a non-linear least squares method. They exhibit biphasic character, with pseudo-first order rate constants of $1.6 \pm 0.6 \times 10^{-2}$ sec^{-1} and $1.7 \pm 0.5 \times 10^{-3}$ sec^{-1} at pH 7.5, 23°C. Fractional amplitudes associated with the rate constants are 25% for the fast and 75% for the slower rate. These results suggest that there are two classes of -SH groups. Pyrene maleimide, which is virtually non fluorescent in solution, becomes fluorescent when it reacts with proteins. The kinetics of the appearance of pyrene fluorescence with Enzyme I in 100 mM potassium phosphate buffer, pH 7.5 and 1 mM EDTA were also biphasic with rate constants similar to that of the DTNB reaction. There is spectral overlap between tryptophan emission and pyrene absorption. Resonance energy transfer was observed via quenching of tryptophan fluorescence. The kinetics of this quenching were obtained by a steady-state fluorescence. Nanosecond decay parameters obtained during the course of the reaction will also be presented and discussed, as will emission anisotropy decays. This work was supported by NIH grant No. GM-11632.

T-Pos91 FLUORESCENCE LIFETIME DISTRIBUTIONS IN EEL TROPONIN C AND STRUCTURALLY HOMOLOGOUS PARVALBUMINS. †Francois, J.-M., *Sedarous, S., †Gerday, C., °Gratton, E., and *Prendergast, F. G. *Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN; †University of Liege, Belgium; °Department of Physics, University of Illinois, Urbana, IL.

Troponin C from the European eel (Anguilla anguilla) has a single tryptophan residue, and 10 phenylalanines but has no tyrosine. The tryptophan fluorescence emission exhibits marked changes in spectrum, lifetimes and anisotropy upon binding Ca(II), Mg(II) and lanthanides. The fluorescence emission spectra of this protein and homologous tryptophan containing parvalbumins shift from ca 350 nm for the proteins devoid of Ca^{++}, to 320-326 nm when Ca^{++} ions are bound, and the steady-state anisotropy increases. To measure fluorescence lifetimes, we have used multiple frequency phase fluorometry which employed the harmonic content of a synchronously pumped, mode-locked and cavity dumped Nd:YAG (Spectraphysics #3000) laser as an excitation source. The frequency was quasi-continuously varied from 4 MHz to 500 MHz by driving the cavity dumper with a Hewlett-Packard frequency synthesizer which coupled the driving frequency of the cavity dumper with that at the Hamamatsu R928 photomultiplier tubes. Data on fluorescence lifetimes were first analyzed by use of a standard exponential approach and subsequently by use of a distribution analysis (Alcala, R., Gratton, E., and Prendergast, F. G., Biophysical Journal, submitted for publication). Exponential analysis for all forms of the proteins suggested biexponential fluorescence decay, whereas the distribution analysis showed a broad distribution of τ for all the proteins (metal ion free and metal complexed) and the profiles were generally similar. The rationale for the latter type of analysis will be discussed. Supported by GM 34847.

T-Pos92 DYNAMIC ANALYSIS OF WATER ACCESSIBILITY TO TRYPTOPHAN IN RNAse-T_1. Axelsen, P. H. Haydock, C., and Prendergast, F. G. Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN.

Small non-polar molecules are capable of quenching the fluorescence of TRP-59 in RNAse-T_1. Further data suggest that this residue is buried amongst other hydrophobic residues and has limited water accessibility. In order to understand the structural basis for these results, we have applied Connolly's Molecular Surfacing algorithm (Connolly, M. L., 1983, Science 221:709-713) to the X-ray crystal structure of RNAse-T_1 (Heinemann, U., and Saenger, W., 1982, Nature 299:27-31) and molecular dynamics simulations computer generated by CHARMM (Brooks, B. R., et al., 1983, J. Comp. Chem. 4:187-217). Preliminary calculations for the crystal structure show that TRP-59 is largely buried among other residues, although "pits" approximately 3 to 5 Å deep permit a 1.4 Å radius probe sphere to contact portions of both tryptophan rings. Several types of dynamic simulation show marked changes of TRP water accessibility over time. Fluorescence data are interpreted in light of these simulations. (X-ray crystal coordinates kindly supplied to F.G.P. by Prof. W. Saenger.) This work supported by GM 34847.

T-Pos93 ELECTROSTATIC INTERACTIONS OF THE TRYPTOPHAN RESIDUE IN STREPTOMYCES GRISEUS PROTEINASE A AND RIBONUCLEASE T_1. Liebman, M., and Prendergast, F. G. Department of Physiology and Biophysics, Mt. Sinai Medical School, NY, and Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN.

The properties of the fluorescence of the tryptophan residue in proteinase A from Streptomyces griseus and of ribonuclease T_1 from Aspergillus oryzae are distinctly different. Yet, molecular graphics depictions of the environs of the tryptophan shows the indole moiety in each protein to be "buried" to about the same extent, in other words, to exhibit very similar water accessibility as determined by use of the Connolly algorithm (Connolly, M. L., 1983, Science 221:709-713). Moreover, a simple examination of the nearest neighbors ($\leq$ 5 Å) does not provide any clear clues to explain the difference in fluorescence between the proteins. To probe the molecular basis of the fluorescence, we examined in detail the molecular interactions of the indole side chain with the protein matrix in each protein. This included calculation of dipole-dipole, charge-dipole, and charge-charge terms, and determination of molecular electrostatic potentials for the tryptophan environs. The data were displayed by use of two-dimensional linear distance and interaction energy plots. The results show a distinct disparity in the pattern of electrostatic interactions in the two proteins, the most striking being in the dipolar interaction terms. The tryptophan in ribonuclease T_1 was found to be in an apparently structurally restricted, electrostatically more stable environment than the tryptophan in Streptomyces griseus proteinase A. The significance of these findings to the apparent differences in the fluorescence will be discussed. Supported by GM 34847 to F.G.P.

T-Pos94 MULTIFREQUENCY PHASE/MODULATION FLUORESCENCE MEASUREMENTS WITH RIBONUCLEASE T_1, ALCOHOL DEHYDROGENASE, AND SELECTED TWO-TRYPTOPHAN PROTEINS. Maurice R. Eftink,[1] Zygmunt Wasylewski,[2] and Camillo A. Ghiron,[3] [1]Department of Chemistry, University of Mississippi, USA, [2]Department of Biochemistry, Jagiellonian University, Krakow, Poland, and [3]Department of Biochemistry, University of Missouri, USA.

Using multifrequency (10-200 MHz) phase/modulation fluorescence measurements, we have studied the effect of temperature, ligand binding, and solute quenchers on the fluorescence lifetime of tryptophan residues in selected proteins. For Ribonuclease T_1 we have confirmed the two lifetime fit (Chen, Longworth, and Fleming, to be published) for this protein at pH 7.4 using the frequency domain method and, from temperature dependence studies, we have determined the activation energies of 1.1 kcal/m for the long lifetime (3.7 nsec) component and 4 kcal/m for the short (1 nsec) component. The origin of the biexponential decay for Ribonuclease T_1 must relate to different conformational states, but with certain two-tryptophan proteins the most obvious interpretation of a biexponential decay is to assign lifetimes to the individual residues. This is the case for alcohol dehydrogenase, which Brand and coworkers and our group have studied extensively. We have now measured phase resolved spectra and the temperature dependence of the lifetimes for Trp-314 (buried at the subunit interface) and Trp-15 (surface) of this protein and its binary (with NAD) and ternary (with pyrazole or trifluoroethanol) complexes. We have also performed similar studies with the two tryptophan proteins, yeast 3-phosphoglycerate kinase, Staphylococcal aureus metalloprotease, and spinach ribulose-5-phosphate kinase. This work was supported by NSF grant DMB-8511569.

T-Pos95 **TRYPTOPHAN TO NADH ENERGY TRANSFER IN LIVER ALCOHOL DEHYDROGENASE. A PHASE/MODULATION FLUORESCENCE STUDY.** M. R. Eftink, Department of Chemistry, University of Mississippi, University, MS 38677.

Resonance energy transfer occurs between tryptophanyl residues and bound NADH in the ternary complex formed between liver alcohol dehydrogenase, NADH, and isobutyramide (Theorell and Tatemoto, ABB 142, 69). We have performed frequency domain lifetime studies and steady state fluorescence anisotropy studies with this system. When excited directly (340 nm), the fluorescence of bound NADH decays with a single lifetime of 4.4 ns, and has a high anisotropy (r = 0.33). When excited indirectly (i.e., trp to NADH energy transfer) at 290 nm, the decay of NADH is very non-exponential. In fact we find that the apparent phase lifetime surpasses the apparent modulation lifetime at frequencies above 10 MHz, and the phase angle actually exceeds 90° at ~100 MHz. This pattern is indicative of an excited state process (Lakowicz and Balter, Biophys. Chem. 16, 79) -- resonance energy transfer in this case. By adding iodide, which selectively quenches the surface trp-15 of LADH, we are able to focus only on the energy transfer process from the buried trp-314 of the protein. The sensitized (excitation at 300 nm) fluorescence anisotropy of bound NADH is found to be 0.27. This is a high value and indicates that the emission moment of the donor (trp-314) and that of the acceptor (NADH) are nearly parallel. This work was supported by NSF grant DMB-8511569.

T-Pos96 CORRELATION OF ENVIRONMENTAL RELAXATION AND INTERNAL MOTION FOR TRYPTOPHAN RESIDUES OF PROTEINS. Iain Johnson, Dan Harris, Bruce Hudson. Department of Chemistry, University of Oregon, Eugene, Oregon 97403.

Tryptophan fluorescence decay attenuation at long wavelengths (>400 nm) associated with environmental relaxation around the excited state is often observed in proteins. Data representing several proteins including T1 ribonuclease and mutants of T4 phage lysozyme indicate that this effect is enhanced as the internal mobility of the tryptophan residue (measured by the fluorescence anisotropy decay) increases. The accompanying reduction of the observed fundamental anisotropy (r_o), which may be of either photophysical or rotational origin, is discussed. Corresponding effects are shown by the model fluorophore N-acetyltryptophanamide (NATA) in viscous solvents at low temperatures. The fluorescence decay of NATA in propylene glycol at -7.5°C exhibits a clear negative preexponential factor demonstrating the relaxational nature of the excited state process. These data will be compared to previously reported phase-modulation fluorescence results. Contributions of this "general" mechanism leading to complex tryptophan fluorescence decay are evaluated in relation to overlapping effects which are more protein specific.

T-Pos97 **ENVIRONMENTAL STUDIES OF TYROSYL AND TRYPTOPHYL RESIDUES OF MAMMALIAN MYOGLOBIN WITH UV RAMAN SPECTROSCOPY.** S. J. Alex, S. A. Asher, Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260.

UV Resonance Raman spectra of Horse and Sperm Whale myoglobin (Mb) when excited selectively between 230 and 245 nm show well-defined peaks between 1500-1600 cm^{-1}, that are readily assigned to the ν_8 Raman modes of tyrosyl and tryptophyl residues. No interferences from phenylalanine vibrations are detected.

In the case of tyrosyl residues, dramatic changes in intensity and small frequency shifts are observed when compared to end-blocked aromatic amino acids in aqueous stoichiometric mixtures that mimic the aromatic amino acid composition of the myoglobin. Examination of the effects of pH and protein structural pertubants on the ν_8 Raman bands, demonstrates that the intensity changes derived from differences in the nature of hydrogen bonding of the phenolic groups. The presence of this kind of interaction is clearly illustrated with the Raman spectra recorded with a 245 nm excitation wavelength, where only tyrosinate and tyrosine strongly hydrogen bonded via the OH group dominate the raman spectra. No intensity and frequency shifts are observed from tryptophyl residues, this is consistent with the fact that the environment of tryptophans is not pH dependent in these myoglobins.

Similar Raman studies of aromatic amino acids on hemoglobin characterize the role of aromatic amino acids during the R ⇌ T transition in the hemoglobin cooperativity mechanism.

T-Pos98 A RESONANCE RAMAN STUDY OF THE ELECTRONIC PROPERTIES OF METAL-ANTHRACYCLINE COMPLEXES, Todd S. Koch and Richard P. Rava, State University of New York at Binghamton, Department of Chemistry, Binghamton, New York 13901

Metal ions have been known to interact with the anthracycline antibiotics for over 20 years since the observation of visible absorption spectral changes in the drugs upon addition of particular ions. Though the metal ions have been implicated as co-factors in the anti-cancer and cardiotoxic activity of the drugs, some metal chelated anthracyclines have shown reduced toxic properties. We have studied the resonance Raman spectroscopy of metal anthracycline complexes in order to fix the electronic and vibrational properties of these systems, and understand the activity of these drugs. Resonance Raman excitation profiles on various metal chelated antibiotics have been carried out, and electronic and vibrational calculations undertaken. The results indicate the relationship of the electronic properties of different metal-drug systems to the redox abilities of the complexes, and the possible relevance of these properties to the physiological activity of these drugs.

T-Pos99 ORIENTATION OF THE CONTRACTILE PROTEINS OF MUSCLE FIBERS BY RAMAN AND INFRARED SPECTROSCOPY. J.P. Caillé, M. Pigeon-Gosselin, F. Dousseau and M. Pézolet. Département de Biophysique, Université de Sherbrooke, Sherbrooke and Département de Chimie, Université Laval, Québec, Canada.

Raman spectra of glycerinated barnacle muscle fibers, incubated in a relaxing solution, were obtained with the long axis of the fiber parallel and perpendicular to the electrical field of the incident light. First it was established that the intensity of the peak at 1003 cm^{-1} observed in the Raman spectrum of these fibers and assigned to phenylalanine is not affected by the orientation of the sample, so that this peak was used as an internal intensity standard. With the electric field perpendicular to the long axis of the muscle fiber the intensity of the amide I is reduced and shifted to higher frequency (1656 cm^{-1}). The difference spectrum (electrical field $\parallel - \perp$) contains a symmetrical band centered at 1646 cm^{-1} characteristic of the α-helix conformation. This difference in the Raman scattering at 1646 cm^{-1} is due to the orientation of the α-helical segments of myosin, paramyosin and tropomyosin which stand at a small angle (10°) with respect to the axis of the fiber. There is also residual Raman scattering intensity in the difference spectrum at 1452 cm^{-1} (CH_2-bending). Since this band is not influenced by the conformation of the proteins it is proposed as a probe for modification of orientation of the proteins in this system. When these measurements were performed in D_2O the contribution of the β sheet and the disorder conformation to the amide-I band could be obtained after substraction of the α-helix amide-I band of the spectrum with the perpendicular orientation. The amide-III band was observed in infrared spectroscopy and the effect of orientation on this vibration mode was also observed.

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T-Pos100 MEASUREMENT OF THE VI CAPSULAR POLYSACCHARIDE, AN AMINO-URONIC ACID POLYMER, BY IR SPECTROSCOPY AND COLORIMETRIC REACTION . Shousun Chen Szu and Audrey Stone, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Development, NIH, Bethesda, MD 20814

The Vi capsular polysaccharide, a potential protective antigen of Salmonella typhi the causative agent of typhoid fever, is composed of linear homopolymer of α(1-4)2-deoxy-2N-acetyl galacturonic acid, variably O-acetylated at the C3 position. The polysaccharide and its protein conjugate are investigational vaccines. Due to its chemical structure, traditional colorimetric reactions for sugar are not valid. An analytical assay was developed to determine the polysaccharide concentration in these vaccines. Fourier transformed infared spectra were recorded with a Nicolet 5000 spectrophotometer. Polysaccharide solution containing KBr was freeze-dried and pressed into thin discs. Concentration of Vi polysaccharide were determined from the intensities of the C=O stretching band near 1750cm-1 and the C-O vibration near 1190cm-1. The amont of Vi, determined by this method, ranges from 0.5 mg to 1 mg.

In addition, Vi polysaccharide can be titrated in microgram quantities by means of the metachromatic reaction with dyes, such as Acridine orange bound to the regularly spaced negative charges on the Vi. The titration is performed on solutions of the polysaccharide as contrasted with its measurement in the solid form by the FTIR determination.

T-Pos101 DISTINCT PHYSICAL CONFORMATIONS IN DNA ADDUCTS OF THE (+) AND (-) ENANTIOMERS OF ANTI-BENZO(A)PYRENE DIOL EPOXIDE AS PROBED BY OPTICAL DETECTION OF MAGNETIC RESONANCE.
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The (+) and (-) enantiomers of 7,8-dihydroxy-9,10-epoxybenzo(a)pyrene (BaPDE) show quite distinct mutagenic and tumorigenic activities, and it has been suggested that these differences in biological activity might be related to differing physical conformations of the aromatic pyrene residue at the DNA binding site. We have used optically detected magnetic resonance (ODMR) to probe the local environment of the pyrene chromophore in these complexes. In our application, the phosphorescence of the pyrenyl excited triplet is observed while pumping the zero field splittings with microwaves; at resonance, a change in phosphorescence intensity is generally observable, allowing these environmentally sensitive parameters to be determined. The phosphorescence wavelengths and zero field splittings of the (+) adduct are found to agree closely with those shown by the benzo(a)pyrene tetraol in the same solvent, which is our model for a solvent exposed adduct. By contrast, the phosphorescence peak of the DNA adduct of the (-) enantiomer is red shifted by 10 nm, and the zero field splitting frequencies are lowered, which implies substantial base stacking interactions with the pyrenyl chromophore. Thermal denaturation produces triplet state properties suggestive of residual base stacking interactions in both single stranded adducts, while enzymatic digestion to individual deoxyribonucleoside adducts yields spectra which agree with the solvent exposed tetraol. These ODMR results thus imply a predominance of solvent exposed binding sites in the (+) adduct, and "quasi-intercalative" sites in the (-) adduct, in agreement with other recent spectroscopic studies. Work supported by DHHS-NCI Grant CA-20851.

T-Pos102 SITE SELECTION SPECTROSCOPY OF HORSE RADISH PEROXIDASE J.M. Vanderkooi, K.-G Paul and H. Koloczek, Dept. of Biochemistry & Biophysics, School of Medicine, Univ. of Pennsylvania, Philadelphia PA 19104 and Dept. of Physiol. Chem., Univ. of Umea, Umea, Sweden.

Resolved emission spectra of iron-free horse radish peroxidase (HRP) are obtained using site-selection conditions (i.e., narrow laser band excitation and low temperatures). Details of the emission spectra and the extent of inhomogeneous broadening differ with different HRP isoenzymes and further vary with the addition of substrate, indicating that the polypeptide chain composition and structure influence the spectra. The two tautomers of the porphyrin of HRP (which arise from the two possible positions of the hydrogens on the pyrole nitrogens) give distinct emission spectra. Prolonged illumination of one tautomer give rise to the appearance of the other with the simultaneous bleaching of the form that is illuminated. . These results are interpreted in terms of an excited state isomerization reaction which is occuring at temperatures close to absolute zero. The use of site selection spectroscopy will be discussed in terms of current understanding of protein structure and dynamics.

This work was supported by NSF Grant PCM84-0084.

T-Pos103 THE CIRCULAR DICHROISM OF α HELICES. DETERMINATION OF THE IMPORTANT GEOMETRIC AND ELECTRONIC FACTORS. Mark C. Manning and Robert W. Woody, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.

The matrix method for the CD calculation of polypeptides has been extended to include four peptide-localized transitions ($n\pi^*$, $\pi_0\pi^*$, $\pi_+\pi^*$, and $n'\pi^*$). Inclusion of higher energy transitions ($\lambda < 140$ nm) yields improved agreement with experiment. All ground state charges and transition moments were obtained from CNDO/S calculations on model peptides using both orthogonal and Slater-type basis sets. For α-helical structures, the rotational strength of the $n\pi^*$ transition is acutely sensitive to the ground state charge distribution. Both Lowdin and Mulliken population analyses were examined. Results were similar except that the Mulliken charges display an increased polarity in the N-H bond, leading to greater $n\pi^*$ intensity. Even more important is the correct description of the lone pairs on the carbonyl oxygen. Inclusion of hybridization effects significantly improves the CD calculations. The sensitivity of the calculated spectra of α helices to geometric factors is pronounced. Choice of the O-C-N bond angle is critical. Dependence on the ϕ and ψ torsional angles is also large. Hydrophilic helices ($\phi \simeq -65°$, $\psi \simeq -45°$) display weaker $n\pi^*$ transitions and a decreased intensity in the positive $\pi\pi^*$ band. In addition, this positive band is shifted to longer wavelengths. (Supported in part by USPH GM 22994, and a grant of computer time from Colorado State University).

T-Pos104 EFFECT OF ELECTROSTATIC INTERACTIONS ON TRANSITION MOMENT DIRECTIONS IN CRYSTALS OF SIMPLE AMIDES. Sundaram Devarajan and Robert W. Woody, Dept. of Biochemistry, Colorado State University, Fort Collins, CO 80523.

The direction of the electric dipole transition moment for the first $\pi\pi^*$ transition is a critical parameter in theoretical calculations of the absorption and circular dichroism of polypeptides. Accurate experimental data are available for only two molecules, obtained by Peterson and Simpson (J. Amer. Chem. Soc. 77, 3929 (1955)) from polarized absorption measurements on single crystals of myristamide (I) and N,N'-diacetylhexamethylenediamine (II). We have carried out CNDO/S calculations on simpler models of these molecules, acetamide and N-methylacetamide, respectively. The transition moment directions obtained differ from experiment by 15° in the case of I and by 18° in the case of II. The CNDO/S wave functions have been used to calculate the effects of the electrostatic fields induced by the surrounding molecules on the excited state energies and transition moment directions. Inclusion of these environmental effects causes the theoretical transition moment directions to rotate toward the experimental directions, reducing the discrepancy to 9° for I and 12° for II. Electrostatic effects in the crystals also lead to a large blue shift in the $n\pi^*$ and $n'\pi^*$ transition energies, where n' is the second lone-pair orbital on the carbonyl oxygen. These shifts cause the $n'\pi^*$ transition, predicted to be lower or nearly equal in energy to the first $\pi\pi^*$ transition, to shift to energies well above the $\pi\pi^*$ transition. Calculations have also been performed for diketopiperazine and N-acetylglycine, crystals for which rudimentary transition moment direction data are available. (Supported in part by USPH GM 22994.)

T-Pos105 AB INITIO QUANTUM MECHANICAL CHARACTERIZATION OF THE ELECTRONIC EXCITED STATES OF NUCLEIC ACIDS. J.D. Petke & G.M. Maggiora, The Upjohn Company, Kalamazoo, MI 49001.

The electronic excited states of cytosine, thymine, uracil, adenine and guanine have been computed from large-scale ab initio configuration interaction calculations which employ an extended basis set consisting of double-zeta, polarization, and diffuse functions. In general, computed $\pi \rightarrow \pi^*$ transition energies are high compared with experiment (ca. 1-2 eV), but the relationship of specific transition energies, oscillator strengths, and polarizations to the observed spectral features of the molecules has provided a basis for assigning and analyzing their spectral transitions. The spatial extent of the electron distributions of the two lowest $^1(\pi,\pi^*)$ states are similar to that of the ground state (i.e. "valence-like"), while many of the higher-lying $^1(\pi,\pi^*)$ states appear to possess considerably more diffuse electron density distributions (i.e. "Rydberg-like"). The location of $^1(n,\pi^*)$ states will also be reported.

T-Pos106 THEORETICAL INVESTIGATIONS OF THE SPECTROSCOPIC PROPERTIES OF BENT α-HELICES. Thomas M. Cooper, Mark C. Manning and Robert W. Woody, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.

Recent work by Yamaoka, et al. (JACS 108:4617-4625 (1986)) describes electric linear dichroism spectra of the α-helix which appear to be inconsistent with the splitting of the 190 nm $\pi\pi^*$ absorption band into parallel and perpendicular components as predicted by exciton theory. To rationalize these observations, we have investigated the effect of systematic bending of the α-helix on its calculated spectroscopic properties. We compared the Pauling α-helix with a bent helix as described by Blundell, et al. (Nature 306:281-283 (1983)) i.e., with "hydrophobic" ϕ, ψ values on one side of the helix and "hydrophilic" ϕ, ψ values on the other. An exciton model incorporating the amide $\pi\pi^*$ transition at 190 nm was used to calculate the parallel and perpendicular components of absorption, and the reduced linear dichroism spectrum. A Pauling helix, 200 residues long, exhibited the expected splitting between parallel and perpendicular absorption bands. In contrast, a bent helix of 200 residues exhibited a 90% decrease in the intensity of the reduced linear dichroism spectrum as compared to the Pauling helix. These results parallel the experimental data published by Yamaoka, et al., suggesting that bending of the α helix in solution can reconcile these data with Moffitt's theory. (Supported in part by USPH GM 22994, and a grant of computer time from Colorado State University).

T-Pos107 A BRILLOUIN SCATTERING STUDY OF THE HYDRATION OF LI- AND NA-DNA FILMS. T. Weidlich, S.A. Lee, S.M. Lindsay, J.W. Powell[+], N.J. Tao, G. Lewen and A. Rupprecht[*] Department of Physics, Arizona State University, Tempe, Arizona 85287, [+]MPI, Stuttgart, FRG and [*]Arrhenius Laboratory, University of STockholm, Sweden.

We have used Brillouin spectroscopy to study the velocities and attenuation of acoustic phonons in wet-spun films of Na-DNA and Li-DNA as a function of the degree of hydration at room temperature. Taken together with x-ray, optical, gravimetric and swelling data, we use these experiments to separate the various hydration processes: mass loading (tight water binding), relaxation (viscous coupling) and potential softening (due to electrolyte dilution). We extract intrinsic elastic constants for hydrated Na-DNA of c_{11} = 1.0 x 10^{11} dynes/cm^2 and c_{33} = 5.7 x 10^{10} dynes/cm^2 which corresponds to E = 1.1 x 10^{10} dynes/cm^2 (with Poisson's ratio, σ = 0.44). The negative elastic anisotropy is evident in the macroscopic properties of the films which we believe is evidence of strong interhelical bonds. The acoustic losses are accounted for by relaxational coupling to the hydration shell. (Work supported by NSF PMC8215433, ONR N00014-84-C-0487 and EPA 68-02-4105.)

T-Pos108 EVIDENCE FOR ANTIGEN-INDUCED CONFORMATIONAL CHANGES IN ANTI-GALACTAN ANTIBODIES FROM PHOSPHORESCENCE SPECTROSCOPY by C. P. J. Glaudemans, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH Bethesda, Maryland, and Z. Li and W. C. Galley, Department of Chemistry, McGill University, Montreal, Quebec.

In that changes in the protein fluorescence accompany the binding of antigen to the Fab fragments of anti-galactan antibodies and X-ray data implicate tryptophan residues close to- or in the combining sites of a number of antibodies, we have initiated a study of short-range interactions within these systems employing triplet state spectroscopy. Spectra and decays of the intrinsic phophorescence of the Fab fragment of the anti-galactan antibody J-539 were obtained in rigid solution at 77K and above. While the heterogeneous tryptophan component of the emission spectrum was found to undergo only minor changes on antigen binding, the decay times were more discernably perturbed. In the absence of antigen the decay of the trypophan component of the phosphorescence was non-exponential, but in most cases could be approximated as the sum of two exponentials. While the slower component of the decay (4.1 - 4.8 sec.) approached values found for tryptophan in most proteins and peptides, the smaller rapid component possessed a lifetime (0.8 sec.) which was clearly anomalous. The short tryptophan lifetime which is seen with lysozyme and Ra5 ragweed allergin appears to derive from the proximity of aromatic side chains to disulfide bridges. On antigen binding the slow component in the decay was essentially unchanged, but the anomalous decay time was significantly increased. This decrease in the magnitude of the perturbed decay rate implies an increase in the relative separation of one or more tryptophan side chains to disulfides in the Fab fragment resulting from a conformation change on antigen binding.

T-Pos109 ELECTRONIC TRANSITIONS IN BIOLOGICAL MOLECULES: THEORETICAL SIMULATION OF ENVIRONMENTAL EFFECTS. Ilich, P., Haydock, C., Axelsen, P.H., and Prendergast, F.G. Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905.

In the energy operator expression for a spectroscopic observable,

$$H_{eff} = H - \frac{i}{2}\Gamma$$

the energy position operator H has been statically modified by an external field. Scalar, vector and tensor properties corresponding to electron energies, electron transition moments and nonlinear optical susceptibilities, respectively, were calculated by semiempirical quantum mechanical algorithms. A variety of molecules of direct and indirect biological importance was treated in this way and results were analyzed in terms of electronic and molecular structure properties of substrate and geometries of external fields. For specific cases the external field geometries were generated by molecular dynamics simulation calculations of concrete protein structures. The results were discussed in terms of conformational and solvation effects in protein photophysics. Supported by GM 34847.

T-Pos110 THE GROUND AND EXCITED-STATE INTERACTIONS OF EQUILENIN AND 17 β-DIHYDROEQUILENIN WITH TRIETHYLAMINE. A.Örstan[1], W.R. Laws,[2] and J.B.A. Ross.[1] [1]Department of Biochemistry and [2]The Center for Polypeptide and Membrane Research, The Mount Sinai School of Medicine, 1 Gustave L Levy Place, New York, N.Y. 10029.

We previously used the fluorescent steroid equilenin to probe the steroid-binding site of the human and rabbit sex steroid-binding protein (SBP) (Biochemistry 25, 2686 (1986)). The red-shifted fluorescence emission spectrum of the SBP-bound equilenin resembles that of the hydrogen-bonded equilenin-triethylamine (TEA) complex in cyclohexane, suggesting that the steroid-binding site of SBP is a low-dielectric cavity containing a proton acceptor. To further understand the steroid-binding site of SBP, we have investigated the ground and excited-state interactions of equilenin and 17 β-dihydroequilenin with TEA in cyclohexane and toluene, using absorption and fluorescence methods. The formation of the equilenin-TEA complex red-shifts the absorption spectrum of equilenin in either solvent about 5 nm. While the fluorescence emission peak of the complex in cyclohexane is at 370 nm, it is near 415 nm in toluene, resembling ionized equilenin in high-pH aqueous solution. In a 20% solution of toluene in cyclohexane, the emission spectrum of the equilenin-TEA complex has two peaks near 370 and 400 nm. The data indicate that while the ground-state interactions of the steroids with TEA are similar in both solvents, the excited-state behavior is solvent dependent. Fluorescence decay kinetics are complex, indicating that there is also an excited-state reaction between equilenin and TEA. (Supported by NSF Grant DMB-8516318 and NIH Grant HD-17542)

T-Pos111 A STUDY OF RED BLOOD SICKLE CELLS USING DIFFERENTIAL POLARIZATION MICROSCOPY
David A. Beach, K. Sam Wells, Carlos Bustamante. University of New Mexico Chemistry Dept. Albuquerque, New Mexico 87131

A differential polarization microscope has been built that uses a photoelastic modulator as the polarizing element, an image dissector tube camera and a phase-lock detector to generate differences from images obtained using two orthogonal polarizations of the light. The images are formed simultaneously pixel by pixel. The signal-to-noise ratio of the resulting differential image can be controlled by the operator, by the choice of the stay-put time of the dissector camera at every pixel location.

This newly designed differential polarization microscope has been applied to the study of the spatial distribution of hemoglobin polymer inside red blood sickle cells. This finding, first reported by Mickols et al., has been thoroughly confirmed by our measurments. In particular, since these measurments have been done with quite distinct and independent microscope designs, a comparison of our data with these authors' data lends much support to the idea that there are certain well-defined and repeatable patterns of polymerization inside the red blood sickle cells. The theory of differential polarization imaging recently derived in our laboratory is used to construct spatial models for the observed patterns.

T-Pos112 NANOSECOND TIME-RESOLVED FLUORESCENCE: A TOOL FOR CHEMICAL KINETIC STUDIES. Dana G. Walbridge, Jay R. Knutson, Myun K. Han and Ludwig Brand. The Department of Biology, The Johns Hopkins University, Baltimore, MD 21218 and the Laboratory of Technical Development, NHLBI, NIH, Bethesda, MD 20892.

A monophoton counting fluorescence decay instrument has previously been used to measure the kinetics of the acid denaturation of alcohol dehydrogenase on the time scale of minutes (Walbridge et al., Biophys. J., 37, 393A, 1982). The instrument was modified so that a series of sequential decay curves could be obtained and rapidly transferred to a computer. The information available from this data is greatly enhanced by the use of global analysis methods (Knutson et al., Chem. Phys. Lett., 102, 501, 1983) in which one or more decay parameters are linked between decay curves. Nanosecond time-resolved fluorescence measurements allow one to decompose the emission into its dynamic components which are often more sensitive to protein conformation than time averaged values. The monophoton instrument has now been improved by addition of a synchronously pumped, mode-locked, cavity-dumped dye laser as a light source. The high repetition rate of this source has enabled us to measure decay curves with only a 2 second accumulation time. Rates of reactions can thus be measured over the time course of a few seconds or less. Using our previous time resolution only lifetimes of 2 and 5 nanoseconds were observed during denaturation of alcohol dehydrogenase. With our present capability, lifetimes akin to the native enzyme (3.6 and 7.4 nanoseconds) were detected during the early stages of denaturation. Other examples of rapid kinetic studies will be presented. Supported by NIH grant GM11632.

T-Pos113 TIME-RESOLVED FLUORESCENCE OF INTRACELLULAR QUIN-2. John Wages, Jr., Beverly Packard, Michael Edidin, and Ludwig Brand. Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Quin-2, the first in a series of fluorescent analogues of the calcium-selective chelator EGTA, has been extensively employed in the study of cellular processes involving calcium. Neither these studies nor those using the newer dyes fura-2 and indo-1 has explored the dimension of fluorescence decay on the nanosecond time scale although the information provided by such techniques could help resolve such questions as possible dye or free calcium heterogeneity or compartmentalization within cells. Therefore, we have begun to characterize the time-resolved emission from living quin-2-loaded cells.

Madin-Darby bovine kidney (MDBK) cells adherent to coverslips were loaded with quin-2 using its acetoxymethyl ester. The fluorescence decay from these cells was analyzed by a sum of exponentials, two components of which were similar to the lifetimes of quin-2 and its calcium complex in solution (These measured lifetimes were 7.4 ns for the calcium complex and 0.7 ns for the free quin-2 in solution at 37° C.). Addition of ionomycin followed by $MnCl_2$ quenched both components, in good agreement with the observed quenching by Mn^{2+} of both dye species in solution.

The observed difference in lifetimes of quin-2 has allowed reproducible resolution of both dye species in calcium EGTA buffers. On the other hand, the lifetimes of the calcium chelated and free forms of fura-2 and indo-1 differ by comparatively little.

T-Pos114 RESOLUTION OF TWO-COMPONENT FLUORESCENCE EMISSIONS BY PHASE-SENSITIVE DETECTION AT MULTIPLE FREQUENCIES, by S. Keating-Nakamoto, H. Cherek and J. Lakowicz, University of Maryland, School of Medicine, Department of Biological Chemistry, Baltimore, Maryland 21201.

Phase sensitive emission spectra recorded on a variable frequency instrument were used to determine the lifetimes and steady state spectra of the fluorophores in two component mixtures. The analysis does not require any previous knowledge of the component lifetimes, fractional intensities or the emission spectra. Phase sensitive spectra are recorded at several frequencies and arbitrarily chosen detector phase angles. The data are analyzed using non-linear least squares to recover the lifetimes and wavelength-dependent fractional intensities. The fractional intensities determine the emission spectrum and relative intensity of each component in the mixture. Using this technique we resolved two component mixtures with lifetimes that differed about 2-fold, of fluorescein and 9-aminoacridine; 2(p-toluidinyl)naphthalene-6-sulfonic acid and 6-propionyl-2-dimethylaminonaphthalene; and N-acetyl-L-tyrosinamide and N-acetyl-L-tryptophanamide. Analysis of simulated data is also presented to illustrate the requirements for a satisfactory resolution. For simulated two component mixtures, the components can be resolved if the lifetimes differ by at least 2-fold even with extensive overlap of the emission spectra.

T-Pos115 GLOBAL ANALYSIS OF FLUORESCENCE DATA: SOME EXTENSIONS

Jay R. Knutson, Laboratory of Technical Development, NIH/NHLBI, Bethesda, MD 20892

The simultaneous optimization of fitting parameters from multiple, related fluorescence experiments ("global analysis": cf. Knutson et al., Chem. Phys. Lett. 102:501, 1983) has become a popular tool for analyzing complex luminescence data. Multiple decay times, associated spectra (DAS), multiple rotation rates (eg., for axial ratios) and macromolecular equilibria have all been quantitated by this approach. This overdetermination has recently been extended to some other problems:

1. Distributed decay profiles. A set of three-parameter lifetime distribution functions can now be tested with linkage (uses chain rule on a mesh of convolutions, kept in memory, to speed computation). This program is much less susceptible to a weakness of non-global distribution fitters - assignment of broad, ill-defined distributions to simple discrete decay systems.
2. The analysis of many brief (noisy) decays. As suggested by Selinger and Harris, Poisson statistics can lead to weighting bias (errors in lifetimes) when curves containing many low-count channels are fit. Weighting via $(fit)^{-1}$ near the optimum provides a first-order fix. This modification is helpful in cases of rapid scanning (global DAS) and kinetic decay studies (see Walbridge et al, this volume). In laser instruments like ours, very rapid data collections (as short as ≤100ms) become usable. With multishot averaging, even "stopped-flow" lifetimes are tenable.
3. Simple extensions to analyze transient quenching (decays and steady state heterogeneity) and transforms of each case for phase fluorometry will also be discussed.

T-Pos116 ANALYSIS OF COMPLEX TIME-RESOLVED FLUORESCENCE DECAY DATA.

Peter Bayley & Stephen Martin, Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA.

We have examined the ability of a typical analysis of multiple exponentials to identify two types of potential complexity in simulated experimental data to which statistical noise is added: a) a continuous Gaussian distribution of exponential decays, convoluted with a 200ns FWHM profile; b) a single exponential with contributions from a diffusional (sqrt.t) term. Each simulated decay curve is analysed by non-linear least squares (Marquardt algorithm), with statistical criteria of a) residuals; b) χ^2; c) autocorrelation; d) Durbin-Watson, DW.

With a Gaussian width of 10% of the mean value (5ns), the data deviates from a single exponential only if the peak counts exceed 25000, and a two-exponential fit is adequate even at a 30% width, for data to a peak of 25000. Multiple exponentials can readily describe the diffusional case; highly accurate data at short times is essential for the analysis.

The general conclusion is that the analysis of complex decay curves is necessarily highly degenerate and ultimately model-dependent. In both cases studied, additional information is required to justify the choice of physical model, and the need for statistical significance is emphasized.

T-Pos117 COMPLEX ANISOTROPY DECAYS: ANALYSIS IN TERMS OF MULTIPLE ENVIRONMENTS.

Richard D. Ludescher, Linda Peting, Suzanne Hudson and Bruce Hudson, Department of Chemistry, University of Oregon, Eugene, Oregon 97403.

Previous simulation calculations [Biophys. J. 45, 379a]of the behavior expected for the decay of the fluorescence anisotropy expected under conditions of heterogeneous environments have been extended to include the problem of the unique interpretation of such data. The simulations emphasize those cases where environments that are noninterconverting on the fluorescence time scale exhibit distinct fluorescence lifetimes and dynamics behavior. The resulting anisotropy decays are time dependent even when there is no reorientational motion on the time scale of the measurements. It is found that those anisotropy curves that start from a high initial value and show upward curvature at long times [cf. Hundson et al., in "Applications of Fluorescence in the Biological Sciences", D.L. Taylor et al., editors (Alan R. Liss, 1986)] can be uniquely analyzed in terms of the properties of the two environments. In particular, it is argued that anisotropic rotation cannot give rise to this type of behavior. On the other hand, curves that are monotonically decreasing can, in some cases, be interpreted equally well in terms of heterogeneous behavior or homogeneous behavior with a complex lifetime decay and multiple relaxation times for the anisotropy.

T-Pos118 TRANSIENT EFFECTS IN QUENCHING DETECTED BY HARMONIC-CONTENT FREQUENCY-DOMAIN FLUOROMETRY. M.L. Johnson, University of Virginia, Department of Pharmacology, Charlottesville, J.R. Lakowicz, N. Joshi and I. Gryczynski, University of Maryland, Department of Biological Chemistry, Baltimore.

We used harmonic-content frequency-domain fluorometry to investigate the quenching of indole fluorescence by iodide and acrylamide. The frequency-response of the fluorescence emission was measured over a frequency range from 10 to 2000 MHz. In the absence of collisional quenching the decay of indole is dominately a single exponential. The intensity decay became increasingly non-exponential in the presence of collisional quenching by iodide or acrylamide. We attribute the complex decays to transient effects as predicted originally by Smoluchowski. At low quencher concentrations the non-exponential component is accounted for by a decay law of the form $\exp(-t/\tau - 2b\sqrt{t})$, which is known to be an approximate form of the more complete expression. In water solutions at concentrations of quencher above 0.1 M this $\sqrt{t}$ decay law does not completely account for the data. The data and theory are in better agreement when using the complete expressions for the "radiation" boundary conditions. However, in aqueous solutions above 0.5 our experimental resolution is adequate to detect deviations from even the more complete theory. Evidently, the resolution of the frequency-domain method is adequate to recover the complex subnanosecond decays found at high concentrations of quenchers. Extension of this theory and analysis to buried residues in proteins should allow determination of diffusion rates of quenchers within proteins.

T-Pos119 **STANDARDIZATION OF FLUOROPHORES USING PHASE FLUOROMETRY.**
Richard B. Thompson and Enrico Gratton*; Bio/Molecular Engineering Branch, Code 6190, Naval Research Laboratory, Washington, DC, 20375-5000; and *Dept. of Physics and Laboratory for Fluorescence Dynamics, University of Illinois, Urbana, IL, 61801.

Measurement of fluorescence lifetimes, like any other analytical procedure, requires the use of standards for instrument calibration and comparison. Scattering materials have often been used for this purpose because their "lifetime" is accurately known. It is well known that scatterers can introduce various artifacts into lifetime measurements, particularly the "color effect" seen in photomultiplier tubes. Standard fluorophors are commonly used to minimize these artifacts by matching the emission wavelength of sample and standard. The accuracy with which the lifetimes of the standard fluorophors are known can limit the accuracy of decay measurements. Unfortunately, there is no wide agreement on the lifetimes of the standards (to <3%), even though the precision of current pulse and multi-frequency phase instruments is perhaps ten-fold better. This variation may be due to differences in instruments and/or sample preparation. We propose a phase method for accurately determining the lifetimes of standard fluorophores in situ, without assuming any standard value. The basis of the method is that the frequency-dependent phase angle differences and demodulation ratios between two monoexponentials describe simple functions that uniquely determine the two decays. Thus we measured phase differences and demodulation ratios between pairs of standard fluorophors, and fit the data by assuming only that both were monoexponential. Results of these measurements on commonly used standards and a separate test of the method's accuracy will be shown. **This work was supported by PHS IT-41-RR-03155 (E. G.) and DARPA (R.B.T.); R.B.T. was the recipient of an N.R.C. Associateship. The authors are grateful to Jay Knutson, David Jameson, and Bernard Valeur for helpful discussions.**

T-Pos120 STRATEGIES FOR USING "SPARSELY SAMPLED" INSTRUMENT FUNCTIONS IN FLUORESCENCE LIFETIME DETERMINATION

Gary R. Holtom, Regional Laser and Biotechnology Laboratories, University of Pennsylvania (introduced by Ponzy Lu).

In the last few years the response time possible using time-correlated single photon counting methods has improved markedly. While the possibility of resolving decay times and rotational motions less than 100 ps is welcome, significant problems appear when fast and slow components must be resolved simutaneously, as is often the case with real biological systems. For example, most macromolecules have very fast local motions and much slower rotational diffusion involving the entire molecule.

Collecting data over a time window of 20 to 50 ns, and maintaining a reasonable data set of 512 or 1024 points results in a channel width of 20 to 100 ps. With the fastest detectors, virtually all of the counts in the instrument function are compressed to 2 to 5 channels. Changes in counts can be an order of magnitude between adjacent points. This sparse sampling results in numerical difficulties when performing iterative reconvolution. The traditional simple method for convolution produces characteristic errors in modeling the early parts of the fluorescence decay curve, which can be easily confused with experimental artifacts such as a wavelength dependence on transit time in the detector. The origin of these errors and strategics for improving the accuracy of the fitted curve are described. Examples with model systems are presented.

This work was supported by NIH Grant RR-01348.

T-Pos121 RAPID FLUORESCENT EXCITATION SPECTROSCOPY USING AN ACOUSTO-OPTIC TUNABLE FILTER. Ira Kurtz[1], Ron Dwelle[2], Patrick Katzka[2]. Department of Medicine, UCLA School of Medicine, Los Angeles, California[1]; Litton Applied Industries, Sunnyvale, California[2].

A new rapid method for monitoring fluorescent excitation spectra from optical pH or Ca^{2+} probes using an acousto-optic tunable filter (AOTF) has been developed. The AOTF consists of a birefringent crystal bonded to a piezoelectric transducer. Acoustic waves are generated in the crystal by an applied radio frequency. For a given acoustic frequency, only a narrow band of optical frequencies will be diffracted. Unlike normal Bragg diffraction, which is very sensitive to the angle of incidence and can be used only with collimated light, the AOTF has a large angular aperture (up to 28 degrees), and can be used with an uncollimated broadband white light source. Using this device and a xenon-mercury arc lamp, excitation spectra of the fluorescent pH probe BCECF from 440 nm to 508 nm have been acquired in 1/60 sec (spectral resolution $\sim$ 1 nm). In addition, rapid alternation between two excitation wavelengths for excitation rationing can be performed in 20 μs. Spectral imaging is also possible with a resolution of 100 lines/mm.

T-Pos122 HIGH RESOLUTION TEMPERATURE-DEPENDENT FLUIDITY PROFILE OF RENAL BRUSH BORDER AND BASOLATERAL MEMBRANES. Herbert Y. Lin, Nicholas P. Illsley, Pei-Yuan Chen and A.S. Verkman. Cardiovascular Research Institute, Univ. of Calif., San Francisco, CA 94143.

Computer-based temperature control and data acquisition systems were added to an SLM 4800 fluorimeter to perform automated temperature scans of static and dynamic fluorescence parameters with 0.1°C resolution. The fluidity characteristics of renal proximal tubule cell membranes were examined using diphenylhexatriene (DPH) and cis and trans parinaric acids (cPnA, tPnA) inserted into purified brush border (BBMV) and basolateral membrane (BLMV) vesicles isolated from rabbit renal cortex. In triplicate temperature profiles (10-50°C, 400 data points), the steady-state anisotropy (r) of DPH corrected to infinite dilution fitted closely to double connected lines (4 parameter fit) with slopes of -1.48 ± 0.10 $°K^{-1}$ (<28°C) and -1.95 ± 0.08 $°K^{-1}$ (>28°C) [BBMV] and -1.66 ± 0.07 $°K^{-1}$ (<23°C) and -2.21 ± 0.05 $°K^{-1}$ (>23°C) [BLMV]. The temperature at which discontinuities in slope occurred (T_c) were reproducible to within 1°C in multiple experiments. Lateral fluid and solid phase domain structure was studied from the temperature profile of cPnA and tPnA fluorescence lifetimes determined from heterogeneity analysis of 3 frequency phase and modulation data. Two lifetimes were detected at all temperatures, indicating coexistent solid (18-22 ns) and fluid (2-5 ns) domains. In both membranes, the fraction of solid phase phospholipid decreased (0.75-0.25, BBMV; 0.33-0.20, BLMV) with increasing temperature (10-40°C). Both membranes showed a broad temperature range (10-30°C) over which the fluid/solid composition varied montonically. These studies indicate that the basolateral membrane is significantly more fluid than the brush border membrane, due to an increased proportion of fluid-phase phospholipid at all temperatures.

T-Pos123 SYNCHROTRON LIGHT SOURCE EXCITATION STUDIES OF THE FLUORESCENCE OF POLYCYCLIC AROMATIC CARCINOGEN-DNA COMPLEXES. David Zinger* and Nicholas E. Geacintov**, *Biology Department, Brookhaven National Laboratory, Upton, New York and **Chemistry Department, New York University, New York, New York.

The fluorescence of aromatic fluorophores bound to nucleic acids can be extremely sensitive to their microenvironment. This property can be exploited to obtain information on the conformational characteristics of carcinogen-DNA complexes. Fluorescence quenching techniques are valuable for determining whether the carcinogenic moieties are buried within the hydrophobic regions of biomacromolecules (e.g. intercalation sites, or whether they are accessible to the solvent environment (e.g. external adduct conformations). In order to accurately interpret fluorescence quenching data, the fluorescence lifetimes must be known. Unfortunately, carcinogen-DNA adducts are characterized by low fluorescence yields and a heterogeneity of adducts. This heterogeneity can be characterized only if the fluorescence decay profiles can be measured with reasonable accuracy. The synchrotron light source, because of its high repetition rate (52 MHz), high fluence/pulse, pulse stability, and wavelength tunability, is particularly suitable for measuring fluorescence decay profiles of samples of low quantum yields and low photochemical stability. These techniques were applied to a study of adducts derived from the binding of the highly tumorigenic (+) enantiomer of 7β,8α-diol-9α,10α-epoxybenzo(a)pyrene and its biologically inactive mirror image form, the (-) enantiomer, to DNA. Significant differences in the conformation of the covalent adducts derived from these two enantiomers were found utilizing fluorescence techniques.

Work supported by the Department of Energy.

T-Pos124 **LIGHT PROPAGATION IN HUMAN TISSUES: THE PHYSICAL ORIGIN OF THE INHOMOGENEOUS SCATTERING MECHANISMS.**

W. E. Blumberg, Department of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, N.J 07974

Passing red or near infrared light through the human breast or other accessible parts of the body reveals vague, low-contrast "shadows" cast by tumors of sufficient size and appropriate depth. Diagnostic modalities based on this phenomenon are termed diaphanography. Light scattering and absorption properties of normal breast tissue as well as several types of tumors occurring in the breast have been measured between 700 and 1100 nm. Scattering occurs because the tissue is a medium of randomly inhomogeneous refractive index. The autocorrelation function in k space is made up of two parts: one of short distance scale (about 0.3 nm) and the other of longer scale (about 5-10 nm). The proportion of these contributions varies with tissue type. Values of the correlation length l for the direction of propagation vary from about 700 to 2000 microns. This length increases with increasing wavelength and is longer for normal tissue than for tumor tissue. The propagation has become almost completely diffusive after 2 mm travel in all these samples at all wavelengths. On the other hand, absorption (expected from hemoglobin at the shorter wavelengths and from water at the longer wavelengths) is negligible at these distances. The transmission coefficient of a conservative object of thickness t is given by l/t, and a highly scattering object such as a tumor can cast a shadow without absorbing any light. Thus differential scatter in this wavelength region is much more important in image formation than is absorption, and it is the form of the autocorrelation function which determines all these properties.

T-Pos125 LUMINESCENCE PROBES OF OXYGEN CONCENTRATION.

J.M. Vanderkooi, G. Maniara and D.F. Wilson, Dept. of Biochemistry & Biophysics, School of Medicine, University of Pennsylvania, Philadelphia PA 19104.

An understanding of cellular oxygen delivery has been limited by uncertainties in both the oxygen concentration dependence of cellular metabolism and intracellular oxygen concentration. We describe the use of triplet state probes and simple instrumentation which enable the determination of molecular oxygen concentration in aqueous medium from air saturation (250 uM) to nM concentration. The method is based upon the oxygen dependent quenching of luminescence from long-lived, spin-forbidden excited states of lumiphores. Because lifetime and not intensity is measured, the technique is especially suitable for measuring oxygen in biological materials where absorption and turbidity is high. Probes (metal derivatives of water-soluble porphines and halogenated derivatives of fluorescein) were chosen with these properties: 1. their phosphorescence is sensitive to oxygen, following simple Stern-Volmer kinetics, 2. they are chemically stable, 3. their emission is in the red or near IR where biological tissues absorb little, and 4. they are non-toxic. Oxygen consumption by mitochondria will be used to demonstrate the technique.

Supported by NIH Grant GM-36393.

T-Pos126 **I. CO Stretching Frequency As a Probe of Relaxation in Sperm Whale Carboxymyoglobin.**
Anjum Ansari, Joel Berendzen, David Braunstein, Benjamin R. Cowen, Hans Frauenfelder, Mi Kyung Hong, Icko E.T. Iben, Pál Ormos, Todd B. Sauke, Alfons Schulte, Peter J. Steinbach, Robert D. Young, Departments of Physics and Biophysics, University of Illinois, Urbana, IL 61801.

We have measured the stretching frequency (1900–1980 cm^{-1}) of CO bound to the heme iron of sperm whale myoglobin. FTIR spectra were collected in both a 75% glycerol-water solution and in a solid poly(vinyl) alcohol (PVA) matrix over the temperature range 10 to 300K. PVA immobilizes the protein surface at all temperatures. Four IR lines, denoted A_0 to A_3, are observed.

In glycerol-water, the peak positions at 10K are A_0 at 1967 cm^{-1}, A_1 at 1947 cm^{-1}, A_2 at 1941 cm^{-1} and A_3 at 1930 cm^{-1}. The lines are best fit by Voigtians, *i.e.* Gaussian distributions of Lorentzians. The glycerol-water sample showed essentially no change in the relative population of the substates between 10 and 160K. Between 160 and 200K the populations of A_1 and A_3 increase, with comparable decreases in A_0 and A_2. Over the temperature range 10–300K, the peak position of each line shifts slightly. In the case of the MbCO-PVA sample, the relative intensities of the "A" lines are temperature-independent.

Comparison of the glycerol-water and the PVA data implies that rearrangement of the ligand-heme geometry involves major conformational changes that include motion of the protein exterior. This behavior may indicate a slaved glass transition between the solvent and the protein.

T-Pos127 **III. Infrared Measurements of CO Rebinding to Myoglobin Following Photolysis.**
Anjum Ansari, Joel Berendzen, David Braunstein, Benjamin R. Cowen, Hans Frauenfelder, Mi Kyung Hong, Icko E.T. Iben, J. Bruce Johnson, Pál Ormos, Rahul Pandharipande, Todd B. Sauke, Reinhard Scholl, Alfons Schulte, Peter J. Steinbach, Robert D. Young, Departments of Physics and Biophysics, University of Illinois, Urbana, IL 61801.

Employing FTIR spectroscopy at the stretching frequencies of CO, we have measured the kinetics of CO rebinding to myoglobin following flash-off. Experiments were conducted in the time range 10–10^5s at temperatures below 100K. The four CO lines, which indicate four protein substates (see abstract I), all rebind nonexponentially in time but with different rate distributions. The kinetic parameters are analyzed and compared to the values obtained from equivalent experiments done in the Soret absorption band.

At 40K the nonexponential time dependence persists to at least 10^5s. Multiple flash experiments, where the CO ligands are flashed off repeatedly after partial rebinding, show that there is no pumping from one conformational substate to others. These results contradict the evaluation by Chance *et al.*[1] of their flash-photolysis experiments at 40K. Computer simulation indicates that their data can be explained by our model.

[1]Chance, B., Powers, L., Zhou, Y-H., and Naqui, A., *Bull. APS*, **31**, 3, 1986, Abst. EP1, pp. 386.

T-Pos128 **II. Effects of Pressure on the Heme-carbonyl Substates of Sperm Whale Myoglobin.**
*Anjum Ansari, Joel Berendzen, David Braunstein, Benjamin R. Cowen, Hans Frauenfelder, Mi Kyung Hong, Icko E.T. Iben, Pál Ormos, Todd B. Sauke, Alfons Schulte, Peter J. Steinbach, Robert D. Young**, Departments of Physics and Biophysics, University of Illinois, Urbana, IL 61801.

We have studied the pressure dependence of the infrared spectrum of CO bound to sperm whale myoglobin in the frequency range from 1910 cm^{-1} to 1990 cm^{-1}. The sample was pressurized with helium gas in a cell with sapphire windows. At room temperature the spectrum can be resolved into three lines: A_0 at 1966 cm^{-1}, A_1 at 1945 cm^{-1} and A_3 at 1932 cm^{-1}. Pressure from 1bar to 1.7kbar results in significant population redistribution of the three substates. The pressure dependence of the fractional substate populations indicates that the A_0 substate has the smallest volume, A_1 is larger by about 20 cm^3mol^{-1} and A_3 by about 40 cm^3mol^{-1}. These substates have been interpreted as structurally different orientations of the iron-carbonyl bond[1]. The redistribution is consistent with substates that are rapidly interconverting at temperatures above about 180K (see abstract I).

[1] Makinen,M.W., Houtchens,R.A., and Caughey,W.S., *Proc. Natl. Acad. Sci. USA*, **76**:6042, 1979. *Permanent address:Dept. of Physics,Illinois State Univ., Normal IL

T-Pos129 Photodynamics of Ni-porphyrins: Transient Raman Studies of Model Complexes and Reconstituted Hemoglobin and Myoglobin. E.W. Findsen,[1] B.A. Crawford,[1] J.A. Shelnutt[2] and M.R. Ondrias,[1] [1]Dept. of Chemistry, University of New Mexico, Albuquerque, NM 87131 and [2]Div. 6254 Sandia Natl. Labs, Albuquerque, NM 87185

In proteins, metalloporphyrins perform a wide variety of electron transfer, ligand binding and catalytic functions. These diverse functions reflect the large influence of the protein matrix upon porphyrin reactivity. It is, therefore, instructive to study the response of model metalloporphyrins' reactivity and dynamics to environmental influences in order to help understand the modulation of their reactivity and function in a protein matrix.

We report the transient photodynamics of several nickel porphyrins in noncoordinating solvent systems. In these systems, the 4-coordinate ground state (A_{1g}), undergoes a transition characterized by the promotion of one of the $d_{z}2$ electrons of the nickel to the dx^2-y^2 orbital. The formation of this excited state is observed by the appearance of several new modes in the transient Raman spectra which are the core-size sensitive ($^*\nu_3$, $^*\nu_2$ and $^*\nu_{10}$) and porphyrin Π^* electron density sensitive ($^*\nu_4$) modes of the excited state. The excited state Raman spectrum is dependent on the solvent environment, while the 4-coordinate ground state Raman spectrum is not. Solvent effects are also evident in the intensity ratios of the α and β absorption bands. These results are interpreted in terms of the effect of solvent properties upon the porphyrin electronic orbitals. A related study observed the effects of low temperature upon the photodynamic response of 4- and 6-coordinate nickel porphyrins and Ni-protoporphyrin reconstituted hemoglobin and myoglobin. (Supported by the NIH (GM33330) and Sandia Natl. Labs)

T-Pos130 NANOSECOND LIGAND RECOMBINATION KINETICS OF HUMAN HEMOGLOBIN AND ITS SUBUNITS. John S. Olson, Ronald J. Rohlfs, and Quentin H. Gibson, Department of Biochemistry, Rice University, Houston, TX 77251 and Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14653.

The rebinding of CO, O_2, NO, methyl, ethyl, n-propyl, and n-butyl isocyanide to isolated α and β subunits and intact hemoglobin at pH 7, 20° was examined both during and after a 30-ns dye laser pulse. The resultant absorbance changes were analyzed in terms of a 3-step reaction scheme: $HbX \rightleftharpoons B \rightleftharpoons C \rightleftharpoons Hb+X$. Four major conclusions were obtained. First, the nanosecond recombination time courses for intact, fully liganded hemoglobin can be approximated by the sum of time courses for the isolated α and β chains. Second, our analyses predict that the initial geminate recombination phases for the NO and O_2 complexes of hemoglobin should exhibit half-times equal to ~17 ps and ~440 ps, respectively, which are in excellent agreement with the more direct picosecond measurements of Cornelius et al. (1983, J. Mol. Biol. **163**, 119-128) and Friedman et al. (1985, Science **229**, 187-229). Third, for both subunits the rate and equilibrium constants for the formation of the CO geminate state (i.e., k(Hb→B) and K(Hb⇌B)) are 4-6 times smaller than the corresponding parameters for the O_2 state, which demonstrates that the distal pocket in hemoglobin can discriminate between the gaseous ligands in the absence of iron bond formation. Fourth, the major kinetic differences between the subunits are that the initial geminate recombination rates for all ligands are 2-3 times greater in α chains whereas the intermediate states (i.e., B and C) are both kinetically and thermodynamically more stable in β chains. (Supported by Grants GM 14276 (NIH), GM 35649 (NIH), PCM 830845 (NSF), and C-612 (Robert A. Welch Foundation).

T-Pos131 **STRUCTURE OF THE GEMINATE STATES OF PHOTOLYZED CARBOXY-MYOGLOBIN AT 4 AND 40K.** L. Powers, AT&T Bell Labs, Murray Hill, NJ, B. Chance, A. Naqui, Y-H. Zhou, and M. Chance, Dept. Biochem. Biophys., U. Penn. Med. Sch., Phila.,PA.

The local structure of the iron heme and CO ligand of the geminate state at 4K [Biochem. *22*, 3820 (1983)], the slowly recombining geminate state at 40K produced by optical pumping [Biophys. J. *49*, 537a (1986)], as well as deoxy- and carboxymyoglobin at both temperatures were compared using x-ray absorption spectroscopy. Although the structure of these geminate states differ significantly from the deoxy state, their iron heme structures are identical having an average Fe-Np (pyrrole N) distance of 2.03±0.01Å and an Fe-Ne (proximal N) distance of 2.20±0.02Å. The distinguishing feature is the displacement of the CO from the iron. In the 4K geminate state, the C of CO is 1.97±0.02Å from the iron making an Fe···CO angle of ~115° [Biochem. *23*, 5519 (1984)], while it is ~0.7Å further away in the 40K geminate state with the atoms of CO at 2.58±0.04Å and 3.42±0.06Å. The fact that the CO can be observed in the 40K geminate state in these studies indicates that the CO position is highly localized. Using computer graphics modeling and the 1.5Å resolution crystallographic data for carboxymyoglobin at 260K [Kuriyan et al., J. Biol. Chem. in press], a crevice has been identified on the protein side of the pocket which can comfortably house the atoms of CO at these distances and restricts the CO rotation to ±10°. These results suggest that there are discrete way-stations on the ligand route to the iron heme and as such represent barriers to recombination along the ligand trajectory. Resonance Raman studies [Friedman et al. this meeting] support these conclusions. Supported in part by NIH Grant HL18707.

T-Pos132 **MYOGLOBIN RECOMBINATION AT LOW TEMPERATURES: TWO PHASES REVEALED BY FTIR SPECTROSCOPY.** M. Chance, B. Campbell, and J. Friedman, AT&T Bell Laboratories, Murray Hill, N.J., and R. Hoover, Howard University, Wash., D.C.

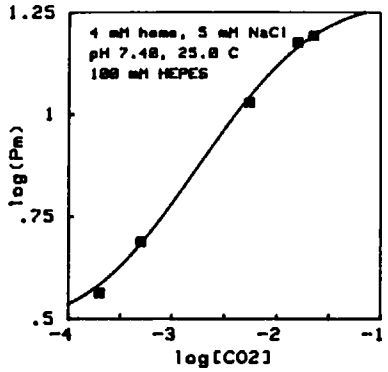
The low-temperature recombination of CO with myoglobin was studied using Fourier transform infrared spectroscopy. The bound state of MbCO has two distinct conformers observed at 1925.5 and $1945cm^{-1}$ with an intensity ratio of 1 to 8. The recombination of these bands after complete photolysis at 10K followed by a temperature jump shows distinct kinetics for the two bands. The $1945cm^{-1}$ band apparently follows the non-exponential kinetics originally described by Frauenfelder et. al. (Austin et. al., Biochemistry, 1975, v.14, p.5355-5373.) But, the $1925.5cm^{-1}$ band does not appear appreciably below 70K and appears with simple exponential kinetics at 80K and above. In fact, at 70K the $1945cm^{-1}$ band is fully recovered after one hour, while no detectable amount of the $1925.5cm^{-1}$ band has yet appeared. The results of this study indicate that the photoproduct population may not have a smooth distribution of activation energies as proposed by Austin et. al. The two distinct bands react at significantly different temperatures implying that the bands have either different activation energies or different rate prefactors. We present kinetic recombination data for both the slow ($1925.5cm^{-1}$) and the fast ($1945cm^{-1}$) phases as a function of temperature.

T-Pos133 TIME-RESOLVED AND CW RAMAN STUDIES OF CONFORMATIONAL HETEROGENEITY AND RELAXATION IN PHOTODISSOCIATED LIGANDED MYOGLOBINS AT CRYOGENIC TEMPERATURES. B. F. Campbell and J. M. Friedman, AT&T Bell Laboratories, Murray Hill, New Jersey. Time resolved and cw resonance Raman studies comparing deoxy myoglobin and photodissociated liganded myoglobins over a wide range of temperatures (1.6-230K) have been used to address several fundamental issues. In addition to deoxy and carboxy sperm whale myoglobin, other myoglobins (elephant and tuna) and ligands (N-butyl isocyanide) were used to expose the sensitivity of specific heme Raman bands to distal and peripheral influences. On the basis of such studies, it is concluded that the shifted core marker band (ν_2) in the deoxy photoproduct is not due to a non-fully out of heme plane iron but is derived instead from ligand induced changes in the distal heme pocket. A comparison of the cw and time resolved (10ns) spectra at 160K indicates that the cryogenically trapped intermediates of photodissociated COMb may not be relevant in the higher temperature reaction. A potentially significant influence of the photodissociated ligand upon both structural relaxation and heme structure is indicated from a comparison of the temperature dependent changes in the spectra of the photoproduct derived from COMb and N-butyl isocyanide Mb. Studies of hysteresis and Raman hole burning effects are in progress. These studies are being used to examine the dynamics associated with conformational heterogeneity especially in the regime of the "glass transition" at $\sim$180-200K.

T-Pos134 RESONANCE RAMAN SPECTRA OF CHEMICALLY MODIFIED HEMOGLOBINS, Frank J. Bruzzese and James A. Dix, Department of Chemistry, State University of New York, Binghamton, New York, 13901 and L. C. Cerny, Masonic Medical Research Laboratory and Utica College of Syracuse University, Utica, New York 13501.

Chemically modified hemoglobins (Hb) have been studied using Resonance Raman (RR) spectroscopy in a search for the distribution of free energy of cooperativity upon oxygen binding. Hb was modified by substituting glyoxalic acid (gly) or 1,2-cyclohexadione (cyclo) for naturally occurring 2,3-diphosphoglycerate. The modified Hb was subsequently crosslinked with a hydroxyethyl starch-polymeric trialdehyde complex (HES). All modified Hb showed biological activity with modified oxygen uptake cooperativity. RR spectra obtained with Sôret excitation indicated that the Fe spin state marker, occuring at 1586 cm^{-1} in stripped Hb, was shifted to lower frequencies by the chemical modifications: HES-Hb-cyclo, 1577 cm^{-1}; Hb-cyclo, 1578 cm^{-1}; Hb-gly, 1580 cm^{-1}; HES-Hb-gly, 1584 cm^{-1}. These results imply that the iron atom of the modified Hb is further removed from the porphyrin plane than the iron of unmodified Hb. Oxygen saturation curves of modified Hb show sigmoidal character, although less pronounced than that of unmodified Hb. The cooperativity in oxygen uptake seen with HES-Hb-cyclo was larger than that seen with HES-Hb-gly. Since HES-Hb-cyclo exhibited the largest shift in RR spin state marker, our results suggest a correlation between the Fe-porphyrin distance and cooperativity in oxygen binding. Supported in part by the American Heart Association, Broome County, New York State Chapter.

T-Pos135 **CARBON DIOXIDE AND OXYGEN LINKAGE IN HUMAN HEMOGLOBIN TETRAMERS,** **Michael L. Doyle, Enrico Di Cera, Charles H. Robert and Stanley J. Gill, Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309.**



Oxygen binding curves were measured on samples of highly concentrated hemoglobin (4 mM) and over a range of fixed CO_2 partial pressures (4 to 540 torr). Such high concentrations allow estimation of the heterotropic effects of CO_2 on O_2 binding to the essentially pure tetrameric state of hemoglobin. High precision binding curve measurements were made using a thin-layer apparatus which measures changes in oxygen fractional saturation upon logarithmic changes in the O_2 partial pressure. Gas mixtures of CO_2-N_2 and CO_2-O_2 were prepared gravimetrically. The overall shift in the O_2 binding curve is shown to the left as the change in the p_m with respect to the activity of CO_2. The theoretical curve was generated according to an allosteric model employed in a simultaneous fit of all the binding curves. The model is based on a wide range of structural and functional results. (Supported by NIH grant HL 22325.)

T-Pos136 **INFRARED STUDIES OF DES-HIS AND IODOACETAMIDE DERIVATIVES OF HUMAN CARBOXYHEMOGLOBIN.** K.A. Powell, F.G. Fiamingo, and J.O. Alben. Dept. of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210.

FT-IR spectroscopy was used to study the sulfhydryl absorption bands due to α-104, β-112, and β-93 cysteine residues in the des-His and iodoacetamide (IAA) derivatives of HbCO, and normal HbA(CO) (all provided by Dr. Max F. Perutz). We observed a change in the α-104 center frequency in both the des-His and IAA derivatives from HbA(CO). A change in absorptivity, but not frequency, of the β-93 sulfhydryl was observed in the des-His derivative relative to HbA(CO). These results imply that there is no hydrogen bonding associated with the β-93 cysteine in the F - H pocket, but that the position of the sulfhydryl relative to the benzene ring of the β-145 tyrosine is altered in the des-His derivative. In both derivatives, effects of a small change in protein conformation, remote from the heme, were observed at the coordinated ligand. Changes in the CO stretching frequency and band narrowing of both the des-His and IAA derivatives of HbCO were observed relative to HbA(CO), where for the CO center frequencies: HbA > Hbdes-His > HbIAA, and for the bandwidths: HbA > HbIAA > Hbdes-His. This suggests that the β-chain heme-CO is affected more than the α-chain in these modified β-chain hemoglobin derivatives. These observations show that both the cysteine sulfhydryl and the CO stretching bands are sensitive molecular probes of changes in protein tertiary and quarternary structure. (Supported in part by NIH and AHA.)

T-Pos137 **LOCAL MOLECULAR TEMPERATURE IN CARBOXY-HEMOGLOBIN PHOTOLYZED AT LOW TEMPERATURE.** V.M. Molleran, F.G. Fiamingo, A.A. Croteau, and J.O. Alben. Dept. of Physiological Chemistry, The Ohio State University, Columbus, Ohio, 43210.

Photodissocation of carboxy-hemoglobin shifts the infrared CO absorption band from 1951 cm^{-1} (Fe-CO, A_1 state) to 2130 and 2117 cm^{-1} (B_1 and B_2 states of *CO, respectively). The ratio of B-state band intensities is dependent upon both sample temperature and photolyzing light intensity. The B_2 band disappears above 25 K, but represents the dominant B-state at 10 K. The B_2/B_1 intensity ratio varied from 1.5 at 10 K to 0 above 25 K. When the sample holder temperature was 10 K and the photolyzing light intensity was increased, the B_2/B_1 intensity ratio decreased, suggesting an increase in local temperature near the photodissociated *CO. Infrared vibrational changes were also observed between 2550 and 2600 cm^{-1}, indicating structural changes in the sulfhydryl groups of both the α-104 and β-93 cysteine residues. These protein motions were observed at sample holder temperatures below 30 K, and under conditions of low light intensity, including light intensity that was sufficiently low that only half of the total HbCO was photodissociated in an optically thin sample (A_{568}=1.5). These results suggest that photolyzing light can cause significant local heating of the protein, and indicate some of the pathways through which this energy can be dissipated. These studies emphasize the need for low photolyzing light levels and efficient sample cooling techniques at low temperatures. (Supported in part by NIH and AHA.)

T-Pos138 MULTIPLE-PROBE ANALYSIS OF THE RATE OF CONFORMATIONAL CHANGE IN HEMOGLOBIN A

Anthony J. Martino, Alison J. Graf, and Frank A. Ferrone, Department of Physics and Atmospheric Science, Drexel University, Philadelphia, PA 19104.

We have modified our existing modulated excitation apparatus (Biophys. J., 1985, 48, 269-282) to permit the rate of conformational change between R and T quaternary structures with three ligands bound to be monitored with multiple optical probes. In the most novel application, we have monitored the modulated fluorescence quenching of the DPG-analog, 8-hydroxy-1,3,6 pyrene trisulfonic acid (PTS). Modulated photolysis is provided by a dye laser, pumped by an argon ion laser, which also provides the source for the DC fluorescence excitation. In this configuration, two computer controlled monochromators permit both modulated absorbance and fluorescence to be measured without change in detector phase shift. This allows the ligand-rebinding signal to be phase nulled, in turn permitting us to use the phase of the fluorescence signal as a direct measure of the rate of conformational change. The use of reflecting objectives and quartz optics also allows us to utilize the near-UV absorption signals arising from the $\alpha_1\beta_2$ interface which have been previously assigned as structural markers. The results of measurements using these probes can be compared with the rates measured using heme absorption measured in the Soret, with the possibility of determining whether the conformational change rates in the allosteric core are the same as those in the interchain interfaces.

work supported by NIH grant AM30239.

T-Pos139 INTRINSIC FLUORESCENCE OF CARP HEMOGLOBIN: A STUDY OF THE R --> T TRANSITION.

Rhoda Elison Hirsch* and Robert W. Noble+, Albert Einstein College of Medicine, Bronx, N.Y. 10461* and SUNY at Buffalo, VA Medical Center, Buffalo, N.Y. 14215+.

It has been demonstrated that the intrinsic fluorescence of Hemoglobin A (Hb A) is a function of quaternary structural change (a,b). The hemoglobin from the carp, Cyprinus carpio, is a good model with which to explore this conclusion, since this Hb can be switched to its T-state, even when saturated with ligand, by lowering the pH<6 in the presence of IHP(c). Using front-face fluorometry, we find that: (1)Carp Hb exhibits an intrinsic fluorescence signal of three-fold greater intensity with an emission maximum at shorter wavelengths (Δ 4nm) than human Hb A. (2)In the presence of IHP, the fluorescence intensities of met and CO-carp Hb start to increase below pH 6.8. The maximal fluorescence change occurs at pH 5.9 with an increase of 8.6% with CO-carp Hb. The intensity change of the met form is less than the CO form. (3)In the absence of IHP, a small but consistent decrease in the fluorescence intensity as a function of pH is observed. These observations respectively suggest: (1)carp Hb has more tyrosines and tryptophans located in exposed areas of the molecule than Hb A; (2)the decrease in intensity as a function of pH may result from the formation of a charge-transfer complex between a Trp and His(d): the amino acid sequence of carp Hb locates α 45 His next to α 46 Trp, suggesting that α46 Trp may be a significant emitter of fluorescence in carp Hb; and (3)the fluorescence changes are a function of quaternary structural change. (a)Hirsch & Nagel(1981)JBC 256:1080;(b)Itoh et al.(1981)BBRC 100:1259;(c)Tan & Noble (1973)JBC 248:7412;(d)Irace et al.(1981)Biochem. 20:792. Supported by American Heart Association NYC Affiliate & NIH HL21016 (REH); and NIH HL12524 & funds from the Veterans Administration (RWN).

T-Pos140 FLUORESCENCE MEASUREMENTS OF THE BINDING OF NADPH BY HUMAN AND SHEEP HEMOGLOBINS.

Satie Ogo, Aldo Focesi, Jr., Robert Cashon, Celia Bonaventura and Joseph Bonaventura, Marine Biomedical Center, Duke University Marine Laboratory, Beaufort, NC 28516.

We have previously shown that the nicotinamide adenine dinucleotides act as heterotropic allosteric effectors of hemoglobin function. We have extended these studies and have made use of the differential fluorescence of free and bound NADPH to further characterize the interactions of this effector with hemoglobin of varied species in various states of oxidation and ligation. Our results are consistent with the analysis of oxygen binding curves in that NADPH is bound with high affinity to deoxy Hb A. The fluorescence technique allowed us to demonstrate significant but lower affinity binding by both oxy and oxidized Hb A. The fluorescence enhancement brought about by addition of inositol hexaphosphate provides further evidence that NADPH binds at the anion binding site formed by the beta chain charge cluster. In sheep hemoglobin the affinity of the beta chain charge cluster for organic phosphates is lessened relative to small anion effects, with the consequence that the low affinity for oxygen brought about by inositol hexaphosphate can be largely abolished by chloride concentrations as low as 0.1 M. Fluorescence studies of NADPH binding to sheep hemoglobin reflect this decreased affinity of the binding site for organic phosphates, although parallel oxygen binding experiments show that NADPH does act as an allosteric effector. We have suggested that the preferential binding of the reduced forms of the nicotinamide dinucleotides to hemoglobin may affect cofactor-dependent metabolic processes in red blood cells. This physiological consequence may perhaps be looked for with more success in sheep red cells than in human red cells due to their significantly lower concentrations of 2,3-diphosphoglycerate.

T-Pos141 EFFECTS OF MONOMER-TETRAMER SELF-ASSEMBLY ON LIGAND BINDING KINETICS OF HEMOGLOBIN β SUBUNITS. John S. Philo, Jeffrey W. Lary, and Todd M. Schuster, Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268

In 1978 Valdes & Ackers showed that assembly of human Hb β subunits into tetramers significantly raises their affinity for O_2 and CO. We have investigated the kinetic consequences of this "quaternary enhancement" phenomenon by performing laser photolysis kinetic experiments on βO_2 and βCO solutions over a wide range of protein concentration, in the "Ackers' buffer" (.1 M Tris, .1 M NaCl, 1 mM EDTA, pH 7.4, 21.5°C). For O_2 binding we find that self-assembly has no effect on the association rate constant, k', which implies that the ≃4 fold higher affinity of β_4 is due entirely to a decrease in the O_2 dissociation rate.

In contrast, for CO binding at all protein concentrations we observe 2 binding phases differing in association rate by about 2.5. The proportion of the faster phase increases at higher protein concentrations. However, the concentration dependence of this kinetic data, and of changes in the absorption spectrum, does not fit a $4\beta \rightleftarrows \beta_4$ or $4\beta \rightleftarrows 2\beta_2 \rightleftarrows \beta_4$ association model if each assembly state has a single binding rate. Rather, we believe the data indicate that β chains have 2 conformations (corresponding to the 2 different binding rates), and that self-assembly merely shifts the equilibrium between the 2 conformers toward the faster binding form. That is, both conformers occur in tetramers, and both may occur in monomers. We believe this is the first time that significant functional heterogeneity has been observed within a single type of Hb subunit. The possibility that similar functional heterogeneity occurs with the β subunits of Hb A tetramers must now be considered. (Supported by NIH HL-24644)

T-Pos142 ENZYMATIC FUNCTIONS OF MYOGLOBIN. Franklin Mauri, Giulia Ferruzzi, Celia Bonaventura, and Joseph Bonaventura. Marine Biomedical Center, Duke University Marine Laboratory, Beaufort, NC 28516.

Myoglobin (Mb), well known for its role in reversible oxygen binding, was studied with regard to its ability to act as a catalyst in the hydroxylation of aromatic substances. J.J. Mieyal and coworkers have used a reconstituted live microsomal cytochrome P450 system to demonstrate that methemoglobin A can efficiently substitute for the native P450 monooxygenase. We have focused on the ability of a highly purified sperm whale met Mb to carry out the hydroxylation reaction when it is the sole protein present in the reaction mixture. We find that this Mb preparation is able to catalyze a very efficient hydroxylation reaction. The reaction mixture consists of NAD(P)H, oxygen, aniline and the met Mb. Para-aminophenol is the sole product of the reaction. Para-aminophenol can be generated at a rate of 2 n moles/min, with a turnover number of 0.5 n moles produced/min - n mole of heme. The rate of product formation is dependent on pH, temperature and the concentration of each reactant. Product inhibition can also occur. The high efficiency of the reaction is of particular interest in that a side reaction can occur in the absence of aniline in which NAD(P)H oxidation is coupled to reduction of met Mb. This coupled oxidation/reduction does not occur at a detectable rate in the presence of superoxide dismutase or in the absence of oxygen. When aniline is present, the hydroxylation reaction is highly preferred. Although it has not yet been shown that hydroxylation reactions catalyzed by Mb have physiological significance, our results suggest that the simple reaction system described above can be used to clarify the factors which control electron flow in an enzymatic reaction having multiple possible pathways.

T-Pos143 INTERMEDIATE STATES IN THE COORDINATION OF METHEMOGLOBIN. P. Kuppusamy, A. Levy and J.M. Rifkind. National Institutes of Health, National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224.

Previous studies demonstrate that bishistidine complexes are reversibly formed above 200 K for both deoxyhemoglobin and methemoglobin (A. Levy & J.M. Rifkind, Biochemistry 24, 6050-6054, 1985). By following the time course for the formation of these methemoglobin complexes as a function of pH it has been possible to identify an intermediate state thought to involve interactions of both the distal histidine and the exogenous ligand. As a function of increasing pH, this intermediate is converted from a complex with ligand field characteristics of a nitrogenous ligand to that of an oxygen ligand. These results suggest simultaneous coordination of the exogenous ligand and the distal histidine. At low pH the interaction with the distal histidine is greater than the weak water ligand while at high pH the strong hydroxide ligand dominates the ligand interactions. The possible role for these intermediates in controlling ligand binding reactions of hemoglobin will be discussed.

T-Pos144 SANS STUDIES OF INTERACTING Hb IN INTACT ERYTHROCYTES. R. Nossal and S. Krueger, Physical Sciences Lab., DCRT, NIH, Bethesda, Maryland 20892.

Small angle neutron scattering (SANS) was used to investigate interaction forces between hemoglobin (Hb) molecules contained within red blood cells. Studies on both intact cells and membrane preparations enabled us to identify the scattering separately attributable to cell membranes and intracellular Hb. A series of D_2O-H_2O contrast variation measurements were made in order to establish conditions for which the cell membrane is invisible (ca. 15% D_2O). Measurements then were performed to examine changes in intermolecular Hb interactions occurring when the cells are contracted or swollen by varying the ionic strength of the buffer in which the cells are suspended. The scattering cross-sections were fitted to structure factors computed by a mean spherical approximation (R. Nossal, C. Glinka and S-H. Chen. Biopolymers 25, 1157 (1986)), and molecular parameters thereby extracted. Oxygenation studies on normal cells were performed, and results contrasted with those of similar studies of erythrocytes obtained from sickle cell disease patients.

T-Pos145 POLYMERS OF MIXED FUNCTIONAL HEMOGLOBIN MUTANTS

William A. McDade
The University of Chicago, Department of Molecular Genetics and Cell Biology

Hemoglobin S (Beta 6 glu- val; HbS) is a variant protein produced by individuals with sickle cell anemia. In stirred, purified solutions of HbS, crystallization occurs through a series of polymeric intermediates which include fibers identical to those found within sickled erythrocytes. Hemoglobin O-arab (Beta 121 glu- lys; HbO-arab) is another variant protein that may occur with HbS in heterozygous individuals. The route of crystallization in mixtures of these two types of hemoglobin appears to differ in several respects from that for HbS alone. Angle layer aggregates, similar to the type described for actin filaments, were found to occur by the interaction of fibers composed of mixtures of the unhybridized HbS and HbO-arab tetramers. The saturation concentration for the development of polymers was significantly higher for the mixture than for purified HbS alone. Crystallization occurred over several days in contrast to the eight hours it takes to crystallize pure HbS. However, macrofibers, the low pH pathway intermediates found in pure HbS solutions of pH less than 6.6, were also produced in mixtures of HbS and HbO-arab under similar conditions. The composition of the polymers was examined to determine the percentage incorporation from each mutant type. This work is supported by HL 30121, HL 226654 and PHS 5T32 GM07281.

T-Pos146 DIELECTRIC CONSTANT AND VISCOSITY OF HEMOGLOBIN S IN GEL STATE

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Philadelphia, PA. 19104-6392

The gelation of hemoglobin S entails the dramatic increase in viscosity because of the interlocking of individual molecules. In spite of this, the rotational freedom of Hb S is not affected significantly by gelation. The rotation of Hb S in gel was first studied by spin label technique and it was found that electron spin resonance signals of labeled Hb S was not affected by gel formation. Another method to investigate the rotational characteristics is dielectric relaxation technique. When an electrical field is applied and if the torque is sufficient to overcome the viscous drag of the solvent, the polarized state is created. The rate of polarization depends on the viscosity of the medium. If polar molecules are locked in the lattice of semi-crystalline gels, the rate of polarization will be very slow or Hb S molecules may not even be able to respond to applied fields. Thus the lattice of Hb S gel is rigid, the dielectric constant of Hb S will decrease considerably upon gelation. Our experiments, however, did not reveal this predicted change in the dielectric constant. This result indicates strongly that Hb S molecules are highly rotational even in gel state in spite of hydrophobic contacts with nearest neighbors. Using Eyring's theory, we calculated the activation enthaly of the rotation of Hb S in gel. We found a value of 6 Kcal/mole. This value is only twice as that in solution. This observation suggests that Hb S gels are mechanically very soft and that individual Hb S molecules are highly rotational. This research is supported by ONR N00014-85-K-0517.

T-Pos147 A LINEAR DICHROISM MICROSCOPY STUDY OF THE PERCENTAGE OF ALIGNED HEMOGLOBIN DEPENDENCE ON SPEED OF DEOXYGENATION AND PATIENT HETEROGENEITY, MAESTRE, M.F., (1), MICKOLS, WM. (2), CORBETT, J.D. (2), EMBURY, S.H. (3), LAWRENCE BERKELEY LABORATORY, DIVISION OF BIOLOGY AND MEDICINE (1), DEPT. OF CHEM. UNIV. OF CAL. BERKELEY (2), SAN FRANCISCO GENERAL HOSPITAL (UCSF) (3)

Linear dichroism imaging of sickle red cells shows the amount and distribution of aligned hemoglobin polymers within intact sickle red blood cells (PNAS, 82(1985), p 6527). A large population of cells were studied (1086) and distinct morphological classes emerged based on the distribution of aligned hemoglobin in the cell. Different numbers of domains of aligned polymer are clearly seen as well as some new special classes not seen before. Two different rates of deoxygenation were employed. From slow (a linear decrease from room oxygen tension to 0% oxygen over one hour) to fast deoxygenation (direct injection of oxygenated cells into deoxygenated buffer) only a small difference was seen in the average percent aligned hemoglobin per cell; 10.7% for slow and 8.9% for fast. If the class of cells designated zero domain cells are eliminated from the cell population the percent aligned hemoglobin values become identical for the different speeds of deoxygenation as do the percentages of cells in each class. The percentage of these zero domain cells was 15 percent higher for the faster rate of deoxygenation. Of the three patients studied one showed no change in their percentage of aligned hemoglobin with speed of deoxygenation while another showed a large difference. Other more subtle patient heterogeneities were also seen. Since there is a difference of about 1000 in the rates of deoxygenation, and we see such a small difference in the amount of aligned hemoglobin, we conclude that we are looking at a thermodynamically controlled process under these conditions. We are investigating whether preformed polymerized Hb forms extra nucleation sites.

T-Pos148 KINETICS OF THE GROWTH OF DOMAINS OF SICKLE HEMOGLOBIN Soumen Basak, Frank A. Ferrone, Jin Tong Wang, Anthony J. Martino and Huan Xiang Zhou, Department of Physics & Atmospheric Science, Drexel University, Philadelphia, PA 19104

We have monitored the growth of domains of sickle hemoglobin polymers using temporally and spatially resolved light scattering and birefringence, measured pseudo-simultaneously on the same 50 μm square area. Polymerization was induced and indefinitely maintained by photolysis of HbCO using an argon ion laser; intensity scattering and birefringence (measured as intensity transmitted through crossed polarizers) were measured using an SIT vidicon interfaced to a computer. Polymer concentration, as inferred by light scattering, grew relatively uniformly in all directions, with a radial profile well described by a gaussian, which increased in amplitude and width. A small anisotropy in the form of a 2-fold symmetry was initially present in the pattern, and in all cases, diminished with time. The scattering intensity also exhibited notable overshoots. Birefringence developed at the same time as the overshoots appeared, with the same rate of growth as the diminution of scattering. The radial density of *aligned* polymers, as inferred by birefringence, was also gaussian, and it too grew in amplitude and width, beginning with a radius much smaller than the scattering radius measured at that time. The birefringence pattern was notably anisotropic, initially appearing as two lobes. If allowed to incubate, a third and finally a fourth lobe appeared until a characteristic "Maltese cross" pattern formed. We interpret our results as evidence for a separate alignment transition in sickle hemoglobin polymer formation.

Work supported by NIH grant HL21802.

T-Pos149 CALCULATIONS OF THE ANGULAR DEPENDENCE OF LIGHT SCATTERING FROM A DILUTE SOLUTION OF SICKLE HEMOGLOBIN POLYMERS. Marilyn F. Bishop, Department of Physics, Virginia Commonwealth University, Richmond, Virginia 23284.

We have calculated the angular dependence of the intensity and polarization of linearly polarized light scattered from a dilute solution of long sickle hemoglobin polymers, where we assume the polymers to be long rigid cylindrical rods. The angular dependence that we calculate by a method developed by L. D. Cohen, R. D. Haracz, A. Cohen, and C. Acquista [Applied Optics, 21, 742 (1983)] is more complicated than one would obtain from the Rayleigh-Debye method, as is commonly used. [See, for example, B. J. Berne, J. Mol. Biol. 89, 755 (1974) or C. Tanford, Physical Chemistry of Macromolecules (Wiley, New York, 1961), p. 297.] This is because, for filaments long compared with the wavelength of light, the polarizability of a filament is anisotropic due to its geometry, with the ratio of the perpendicular to parallel components approximately equal to $2/(m^2+1)$, where $m \cong 1.14$ (for a hemoglobin solution of 40 g/dl) is the ratio of the index of refraction of the filament to that of the solution. For incident light polarized parallel to that of Rayleigh scattering, for near forward or backward scattering, the intensity of scattered light for incident polarization along the cylinder axes is only 75% of that when the incident electric field is perpendicular to the cylinder axes, while for Rayleigh scattering these two would be the same. The intensity of scattered light polarized in a direction that Rayleigh scattering is absent is about 100 times smaller than that polarized the same as Rayleigh scattering.

This work was supported by N.I.H. Grant Number HL38614.

T-Pos150 COMPLETE CYANIDE ION KINETIC STUDIES AND PROTON NMR ASSIGNMENTS OF GLYCERA DIBRANCHIATA HEMOGLOBIN FRACTION II.
Jan Mintorovitch and james D. Satterlee. University of New Mexico,Department of Chemistry. Albuquerque, New Mexico 87131.

The monomer hemoglobin fraction from the marine annelid Glycera Dibranchiata consists of at least 3 major fractions, one of which has been shown to possess a three-dimensional structure similar to vertebrate hemoglobins. However, unlike vertebrate hemoglobins this protein possesses the exceptional primary sequence substitution in which the distal histidine (E7) is replaced by leucine.

The results which will be presented here are from our complete studies of the kinetics and thermodynamics of CN^- (as KCN) binding to the oxidized (met) form of fraction II in comparison to Sperm - whale met Myoglobin. Our values for Myoglobin were in accordance with the literature values. For the Glycera Hemoglobin, however, the results were not comparable to previously published data. Competing eqilibria and a possible protonation of the ligand could account for this.

Proton NMR assignments in the met-cyano (low spin) form have partiaaly been made by using specifically deuterium labelled protoporphyrin IX,(in collaboration with Prof. Kevin Smith, University of California, Davis) and by ^1H -^1H nuclear Overhauser effects.Assignments which have been made are of the heme resonances and resonances of amino acids in proximity to the heme.

T-Pos151 PROTON NMR STUDIES OF CYTOCHROME C ISO-1 MONOMER AND DIMER FROM SACCHAROMYCES CEREVISIAE. Susan J. Moench and James D. Satterlee, Department of Chemistry, University of New Mexico, Albuquerque, NM 87131.

Saccharomyces cerevisiae synthesizes two molecularly distinct cytochromes c which have been termed iso-1 and iso-2. The presence of a single free sulfhydryl group near the carboxy- terminal end of iso-1 cytochrome c enables the protein to form disulfide-linked dimers. The monomer and dimer forms of iso-1 cytochrome c can be separated by gel filtration or ion exchange chromatography. Proton NMR studies have revealed that many of the resonances of the heme of the dimer are shifted relative to the corresponding heme resonances of the monomer indicating that dimerization perturbs the heme environment of the protein. It is therefore possible to spectroscopically follow the monomer to dimer conversion under conditions which favor intermolecular disulfide bond formation. The proton NMR spectrum of the iso-1 monomer specifically blocked at the free sulfhydryl group with iodoacetamide has also been characterized. Proton hyperfine resonance assignments have been made for the iso-1 monomer and dimer using the technique of nuclear Overhauser enhancement.

T-Pos152 PROTON NMR ASSIGNMENTS USING THE ONE DIMENSIONAL NUCLEAR OVERHAUSER EFFECT IN THE PARAMAGNETIC HEME PROTEINS CYTOCHROME c PEROXIDASE, CYTOCHROME c AND THEIR MOLECULAR DOCKING COMPLEX. James D. Satterlee*, Susan J. Moench* and James E. Erman, Departments of Chemistry, *University of New Mexico, Albuquerque, NM 87131 and Northern Illinois University, DeKalb, IL 60115.

The proton nuclear Overhauser effect is a valuable method for making assignments in cytochrome c peroxidase (ccp, native and cyanide ligated ferric form) and horse ferri-cytochrome c. Complete proton hyperfine resonance assignments have been made for these two proteins and thereby allows the effect of intermolecular complex formation to be interpreted. This type of complex formation is postulated to represent the docking-electron transfer complex formed by these two types of proteins in yeast mitochondria. Comparisions with other protein-protein complexes will be made. The results show that the ccp-cytochrome c complex is unique, in comparision to complexes between cytochrome c and smaller proteins.

T-Pos153 AN APO-MYOGLOBIN-HEME D_1 COMPLEX AS A MODEL FOR THE CATALYTIC CENTER OF PSEUDOMONAS CYTOCHROME OXIDASE Steup, Margaret B. and Muhoberac, Barry B., Department of Chemistry, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.

A heme-protein complex is formed by the replacement of the iron-porphyrin of myoglobin with the iron-chlorin (heme d_1) extracted from Pseudomonas cytochrome oxidase by acid-acetone. The complex has been characterized by optical absorption spectroscopy and assayed for oxidase activity using a variety of different electron-donor systems. The oxidized and dithionite-reduced complex as well as ligand-bound forms exhibit characteristic optical absorption spectra which are similar to components of those of the intact oxidase. A comparison of spectra from the model complex (M.C.) with those of the intact oxidase (P.C.O.) is as follows: CN^--bound oxidized; M.C. 410 (shoulder), 445, 630 nm., P.C.O. 412, 450 (sh.), 632 nm.; dithionite-reduced, M.C. 416 (sh.), 457, 630 nm., P.C.O. 416, 460 (sh.), 626 nm.; CN^--bound dithionite-reduced, M.C. 425 (sh.), 441, 470 (sh.), 628 nm., P.C.O. 418, 440 (sh.), 475 (sh.), 626 nm.; imidazole-bound dithionite-reduced, M.C. 415 (sh.), 454, 628 nm., P.C.O. 416, 460 (sh.), 626 nm. The relative absorption band intensities of the complex are consistent with those expected of a model representing the heme d_1 portion of the oxidase. Assays for oxidase activity using cytochrome c-551 show that the apo-myoglobin-heme d_1 complex has approximately 10% of the activity of Pseudomonas cytochrome oxidase. In assays incorporating different electron donors such as ascorbic acid with PMS, the intact oxidase displays much lower activity while the new complex exhibits up to 60-90% of this reduced activity. The similarity of spectral characteristics together with the minimal oxidase activity emphasizes the importance of donor-acceptor compatibility in the electron-transport system versus the identity of the heme.

T-Pos154 EXAFS RESULTS SUGGEST THAT METHYLMERCURY TRAPS CYTOCHROME C OXIDASE IN THE PULSED FORM.

D. Bickar, L. Powers and B. Chance. Smith College, Bell Laboratories and the Univ. of Pennsylvania.

Methylmercury is an inhibitor of cytochrome c oxidase under steady-state turnover conditions (A. Mann & H. Auer (1980) J. Biol. Chem. 255, 454-458) but an activator of cytochrome c oxidase during its initial substrate utilization (D. Bickar, J. Bonaventura, H. Auer and M. T. Wilson (1984) Biochem. 23 680-684). Bickar et al. suggested that this unusual effect occurred because the methylmercury was able to trap the cytochrome c oxidase in a state resembling the "pulsed" form a form of the enzyme usually prepared by reduction and reoxidation. They also proposed that if this was the case, the methylmercury was probably binding near cytochrome a_3, and should be detectable by copper and/or iron EXAFS of cytochrome oxidase. Our examination of the X-ray absorbance of cytochrome oxidase shows that the methylmercury-treated enzyme, in a agreement with the prediction of Bickar et al., is in a pulsed-like conformation, with the methylmercury bound near the copper and iron atoms of cytochrome a_3. These results support the conclusion that different forms of cytochrome oxidase, with different initial kinetic behaviors are the result of the liganding of the iron and copper of cytochrome a_3 (A. Naqui, C. Kumar, Y. Ching, L. Powers and B. Chance (1984) Biochem. 23, 6222-6227).

T-Pos155 STRUCTURE AND REDOX CHEMISTRY OF OXOPORPHYRINS. K.M. Barkigia, C.K. Chang, E. Fujita, L.K. Hanson, A.M. Stolzenberg, and J. Fajer. Department of Applied Science, Brookhaven National Laboratory, Upton, New York 11973.

The recent proposal by Chang[1] that a dioxoisobacteriochlorin comprises the green prosthetic group of dissimilatory nitrite reductases has prompted investigations[2,3] of this poorly characterized family of porphyrin derivatives substituted with keto functions at the peripheral β positions.

Single crystal structural determinations of free base and Cu(II) dioxoisobacteriochlorins, and of a free base dioxobacteriochlorin offer the first structural parameters for dioxoporphyrins. Bond distances parallel those found in other hydroporphyrins.

The redox chemistry of the oxoporphyrins is noteworthy in that the compounds exhibit ring oxidation potentials very similar to those of the parent porphyrin, in contrast to those of hydroporphyrins which are easier to oxidize than the porphyrin by as much as 0.6V. The dioxoisobacteriochlorins are also easier to reduce than porphyrins. The Fe(II)-Fe(III) couples shift as well so that metal centered reductions become easier. The redox trends and optical properties of the oxoporphyrins are readily explained by extended Hückel calculations. The calculations also provide reasonable descriptions of the observed spin densities of π radicals of the oxo compounds, and correctly predict electron abstraction from the "a_{1u}" orbital, as observed in other hydroporphyrins.

This work supported by the Chemical Sciences Division of the U.S. Department of Energy.

(1). Chang, C. K. J. Biol. Chem. 260, 9520 (1985). (2). Chang et al. J. Am. Chem. Soc. 108, 1352 (1986). (3). Stolzenberg et al. Inorg. Chem. 25, 983 (1986).

T-Pos156 REDUCTION OF CYTOCHROME c PEROXIDASE COMPOUND I BY FERROCYTOCHROME c: A TRANSIENT STATE KINETIC STUDY. F. E. Summers and J. E. Erman, Department of Chemistry, Northern Illinois University, DeKalb, IL 60115.

The reaction between yeast cytochrome c peroxidase compound I and horse heart ferrocytochrome c has been investigated at pH 7.5 in a 4.1 mM phosphate buffer (10 mM ionic strength) at 5°C by stopped-flow techniques. At low enzyme and cytochrome c concentrations, the reduction of compound I occurs in two distinct phases. Spectral analysis indicates that compound I is reduced by cytochrome c to the native enzyme via an intermediate which has a spectrum essentially identical to that of the native enzyme. Under pseudo-first order conditions with cytochrome c in excess, the fast phase of the reaction is first order in enzyme and shows saturation behavior with respect to cytochrome c. The cytochrome c dependence can be described by a mechanism involving complex formation followed by intramolecular electron transfer. Values of 7.4 μM and 350 s^{-1} have been obtained for the equilibrium dissociation constant of the ferrocytochrome c - compound I complex and the electron transfer step, respectively. The rate of the second phase of the reaction in independent of cytochrome c and first order in enzyme concentration. The enzyme concentration dependence of the slow phase suggests that biomolecular reactions between enzyme species are involved in the reduction of the intermediate to the native ferric state by ferrocytochrome.

T-Pos157 SALT DEPENDENCE OF THE SOLUTION STRUCTURE OF OXIDIZED AND REDUCED CYTOCHROME c STUDIED BY SMALL ANGLE X-RAY SCATTERING. Jill Trewhella, Elizabeth H. Curtis and Douglas B. Heidorn, Life Sciences Division and Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM 87545.

While the X-ray crystal structures of cytochrome c show the reduced and oxidized forms to be very similar, there is considerable evidence to suggest that the interior of the molecule in solution is much more accessible to solvent in the oxidized compared with the reduced state. This observation has led many investigators to conclude that while the structures of the two forms are similar, their dynamics are different. The oxidized form may undergo more, low frequency, large amplitude motions than the reduced form. Inelastic neutron scattering measurements (1), however, could not detect any difference in dynamics in the range 1-250 cm^{-1} upon change in oxidation state in solution. Since the crystal structures of cytochrome c were derived from crystals grown in very high salt concentrations it seemed pertinent to investigate the effects of such high concentrations of salt on the solution structures of oxidized and reduced cytochrome c. We have done small angle X-ray scattering on oxidized cytochrome c and have observed a dramatic decrease in the radius of gyration with increasing salt concentration (at 200 mM NaCl the radius of gyration has decreased by 7%). We are now measuring the salt dependence of the reduced form, and our preliminary data also show a reduction in radius of gyration with increasing salt, but the effect appears to be smaller.

(1) Cusack, S., Smith, J., Finney, J., Karplus, M. and Trewhella, J., Physica (1986) 136B:256-259.

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T-Pos158 DEPENDENCE OF CYTOCHROME a_3-NO SPECTRA ON THE ENZYME REDOX LEVEL IN NITROSYL CYTOCHROME OXIDASE. Satish Singh, Yuan-chin Ching, Massimo Sassaroli and Denis L. Rousseau. AT&T Bell Laboratories, Murray Hill, NJ 07974

We report the first resonance Raman studies of NO-bound cytochrome oxidase. Resonance Raman scattering and optical absorption spectra have been obtained on the fully reduced enzyme ($a^{2+}a_3^{2+}NO$) and the mixed valence enzyme ($a^{3+}a_3^{2+}NO$). Clear vibrational frequency shifts are detected in the lines associated with cytochrome *a* in comparing the two redox levels. For example, in the resonance Raman spectra of the fully reduced enzyme obtained with 406.7 nm excitation, a strong line appears at $\sim$1611 cm^{-1} from cytochrome a^{2+} whereas in the mixed valence preparation a strong cytochrome a^{3+} line appears at $\sim$1647 cm^{-1}. Similarly, with 441.6 nm excitation the fully reduced preparation yields a spectrum similar to that of carbon monoxide bound cytochrome oxidase and is dominated by the spectrum of reduced cytochrome *a*. In contrast, in the mixed valence preparation the spectrum has no reduced cytochrome *a* lines. For both types of NO-bound sample a line appears at $\sim$545 cm^{-1}, a frequency similar to that found in NO-bound hemoglobin and myoglobin and assigned as an Fe-NO stretching or bending mode. The carbonyl line of the formyl group in the fully reduced NO-bound enzyme appears at $\simeq$1666 cm^{-1} in the resonance Raman spectrum. In the mixed valence preparation the frequency of the carbonyl line increases by 1.2 cm^{-1} to $\simeq$1667 cm^{-1}. Thus, this mode in cytochrome a_3^{2+} NO is sensitive to the enzyme redox state. In the absence of any direct evidence about the oxidation state of the Cu_B, we conclude that this sensitivity may result from either a heme-heme interaction, or a copper-heme interaction.

T-Pos159 A NOVEL ROLE OF HEPATIC CYTOCHROME P-450 IN THE CONVERSION OF LEUKOTRIENE A_4 TO LEUKOTRIENE B_4. Josef Gut and James R. Trudell, Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Leukotriene A_4 (LTA_4) was converted to leukotriene B_4 (LTB_4) by human liver microsomes under anaerobic conditions. The reaction was dependent on time and on both protein and substrate concentration and was not supported by heat- or acid-inactivated microsomes. Kinetic analysis of the reaction revealed apparent Michaelis-Menten type behavior (app. Km ~20 uM). Unlike usual cytochrome P-450 (P-450) substrate hydroxylation reactions, LTB_4 formation from LTA_4 was not NADPH dependent, was readily catalyzed by microsomes with P-450 in its oxidized (Fe^{3+}) state, and was inhibited by O_2. Thiol compounds, particularly propanethiol, which replaces the naturally occurring sixth ligand of the ferric P-450 heme group, as well as piperonyl butoxide, a classical inhibitor of P-450 functions, interfered with LTB_4 formation. The computer molecular modeling program XICAMM was used to search for a low energy conformation of LTA_4 which can bind to the substrate binding site of P-450 and in which opening of the epoxide ring and migration of the double bonds occur without significant movement of the carbon skeleton, resulting in the formation of the essential 6-cis double bond of LTB_4. We suggest that in analogy to a number of hydroperoxide decompositions, the central Fe^{3+} of the heme group of oxidized P-450 can act as a Lewis acid-type catalyst in the conversion of the allylic epoxide LTA_4 to LTB_4.

T-Pos160 **BINDING OF FERRICYTOCHROME c TO PORPHYRIN CYTOCHROME c PEROXIDASE: DEPENDENCE UPON IONIC STRENGTH.** **L. B. Vitello and J. E. Erman, Department of Chemistry, Northern Illinois University, DeKalb, IL 60115.**

The binding of horse heart ferricytochrome c to yeast cytochrome c peroxidase in which the heme group was replaced by protoporphyrin IX was determined by a fluorescence quenching technique (Leonard, J. J. and Yonetani, T. (1974) Biochemistry 13, 1465-1468). The binding was investigated at pH 6.0 in 10 mM cacodylate buffer with ionic strength adjusted with KNO_3. Ionic strengths varied between 3.5 mM and 1.0 M. No detectable binding was observed at 1.0 M ionic strength although there was a decrease in fluorescence intensity due to the inner filter effect. The data at all ionic strengths were corrected for the inner filter effect by dividing by the relative intensity of 1.0 M ionic strength solutions containing identical concentrations of ferricytochrome c and porphyrin cytochrome c peroxidase. Significant quenching was observed between 3.5 mM and 100 mM ionic strength which was attributed to complex formation between the two proteins. Equilibrium dissociation constants calculated from the data for a single binding site model give values which are in agreement with those determined for ferricytochrome c and the native enzyme by difference spectrophotometry (Erman, J. E. and Vitello, L. B. (1980) J. Biol. Chem. 255, 6224-6227).

T-Pos161 LIGAND STRUCTURE OF EPR-UNDETECTABLE COPPER IN CYTOCHROME OXIDASE. Linda S. Powers, Y. Ching, Bell Telephone Laboratories, Murray Hill, NJ 07974, Britton Chance, University of Pennsylvania, Philadelphia, PA 19104, and David C. Wharton, Northeastern University, Boston, MA 02115.

The studies of the copper redox sites of cytochrome oxidase have been continued to include the preparation preferentially depleted of the EPR-detectable copper (Cu_a) by means of a short exposure to bathocuproine sulfonate at pH 5.0 (1). This preparation of the oxidase retains at least 75% of the EPR-undetectable copper (Cu_{a3}) but has no near IR absorption band and, of course, no EPR signal attributable to copper, indicating that the residual copper remains spin-coupled to the iron of cytochrome a_3. X-ray absorption spectroscopy of the remaining copper atom shows that 1) the Cu_{a3} is 3- or 4-coordinate with at least one and perhaps two of the ligands being S and the others being N, or possibly O; 2) the Cu-Fe distance of 3.75 $\pm$ 0.05 Å that is observed with the intact oxidase is preserved; 3) the geometry of the Cu_{a3} is less tetrahedryl than in the oxidized "resting" state of the untreated enzyme. These results are consistent with those reported previously (2).

(1) Weintraub, S.T. and Wharton, D.C. J. Biol. Chem. 241, 1669-1676 (1981).
(2) Powers, L., Chance, B., Ching, Y., and Angiolillo, P. Biophys. J. 34, 465-498 (1981).

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T-Pos162 THE COMPLETE PRIMARY STRUCTURES OF THE CHAINS FORMING THE TRIMER IN THE HEMOGLOBIN OF THE EARTHWORM, LUMBRICUS TERRESTRIS.

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Hemoglobin (Hb) of the oligochaete, Lumbricus terrestris, has 4 major chains in equal proportions designated a, b, c and d. Each forms a heme-containing subunit. Chains a, b and c form a disulfide-linked trimer. The sequence of the non-trimer chain d has been completed by Shishikura,F., et al., J. Biol. Chem. submitted, (1986). The amino acid sequences of chains a and c have now been determined. The sequence of chain b reported earlier (Garlick, R.L. and Riggs A.F., J. Biol. Chem. 237, 9005-9015 (1982)) has been redetermined. Comparison of these chains with those of the polychaetes, Tylorrhynchus heterochaetus (Suzuki, T. and Gotoh, T., J. Biol. Chem. 261, 9257-9267 (1986)) and Glycera dibranchiata and of vertebrates suggests that (i) C2 Pro, CD1 Phe, E7 His and F8 His are conserved in the Lumbricus chains, (ii) the H helices of L. terrestris and T. heterochaetus appear to be about 3 residues shorter than those of vertebrate Hbs, (iii) these annelid Hbs lack D helices and possess additional residues at the AB and EF corners, (iv) the residues believed to form the heme-pocket are very similar to those in other Hbs. The results show that the four major chains of the Hb of L. terrestris have the "myoglobin fold". The positions of all ten cysteinyl residues in the trimer are identical in both L. terrestris and in T. heterochaetus. This suggests that the -S-S- bonds have the same arrangement in the Hbs of both species. The inter- and intra-chain -S-S- positions are now being determined. (This work has been supported by grants NSF DNB-85-2857 and Welch Foundation F-213).

T-Pos163 DISSOCIATION OF FIBRIN OLIGOMERS AND FINE FIBRIN CLOTS BY GLY-PRO-ARG-PRO.
Akira Shimizu, Günther F. Schindlauer, and John D. Ferry, Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. (Intr. by Hyuk Yu.)

After the fibrinopeptides A and B are released from fibrinogen by thrombin, the resulting fibrin monomer polymerizes spontaneously to form oligomers which grow linearly to protofibrils and eventually produce a three-dimensional gel network, the fibrin clot. Oligomers are partly dissociated by the tetrapeptide Gly-Pro-Arg-Pro (which, Laudano and Doolittle showed, binds to fibrinogen and inhibits polymerization) at concentrations of the order of 3 to 20 mM. A "fine" clot (i.e., formed at pH 8.5, ionic strength 0.45, with minimal lateral aggregation of the protofibrils), if unligated (i.e., without the covalent bonds introduced by fibrinoligase when it is present), is strikingly modified when Gly-Pro-Arg-Pro is introduced by diffusion at concentrations of the order of 1 to 4 mM. The shear elastic modulus decreases, and viscoelastic creep rate and irrecoverable deformation under constant stress are enormously increased, so the clot appears to flow with finite viscosity. The snake venom enzymes ancrod and batroxobin release only the A fibrinopeptides; the resulting α-fibrin or des-AA fibrinogen monomers also polymerize and form clots. The α-fibrin oligomers are much more susceptible to dissociation by Gly-Pro-Arg-Pro, at concentrations from 0.3 to 1 mM. The α-fibrin fine unligated clots have low elastic moduli and high creep rates even without Gly-Pro-Arg-Pro, but introduction of the peptide at 0.1 to 0.3 mM decreases the viscosity enormously. This effect can be interpreted as due to severance of network strands by dissociation of intermonomer junctions which is much more rapid for α-fibrin than for ordinary αβ-fibrin formed by thrombin in which both A and B interaction sites participate in the structure. Ligation ("cross-linking") by fibrinoligase (Factor XIII a) completely eliminates viscoelastic creep and recoverable deformation.

T-Pos164 CALCIUM-INDUCED SOLUBILITY OF THE CASEINS: A THERMODYNAMIC LINKAGE APPROACH
Harold M. Farrell, Jr., Thomas F. Kumosinski, Paul Pulaski and Marvin P. Thompson (Intr. by H. Pessen), USDA, Agricultural Research Service, Eastern Regional Research Center, Phila., PA 19118

The phenomena of salt-induced precipitation of proteins in general (salting-out), and the resolubilization of these proteins at higher salt concentrations (salting-in), have never been accorded a fully quantitative interpretation. A new approach based on the concepts of Wyman's Thermodynamic Linkage has been successfully tested on the caseins, using Ca^{+2} and other cations under various environmental conditions. It is now possible to describe salting-out by a salt-binding constant, k_1, and the number of moles of salt bound per mole of protein, n, while salting-in is described by the corresponding constants k_2 and m. Results of calcium-induced solubility profiles of α_{s1}-casein A (α_{s1}-A), α_{s1}-casein B (α_{s1}-B) and β-casein C (β-C) at 37°C, where hydrophobic interactions are maximized, showed no salting-in behavior and yielded k_1 and n values of 157 L/mole and 8, 186 L/mole and 8, and 156 L/mole and 4, respectively. At 1°C, where hydrophobic interactions are minimized, β-C is totally soluble at all Ca^{+2} concentrations, while α_{s1}-B and α_{s1}-A were now found to have salting-in parameters, k_2 and m, of 2.5 L/mole and 4, and 13 L/mole and 8, respectively. k_1 remained essentially independent of temperature. The influences of ionic strength (KCl) and various other cations on the 1°C solubility profiles of α_{s1}-A were studied. The changes observed in k_1 and k_2 are consistent with the concept of electrostatic, phosphate- and carboxylate- ligand coordination leading to changes in protein solubility.

T-Pos165 INITIAL ANALYTICAL ULTRACENTRIFUGATION STUDIES OF SOLUBILIZED RABBIT THROMBOMODULIN.
Paul T. Winnard and Thomas M. Laue, Department of Biochemistry, University of New Hampshire, Durham, NH 03824.

Thrombomodulin (TM) is an endothelial cell surface receptor for thrombin and is an essential component in the protein C-mediated anticoagulation pathway. Active TM can be purified in the presence of non-ionic detergents. SDS-gel electrophoresis indicates a TM monomer Mr=75,000, while gel permeation chromatography suggests a protein detergent complex Mr 650,000. Lubrol-PX-solubilized, homogeneous (by SDS gel electrophoresis) rabbit TM has been examined by equilibrium sedimentation in 0.05 M Tris (pH 7.6), 0.1 M NaCl, in the presence of 0.01 to 0.23% Lubrol PX, and with D_2O (20% to 72%) to vary the solvent density. Samples were analyzed at speeds of 7.2, 12, 16, 20, 24 and 28 thousand rpm. Preliminary analyses suggest: 1) Lubrol-solubilized TM exists as monomers and oligomers, 2) the oligomers are not in rapid equilibrium with the monomer, 3) large amounts of detergent (> 1 g-detergent/g-protein) are bound for each protein monomer and 4) increasing the detergent concentration favors the formation of smaller protein oligomers. In addition to aggregation-state heterogeneity, there is some evidence for heterogeneity in the extent of detergent binding. Sedimetation velocity profiles show a single broad boundary, again suggesting heterogeneity in these samples.

T-Pos166 THROMBIN-HEPARIN INTERACTIONS: A LARGE LIGAND ON A SMALL LATTICE.
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Since the work of McGhee and von Hippel (JMB 86:469) the pertinence of "overlapping sites" to protein-nucleic acid interactions has been widely recognized. Inexplicably, it is not generally appreciated that these same considerations apply to the interactions of blood-clotting proteins with heparin. One difficulty with the small lattice problem is that "end-effects" cannot be ignored, which has required the explicit use of combinational expressions. I have derived a reasonably simple closed-form alternative. Analysis of experimental data for thrombin-heparin interactions (heparin MW about 10,000) suggests that thrombin covers 4 disaccharide units of the heparin chain, with a dissociation constant of 4.2 μM. In contrast, analysis according to a stoichiometric or specific site model leads to an inference of 2.4 sites (i.e, a spacing of about 6 disaccharide units) with a dissociation constant of 2.1 μM. The qualities of the fits are indistinguishable. Plausibility arguments are presented for the overlapping site model.

T-Pos167 THERMODYNAMIC PARAMETERS FOR ASSOCIATION OF THE GROUP I METAL CATIONS WITH GRAMICIDIN A.
Roger E. Koeppe II, James F. Hinton, Juan Fernandez, Dikoma Shungu, William L. Whaley, & Francis S. Millett, Dept. of Chemistry and Biochemistry, Univ. of Arkansas, Fayetteville, AR 72701.

We have previously reported equilibrium association constants for Tl^+, Li^+, Na^+, K^+, Rb^+ and Cs^+ with the gramicidin A channel, determined by competition studies and Tl^+-205 NMR spectroscopy [Biophys. J. 50, 539-544 (1986)]. We now report further investigations of the cation selectivity of gramicidin A in the form of these thermodynamic parameters:

Cation	ΔH (kcal/mol)	ΔS (cal/mol-deg)	ΔG (kcal/mol)
Li^+	-2.7	-2.0	-2.1
Na^+	-2.9	-2.6	-2.2
K^+	-3.2	-2.6	-2.3
Rb^+	-3.8	-4.8	-2.4
Cs^+	-4.1	-5.4	-2.4
Tl^+	-6.8	-9.8	-3.8

These results are for gramicidin A packaged in aqueous dispersion of lysophosphatidylcholine. The association constant for thallium to gramicidin in this lipid agrees with the first association constant for thallium to gramicidin channels in vesicles of dimyristoylphosphatidylcholine [Biochemistry 25, 6103-6108 (1986)]. We therefore presume that the equilibrium binding properties of the other ions may be similar for the two lipid systems. Ion association is in all cases favored by the enthalpy term and opposed by the entropy term. The results indicate that a small difference in binding energy is effectively magnified by the channel into a larger difference in the overall rate of transport when two cations are compared.

T-Pos168 COMPARISON BETWEEN FUNCTIONAL ENERGETICS AND QUATERNARY STRUCTURES FOR INTERMEDIATE STATES OF HUMAN HEMOGLOBIN.
Francine R. Smith, David Gingrich, Brian M. Hoffman and Gary K. Ackers, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218 and Department of Chemistry, Northwestern University, Evanston, IL 60201

Understanding the detailed mechanism of cooperative oxygenation by human hemoglobin requires information on the structures and functional energetics of tetrameric molecules at the intermediate states of ligation. An x-ray crystallographic structure was recently determined for the intermediate form of hemoglobin in which both β subunits contain Mn(II) protoporphyrin IX (an analog of the unligated heme) while the α subunits contain normal hemes ligated with carbon monoxide [Arnone et al. (1986) J. Mol. Biol. 188: 693]. The results showed that this species could crystallize in a deoxy ("T") quaternary structure. We have determined the cooperative free energy in solution for this same species, along with those of the corresponding unligated and fully-ligated tetramers. We find the functional energetics of this intermediate species to be identical with fully-ligated ("R" state) hemoglobin, in sharp contrast to behavior expected from the crystallographic results. Cooperative free energies are also presented for other intermediate forms of the Mn(II)/Fe(II)-CO system.

Supported by grants from the National Institutes of Health and the National Science Foundation. F.R.S. acknowledges a Postdoctoral Fellowship from the American Heart Association.

T-Pos169 STABILITY OF ABNORMAL HUMAN HEMOGLOBIN: VARIATION IN STABILITY AND EFFECTS OF CROSS-LINKING. Kenneth W. Olsen, Thao Yang, Thomas Corso and Lilly Swiersz, Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Road, Chicago, IL 60626.

The variation in stability of abnormal human hemoglobins has been studied by thermal denaturation. The effects of changing the oxidation state of the heme iron and of cross-linking the protein with a variety of reagents were studied. The denaturations were done in 0.01 M MOPS, pH 7, containing 0.9 M guanidine. For the methemoglobin experiments the samples were first oxidized with $K_3Fe(CN)_6$ and the buffer also contained 1 mM NaCN. The changes in absorbance between 200 - 650 nm were monitored by a diode array spectrophotometer while the sample was heated at a rate of 0.3°C/min from 25° to 70°. The methemoglobins tested had increasing stability in the following order: Hb S < Hb New York <= Hb O Indonesia < Hb Andrew - Minneapolis = Hb A < Hb A_2. The T_m for met-Hb A was 42°, while those for the abnormal hemoglobins were 39° to 41°. Carbonmonoxy hemoglobins showed a similar order of stability, but they had transition temperatures that were approximately 10° higher than those of the methemoglobins. A diaspirin cross-linking reagent, which introduces a fumarate cross-link between the two Lys 82 (Walder et. al., Biochemistry (1979) 18:4265), produced a higher temperature transition in both the abnormal and normal hemolgobin. The T_m for cross-linked met-Hb A was 57°, while those of the cross-linked abnormal hemoglobins varied from 54° to 56°. Cross-linking of HbA with a series of different length imidates showed that these reagents could also stabilize the molecule but that the degree of stabilization depended on the cross-linker:hemoglobin ratio.

T-Pos170 ANISOTROPY DECAYS OF MELITTIN FROM 2 GHz FREQUENCY-DOMAIN FLUOROMETRY AND GLOBAL ANALYSIS OF QUENCHED SAMPLES. Joseph R. Lakowicz, H. Cherek, I. Gryczynski and N. Joshi, University of Maryland, Department of Biological Chemistry; Baltimore, Maryland and M.L. Johnson, University of Virginia, Department of Pharmacology, Charlottesville, Virginia, U.S.A

Enhanced resolution of rapid and complex anisotropy decays was obtained by measurement and analysis of data from progressively quenched samples. Collisional quenching by acrylamide was used to vary the mean decay time of indole or melittin fluorescence. Anisotropy decays were obtained from the frequency-response of the polarized emission at frequencies from 4 to 2000 MHz. Quenching increases the fraction of the total emission which occurs on the subnanosecond timescale, and thereby provides increased information of local motions in proteins. For complex anisotropy decays, such as those due to both local motions and overall protein rotational diffusion, superior resolution is obtained by global analysis of data from quenched and unquenched samples. The method greatly reduced the uncertainty of the two correlation times of indole in propylene glycol, 140 and 720 ps. The method was applied to melittin in the monomeric and tetrameric forms. With increased quenching, the anisotropy data showed a decreased contribution from overall protein rotation, and increased contribution from picosecond tryptophan motions. The tryptophan residues in both the monomeric and the tetrameric forms of melittin displayed substantial local motions with correlation times near 0.16 and 0.06 ns, respectively. The amplitude of the local motion is two-fold less in the tetramer. These highly resolved anisotropy decays should be valuable for comparison with molecular dynamics simulation of melittin. The data are not consistent with a 2 ps correlation time for either the monomer or the tetramers.

T-Pos171 ELECTRIC BIREFRINGENCE STUDIES OF TROPOMYOSIN. Charles A. Swenson and Nancy C. Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

The inherent head-to-tail aggregation of skeletal tropomyosin in solution at low ionic strength has precluded a definitive electric birefringence study of the monomeric species. We have studied the molecular properties of non-polmerizable tropomyosin obtained by treatment of rabbit skeletal tropomyosin with carboxypeptidase A. Birefringence measurements were made as a function of concentration, electric field strength and pulse length. No concentration dependence was noted in the range of 6-150 ug/ml. The rotational relaxation time was independent of concentration and its magnitude is consistent with tropomyosin being a rigid rod in aqueous solution. Orientation occurs primarily by a permanent dipole mechanism. Estimation of the permanent dipole moment was made by combination of results from reversing field experiments and from an analysis of the effect of field strength on the steady state value of the birefringence. The dipole moment will be compared with the value obtained for intact tropomyosin. Supported by grants from NIH, GM-29690, and AHA.

T-Pos172 DIFFERENTIAL MELTING OF CALMODULIN, A.L. Jacobson, Division Biochemistry, Chemistry Dept., University of Calgary.

Differential melting of portions of the calmodulin molecule are observed. Two major domains of the molecule are well separated and the effect of pH, ionic strength and specific ions can be determined on each portion. A third domain can be determined by computer separation of the thermogram. In the absence of strongly binding divalent cations (EGTA and DTT present), pH and specific ion effects both determine the melting temperatures. At pH >8, tetramethyl ammonium chloride destabilizes the molecule, while there is little effect between pH 4 - 7. While there is little effect of the added monvalent ion in the low pH range, the melting temperatures are pH dependent. At comparable ionic strength, sodium chloride has a small stabilizing effect on the entire pH zone (4-9). At high pH, ethanol has a stabilizing effect (compare to tetramethyl ammonium chloride) while at lower pH, ethanol has a destabilizing effect and the lower melting domain has a higher enthalpy of denaturation than the higher melting domain. This behavior is the reverse of the samples in sodium chloride.

This data illustrates that specific portions of the calmodulin molecule melt independently and are influenced by their environment in a different manner.

T-Pos173 SEDIMENTATION EQUILIBRIUM STUDIES OF BOVINE BRAIN PROTEOGLYCAN. P. Gonzaga and L. Vitello, Department of Chemistry, Northern Illinois University, DeKalb, IL 60115.

Proteoglycan (PG) molecules consist of a protein core to which several glycosaminoglycan (GAG) chains are covalently attached. They are present in most tissues in association with hyaluronic acid (HA). The proteoglycans characterized were those isolated from delipidated brain by aqueous extraction and fractionated by anion exchange chromatography and gel filtration. Preliminary results on the fraction isolated gave a 0.17 uronic acid: protein ratio with uronic acid contributed mostly by the GAG, chondroitin sulfate and approximately 10% by HA. This aggregation with HA was studied by determining the apparent molecular weights of the aggregate and the HA-free proteoglycan using sedimentation equilibrium centrifugation. The HA in the isolated proteoglycan was degraded by streptomyces hyaluronidase treatment. The concentration dependence of the molecular weights was determined in 4M guanidine hydrochloride (Gdn HCl) & in 0.2Msodium acetate (NaOAc). Centrifugation was performed following the meniscus depletion method and sedimentation was monitored by interference optics. The molecular weights obtained for the HA-free proteoglycan in the two solvents were 78000 in 0.2 M NaOAc and 74400 in 4M Gdn HCl. These values are in close agreement, indicating the monomeric nature of the sample. The aggregate gave an average molecular weight of 98700 in the associating solvent, NaOAc, confirming the molecular association with HA. It gave a lower value in the dissociating solvent, Gdn HCl indicating a certain degree of dissociation. The apparent molecular weights were computed using a partial specific volume of 0.59 ml/g as calculated from the weighted contribution of protein and carbohydrate in the molecule.

T-Pos174 Protection of Oncogene Product SC1N p21 Against Methionine Oxidation. Martin Poe, Joseph K. Wu and Brian M. McKeever. Department of Biophysics, Merck Sharp & Dohme Research Labs, Merck & Co., Inc., Rahway, NJ 07065

The oncogene product SC1N p21, a hybrid of viral Harvey ras p21 and yeast SC1N p21 [Temeles et al., Nature 313 700 (1985)] which has 6 methionine and no cysteine residues, was found to be microheterogeneous after purification, when chromatographed in nondenaturing polyacrylamide gels. Quantitive amino acid analysis of the two principal components (purified by isoelectric focussing) with isoelectric points (pI) of 4.28 and 4.44, respectively, gave 1.25 ± 0.2 and 1.76 ± 0.3 moles of methionine sulfoxide per mole p21. The narrow, three-proton resonances between 2.0 and 2.5 ppm downfield from internal trimethylsilylpropionate in the 400 MHz ^{1}H-NMR spectrum of binary, one-to-one SC1N p21:GDP were used to measure methionine oxidation in samples protected against methionine oxidation by either: 1) 5 mM $MgCl_2$ 1 mM dithiothreitol 2) 5 mM $MgCl_2$ or 3) 1 mM EDTA, all three solutions with 100 mM NaCl 20 mM sodium phosphate pH 7.5 and 0.01 mM GDP at 4°C in the dark. Solution 3 showed essentially no methionine oxidation after 3 weeks while solutions 1 and 2 had two and three residues oxidized, respectively.

T-Pos175 THE MECHANISM OF pH-INDEPENDENT PEPTIDE HYDROGEN ISOTOPE EXCHANGE. Roger B. Gregory. Department of Chemistry, Kent State Uiversity, Kent, OH 44242

A kinetic analysis of hydrogen isotope exchange in model amides and polypeptides has been undertaken to determine whether pH-independent exchange involves direct proton abstraction by water ($Z + H_2O$) or proceeds via an imidic acid intermediate ($ZH^+ + OH^-$). Proton activating factors (paf) and deprotonating factors (dpf) have been calculated and the relative rates of the two kinetically equivalent paths have been estimated by assuming ideal behavior (i.e. $paf_{H_2O} = paf_{OH}$). Although this analysis cannot determine which of these reaction paths is the more important, it is argued that $Z + H_2O$ makes the larger contribution to isotope exchange in peptides. The peptide system is actually non-ideal (i.e. $paf_{H_2O} > paf_{OH^-}$) and this causes the analysis based on ideal behavior to underestimate the contribution from $Z + H_2O$. Analysis of exchange data for proteins indicates significant contributions to exchange from pH-independent reactions, which are often greater than those observed in solvent-exposed model peptides. This enhancement of the $Z + H_2O$ reaction suggests that the charged catalyst species are preferentially excluded from the protein interior relative to water. This conclusion is consistent with catalyst penetration models but is not easily explained by local unfolding models of exchange for which the relative contributions from H^+, OH^- and H_2O catalyzed reactions would be expected to be similar to those found in random coil polypeptides like poly-D,L-alanine. Supported by NSF Grant DMB85-18941.

T-Pos176 WESTERN BLOTTING OF PROTEINS: USE OF A NEW MEMBRANE SUBSTRATE WITH HIGH SENSITIVITY PROTEIN AND IMMUNOCHEMICAL STAINING. Charles Vecoli, David A. Hicks and Malcolm G. Pluskal (Intr. by Stanley J. Masiak). Integrated Separation Systems, 44 Mechanic St., Newton, MA 02164 and Biochemistry Dept., Millipore Corporation, 80 Ashby Road, Bedford, MA 01730.

Blotting of proteins to a new membrane substrate will be described for a range of analytical gel systems. The membrane is mechanically stable and hydrophobic in character.

Transfers will be illustrated for SDS-PAGE gels using a total heart muscle extract, and 2D-PAGE with IEF in the first dimension for and E.coli extract. High molecular Wt. proteins (67-669K) were transferred from a non-denaturing gel systems. Low molecular Wt. proteins (1.4-20K) were also blotted from a SDS-PAGE system. The proteins transferred were stained with India Ink and Aurodye staining protocols with enhanced sensitivity over nitrocellulose and gave very low background staining. Immunostaining was also carried out using alkaline phosphatase and glucose oxidase conjugated detector antibodies, giving high sensitivity with low background staining. Data will be presented comparing this new membrane to existing protein blotting substrates.

T-Pos177 A RECONSIDERATION OF PROTEIN UNFOLDING. Rufus Lumry, University of Minnesota, Minneapolis, MN 55455.

For many proteins the rate-limiting step in unfolding is probably cooperative disruption of strong, dominant structural elements ("knots"; (The Fluctuating Enzyme, John Wiley, 1986, Chap. 1.). If correct, some consequences for unfolding processes in water follow: 1. Knot disruption "unlocks" native structure allowing expansion and water uptake to a reasonably well-defined state with about twice native volume and high motility. 2. The consequent increase in dielectric constant is primarily responsible for the large increase in heat-capacity, there being little increased exposure of internal residues to bulk water. 3. Varying amounts of persistent structure remain embedded in the liquid net. Retention is high when one knot remains intact, e.g., RNase S has lost one knot of RNase A but retains many native properties; thermally denatured chymotrypsin A is half unfolded. 4. The Gibbs-Duhem relationship among interfacial area A, volume V and water content is of central importance in protein processes, e.g., alterations in the free energy of A force alterations in that of V producing changes in packing geometry and potential energy, and fluctuation dynamics (internal viscosity). 5. The A-V relationship depends on relative proportions of hydrophilic and hydrophobic groups in the interface. "Hydrophobic hydration" enters significantly only through interface interactions; these are not well-modeled by small solutes in bulk water. The preferential uptake of amphipathic solutes during expansion superficially reflects the latter. 6. Changes of charge, solvent composition and association with macromolecules produce internal anisotropic alterations which are often both thermodynamically and kinetically large and may be large geometrically. The mother liquors used in diffraction studies effect strong compression as perhaps do crystal forces.

T-Pos178 THE KINETICS OF LOW TEMPERATURE UNFOLDING AND REFOLDING OF A VARIANT OF BACTERIOPHAGE T4 LYSOZYME CONTAINING A DISULFIDE BRIDGE FROM C3 TO C97

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A variant of T4 lysozyme (C3T54) which has a disulfide bridge from C3 to C97 and contains no free sulfhydryls [L.J. Perry & R. Wetzel, Biochem. 25, 733-739 (1986)] has been found to reversibly unfold at low temperatures in the presence of denaturants. The equilibrium aspects of the low temperature unfolding reaction of proteins has recently been investigated in detail [P.L. Privalov, et al., JMB 190,487-498 (1986)].

Here, we use circular dichroism at 223 nm to determine the kinetics of the unfolding and refolding reaction for the cold-induced denaturation of this protein. We find the kinetics for this reaction to be quite slow below zero degrees C and independent of the concentration of protein. The transition is highly cooperative with no intermediates detectable within the transition zone. The Arrhenius energy of unfolding in 3 M Gdn-HCl is 13.5 Kcal/mol. The activation energy of refolding is 40.5 Kcal/mol evaluated at zero degrees C and has obvious dependence on temperature.

We are also investigating the dependence of macroscopic and microscopic rate constants of this reaction on the concentration of denaturant and the pH. (Supported by PHS Grant GM20195 and NSF Grant DMB 8609113.)

T-Pos179 THERMODYNAMIC LINKAGE OF CALCIUM BINDING TO COLLOIDAL BOVINE CASEIN MICELLES

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The caseins are thought to occur in milk as complexes of protein aggregates connected by calcium salt bridges, resulting in particles of approximately 650 A radius as determined by electron microscopy. The protein aggregates themselves have been hypothesized to result from mainly hydrophobic interactions. The fractional concentration of casein micelles of radius of approximately 650 A (F_{650}) can be obtained in a casein and Ca^{+2} solution by centrifugation of 2600 x g. Thus, the F_{650} with varying Ca^{+2} concentrations can be determined and analyzed using Wyman's Thermodynamic Linkage Theory. Ca^{+2} induced F_{650} profiles under various conditions were obtained experimently for model micelles consisting of only α_{s1}-B and κ-casein as well as α_{s1}-A and κ-casein, eliminating the complications arrising from β- and minor casein forms. Analysis of α_{s1}-A micelle profiles yielded a salting-out binding constant k_1 and the number of moles of Ca^{+2} bound per mole of protein, n, as well as salting in parameters, i.e., k_2 and m. No variation of k_1, n, k_2 or m was found with increasing amounts of κ-casein. From the variation of the F_{650} capable of being salted-in verses amount of added κ-casein an association constant of 4 L/g could be calculated for the complexation of α_{s1}-A and κ-casein. For the α_{s1}-β and κ-casein micelle F_{650} verses Ca^{+2} profiles, additional salting-out parameters, i.e., k_3 and q were added to the existing k_1, n, k_2 and m parameters in order to fully describe the system. Furthermore, the value of k_3 decreased with increasing concentration of κ-casein. These results will be contrasted with those obtained from calcium induced F_{650} profiles of dephosphorylated α_{s1}-β casein in order to determine the protein sites responsible for these Ca^{+2} induced quaternary structural effects.

T-Pos180 THERMODYNAMIC AND KINETIC LINKAGES BETWEEN LIGAND BINDING AND FOLDING OF THE CATALYTIC SUBUNIT OF *E. COLI* ASPARTATE TRANSCARBAMYLASE.

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Substrates and substrate analogs increase the melting temperature of the catalytic subunit (c_3), while the regulatory nucleotides ATP and CTP have the opposite effect (Edge *et al*, **Biochemistry**, 24, 5899 (1985)). Although these observations suggest that substrates and nucleotides bind preferentially to the folded and unfolded forms of the protein respectively, these data are somewhat inconclusive because thermal denaturation is not experimentally reversible under the conditions used. We have therefore begun to examine the effects of ligands on reversible denaturation in urea and guanidinium-HCl. Unfolding has been monitored by analytical gel chromatography coupled to postcolumn derivatization with o-phthalaldehyde and by ultraviolet difference spectroscopy. Results suggest that the two probes monitor different processes. In chromatographic experiments carried out at 7°C and pH 8.3, the values of K_d obtained in 0 and 7M urea differ by 21%. As is the case in thermal denaturation, the trimer appears not to dissociate. The midpoint of the transition in the absence of ligand is 3M urea. Saturating concentrations of PALA and carbamyl phosphate + succinate increase the K_d value obtained in 3M urea by only 4% and 1.5%, respectively, while 0.1M ATP and 0.25M NaCl have no effect. In contrast, both $2x10^{-8}$M PALA and 0.25M NaCl in 4M urea eliminate the ultraviolet difference spectrum associated with urea-induced unfolding at pH 8.3, 25°C. PALA has no effect on the difference spectrum produced by guanidinium-HCl, probably because the high ionic strength prevents its binding. Similar experiments with regulatory subunit (r_2) and kinetic experiments with both c_3 and c_6r_6 are planned. Supported by NIH grant AM-17335.

T-Pos181 STABILIZATION OF PHOSPHOFRUCTOKINASE WITH ORGANIC SOLUTE/TRANSITION METAL MIXTURES DURING FREEZE-THAWING AND FREEZE-DRYING. John F. Carpenter, Lois M. Crowe and John H. Crowe, Department of Zoology, University of California, Davis, CA 95616.

Phosphofructokinase (PFK) purified from rabbit skeletal muscle is fully inactivated after freeze-thawing or freeze-drying and rehydration. In the presence of 500 mM trehalose, maltose or sucrose, up to 80% of the pretreatment activity is recovered. Proline and trimethylamine-N-oxide also protect the enzyme during freeze-thawing, but afford no stabilization during freeze-drying. Glucose, glycine, 4-hydroxyproline, inositol and glycerol at concentrations up to 500 mM are relatively ineffective at protecting PFK during either treatment. However, in the presence of ionic zinc (< 1 mM), the cryoprotection provided by any of the above organic solutes is greatly enhanced. During freeze-drying, in contrast, zinc-induced increases in stabilization are only noted with sugars. During either treatment, this effect is not due simply to the summation of the individual protective capacities of zinc and the organic solute. Zinc alone provides no protection, but greater than 70% of the initial activity can be recovered when zinc is added to an organic solute/PFK preparation, even when the organic solute is at a concentration at which, by itself, it is totally ineffective. The addition of ionic copper, cadmium, nickel or cobalt to trehalose/PFK solutions prior to freeze-thawing or freeze-drying also greatly increases the percentage of activity recovered. In the presence of calcium or manganese, a slight increase in enzyme stabilization is noted, whereas magnesium is ineffective in this respect. (Supported by grant DMB 85-18194 from NSF).

T-Pos182 SOLUBILITY OF XENON GAS IN AQUEOUS-AMINO ACID SOLUTIONS. G.L. Pollack, J.F. Himm, and R.P. Kennan, Physics and Astronomy Department, Michigan State University, East Lansing, MI 48824.

Ostwald solubility (L) of xenon gas, as the radioisotope ^{133}Xe, has been measured as a function of solute concentration, at 25.0°C, in aqueous solutions of 14 amino acids and of sucrose and sodium chloride. The amino-acid concentrations investigated covered almost their entire solubility ranges in water, viz.: alanine (0-1.8M), arginine (0-0.9M), asparagine (0-0.19M), cysteine (0-1.2M), glutamine (0-0.22M), glycine (0-2.1M), histidine (0-0.26M), hydroxyproline (0-2.3M), lysine (0-2.8M), methionine (0-0.22M), proline (0-3.5M), serine (0-0.38M), threonine (0-1.4M), and valine (0-0.34M). In all of the amino-acid systems, in sucrose, and in NaCl at low concentrations, the Ostwald solubility decreases linearly with increasing solute concentration, i.e., $L = L_o - aM$, where $L_o = 0.1057$ is the solubility of ^{133}Xe in distilled water at 25.0°C and a is a constant characteristic of the solute. For L(25.0°C) we obtained: 0.066 in 2.1M glycine solution, 0.055 in 2.8M lysine, 0.055 in 2.0M NaCl, and 0.052 in 2.0M sucrose. In NaCl solutions the decrease in gas solubility is due to the salting-out effect and is associated with a hydration number H≈16. If one postulates that the observed decreases in gas solubility in amino-acid solutions are due to hydration, one can calculate effective hydration numbers for the amino acids under the same assumptions. Some values obtained in this manner are: 0.2 ± 0.5 for arginine, 2.0 ± 0.2 for proline, 4.5 ± 0.4 for hydroxyproline, 6.1 ± 1.1 for lysine, 11.3 ± 0.6 for serine, 13.3 ± 1.0 for glutamine, and 15.1 ± 0.9 for asparagine. Effective hydration numbers for the other amino acids we investigated ranged from about 7 to 8.5. For arginine, glycine, and proline the temperature dependence of this effect, between 5-40°C, was investigated.

T-Pos183 TRYPTOPHAN AT THE STEROID-BINDING SITE OF CORTICOSTEROID BINDING GLOBULIN. M.F.Lulka§, J.B.A.Ross§, R.Kuhn¶. §Dept. of Biochemistry, Mt. Sinai Medical School, City University of New York. ¶Dept. of OB/GYN, University of California, San Francisco.

Corticosteroid Binding Globulin (CBG) binds corticosteroids with high affinity and specificity. CBG from guinea pig serum is a 43kd protein containing 2 tryptophans and 6 tyrosines. In the presence of cortisol (Ka=2.5e+7/M), protein fluorescence is extensively quenched. It has been proposed that resonance energy transfer from tryptophan to the 3-oxo-4-ene chromophore of cortisol is the quenching mechanism (1), since protein fluorescence increases when cortisol is removed or replaced by 5α-pregnane3,20-dione (PD) (Ka=6e+5/M) (2). Our results show that CBG protein fluorescence is from tryptophan, with little, if any, emission from tyrosine. In the CBG/PD complex, the excitation and emission spectra of one tryptophan are red-shifted with respect to the other. Time-dependent fluorescence parameters (phase/modulation) are complex and are emission wavelength dependent. Protein fluorescence is insensitive to collisional quenching by iodide in the CBG/PD complex, and is only slightly sensitive to acrylamide. Removal of PD greatly increases the ability of iodide to quench the "red" tryptophan. These results strongly suggest that at least one tryptophan residue is located in the steroid binding site, and that the two tryptophans are in different environments. Supported by NIH grants HD-17542 (J.B.A.R.) and HD-10328 (R.K.).

1. Stroupe et al., Archives of Biochemistry 168, 473 (1975).

2. Mickelson et al., Biochemistry 18, 2685 (1979).

T-Pos184 AN EQUILIBRIUM STUDY OF THE CONVERSION OF L-PHENYLALANINE TO TRANS-CINNAMIC ACID AND AMMONIA Yadu B. Tewari, Ewa Gajewski and Robert N. Goldberg (Intr. by William T. Yap)

The thermodynamics of the enzymatic conversion (L-phenylalanine ammonia-lyase) of aqueous L-phenylalanine to trans-cinnamic acid and ammonia has been investigated using high-performance liquid chromatography (HPLC). The reaction was carried out in 0.1 M TRIS/HCl buffer containing ammonium chloride over the pH range 7.0 to 7.7, at ionic strengths from 1.0 to 2.1 mol kg^{-1}, and over the temperature range 285 to 316 K. Analysis of the HPLC data using an estimated heat capacity change of 50 J mol^{-1} K^{-1} and an "ion-size" parameter of 1.6 $mol^{-1/2}$ $kg^{1/2}$ leads to an equilibrium constant of 1.16 ± 0.3 mol kg^{-1} and an enthalpy change of 24.8 ± 2.0 kJ mol^{-1} at 298.15 K for the process: L-phenylalanine±(aq) = trans-cinnamic $acid^{-}$(aq) + NH_4^+(aq). The use of these thermodynamic parameters in an equilibrium model for this system allows for the prediction of values of the apparent equilibrium constant as a function pH, temperature, and composition and also of the effects of these parameters on the optimal product yield of L-phenylalanine during its manufacture from trans-cinnamic acid and ammonia. The available thermochemical data for this generic type of reaction can be rationalized in terms of a scheme which views the entropy changes for related processes to be compared and then attributes differences in Gibbs energy changes to differences in enthalpy changes which can be influenced by effects such as resonance stabilizations of the double bonds which are formed.

T-Pos185 **THERMODYNAMIC PARAMETERS FOR THE FORMATION OF BINARY AND TERNARY COMPLEXES WITH LIVER ALCOHOL DEHYDROGENASE.** **Joanna K. Hu and Maurice R. Eftink, Department of Chemistry, University of Mississippi, University, MS 38677.**

Equine liver alcohol dehydrogenase(LADH) is a well studied enzyme that exhibits positive heterotropic cooperativity with respect to ligand binding. It interacts with the coenzyme NAD^+ and small inhibitor molecules such as pyrazole(P) and trifluoroethanol(TFE) to form binary and ternary complexes. Using fluorescence binding techniques, we have characterized the thermodynamic linkage between the binding of these two types of ligands to LADH. The formation of the binary complex, LADH-NAD^+, is known to be pH dependent. We have shown that the formation of the ternary complex, LADH-NAD^+-P, is also pH dependent. Interaction of P with LADH at saturating NAD^+ shows an increase in the binding constant from approximately 1 x 10^6 to 3.2 x 10^7 M^{-1} as pH is increased from 6.5 to 10. Similar studies of the binding of NAD^+ to LADH as a function of P concentration in the pH range 6.5 - 8.5 exhibit an even larger increase in the binding constant from 1.6 x 10^7 to 5 x 10^9 m^{-1}. In order to study the interaction of P with LADH alone, we performed displacement studies using the chelating agent dipyridine(DP). We have shown that the formation of the binary complex LADH-P is relatively pH independent in the range of 5.5 to 10 with the association constant approximately equal to 10^2 M^{-1}. Comparison of the binding of P to LADH alone and the LADH-NAD^+ binary complex demonstrates that a very large - ΔG of coupling exists for this system. We will present similar thermodynamic studies of the formation of the TFE ternary complex. This work is supported by NSF grant DMB-8511569.

T-Pos186 PURIFICATION AND PROPERTIES OF AURACYANIN, A BLUE COPPER PROTEIN FROM Chloroflexus aurantiacus.

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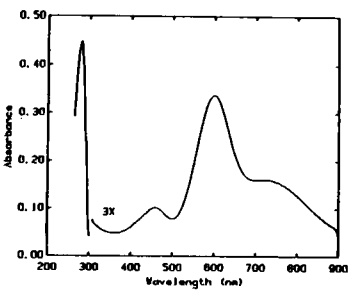
A membrane-bound blue copper protein was isolated from the thermophilic green photosynthetic bacterium Chloroflexus aurantiacus. The protein was obtained from lauryldimethylamineoxide (LDAO) extracted membranes and then purified by DEAE-cellulose and gel filtration chromatography. The protein shows abosorption maxima at 280, 455 and 598 nm with a shoulder at 715 nm. Atomic absorption analysis yields a molar extinction coefficient ε=2900 M^{-1} cm^{-1} at 598 nm. In conjunction with a SDS-PAGE molecular weight of 12,200 and a Lowry protein assay this indicates two moles of copper per mole of protein monomer. X and Q band ESR spectra yielded g_x= 2.024, A_x = 75G, g_y = 2.062, A_y = <40G, g_z = 2.215, A_z= 43. The g and A values are typical of blue copper proteins. Redox titrations of auracyanin gave a midpoint potential of +240 mV (VS NHE) with an n=1 curve. The redox titration and ESR spectra do not give evidence for inequivalence of the coppers. It is not clear whether auracyanin functions in respiratory or photosynthetic electron transport.



Supported by a grant from the USDA Competitive Research Grants Office 84-CRCR-1-1523.

T-Pos187 HYDROLYSIS OF PEROXIDIZED PHOSPHOLIPID BY PHOSPHOLIPASES A_2 AND C.
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Lipid peroxidation modifies membrane structure and function during injury. Since lipid peroxidation by oxygen radicals also alters phospholipid composition of membranes we investigated the relationship between lipid peroxidation and the susceptibility of peroxidized phospholipid to subsequent attack by phospholipases using liposomes of 1-acyl- 2-(1-^{14}C)-linoleoyl-sn-glycero-3-phosphoryl-ethanolamine (^{14}C-PE). When ^{14}C-PE was exposed to air at pH 5.0 or pH 7.0 at 37°C more polar derivatives were formed as determined by thin layer chromatography. Up to twice as much polar lipid was formed at pH 5.0 compared to pH 7.0. Time dependent generation of thiobarbituric acid reactive substances indicated the formation of lipid peroxidation products. Hydrolysis of PE exposed to air by acid-active phospholipase C associated with cardiac sarcoplasmic reticulum was 188% of control PE and chromatographically altered, or peroxidized PE was preferentially degraded. By contrast, hydrolysis of PE exposed to air by exogenously added phospholipases A_2 was reduced approximately 80% compared to control and the chromatographically altered PE was not hydrolyzed. Similarly, the hydrolysis of peroxidized PE by neutral-active bacterial phospholipase C was reduced 95% when compared to control. These studies show that lipid peroxidation may modulate the susceptibility of phospholipid to attack by specific cellular phospholipases and may be an important determinant in membrane dysfunction during injury.

T-Pos188 **MYOFIBRIL BOUND FRUCTOALDOLASE HAS AN APPARENT EQUILIBRIUM CONSTANT DIFFERENT THAN THAT MEASURED IN VITRO.** **C. Hardin, Dept. of Physiology and Biophysics, University of Cincinnati, OH, 45267-0576**

The apparent equilibrium constant of the combined fructoaldolase (EC 4.1.2.13) and triosephosphate isomerase (EC 1.2.1.12) catalyzed reactions is lower when measured in vivo as compared to that measured in vitro (R.J. Connett, J. Biol. Chem. 260 (6):3314-3320). I examined whether the known myofibril binding of fructoaldolase might result in a decreased measured equilibrium constant of the combined reaction. A rat heart myofibril preparation with high intrinsic glycolytic activity was used to study the effects of myofibril enzyme binding on the measured combined equilibrium constant. The combined measured equilibrium was lowered an average of 23% with myofibrils. Equilibrium was approached from the reverse direction using dihydroxyacetone phosphate as substrate resulting in a 15% decrease in the combined equilibrium constant with myofibrils. This decrease was not dependent on the fibril preparation concentration, the recovery of fructose 1,6 diphosphate, or a kinetic limit on reaching equilibrium. A model is presented wherein the enzyme binding to myofibrils provides a locus of lower free energy for the fructose 1,6 diphosphate which reacts with bound enzyme compared to that which reacts with unbound enzyme.

T-Pos189 ENDOR SPECTROSCOPY OF SPIN-LABELED TRYPTOPHAN METHYL ESTER SUBSTRATES AND α-CHYMOTRYPSIN: STRUCTURE AT THE ACTIVE SITE OF ACYL-ENZYME REACTION INTERMEDIATES AND IMPLICATIONS FOR THE REACTION MECHANISM. Gregg B. Wells, Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, Illinois 60637.

Electron nuclear double resonance (ENDOR) spectroscopy and molecular modeling have been used to determine the geometries of L-tryptophan and 5-fluoro-L-tryptophan which are bound in the active site in the form of the acyl-enzyme reaction intermediate of α-chymotrypsin (α-CT) and which are acylated at the amino nitrogen with the spin-label 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxylic acid. The spin-labeled acyl-enzyme was accumulated in solution at pD 4.2 and trapped by freezing in liquid nitrogen. It is shown that electron-nucleus distances can be calculated accurately from anisotropic hyperfine coupling constants under the strong-field, point-dipole approximation. In the free substrates the α-proton and fluorine of the tryptophan are 6.8 A and 9.8 A, respectively, from the unpaired electron. Spectra of the acyl-enzyme formed from the unfluorinated and fluorinated substrates are similar and contain features from the α-proton at 6.5 A and fluorine at 9.8 A and from three groups of protons from the enzyme at 5.0 - 5.4 A, 6.3 A and 8.0 A. Conformations of the free substrates, the conformation of spin-labeled tryptophan in the acyl-enzyme reaction intermediate, and possible assignments of residues responsible for the ENDOR features from the enzyme have been derived by molecular modeling. The features from the α-proton in the free substrate and in the acyl-enzyme are spectroscopically distinguishable and indicate significantly different relative conformations of the spin-label and tryptophan side chain. These changes in conformation which occur upon binding of the substrate to α-CT are analyzed according to stereoelectronic principles. (Supported by NIH GM 21900)

T-Pos190 REVERSIBLE THERMAL DENATURATION OF IMMOBILIZED RHODANESE. Paul Horowitz and Steven Bowman, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

The enzyme rhodanese was immobilized using an N-hydroxymalonimidyl derivative of Sepharose containing a 6-carbon spacer. The number of immobilized, competent active sites was measured by using $^{35}SSO_3^{2-}$ to form an active site persulfide that is the obligatory catalytic intermediate. Soluble enzyme was irreversibly inactivated in 10 minutes at 52°. The immobilized enzyme retained at least 30% of its original activity even after boiling for 20 minutes. The immobilized enzyme had K_m and V_{max} values that were each approximately 3x higher than the corresponding values for the native enzyme. After preincubation at high temperatures, progress curves for the immobilized enzyme showed an induction period of up to 5 minutes before attaining an apparently linear steady state. The pH dependence of the activity was the same for both the soluble and the immobilized enzyme. These results indicate significant stabilization of rhodanese after immobilization, and instabilities caused by adventitious solution components are not important. The results are consistent with models for rhodanese that invoke protein association as a major cause of inactivation of the enzyme. Further, the induction period in the progress curves is consistent with studies that show rhodanese refolding proceeds through intermediate states. (Supported by NIH grant GM25177 and Welch grant AQ723.)

T-Pos191 RAMAN DIFFERENCE SPECTROSCOPY OF ADPR BOUND TO THE CO-ENZYME SITE OF LIVER ALCOHOL DEHYDROGENASE. Dehuai Chen, Kee Woo Rhee, Charlotte Martin, Donald Sloan and Robert Callender, Department of Physics and Chemistry, City College of New York, New York 10031; Kwok To Yue, Department of Physics, Emory University, Alanta GA 30322.

It is well known that the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD^+/NADH) are coenzymes of liver alcohol dehydrogenase (LADH). The adenine moiety of NADH is very important for the binding of NADH to LADH. Recently, (Biochemistry 23, 6480-6483, 1984) we published the Raman spectrum of NADH when it is bound to LADH. Here we report the Raman spectrum of adenosine 5'-diphosribose (ADPR) when it is bound to LADH. These data allow us to assign bands observed in the bound NADH spectrum to either the nicotinamide or the adenosine moieties of NADH. We find that the adenine ring protonates when either NADH or ADPR binds to LADH based on spectral behavior from deuterated samples. This protonation likely results from ion pair formation between the bound adenine moiety and some nearby amino acid residue, stabilizing the LADH/NADH (or ADPR) complex. Such information can form the basis of a molecular understanding of the Theorell Chance mechanism.

T-Pos192 RESONANCE RAMAM STUDY OF THE SUBSTRATE DABA AT THE ACTIVE SITE OF LIVER ALCOHOL DEHYDROGENASE. Charlotte Martin, Dehuai Chen, Kee Woo Rhee, Donald Sloan and Robert Callender, Department of Physics and Chemistry, City College of New York, New York 10031; Kwok To Yue, Department of Physics, Emory University, Atlanta, GA 30322; Robert Vandersteen and Johan Lugtenburg, Department of Chemistry, State University of Leiden, Leiden, Netherlands.

We have obtained the resonance Raman spectra of the aromatic aldehyde DABA (p-dimethylamino-benzaldehyde) and isotopically labelled derivatives when at the zinc containing active site of liver alcohol dehydrogenase (LADH). In agreement with previous investigations [Jagodzinski, P. W. and Peticolas, W. L. (1981) JACS, 103, 234-236; Jagodzinski, P. W. et al (1982) Biochemistry 21, 2193-2202.] we find that the resonance Raman spectrum of DABA in situ is very close to the resonance Raman spectrum of Zn^{2+} complexes with DABA in solution. On the other hand spectral changes that accompany ^2H, ^{13}C, and ^{15}N isotopic labelling of various atoms of DABA are markedly different in the two cases. Our data seriously question the idea that Zn^{2+} complexes with DABA are good models for DABA at the active site of LADH even though X-ray crystallographic studies suggest that substrates closely related to DABA are directly liganded to the Zn^{2+} cation at the active site of LADH. A quantitative analysis is now underway to understand the spectral changes which accompany the isotopic labelling.

T-Pos193 REGULATION OF PLANT CELL WALL ENZYME ACTIVITY: EFFECTS OF DIVALENT CATIONS.
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The cell walls isolated from both potato (storage tissue) and corn roots (transport tissue) were found to contain acid phosphatase activity. In the case of potato, the enzyme activity of EDTA-treated cell walls was stimulated by the presence of Mg^{2+} (up to 10 fold), inhibited by Hg^{2+}, and unaffected by Ca^{2+}. While Ca^{2+} showed no effect on Mg-stimulation, the Hg-inhibition was reduced significantly by the presence of Ca^{2+}. Kinetic analyses indicated that the binding of Ca^{2+} to the cell walls did not alter the negative cooperativity of the acid phosphatase. However, in the presence of Mg^{2+}, the kinetics followed a simple Michaelis-Menten scheme. For corn root cell walls, Mg^{2+} only slightly stimulated the acid phosphatase activity ($\sim$40%) but the protection of Ca^{2+} against the Hg-inhibition was still observed. These data suggest that function linked divalent cation binding may occur at two sites. It appears that Mg^{2+} binding occurs at a site which is different from that for Ca^{2+} and Hg^{2+}. Whether the binding involves only the protein part or the carbohydrate part of the walls or both, remains to be determined.

T-Pos194 ZINC IN BEEF HEART CYTOCHROME OXIDASE. A. Naqui[a,b], L. Powers[c], M. Lundeen[a,b] and B. Chance[a,b]. [a]Dept. Biochem/Biophys, Univ. of Pennsylvania; [b]Inst. Structural & Functional Studies, Univ. City Sci. Ctr., Phila., PA 19104; [c]AT&T Bell Labs, Murray Hill, NJ 07974

It has been reported that cytochrome c oxidase (CcO) from beef heart contains 1 Zn atom and 1 Mg atom as well as 2 Cu and 2 Fe per monomer. We have tested 35 CcO preparations prepared by different methods and can confirm that CcO from beef heart indeed contain Zn of reported ratio of Fe:Cu:Zn=2:2:1. To elucidate the role of zinc: 1) We performed both edge and EXAFS of Zn in resting cytochrome oxidase. Our results show that Zn is in a distorted tetrehedral environment. The EXAFS data is best fitted with 3 S atoms at an average distance of 2.4 A and 1 N(O) atom at 2.08 A. 2) We have performed secondary structure predictions using the Chou-Fasman method of subunits VIa and VIIIc (nomencl. of Buse) which contain 2 S-H goups each. Our analysis suggest that subunit VIa indeed contain a metal binding site with 2 cys and 1 His residues. 3) We are able to remove 33-50% of the Zinc (the Zn:Cu changes from 1:2 to 1:3-4) by incubating CcO in dipicolinic acid, followed by gel filtration. However, this decrease in zinc content does not change the rate of O_2 uptake as compared to a control experiment (29 s^{-1} vs 31 s^{-1}). Trypsin digestion also removed up to 50% of zinc. It is know that trypsin digestion removes several small subunits. Based on these results, we propose that Zn in cytochorme oxidase may play a structual role rather than a catalytic role. This is consistent with the finding that bacterial oxidase of PS3 which contains only 3 subunits does not contain Zn. This is also consistent with the coordination geometry of the Zn site which is similar to the situation in 5-amino laevulinate dehydratase where Zn is known to play a structural role.
SUPPORT: NIH grants HL 31909, RR01633.

T-Pos195 STRUCTURAL CHARACTERIZATION OF BETA-LACTAMASE INACTIVATED BY BETA-LACTAM SULFONES.
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The inactivation of beta-lactamase I by nafcillin, cloxacillin, and penicillanic acid sulfones was investigated. Using 10-^{3}H-nafcillin sulfone as inactivator it was shown that the tritium remained in the inactivated enzyme. In conjunction with spectroscopic studies this demonstrated that the inactive enzyme involved a covalent bond to the intact inactivator. UV absorbance spectra of the inactive enzyme indicated that it contained an enamine. The far-uv circular dichroism spectrum of the inactivated enzyme showed a substantial difference from that of the native enzyme, revealing a significant change in the secondary structure. Isoelectric focussing of the inactivated enzyme showed similar pI values for nafcillin- and cloxacillin-sulfone inactivated enzyme, which differed from that of penicillanic acid sulfone inactivated enzyme. Calculations on the number of exposed charged residues were also consistent with a conformational change on inactivation. Thus the nafcillin sulfone inactivated enzyme consists of an enamine acyl-enzyme which has a different conformation than that of the native enzyme.

T-Pos196 ACTIVATION OF FRUCTOSE 1,6 BISPHOSPHATASE BY DISULFIDE THIOL EXCHANGE: CONFORMATIONAL CHANGES IN THE REGION OF THE ESSENTIAL CYSTEINE RESIDUES AS ANALYZED BY MAGNETIC RESONANCE SPECTROSCOPY.

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Bovine liver fructose 1,6 bisphosphatase is markedly activated by certain disulfides e.g., L-cysine and oxidized glutathione. However, D-cysine appeared to be ineffective. Treatment of the activated enzyme with L-cysteine,glutathione(SH) reduced the extent of activation. A thiol-disulfide exchange reaction with an essential sulfhydryl group on the enzyme forming either a mixed disulfide or an intermolecular protein disulfide could account for enzyme activation. The environment of the essential sulfhydryl group as seen by ESR-Spin Labelling, appears to undergo a significant conformational change following modification of the enzyme by disulfide agents. From the change of the ESR spectra, it is suggested that activation of the enzyme by disulfide agents alters the enzyme to a most favorable conformational state. These finding suggest that modulation of the enzyme by thiol-disulfide exchange may play an important role in enzyme regulation. Circular Dichorism and Solid state NMR Studies are now in progress to characterize the nature of this conformational changes.

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