W-PM-H1

## CROSSBRIDGE STRUCTURE IN QUICK-FROZEN INSECT FLIGHT MUSCLE.

Clive Walter[MP], David Popp[MP], Carmen Lucaveche[D] and Michael K. Reedy[D]. [MP]Max Planck Inst. für Medforschung, Heidelberg, FRG and [D]Duke University, Durham, NC 27710 (Introduced by H. P. Erickson)

Our first plunge-cryofixations of glycerinated insect flight muscle of the waterbug *Lethocerus* show good preservation of crossbridge structure in rigor and relaxed fibers, but leave undecided how faithfully the variable mix of rigor and relaxed features in contracting fibers represents force-generating crossbridges. Thin bundles (1-4 fibers X 6 mm) were mounted horizontally across an inverted "V" of wire, and plunge-frozen at 1 m/sec through 60 mm of upwelling liquid ethane at -184-189 °C. Fibers were Araldite-embedded and sectioned for EM after freeze substitution in acetone (-80 °C; ≥12 hr in 0.2% tannic acid, 3X rinse, then ≥5 hr in sat'd. uranyl acetate; warmed to 20°C, rinsed & held in 100% acetone 2 hr-90 days). So far, well-frozen regions have been scarce in sections from noncryoprotected (CP-) fibers, but were readily found in cryoprotected (CP+) fibers soaked in 10% sucrose before freezing. The good-looking relaxed and rigor bridges encouraged study of CP+ fibers frozen in isometric contraction at pCa 3.0 (1 mM $Ca^{2+}$, 0 EGTA). In parallel mechanical studies, CP- fibers from the same muscle gave steady (minutes) pCa 3.0 active tension = 25-50 mg/fiber in 5 mM MgATP. 50-75% tension suppres-sion by 10% sucrose was offset by lower Mg, 2 mM with 5 mM ATP, restoring force to 66-85% of CP- values. Bridges in our best-fixed contracting CP+ fibers show a mixture of shapes and angles, only rarely rigor-like, and indicate by optical diffraction more 38.7, 19.3 & 23.2 nm and less 14.5 nm ordering than seen in relaxed fibers. Supp NIH MDA DFG

W-PM-H2

## THICK FILAMENT LENGTHS FROM $CA^{2+}$-ACTIVATED, SKINNED *LIMULUS* MUSCLE.

R.J.C. Levine. Department of Anatomy, Medical College of PA., Phila. PA.

Fiber bundles of *Limulus* telson muscle were skinned in 0.5% triton-X100 in mincing solution and some bundles were activated to contract isotonically with $Ca^{2+}$ and ATP. Thick filaments were isolated, negatively stained and photographed. Lengths of intact filaments and fragments (lacking central bare zones) were measured on EM negatives. 480 intact filaments from $Ca^{2+}$-activated muscle averaged 3.3 μm, which was also the mode (range: 2.4 - 5.7 μm). 336 of these were 2.4 - 3.5 μm, and 144, 3.6 - 5.7 μm. 42 filaments were >4.2 μm. Structures <2.4 μm lacked central bare zones. 218 filaments from skinned but unstimulated muscle averaged 4.2 μm (range 3.5 - 6.2 μm). The few fragments found appeared to be filaments severed at the bare zone during homogenization. These data are consistent with our previous results on $K^{+}$-stimulated intact fibers (Levine & Kensler, J. Mol. Biol. 182:347, '85) and *in vitro* MLC phosphorylation of thick filaments isolated from unstimulated muscle (Levine & Woodhead, Biophys J. 49:259a, '87) and support the hypothesis that thick filament shortening in *Limulus* muscle is physiological and occurs by disruption at the filament ends.

Supp. by HHS: HL15835 to the Penna. Muscle Inst.

W-PM-H3

## EVIDENCE FOR AT LEAST THREE DISTINCT CONFIGURATIONS OF ATTACHED CROSSBRIDGES IN SKINNED MUSCLE FIBERS.

L.C. Yu[+], Y. Maeda[#], and B.Brenner[*]. [+]NIH, [#]EMBL, FRG and [*]Universität Ulm, FRG. According to the crossbridge theory of muscle contraction, the structure of the crossbridges, once attached, would undergo transformation during force generation. Therefore, it is of particular interest to study the configurations of the attached crossbridges. We have obtained equatorial diffraction patterns up to [3,0] from relaxed, fully $Ca^{++}$ activated and rigor states at low ionic strength (50mM). Under these conditions, large fractions of crossbridges are attached in all three states. Thus, changes in the equatorial diffraction patterns will not be dominated by changes in the fraction of attached heads, a problem that has complicated the investigation of structural differences between different attached states in the past. Upon activation, $I_{10}$ decreased by 30% while $I_{11}$ was unchanged; a further decrease by 45% in $I_{10}$ from the activated value upon going into rigor but no change in $I_{11}$. In the activated state, $I_{20}$, $I_{21}$ were weaker than the relaxed and rigor values while $I_{30}$ was between the values of those two states. Based on the constancy of $I_{11}$, the non-reciprocal change of $I_{10}$ and $I_{11}$ and the variations in the higher orders, we conclude that differences between the diffraction patterns of the relaxed, the activated states and in rigor cannot be caused by a change in the fraction of crossbridges attached to actin nor by lattice disorder. Instead, the data suggest that average configurations of force generating crossbridges in $Ca^{++}$-activated fibers are different not only from the relaxed but also from the rigor configuration.(Supported by DFG 849/1-2,3; NATO 0769/85).

W-PM-H4

## TEMPERATURE DEPENDENCE OF PRESSURE INDUCED TENSION TRANSIENTS IN SKINNED RABBIT PSOAS FIBRES.

By Michael Geeves, Neil Fortune and Kuda Ranatunga. Intr. by H. Gutfreund, Departments of Biochemistry and Physiology, University of Bristol, Bristol BS8 1TD, U.K.

We have previously shown that the exposure of a skinned rabbit psoas fibre to 100 atm hydrostatic pressure results in a 8% reversible reduction in the calcium activated isometric tension. A rapid (< 1ms) reduction in pressure from 100 to 1 atm results in a 3 phase tension response: (i) a decline in tension in phase with the pressure change (ii) an early recovery of tension at $17s^{-1}$ (iii) a late recovery at 3.0 $s^{-1}$ (12C, Fortune et al. J Physiol in press). The temperature dependence of phases II & III were 28 & 8.3 $kJ.mol^{-1}$ repectively over the temperature range 8-20°C. At lower temperatures phase III showed a greater temperature sensitivity. The presence of 20mM Pi increased the rate of phase II and decreased phase III. The activation energies were 70 and 13 $kJ.mol^{-1}$ respectively.

Supported by the Wellcome Trust and the Royal Society.

W-PM-H5

EVIDENCE THAT CROSSLINKED ACTO-SUBFRAGMENT-1 IS DISSOCIATED BUT TETHERED IN THE PRESENCE OF ATP H.G. Zot, P. Maupin, and T.D. Pollard intro by A.S. Zot, Dept.Cell Biology, Johns Hopkins Medical School, Balt. MD 21205

We have used electron microscopy of rapidly-frozen samples to determine the effect of EDC-crosslinking on structure of weakly bound intermediates in the actomyosin ATPase cycle. In the presence of ATP, the average angle of attachment of S-1 to actin filaments was 40° with a small minority of heads attached at angles ≥90°. When S-1 was covalently attached to actin filaments by EDC, the distribution of attachment angles depended on the ATP concentration: in rigor 85% of the angles were <90° with the distribution centered about 45°-55°; in the presence of ATP the distribution of angles was nearly random except that there was a bias against angles of 80°-100°, <20°, and >160°. Attachment angles of EDC-crosslinked samples negatively stained with uranyl acetate were distributed symmetrically about 45° in rigor and randomly in the presence of ATP as originally reported by Craig et al. (PNAS 1985) and confirmed with frozen hydrated samples (Applegate and Flicker, JBC 1987). One interpretation of these results is that the weakly bound intermediates are actually attached at 45°and that the randomness observed with crosslinked acto-S-1 in the presence of ATP is due to the free rotation of tethered but dissociated heads. This work was supported by grants from the NIH and AHA Florida Affiliate (GM26132-12 and 88G-514).

W-PM-H6

MECHANICS OF GLYCERINATED MUSCLE FIBERS USING THE NONNUCLEOTIDE SUBSTRATES, NANTP AND pNANTP. R. Cooke, K. Franks-Skiba, K. Nakamaye, E. Pate, and R. Yount. Dept. Biochem. Biophys., UCSF; Dept. Chem., Gonzaga Univ.; Dept. Math., Wash. State Univ.; Biochem Biophys. Program, Wash. State Univ.

We have investigated the ability of the nonnucleotide ATP analogs, 2-[(4-azido-2-nitrophenyl)amino]ethyl (and propyl) triphosphates (MgNANTP, MgpNANTP) to support active contraction in glycerinated rabbit psoas fibers. In the absence of Ca, both analogs relax fibers. Despite its dramatically different structure from ATP, MgNANTP produces active tensions (Po), shortening velocities (Vmax), and stiffnesses 2/3 those obtained with MgATP. MgNANTPase is 1/2 that of MgATP. With MgNANTP as substrate, increasing [Pi] decreases Po while increasing Vmax, as was also previously observed with MgATP. The mechanical similarities suggest that MgNANTP binds to the active site of myosin in a manner quite analogous to MgATP, providing additional confidence in conclusions based upon the use of NANTP as a photoaffinity label for the active site. MgpNANTP on the other hand, (differing by only an extra CH2 spacer) does not support active tension or shortening. Supported by USPHS grants HL32145 (RC), DK05195 (RY), and AR39643 (EP).

W-PM-H7

EFFECT OF $Ca^{++}$ ON THE ACTIN-ATTACHMENT OF WEAK-BINDING CROSSBRIDGES IN SKINNED RABBIT PSOAS FIBERS. Th. Kraft+, L. C. Yu#, B. Brenner+. +Universität Ulm, FRG; #NIH. Fiber stiffness and the equatorial reflections [1,0] and [1,1] were used to study the effect of $Ca^{++}$ on the actin affinity of crossbridges in the presence of MgATPγS. If $Ca^{++}$ regulation in muscle acts through steric blocking of the attachment of weak-binding crossbridges, a profound effect of $Ca^{++}$ on attachment of MgATPγS crossbridges is expected. We found, however, that at full saturation of MgATPγS (in the presence of $Ca^{++}$, at least 10 mM and temperature as low as 1°C), $Ca^{++}$ increases the actin affinity of the MgATPγS crossbridges by only 2 to at most 10 fold, in close agreement with earlier biochemical findings. With truly complete saturation, the effect may be even smaller. While the fraction of attached crossbridges is only slightly affected (almost identical equatorial diffraction patterns), the kinetics of reversible actin attachment are very sensitive to $Ca^{++}$. In the presence of $Ca^{++}$, $k^-$ is reduced almost 100-fold, as revealed by stiffness-speed relations. Based on the small effect found in actin binding, $k^+$ apparently is also much reduced. At high temperature, however, large effects of $Ca^{++}$ on stiffness and on equatorial diffraction patterns, or apparent turning on of the contractile system (Brenner, et al., BJ, 53, 1988), were found to be due to incomplete saturation of crossbridges with ATPγS, particularly in the presence of $Ca^{++}$. The small effect of $Ca^{++}$ on the actin-attachment of weak-binding crossbridges is inconsistent with troponin-tropomyosin regulation via steric blocking of crossbridge attachment. (See also Brenner, Yu & Chalovich, abstract, this meeting). (Supported by DFG Br 849/1-2,3).

W-PM-H8

COVALENT CROSS-LINKING OF SINGLE MUSCLE FIBERS INCREASES THE OSCILLATORY POWER PRODUCTION, EXHIBITING AN INSECT RESPONSE. M. Kawai# and K. Tawada*. #Dept. Anatomy, University of Iowa, Iowa City, IA 52242, and *Dept. Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

Single fibers from chemically skinned rabbit psoas were treated with carbodiimide after the rigor state was induced. At 20°C, 22 minute treatment resulted in 18% cross-linking between myosin heads and the thin filament. The preparation was subjected to sinusoidal length oscillations at varying frequencies (0.25-350 Hz), and amplitude and phase shift in tension were resolved. The resulting complex modulus data were analyzed in terms of three exponential processes (A, B, C). The magnitude of process A declined with cross-linking, and oscillatory work increased. The Nyquist plot of the cross-linked fibers were reminiscent of insect fibers. The cross-linked preparations also exhibited oscillatory work at high ionic strength (0.5-1 M) solutions. We conclude that cross-linked myosin heads are capable of producing oscillatory power, that process (A) relates to rearrangements of sarcomeres, and that the enhanced oscillatory power is caused by the stabilization of sarcomeres.

W-PM-H9

RAYLEIGH IMAGING MOLECULAR MOTION IN MUSCLE.
M. Sharnoff and H. Lin, Univ. of Delaware.
The appearance of skeletal fibers in bright-field microscopy is dominated by light scattered from the filamentary backbones, whose great length concentrates their scattering into the forward direction. Crossbridges are short, and behave as Rayleigh scatterers. By abundance, they dominate the light a fiber scatters into angles near 90°. The optical phase distribution of light coherently scattered near 90° is thus determined by the constellation of crossbridge positions. Changes in that phase distribution can be deciphered holographically and tested statistically to determine whether there are patterns in the behavior of optically resolved fiber subregions each containing several thousand crossbridges. We have used a system capable of detecting subnanometric motion (Lin and Sharnoff, accompanying abstract) to record pairs of conjugate, phase-unbalanced holograms [J. Opt. Sci. Am. A 2, 1619 (1985)] made from light scattered near 90° by resting or activated fibers. We are now becoming able to subtract digitally the images reconstructed from any conjugate pair of holograms. In principle, the resulting image is a mapping, by intensity, of the distribution of mean crossbridge displacement during the holographic recording interval. Typical conjugate image pairs and subtractions will be shown.

W-PM-H10

THE MOLECULAR SWITCH IN TROPONIN C
Z. Grabarek, R.-Y. Tan, T. Tao, & J. Gergely, Dept. of Muscle Research, Boston Biomed. Res. Institute, Boston MA, USA

We have synthesized a mutant rabbit skeletal troponin C designated TnC4882, in which Cys-98 was replaced with Leu, while Gln-48 in the loop connecting helix B with helix C and Gln-82 in the N-terminal segment of the central helix were replaced with Cys. On isolation TnC4882 was found to contain a stable intramolecular disulfide bond. In comparison with troponin C isolated from rabbit skeletal muscle (sTnC), TnC4882 has a decreased affinity for $Ca^{2+}$ at the low affinity sites and a decreased affinity for troponin I. Most importantly this oxidized form of TnC4882 is only 20% as effective in activating the ATPase of the TnC-depleted myofibrils as sTnC. Upon reduction of the disulfide bond and carboxyamidomethylation of the cysteine residues the properties of TnC4882 become similar to those of sTnC. Our data are consistent with the theoretical prediction (Herzberg et al. J.B.C. 261,2638,1986) that $Ca^{2+}$-binding to the low affinity sites in TnC induces a change in the disposition of helices B and C with respect to the central helix with accompanying separation between the B-C linker and the central helix. Our data further demonstrate that this transition is a prerequisite for the biological activity of TnC. (Supported by NIH, MDA and AHA)

W-PM-H11

PROPERTIES OF SKINNED MUSCLE FIBERS FROM MYOSIN LIGHT CHAIN 2 DEFICIENT FLIGHTLESS MUTANTS OF DROSOPHILA MELANOGASTER.
M. Yamakawa, J. Warmke*, S. Falkenthal*, D. Maughan. Dept. Physiol. & Biophys., Univ. Vermont, Burlington, VT 05405, and *Dept. Molecular Genetics, Ohio State Univ., Columbus, OH 43210.

We investigate here the functional role of the myosin regulatory light chains (MLC-2) in the indirect flight muscle. Two mutant heterozygotes, *E38* and *DfB81Dp124P* (*124P*), were studied in which MLC-2 levels are reduced (Warmke, *et al.* Genetics 122:139, 1989). Compared to wild type, fibers from both mutants exhibited: 1) a similar steady active tension level, 2) a similar delayed tension rise (with <1% stretch), but 3) a tension and stiffness response less sensitive to $Ca^{2+}$ at submaximal activation. Electron micrographs revealed myofibrillar regions of nonaligned lattice near the periphery (see below). Thus the MLC-2 deficiency affects myofibrillar assembly and reduces calcium sensitivity, factors that may lead to the flightless phenotype. [Supported by NIH R01 DK33833 and GM33270]


W-PM-H12

SARCOMERE-LENGTH CHANGES IN MYOFIBRILS OCCUR IN STEPS AND PAUSES
Marc L. Bartoo, John A. Myers and Gerald H. Pollack, Bioengineering WD-12, University of Washington, Seattle, WA, 98195.

We have previously reported stepwise shortening in isolated single honeybee myofibrils (Bartoo, et. al., *Biophys J*, 53:370a, 1988.). These findings relied on sarcomere-length measurement by a phase locked loop device. We now report a new method of sarcomere-length measurement which confirms our previous findings. Dissected bundles of 2-5 relaxed rat psoas myofibrils were mounted between stiff glass needles. One end was held fixed, while movement of the second needle was precisely controlled by a piezo-electric motor. Magnified phase images of the myofibril striation pattern were projected onto a linear photodiode array. Images were digitized with a time resolution of 3.2 msec and stored in a Macintosh II computer.

We developed an algorithm to measure the average sarcomere length in a short segment of the digitized striation pattern. A segment was defined by choosing two visible points in the image, such as two different A/I junctions. Segments defined in this manner allowed measurement of a consistent set of sarcomeres during imposed changes of myofibril length. We typically measured segments containing 5-10 sarcomeres. During smoothly imposed ramp stretch or release, segments showed reproducible pauses in sarcomere length change. Measurements of the moving needle, or translation of a fixed grid pattern, showed no such pauses. It appears that stepwise length changes are a basic feature of sarcomere motion.

**W-Pos1**

DESTABILIZATION OF STAPHYLOCOCCAL NUCLEASE BY SINGLE AMINO ACID MUTATION THAT BREAKS $GLU^-$-$LYS^+$ SALT BRIDGE. J.-L. You, D. Shortle & T.Y. Tsong. Dept of Biochem, Univ of Minnesota, St. Paul, MN 55108 & Dept of Biol Chem, Johns Hopkins Univ, Baltimore, MD 21205

Glu 75 in WT possibly forms an ion-pair with a Lys linking two $\beta$ sheets and affecting other parts of the protein structure. Replacing it with a Gly (E75G) significantly destabilized the protein. Differential scanning microcalorimetry and CD were used to study its thermal stability and to compare it with that of the WT, in the pH range 4 to 11. The $T_m$ and the $\Delta H$ of unfolding of E75G were 39.2°C and 53 kcal/mol, respectively, at pH 7.0, as compared to 53.3°C and 85 kcal/mol for the WT. The $\Delta C_p$ was 1.8 kcal/mol•K for E75G and 2.3 kcal/mol•K for the WT. At 25°C, the acidic unfolding had a midpoint pH of 5.5 for E75G and 3.8 for WT, and the alkaline unfolding had a midpoint pH of 10.0 for E75G and 10.5 for WT. Other physical properties of E75G will be discussed in reference to data obtained for the WT. [Supported by NIH Grant GM 37304]

**W-Pos2**

THE IRON IN LIPOXYGENASE BY MOSSBAUER SPECTROMETRY. W.R. Dunham, R.T. Carroll, J.F. Thompson, R.H. Sands and M.O. Funk, Jr. Biophys. Res. Div. Univ. of Michigan, Agric. Res. Serv., USDA, Ithaca, and Dept. of Chemistry, University of Toledo.

Incorporation of $^{57}Fe$ into two lipoxygenase isoenzymes from soybeans permitted the use of Mossbauer spectrometry, which showed high-spin Fe(II) in the native enzyme. From model compounds and the sign of the electric field gradients, the most likely environment of the iron is oxygen and nitrogen ligands in approximately octahedral symmetry. Both enriched and natural abundance native enzymes had the same high-spin Fe(II) Mossbauer parameters confirming that the iron environments in the enzymes isolated from cultured seeds and dry soybeans are the same. The spectra (4.2-250K) after oxidation of the iron by the product of lipoxygenase catalysis were extremely broad ($\sim$20mm/s), as were the EPR signals, indicating paramagnetic broadening even at the relatively high temperatures. Treatment of the product oxidized enzyme with linoleic acid (substrate)under anaerobic conditions produces Fe(II) signals, indicating that lipoxygenase iron cycles between the ferric and ferrous states during catalysis.

**W-Pos3**

LIGAND-INDUCED BIPHASIC PROTEIN DENATURATION

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The results of a thermodynamic calculation of the excess heat capacity that incorporates the effects of ligand binding on the two-state, thermal denaturation of a protein are presented. For a protein with a single binding site on the native species and at subsaturating concentrations of ligand, bimodal or unimodal thermograms were computed merely by assuming a larger or smaller ligand association constant, respectively. The calculated thermograms for this simplified case show the salient features of those observed by differential scanning calorimetry for defatted human albumin monomer in the absence and presence of three ligands for which the protein has higher, intermediate, and lower affinity [J. Biol. Chem. 263, 15392 (1988)]. The computation demonstrates that biphasic unfolding can result from a significant increase in the free energy of denaturation (and the transition temperature) during the course of unfolding due to a substantial increase in free ligand concentration caused by the release of bound ligand by denaturing protein.

Such ligand-induced biphasic denaturation, does not relate to macromolecular substructure but derives from a perturbation, during unfolding, of the ligand binding equilibrium, which is coupled to the equilibrium between the folded and unfolded protein species. Thus, this bimodality is not limited to thermally induced unfolding but is operative independent of the means used to effect denaturation and therefore must be considered when studying any macromolecular folding/unfolding reaction in the presence of ligand.

**W-Pos4**

DIFFUSIONAL ASSOCIATION DYNAMICS OF PROTEINS; OVERCOMING THE STRICT STERIC REQUIREMENT.

Scott H. Northrup and Dennis G. Sprous, Tennessee Technological University, Department of Chemistry, Cookeville, Tennessee 38505

Protein-protein association can occur with a bimolecular rate constant on the order of $10^6$ - $10^7$ $M^{-1}s^{-1}$, which is only three orders of magnitude slower than the Smoluchowski limiting rate for orientationally nonspecific encounter of spheres. However, based purely on geometric estimates, requiring complementary functional groups at protein interfaces to encounter to within a couple of Angstroms of tolerance should reduce the Smoluchowski rate by six orders of magnitude. In this study the Brownian Dynamics simulation method is employed to compute the rate of association of neutral spherical model proteins with highly anisotropic reactivity. The association rate is accelerated to that of real systems only when a realistic short-ranged nonspecific hydrophobic attraction term is added which prolongs protein encounters such that rotational diffusion can bring complementary groups into proper stereospecific alignment. Local specific forces between complementary groups at the interface are not sufficient by themselves to account for the observed high rates of protein association.

W-Pos5

## PROTON-LINKED CONFORMATIONAL SWITCHING IN THE PHAGE LAMBDA cI REPRESSOR

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The lysogenic to lytic switch of bacteriophage lambda operates via cooperative binding of cI repressor dimers to specific DNA sites of the operators $O_R$ and $O_L$. Active repressor dimers are in equilibrium with monomers. Using $^{35}S$ labeled repressor (1) we studied the monomer-dimer equilibrium by large zone analytical gel chromatography (2) over the pH range 5-8. We find a dramatic (i.e. 18%) decrease in apparent molecular radius with increasing proton activity between pH 7 and pH 6. Previous work indicates an enhanced contribution of proton absorption to the energetics of repressor-DNA binding in this same pH range (3). These results suggest a hitherto undiscovered regulatory process in the conformational (allosteric) switching of repressor molecules.

(1) Beckett, D. and Ackers, G.K., In preparation.
(2) Ackers, G.K., (1975) The Proteins (Neurath, H. and Hill, R.L., eds.) 3rd ed., Vol. 1. Academic Press, N.Y.
(3) Senear, D.F. and Ackers, G.K., In preparation.

W-Pos6

## NMR AND CD STUDIES OF TRIPLE-HELIX FOLDING.

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The use of different spectroscopic techniques to measure kinetics of protein folding can indicate the existence of intermediates. Folding of the collagen model peptide $(Gly\text{-}Pro\text{-}Pro)_{10}$ was studied by NMR, monitoring intensity of the upfield Gly α H resonance and by circular dichroism (CD), measuring ellipticity at 227nm. CD indicated a fast early step followed by a slower third order reaction, while NMR data monitored only a third order reaction. Both reached 100% of the original helical content after 20 hours. CD, but not NMR, detects a transformation of single chains from a flexible to a more extended structure which accounts for the fast folding step. Both methods monitor the association of chains to form a triple-helix, which gives a third order reaction. The folding of $(Gly\text{-}Pro\text{-}Hyp)_{10}$ was greatly accelerated compared with $(Gly\text{-}Pro\text{-}Pro)_{10}$. We are in the process of examining whether the presence of hydroxyproline accelerates the single chain transformation or chain association.

W-Pos7

## GLYCEROL EFFECT ON ACRYLAMIDE QUENCHING OF PROTEIN FLUORESCENCE,

Maria Punyiczki*, John A. Norman and Andreas Rosenberg, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA, and *Department of Biochemistry, University Medical School, Debrecen, Hungary.

Viscosity dependence of fluorescence quenching reactions in proteins is sometimes used to test the validity of different kinetic models of structural movement of the matrix. Addition of high levels of cosolvent, however, affects the properties of the macromolecule. In order to dissect these chemical effects from the viscosity effects, we have studied the acrylamide quenching of fluorescence from Human Serum Albumin and determined the putative association constant ($\beta k_{eq}$ = 3.25 $M^{-1}$) and static quenching constant (V = 3.57 $M^{-1}$). Increasing glycerol concentration results in increases of both constants, reaching 4.1 and 4.34 respectively, at 60 % glycerol. We have carried out equilibrium dialysis measurements with $^{14}C$ labeled acrylamide in order to determine the concurrent change of excess acrylamide in the protein phase. (supported by NSF/DMB-8704740)

W-Pos8

## THE MECHANISM OF CYTOCHROME C FOLDING.

K.M. Pryse & E.L. Elson (Introduced by John A. Cooper); Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO.

The reversible folding of cytochrome c has been studied by repetitive pressure perturbation kinetics (RPPK) (Clegg, R.M., Elson, E.L. & Maxfield, B.W. (1975) Biopolymers 14, 883). Two folding steps have been characterized over a wide range of pH, ionic strength, and denaturant concentration. A model based on a three-state mechanism can quantitatively account for all of the kinetic and equilibrium data. Kinetic experiments in the Soret and 695 nm absorbance bands show clearly that the axial ligand Met-80 is involved in the slower folding process, and the other axial ligand, His-18, in the faster step. Additional experiments with fluorescence detection of the folding kinetics support an interpretation of the model in terms of known structural elements of cytochrome c. In addition to the rates, the RPPK method provides information about the thermodynamic volume change of each folding process. Under most conditions an increase in pressure causes unfolding ($\Delta V < 0$), but at very low ionic strength, the volume change of the Met-80 step becomes positive, while the other remains negative. Under other conditions (pH = 2.5, 0.1M $Cl^-$) where a state similar to a "molten globule" state has been suggested we observe a change in the mechanism of folding. This work provides new information about the mechanism of folding of cytochrome c, and demonstrates the applicability of the repetitive pressure perturbation kinetics method to protein folding.

W-Pos9

## ELECTRON TRANSFER BETWEEN FERRICYTOCHROME *c* AND FERRO-CYTOCHROME *b*5; DIFFUSION THEORY AND EXPERIMENT.

Robert G. Herbert[1], Scott H. Northrup[1], Lindsay D. Eltis[2], Paul D. Barker[2], and A. Grant Mauk[2]
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The kinetics of association and electron transfer (et) from ferrocytochrome *b*5 and its heme-esterified derivative to ferricytochrome *c* have been recently studied under a variety of solution conditions by anaerobic stopped-flow spectroscopy. Rate constants are quite large ($\sim 10^8 M^{-1}s^{-1}$ at ionic strength $\mu$ = 0.1, pH=7.0) and show a strong inverse dependence on $\mu$, indicating the possible role of protein-protein electrostatic interactions in the important diffusional association stage. We describe how a combination of electrostatic analysis based on xray-determined coordinate sets, static-accessibility modified Tanford-Kirkwood pK calculations, and Brownian dynamics simulation are used to predict the $\mu$ and pH dependence of the et rates, accounting for the coupling of electrostatics and diffusion. The $\mu$ dependence is successfully reproduced when the protein dipoles are aligned along the likely axis of et. Implications of structural and electrostatic properties of proteins on the association and et function is discussed.

W-Pos10

## Calorimetric Analysis of Calcium Binding to Calmodulin

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The binding of calcium ions to bovine brain calmodulin was studied by microcalorimetric titration in 25 mM PIPES, 0.1 M KCl, pH 7.5, over the temperature range from 25 C to 52 C. Although the heats evolved are weakly positive at room temperature, they become strongly negative at temperatures above physiological. The additional information provided by the change with temperature (heat capacity) permits a thermodynamic analysis (extended to dCp/dT) in terms of four macroscopic binding constants. The nature of the macroscopic stepwise binding constants is such as to suggest that site heterogeneity is the dominant factor in the binding isotherm. The heat effects, however, show behavior consistent with a conformational transition (protein folding) centered around the binding of the third calcium.

At this writing, no simple microscopic (molecular) model has been found to describe the data adequately. The various shortcomings of several seemingly plausible models will be discussed, as will any as yet undiscovered successful model.

W-Pos11

## CALCULATIONS OF THE C-PEPTIDE ELECTROSTATICS IN SOLUTION.

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The method based on the Boundary Element technique is used to study electrostatic interactions in the C-peptide in water. It is found that a significant part of the unusual stability of the C-peptide can be accounted for by the Glu2-Arg10 salt bridge found in the ribonuclease A (~2kcal/mole). We do not find any repulsive interactions between the charged groups and the backbone charges of the $\alpha$-helix in water contrary to the predictions from the helix-dipole hypothesis. We find that the conformations of the side chains are often dictated by local interactions, and do not conform to the expectations from this hypothesis. The observed destabilization of the helix by the charge on the N-terminus is found to be determined by better local interactions of this charge with the backbone in the extended conformation than in the folded helix. It is also found that the backbone electrostatics contributes less than half of the energy driving the helix-coil transition.

W-Pos12

## CHARACTERIZATION OF THE *ESCHERICHIA COLI* ASPARTATE RECEPTOR BY DIFFERENTIAL SCANNING AND TITRATION CALORIMETRY.

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The *E. coli* aspartate receptor is a 60 kDa protein, which consists of a single polypeptide chain. The region proximal to the n-terminus is a membrane-spanning, ligand-binding portion, and the region proximal to the c-terminus is a cytoplasmic portion involved in signaling. We have used differential scanning calorimetry (DSC) to further define the domain structure of the receptor. DSC studies of a cloned, soluble c-terminal fragment of receptor (~30 kDa, ref. 1) have revealed the presence of more than one cooperative unit, which can be interpreted as evidence for the presence of more than one domain in this fragment. Missense mutants of the c-terminus, which are 'locked' into either the smooth-swimming or tumbling signaling states (ref. 1), have different patterns of domain stability. Also titration calorimetry has been used to measure the binding of aspartate to the homologous receptor from *S. typhimurium*. Values for the dissociation constant ($\mu$M) for aspartate and the enthalpy of binding (kcal·mol$^{-1}$) were each found to be ~10.

1) Oosawa, K., N. Mutoh and M. I. Simon (1988) *J. Bacteriol.* **170:** 2521-2526.

W-Pos13

CALORIMETRIC AND SPECTROSCOPIC STUDIES OF THE UNFOLDING OF APOLIPOPROTEIN B (ApoB) Mary T. Walsh and David Atkinson, Dept. of Biophysics, Boston Univ. School of Medicine, Boston, MA

ApoB, the structural protein of low density lipoprotein (LDL) and ligand for the receptor-mediated uptake of LDL from the circulation, is a monomeric protein of MW 550 kD. The unfolding of ApoB in LDL, its native lipid environment, and in micelles of sodium deoxycholate (NaDC) has been studied by high resolution calorimetry and circular dichroic spectroscopy. In, LDL ApoB undergoes two transitions. The first is reversible ($T_m$ 57° ΔH 0.20 cal/g ApoB) and involves a tertiary structural change in the entire ApoB molecule with no change in secondary structure. The second ($T_m$ ~74°, ΔH 0.99 cal/g ApoB) corresponds to disruption of LDL and irreversible denaturation of ApoB. In NaDC, ApoB undergoes three transitions. The first two are reversible ($T_m$'s 50°, 56° and ΔH's 1.13 and 2.55 cal/g ApoB) and coincide with secondary and tertiary structural changes. Deconvolution of these two transitions show five 2-state transitions to occur at discrete temperatures, suggesting that individual domains of ApoB may be unfolding independently. Irreversible denaturation of ApoB occurs at $T_m$=67° (ΔH 0.54 cal/g ApoB).

W-Pos15

THE UNIVERSITY OF HOUSTON BROWNIAN DYNAMICS PROGRAM. Malcolm E. Davis, Jeffry D. Madura, and J. A. McCammon, Dept. of Chem., Univ. of Houston, Houston, TX 77204-5641. Brownian dynamics simulations are becoming an important tool for studying bi-molecular encounters. To aid in the application of this technique, we have developed a general purpose Brownian dynamics simulation package with a user friendly interface. The interface allows for easy entry of input parameters, choice of charge models, and the ability to manipulate coordinates and selectively vary parameters. The program takes molecular coordinates and efficiently calculates Brownian trajectories, statistics, and ultimately bimolecular diffusion-controlled rate constants. Provision is made for flexible molecules and complex reaction criteria. The long range electrostatic interactions are determined from a finite difference method, that accounts for the irregularly shaped low dielectric protein interior, atomic partial charges, the high dielectric solvent, and mobile salt ions. In addition to Brownian dynamics, the program can be used for calculations of electrostatic energies and forces important in many areas of biomolecular interest, including binding and $pK_a$ shifts. An application of the program demonstrating its potential will be presented.

W-Pos14

SELF-ASSOCIATION OF SCAFFOLDING PROTEIN FROM BACTERIOPHAGE P22. D.A. Yphantis(1,2), J.Lary(1), P.E. Prevelige(3) and J.King(3) (1)Molecular and Cell Biology Department and Analytical Ultracentrifuge Facility, University of Connecticut, Storrs,CT.06268 (2)Institute of Molecular Biology and Biotechnology, University of Crete, Iraklion, Crete, Greece; (3)Department of Biology, Massachusetts Institute of Technology, Cambridge, MA. 02139

Purified scaffolding protein from bacteriophage P22 of Salmonella Typhimurium was examined by equilibrium ultracentrifugation. Conditions were those used in studies of the regulation of the in vitro polymerization of P22 coat protein subunits into icosahedral procapsid shells. At the lowest protein concentrations (below about 1 g/l) we saw only an apparently homogeneous monomer-trimer equilibrium with a standard free energy of association of about -11.5 kcal/mole of trimer at 21 C. At higher concentrations we observed further massive associations: the system no longer appeared to be simply reversible, with time-dependent polymerization to molecules ranging in size to over 3.5 million (100 monomers). Supported by NSF grant DIR-8612159 and PHS grant pqr-33333.

W-Pos16

BOHR EFFECT ENERGETICS IN SINGLE SITE MODIFIED HEMOGLOBINS G. J. Turner, G. Lew, M. Daugherty, and G. K. Ackers. Deptartment of Biology, The Johns Hopkins University, Baltimore, MD 21218

The proton linked energetics of cooperative oxygen binding has been investigated for a series of human hemoglobins possesing single amino acid alterations. The hemoglobins studied involve sites implicated as critical to cooperativity and the Bohr effect. They include: Cowtown (β146 His→Leu), des-His (β146 deleted), Bunbury (β94 Asp→Asn), Barcelona (β94 Asp→His), Kariya (α40 Lys→Glu), Ypsilanti (β99 Asp→Tyr), Rothschild (β37 Trp→Arg), des-Arg (α141 deleted), and cyanomet ($Fe^{+3}CN$).

The Bohr contributions to the energetics of cooperativity ($\Delta G_{c,Bohr}$) are determined from the difference in energetics of dimer-to-tetramer subunit association for fully ligated and unligated hemoglobins at well defined pH values (0.1M buffer: Tris-base @ pH 7.4-8.5, or Glycine @ pH 8.5-9.5; 0.1M NaCl; 21.5°C). The patterns of the ligated subunit assembly energetics are observed to be strikingly similar to those of normal hemoglobin $A_O$. The patterns of the deoxy subunit assembly energetics show trends similar to $A_O$, i.e. decreasing stability with decreasing proton activity , but with variable shapes. Deoxy Ypsilanti tetramers increase in stability as pH is decreased, suggestive of a reverse Bohr effect.

$\Delta G_{c,Bohr}$ resolved imply: 1) significant structural compensation resulting from single amino acid alterations 2) the Bohr effect can not originiate solely from a few surface histidine residues 3) putative β chain carboxyl terminus ion pairs are not dominant energetic participants in the Bohr mechanism under these solvent condtions.

**W-Pos17**

IDENTIFICATION OF SUBPROCESSES OF PROTEIN LINKAGE SYSTEMS BY COMPENSATION TEMPERATURES. Rufus Lumry, Chemistry Dept., University of Minnesota, Minneapolis, MN 55454. Because of the weak coupling between subprocesses of protein linkage systems and the dynamical characteristics of conformations, linear–free–energy behavior and/or the corresponding linear enthalpy–entropy compensation patterns are nearly ubiquitous. They are characterized by the values of the slopes of the latter, called the "compensation temperatures", Tc. It is found that these fall in a small number of narrow ranges with relatively small overlapping which suggests that each range reflects a single kind of subprocess. So far this is experimentally supported and the range in which a given Tc value falls identifies the responsible subprocess. In this way some subprocesses can be detected as participants in different manifestations of protein behavior. Thus in proteins constructed using the knot–matrix principle processes involving distortion or destruction of knots yield Tc values near 330K; Tc values near 440K indicate matrix subprocesses. The Tc ranges thus far identified and their respective subprocesses, where known, will be presented with a critique of the method. Some ranges vary with the type of protein so the simplicity discussed above may obtain only for knot–matrix proteins; e.g. enzymes, proteinase inhibitors. Free–energy management in the operation of linkage systems is determined by the Tc values of its subprocesses. Those with Tc values close to the operating temperature serve only as coupling devices but they play a major role in determinine conformational dynamics. Some discussion of these matters will be given.

**W-Pos18**

**PROTHROMBIN AND FRAGMENT 1 SELF-ASSOCIATION: REVERSIBILITY, STOICHIOMETRY AND ASSOCIATION CONSTANTS DETERMINED BY ANALYTICAL ULTRACENTRIFUGATION.** [1]Lyons, D.A., [2]Esmon, C.T., [3]Nelsestuen, G.L. and [1]Laue, T.M., [1]Dept. Biochem., U. New Hampshire, Durham, NH 03824, [2]Howard Hughes Medical Institute, Oklahoma City, OK 73104, [3]Dept. Biochem., U. Minnesota, St. Paul, MN 55108 There has been considerable confusion concerning whether or not the self-association behavior of the N-terminal domain of prothrombin (F1) is maintained in intact prothrombin (PT). Bovine and human PT and bovine F1 were examined at 23.3 °C, in 0.1 M NaCl, 50 mM Tris (pH 7.6), 1 mM benzamidine and either 2 mM $Ca^{2+}$ or 50 mM EDTA. Distinctly different association behaviors are observed for these two molecules. PT consistently forms a small quantity of calcium-dependent, non-equilibrating aggregates past dimer. EDTA reverses this aggregation and recalcification results in identical reaggregation. Aggregation is not influenced by the length of exposure to $Ca^{2+}$, by the pathway of recalcification, species or purification protocol. In contrast, F1 exhibits a $Ca^{2+}$-dependent 1:2:4 self-association described by a 2:1 Kd ~750 $\mu$M and a 4:1 Kd ~$1x10^{-10}$ $M^3$. 2 mM $Mg^{2+}$ does not support self-association. Supported by NSF BBS 8615815, NIH R01 HL29807 and AHA 871092.

**W-Pos19**

**EFFECTS OF CALCIUM-BINDING PROTEINS ON PHOSPHOFRUCTOKINASE AND CHARACTERISTICS OF THEIR BINDINGS**

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**Phosphofructokinase (PFK) is a calmodulin (CaM) binding protein. We found that troponin C (TnC), which is homologous to CaM, also binds PFK and effects PFK's catalytic activity, aggregation states and conformational changes as CaM does in most cases. PFK titration of AEDANS-TnC showed that their apparent dissociation constant is comparable to that of PFK-CaM. Fluorescent labels were also used to probe contact regions on TnC and CaM. It is likely that the C-terminal end of the connecting strand of the TnC molecule is close to PFK in the binary complex. Hydrophobic regions of TnC and CaM also possibly play roles in the binding and in the polymerization of PFK. TnC and CaM deactivate PFK through accelerating PFK confomational change as well as through accelerating PFK tetramer dissociation, as implied in the results of activity, light scattering, fluorescence and circular dichroism experiments. The intact molecule of CaM appears to be required to deactivate PFK because neither half of the CaM molecule has an effect on PFK activity.**

**W-Pos20**

**$CA^{2+}$-DEPENDENT INTERACTION OF PROTEIN C WITH THROMBIN AND THE ELASTASE FRAGMENT OF THROMBOMODULIN. ANALYSIS BY ANALYTICAL ULTRACENTRIFUGATION.** [1]Olsen, P.H., [2]Esmon, C.T., [2]Esmon, N.L. and [1]Laue, T.M.1 Dept. Biochemistry, U. New Hampshire, Durham NH 03824 and [2]Howard Hughes Medical Institute, Oklahoma City, OK 73104.
Previous studies have shown that Thrombin (T), when bound to the elastase fragment of thrombomodulin (elTM), activates protein C (PC) maximally in 0.3 mM $Ca^{2+}$. When examined in 0.1 M NaCl, 50 mM Tris (pH 7.65), 1 mM benzamidine, and containing either 2 mM EDTA, 0.3 mM $Ca^{2+}$ or 5 mM $Ca^{2+}$, active-site-blocked bovine T (37.9 kDa), rabbit elTM (33.5 kDa) and PC (either human, 61 kDa or bovine, 58 kDa) are monomeric. Pairwise mixtures of these reveal that the T-elTM complex (Kd< 10 nM) is $Ca^{2+}$-independent, that the PC-T complex (Kd ~180 $\mu$M in EDTA) is weakened significantly by 5 mM $Ca^{2+}$ or by removal of the Gla domain of PC and that the PC-elTM complex (Kd ~400 $\mu$M in EDTA) is weakened slightly by 5 mM $Ca^{2+}$. A tight, ternary complex of T-elTM-PC forms in EDTA, but dissociates in 5 mM $Ca^{2+}$ into PC and T-elTM, suggesting that PC-T and PC-elTM binding energies are additive. Supported by NSF BBS 8615815, AHA 871092 and NIH R37 HL 30340.

W-Pos21

MOLECULAR BASIS FOR MULTINUCLEAR NMR RELAXATION OF WATER IN PROTEIN SOLUTIONS: PROTEIN CONCENTRATION/ACTIVITY FIELD DISPERSION AND pH/pD DEPENDENCES.

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The current model of protein hydration assumes that water molecules bind to the protein surface where they rotate anisotropicably with a slow (ns) and a fast (ps) motion and that they also exchange fast between a 'bound' and a 'free' state. We have analyzed $^{17}O$ and $^{2}H$ NMR data on lysozyme and other proteins at various pH values and in the absence or presence of salt. Relaxation measurements at different magnetic fields permit the calculation of the amount of 'bound' water and the correlation times that characterize its motions, the results being affected by protein activity. The extent of intermolecular protein interactions are estimated from NMR measurements as a function of protein concentration. The effect of protein aggregation on the measured relaxation rates as well as their pH/pD dependence are quantitatively analyzed through thermodynamic linkage. Non-linear regression fittings of the field dispersion of $^{2}H$ NMR longitudinal relaxation require two independent relaxation contributions.

W-Pos23

DIRECT OBSERVATIONS OF LIGAND DYNAMICS IN HEMOGLOBIN BY SUBPICOSECOND INFRARED SPECTROSCOPY:

P.A.Anfinrud,C.Han and R.M.Hochstrasser (Intro. by Leland May) University of Pennsylvania.

The photodissociation of CO from HbCO is studied by means of a femtosecond IR technique. The bleaching of the FeCO absorption and the appearance of a new IR absorption near that of free CO are both observed at 300 fs. The bleach does not recover on the time scale of a few ps but does by ≈ 4% within 1 ns, which suggests that a barrier to recombination is formed within a few ps. The CO spectrum does not change significantly between 300 fs and 1 ns suggesting that the CO quickly finds locations in the heme pocket not more than a few $A^{o}$ from the iron. The de-ligated CO appears in its ground vibrational level. There is evidence that 85 ± 10% of this CO remains in the heme pocket at 1 ns; it probably resides there for 50 ns. The flow of vibrational energy from the heme to the solvent was observed. The heme cools within 1-2 ps while thermal disruption of the surrounding solvent structure requires ≈ 30 ps.

W-Pos22

**Correlation Between Protein Stability and Nonpolar Surface Area,** **Jeff R. Livingstone† and M. Thomas Record, Jr.†, Departments of Chemistry† and Biochemistry††, University of Wisconsin-Madison**

The transfer of non-polar solutes from water to a non-aqueous phase and the folding of globular proteins both exhibit large negative changes in standard heat capacity ($\Delta C_P^o$). Both of these processes remove large amounts of *nonpolar* solvent-accessible surface area (NSA) from water, and the reduction in solute-water interactions is primarily responsible for the thermodynamic similarity of these processes. A recent compilation of available data from several different sources (Spolar, *et al., Proc. Natl. Acad. Sci. USA*, in press) demonstrated that $\Delta C_P^o{}_{tr}$ for the transfer of non-polar solutes from water to the pure liquid state and $\Delta C_P^o{}_{fold}$ for the folding of proteins exhibit the same proportionality to the change in NSA. To extend and quantify these observations for a wider range of hydrocarbon and protein structures using a completely self-consistent method, we have used Connally's analytical molecular surface area method in conjunction with hydrocarbon and protein crystal structures from the Cambridge and Brookhaven databases to determine changes in the the amount of NSA upon transfer and unfolding. Correlations between these calculations and reported values of $\Delta C_P^o{}_{tr}$ and $\Delta C_P^o{}_{fold}$ will be presented and interpreted at a thermodynamic level.

W-Pos24

DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDY OF THE HEAT-INDUCED REVERSIBLE, PARTIAL UNFOLDING OF DODECAMERIC GLUTAMINE SYNTHETASE (GS) FROM E. COLI.

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Evidence for temperature-induced, reversible (two-state) transitions of two domains of GS has been obtained from Trp and Tyr spectral perturbations at pH 7.0 (50°C) in 100 mM KCl and varying [$Mn^{2+}$] (Biochemistry 28, 6281, 1989). Under the same conditions, repetitive DSC scans from 15-67°C at 45°/h (using the MicroCal-2 DSC) with 6-18 mg of GS (620000 $M_r$) in 1 mM $MnCl_2$ showed a maximum endotherm ($T_m$) at 52°C and $\Delta H_{cal}$ = 180 ± 8 kcal (mol dodecamer)$^{-1}$. After DSC experiments, GS was >95% active. Values of $T_m$ were increased 7° by the presence of 150 mM L-glutamine and decreased 8° by increasing [$Mn^{2+}$] to 10 mM. The observed DSC curves can be expressed within experimental error as the sum of two independent partial unfolding transitions with $T_0$ and $\Delta H_{vH}$ parameters from spectral studies. The DSC results indicate that the cooperative unit is the dodecamer (rather than the subunit) and that two independent two-state transitions can adequately account for DSC endotherms. The partial unfolding of GS therefore is highly cooperative.

**W-Poe25**

Fluorescence of the papain:ACRYLODAN complex at 300 K and at 10 K.

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According to molecular dynamics simulation studies a fluorescence label 6-acryloil-2-(N,N-dimethyl)aminonaphthalene, ACRYLODAN [Prendergast et al., (1983) J. Biol. Chem. 258, 7541], covalently bound to Cys25 at the active center of the proteolytic enzyme papain [EC 3.4.22.2] can assume a large number of embedding substates due to a positional freedom within the papain catalytic cleft. Fluorescence emission energy distribution and intensity decay of the papain:ACRYLODAN complex at 300 K and at 10 K were analyzed in terms of our theoretical model [Ilich & Prendergast (1989) J. Phys. Chem. 93:4441] and demonstrate a persistence of certain, cryogenically isolated embedding substates in the averaged, room temperature papain dynamics. The significance of these data is extrapolated to the general problem of the synthetic and natural chromophore environment in photophysical studies of protein structure and dynamics.

**W-Poe26**

MULTINUCLEAR SPIN RELAXATION STUDIES OF MYOSIN HYDRATION AND SELF-ASSOCIATION IN ELECTROLYTE SOLUTIONS.

I.C.Baianu*, A. Mora * and P.J. Bechtel+, University of Illinois at Urbana, Depts. of *Food Science and+Animal Science,*AFC NMR Facility and +Muscle Biology Laboratory, Urbana, IL 61801.

The self-association and hydration behavior of Myosin A and B separated from beef skeletal muscle were studied by NMR over a wide range of hydration and salt concentrations. The multinuclear spin relaxation results were analyzed with a thermodynamic linkage model, assuming that both ion binding and chemical exchange contribute to the measured relaxation rates. The apparent association constants for myosin A, the electrolyte activity and the myosin hydration were determined from nonlinear regression analyses of the nuclear spin relaxation data. The chemical exchange contribution reflects the preferential binding of ions to myosin. Ion-specific effects were observed for the first time by NMR for $Na^+$ and $K^+$. The observed self-association and hydration behavior of myosin A and B is broadly similar to that reported previously for beef skeletal muscle proteins[1].

REFERENCE CITED
1. I.C. Baianu et al.(1988),Biophys.J.53:74a

W-Pos27

**MOLECULAR MODELING AND MECHANICS OF AN INSULIN ANTIBODY** L.J. Nell, J.J Tanner, and S. Subramaniam. Baylor Coll. Medicine & Univ. of Houston, Houston, TX.
A 3-dimensional model of an insulin antibody, 125, was constructed based on conserved or canonical residues in other known crystallographic immunoglobulin structures. Molecular modeling and mechanics were done with the 125 amino acid sequences using QUANTA and CHARMM on a Silicon Graphics 4D70GT workstation and high temperature dynamics done using the CHARMM19X force field of the XPLOR package on a Stellar GS1000. A minimal model was made by scaffolding using crystallography coordinates of the antibody having the highest amino acid sequence homology with 125, HyHEL-5 (84% light chain, 65% heavy chain). Three hypervariable loop turns that are longer in 125 than in HyHEL-5 were modeled separately, incorporated into the HyHEL-5 structure, then amino acid substitutions were made and torsions optimized. Alternate conformations of the structure were obtained using simulated annealing. The 125 models maintain structures conserved in known antibodies, yet have unique antigen binding sites. In this site are many polar amino acids (especially serines) whereas the overall van der Waals surface shape is determined by positions of aromatic side chains. We predict that H bonds are important in binding the insulin A chain loop epitope by 125.

W-Pos28

ELECTRON MICROSCOPY OF MICROCRYSTALS OF THE ANNEXIN, p68 by Barbra L. Gabriel[1,2], Carl E. Creutz[1], and Robert H. Kretsinger[2], Departments of Pharmacology[1], and Biology[2], University of Virginia, Charlottesville, VA 22901

Microcrystals of p68, a calcium sensitive membrane binding protein, were examined by electron microscopy. Negative staining provides a direct view of the a, c lattice face (010 projection). Optical transforms index with periodicities of 64 $A^{-1}$ and 275 $A^{-1}$. The a axis spacings are broad and appear to be bimodal with maxima at 55 $A^{-1}$ and at 77 $A^{-1}$. We attribute this effect to distortions arising from preparation. A prominent meridional reflection on the fourth layer line suggests the presence of a (pseudo) 4-fold screw axis in the c direction. These data agree with those of Newman et al (J. Mol. Biol. 1989 206:213-219). From x-ray diffraction they determined the space group of the crystals to be $I4_1$ with a = b = 68.14 Å and c = 256 Å.

Embedded and sectioned crystals provide projections of the p68 crystals along the c axis. Optical transforms of such cross-sections index with periodicities of 58 $A^{-1}$ by 61 $A^{-1}$ with $\gamma^* = 88°$. These data are inconsistent with a tetragonal or orthorhombic space group. To further test these results p68 crystals will be studied by electron diffraction.

W-Pos29

CRYSTALLIZATION OF COLICIN E1 PORE FORMING FRAGMENT. Patricia Elkins, Andrew Fisher, Rod Merrill, William Cramer and Cynthia Stauffacher, Dept. of Biology, Purdue University, West Lafayette, Indiana, 47907.
Colicin E1 is a bacteriocin whose toxic effect is caused by forming a pore in the cytoplasmic membrane. This protein has multiple functional states, as it exists as a typical soluble protein in the extracellular medium, is transported across the outer membrane of a target cell and then converts to a pore forming protein in the cytoplasmic membrane. This change of state can be mimicked in vitro with a change of pH from 8.0 to 4.0. Fragments of colicin E1 that retain the ability to form pores have been obtained by cleavage with both thermolysin and trypsin. Large single crystals have been obtained from these fragments in the high pH form. Tetragonal rod-shaped crystals grow from PEG 4000 in Tris buffer at pH 7.0-8.5. The crystals grow to as large as 1.5mm in length and diffract to 2.4A. The space group is I4 with unit cell parameters of a = 102.8A, c = 35.6A. Native data for both of these fragments have been collected and heavy atom derivatives are being screened. Very small crystals of the low pH form have also been observed.

W-Pos30

EVIDENCE FOR A LEFT HANDED ALPHA-HELIX IN AN EGG SHELL PROTEIN FROM SCHISTOSOMA MANSONI. C. R. Middaugh*, J. A. Thomson§, H. Ji+, H. Mach+, B. A. Johnson# and J. S. Cordingley+. +Dept. Mol. Biol., Univ. of Wyoming, Laramie, WY 82071; *Merck Sharp & Dohme Res. Labs, West Point, PA 19486; and #Rahway, NJ 07065-0900; §Vertex Pharm., 40 Allston St., Cambridge, MA 02139.
The eggshell of Schistosoma mansoni contains an unusual protein which consists of more than 80 repeats of the pentapeptide GYDKY followed by a his rich region. The synthetic peptide $(GYDKY)_6$ was constructed to model the largest part of this protein and its structure was examined by a variety of physical techniques. Employing a combination of CD, FTIR, NMR, $H^+$ ion titration and computer assisted molecular modeling, the most probable structure of this unusual tyrosine rich repetitive sequence appears to be that of a left-handed alpha-helix. Similar studies of a model his rich peptide are also consistent with such a structure. This is the first evidence for the existence of this type of secondary structure in a naturally occuring protein since it was theoretically postulated by Pauling, Corey and Branson in 1951. (Supported in part by ONR Contract No. N00014-87-K-0480.)

**W-Pos31**

SOLUTION STRUCTURE OF NEURONAL BUNGAROTOXIN. R.E. Oswald[§¥], M. Bamberger[§¥], M. Sutcliffe[¥], R.H. Loring,[¶] R.E. Zigmond,[†] & C.M. Dobson[¥]. [§]Dept. Pharm., Cornell Univ., Ithaca, NY; [¥]Inorganic Chem. Lab., Univ. Oxford, Oxford UK; [¶]Dept. Pharm., Northeastern Univ. Boston MA; [†]Center for Neurosciences, Case Western Reserve, Cleveland OH.

Neuronal bungarotoxin (nBgt) is a minor protein component of the venom of *Bungarus multicinctus* which binds specifically and with high affinity to neuronal nicotinic acetylcholine receptors (nAChRs) in the peripheral nervous system. The purpose of this project is to determine the three dimensional solution structure of nBgt and compare it with the known structures of αBgt and homologous neurotoxins specific for skeletal muscle nAChRs in an attempt to explain the unique specificity of nBgt. From a series of 2D NMR experiments, we have identified the resonances belonging to greater than 90% of the amino acid residues of the protein and identified their position on the sequence. From this, we have assigned greater than 70% of the crosspeaks observed in the NOESY spectrum, which provides information on the distances between specific atoms in the protein. This has provided us with approximately 550 distance constraints for use in structure determination. Using the distance constraints from the NOESY spectra, the dihedral constraints from the COSY spectra, and the known disulfide bonding pattern, we have begun to calculate the 3D structure of the protein using distance geometry, restrained molecular dynamics and energy minimization. Preliminary evidence suggests that the triple stranded antiparallel β sheet found in all other long and short neurotoxins may be considerably distorted in nBgt. Although additional resolution will be required, differences in the 3D structures of nBgt and αBgt seem to exist and may be related to the differences in specificity of the toxins.

**W-Pos32**

STRUCTURAL CHARACTERIZATION OF THE MITOCHONDRIAL CREATINE KINASE OCTAMER

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Mitochondrial creatine kinase, a membrane bound isozyme of the creatine kinase family, isolated from chicken hearts exists as a globular, octameric protein with a molecular weight of 340 kD (Schlegel et. al., J. Biol. Chem. 263: 16942, 1988). Electron microscopy of negatively stained molecules revealed a square shaped projection with a central cavity (Schnyder et. al., J. Biol. Chem. 263: 16954, 1988). After freeze-drying and heavy-metal shadowing at low specimen temperature (-250°C) under ultra-high vacuum ($\leqslant 10^{-9}$mbar), the molecules showed a cross-like surface depression. Image averaging of individual octamers revealed only one projection, indicating that the molecule is a cube-like entity with approximately 10 nm in side length. The fact, however, that under certain experimental conditions linear aggregates of octamers were formed, is indicative for the existence of different molecule faces. This suggests that the octamer has at least two faces which exhibit different physico-chemical properties. The enzyme was 3-D-crystallized and examined by X-ray analysis. Diffraction until 3.2 Å resolution was obtained and two crystal types, having a tetragonal unit cell each, could be distinguished. Electron microscopic studies of negatively stained thin crystal plates as well as metal replicas of freeze-fractured crystals confirmed the arrangement of octamers in the crystal lattice.

**W-Pos33**

**A PRELIMINARY SOLUTION STRUCTURE OF GIANT RAGWEED ALLERGEN 5 (RA5G) BY $^1$H NMR SPECTROSCOPY.** G.L. Warren[*], K. Beshah[#], L. Goodfriend[+], L.J. Neuringer[#], G.A. Petsko[*]. [*]Dept. of Chemistry & [#]Francis Bitter Nat'l Magnet Lab, MIT, Cambridge, MA, 02139 [+]Royal Victoria Hospital, McGill Univeristy, Montreal, Canada.

Ra5S and Ra5G are two small homologous protein allergens for which the human genetics have been well characterized. Careful screening of human populations resulted in identification of individuals which are sensitive to exclusively one or both of these proteins. Because of this cross-reactivity phenomenon we have undertaken structure determination in an effort to better understand how the protein structure is contributing to the human response to these molecules. Most of the sequential resonance assignments have been made for Ra5G using a 10 mM solution at pH 3.5 and 35° C. A preliminary structure calculated using restrained molecular dynamics and tested by back calculating NOE spectra will be presented.

W-Pos34

NEW APPROACH TO HELICAL DIFFRACTION. G. F. Elliott and C.R. Worthington, Open University, Oxford Research Unit, Boars Hill, Oxford, UK. and Carnegie Mellon University, Pittsburgh, PA.

In a recent study [Acta Cryst. A45:645 (1989)] we have obtained an expression for the autocorrelation function of a helix. The Fourier transform of this function gives a new expression for the diffracted intensity and allows the examination of the effects of helical disorders. In order to extend our theory to helices of biological interest, we now consider subunits of arbitrary size. In previous X-ray studies on helical structures it was generally assumed that each subunit contained a series of spheres, either of uniform density or Gaussians. In our formulation the subunit has a three-dimensional distribution of electron density. Consider the myosin filament that has a helical array of subunits. After assuming a model for the myosin subunits the diffracted intensity is expressed in terms of the Fourier transform of the subunit model. This work is preliminary: either, the intensity profiles obtained by computer will be shown, or else, the basic theory on how the intensity profiles can be calculated will be described.

W-Pos35

MODELS OF PHOTON PENETRATION THROUGH THE HEAD. R. Nossal. National Institutes of Health, Bethesda, MD 20892.

It now seems feasible to measure hemoglobin oxygenation in the brain by noninvasive time-resolved optical absorption spectroscopy (Chance et al., PNAS 85, 4971 (1988)). To a first approximation, passage of near-infrared radiation through the head can be modeled by assuming a composite structure consisting of a layer of optically dense material (the skull) overlying a region of lower scattering density but higher absorption (blood-containing tissue). In previous work, several aspects of photon penetration through such layered media were investigated by computer simulations (Nossal et al., Appl. Opt. 27, 3382 (1988)). We now report results of related analytical studies. Mathematical expressions are given for several parameters pertaining to the time course of photon reemission from an irradiated composite tissue. By calculating the depth distribution of reemitted photons, conditions for probing the optical properties of the underlying tissue layer are assessed.

W-Pos36

ANALYSIS OF OMPF MUTANT PORINS FROM ESCHERICHIA COLI. W.J. Rocque and E.J. McGroarty. Michigan State University, E. Lansing, MI 48824.

Porin trimers were isolated from Escherichia coli K-12 strains lacking ompC and lamB and containing either a wild type ompF gene (PLB3261) or the ompF point mutations R82-C or D113-G (OC901 and OC904 respectively). The temperature and pH stabilities of the mutant porins were determined using electrophoretic and scanning calorimetric analysis and compared to that of the wild type. The Tm of the wild type and OC901 porins containing stoichiometric amounts of lipopolysaccharide (LPS) was 78°C compared to 71°C for the OC904 porin. In the absence of LPS the Tm of the porins decreased 5°-10°C. The wild type and OC901 porins, enriched in LPS, denatured between a pH of 3.5 and 4.5 whereas the OC904 porin denatured between 4.0 and 5.0. When the LPS was removed from the proteins the low pH stability decreased by 0.5 pH units. The size exclusion limit of the OC901 and OC904 porins was larger than the wild type protein as determined using a liposome swelling assay. We conclude that LPS stabilizes the OmpF porins and loss of certain charged amino acid residues decreases porin stability and alters the structure of the porin, increasing the channel size.

W-Pos38

TITLE: TOPOLOGICAL ANALYSIS OF HYDROGEN BONDING IN PROTEIN STRUCTURE

AUTHORS: B. Mao, K.C. Chou, G.M. Maggiora
Computational Chemistry, Upjohn Research Labs, Kalamazoo, MICHIGAN

A recent study has shown that topological stereoisomers exist for the polypeptide chain in disulfide-containing proteins that are represented by non-planar graphs. Here we found that a similar topological analysis can be applied to the hydrogen bonding in alpha-helices and beta-sheets within protein molecules, and we described the topological characteristics of chiral properties of protein secondary structure elements. This topological description of the hydrogen bonding in secondary structures may be extended to higher levels of protein structure and may provide a conceptual framework for studying complex protein architecture in general.

W-Pos37

TIME-RESOLVED X-RAY CRYSTALLOGRAPHY OF PHOTOACTIVE YELLOW PROTEIN.

E.B. Blum*, Y. Chen†, K. Moffat*, T.Y. Teng*, Section of Biochemistry, Molecular and Cell Biology* and School of Applied and Engineering Physics†, Cornell University, Ithaca, NY 14853, and D.E. McRee, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Laue diffraction techniques make it possible to collect x-ray data from biological macromolecules with synchrotron radiation in times that are comparable with their reaction times, thus allowing direct observation of structural changes during its course. The photoactive yellow protein (PYP) produced by the anaerobic bacterium, *Ectothiorhodospira halophila* (T.E. Meyer, Biochem. Biophys. Acta 806, p. 175 [1985]) may be ideal for a time-resolved experiment. PYP undergoes a cyclic reaction, with two or three intermediate states, in which the bleaching of its bright yellow color by a light pulse is followed by recoloring in the dark. Its structure has already been determined at 2.4 Å resolution (D.E. McRee, PNAS 86, p. 6533 [1989]).We have built apparatus that will permit us to stimulate single PYP crystals with a laser pulse and collect x-ray difraction data during the photocycle while simultaneously monitoring the optical absorption of the crystal. We have already shown, from optical measurements, that the slowest reaction rate in the crystal is consistent with the rate in solution. Initial x-ray results will be presented.

W-Pos39

CONFORMATIONAL TRANSITIONS OF A COLLAGEN FRAGMENT STUDIED BY CD AND $^1$H-NMR. by N.J. Greenfield*, G.T. Montelione*, J.Tsao*, S. Stein*, E. Smiley# and J. Bonadio#, *Center for Advanced Biotechnology and Medicine, Rutgers Univ., Piscataway, NJ 08854 and #Howard Hughes Medical Inst., The Univ. of Michigan, Ann Arbor, MI 48109.

Osteogenesis imperfecta is a family of inherited connective tissue disorders that result from mutations in type I collagen. As part of a study of the effects of mutations on collagen structure and function we have synthesized a fragment of the C-terminus of the α1 chain of human Type I collagen ($NH_2$-IGPPGPRGRTGDAGPV-$GPP_{OH}GPP_{OH}GPP_{OH}GPP_{OH}GPP_{OH}$SA-COOH). We have examined the CD and NMR spectra of this fragment as a function of temperature and peptide concentration in water. Below 7 °C the peptide has a CD pattern similar to native collagen and poly-L-proline II with a small positive band at 223 nm and a large negative band at 198 nm. When the material is heated the positive band at 223 nm disappears and the negative band diminishes and shifts to 203 nm. The kinetics of unfolding are rapid, cooperative and independent of concentration with a $T_M$ of approx. 17 °C at pH 5.0. In contrast the refolding is slow and displays a large dependence on both concentration and temperature. The conformational transition was also monitored by $^1$H-NMR. The folded form exhibits slowly exchanging amide protons indicative of an ordered structure. The folded and unfolded forms of the peptide are in slow chemical exchange.

W-Pos40

## CHARACTERIZATION OF STRUCTURE-FUNCTION RELATIONSHIPS IN RECOMBINANT HUMAN INTERLEUKIN 4 THROUGH SPECIFIC CHEMICAL MODIFICATION.

W. T. Windsor, R. Syto, J. Durkin, H. V. Le, S. Tindall, and P. P. Trotta. Biotechnology-Biochemistry, Schering-Plough Research, Bloomfield, N.J. 07003.

Human Interleukin 4 (huIL-4) is a pleiotropic lymphokine that exhibits biological activity on a diverse range of lymphoid and myeloid cells. The effect of specific chemical modification on in vitro biological activity, as measured in a T-cell proliferation assay, and on structure has been examined for purified Chinese hamster ovary-derived recombinant human interleukin 4. N-bromosuccimide modification of Trp-91 and tetranitromethane modification of Tyr-124 in the folded protein causes a 50% and 20% decrease in biological activity, respectively. Modification of each residue requires only a 3:1 ratio of modifier:huIL-4, which suggests each residue is exposed to the solvent. The second tyrosine, $Tyr^{56}$, could only be modified in the presence of a denaturant and modification caused the protein to lose nearly all biological activity. Reduction of the three disulfide bonds followed by alkylation of cysteine residues results in complete loss of biological activity. Large changes in the protein's secondary structure, based on far-UV circular dichroism, were observed only for the cystine and $Tyr^{56}$ modified proteins which suggests these moieties are important for the protein's structural stability. Modification of Trp-91 and Tyr-124 may have altered the protein's activity by disrupting specific functional interactions or by modification-induced tertiary changes.

W-Pos41

## DISULFIDE BOND ASSIGNMENT OF MAMMALIAN CELL-DERIVED RECOMBINANT HUMAN INTERLEUKIN 4.

William T. Windsor, Rosalinda Syto, James Durkin, Pradip Das, Paul Reichert, Birendra Pramanik, Steve Tindall, Hung V. Le, James Labdon, T. L. Nagabhushan and Paul P. Trotta. Schering-Plough Research, Bloomfield, NJ 07003

Human interleukin 4 (huIL-4) is a T-cell derived lymphokine that elicits pleiotropic biological activity on various cell types of lymphoid and myeloid origin. IL-4 is the only known lymphokine containing 6 cysteine residues in its mature sequence. Titrations using 4,4'-dithiodipyridine as well as disodium 2-nitro-thiosulfobenzoate on recombinant huIL-4 (rhuIL-4) derived from chinese hamster ovary cells indicates all of the cysteines are involved in disulfide linkages. Assignment of disulfide pairing was accomplished by determining the N-terminal amino acid sequence of tryptic-digested HPLC fractions containing disulfide-linked peptide fragments as well as by FAB-mass spectrometry of whole tryptic digests. From these studies we have made the following disulfide pair assignment: $C_3$-$C_{127}$, $C_{24}$-$C_{65}$, $C_{46}$-$C_{99}$. We postulate that the high thermodynamic stability of E. coli-derived rhuIL-4 that we have previously reported (1) arises, at least in part, from these three disulfide linkages. (1) Windsor, W.T., Syto, R., Nagabhushan, T.L., Trotta, P.P., Le, H.V. FASEB J. (1988) 2 A1337.

W-Pos42

## ROTATIONAL DIFFUSION AND FLEXIBILITY OF MYELIN BASIC PROTEIN,

MW Nowak and HA Berman, SUNY Buffalo, Buffalo, NY, 14260

This paper characterizes myelin basic protein (MBP) flexibility by measuring rates of acrylamide quenching of fluorescence and anisotropy decay of the single tryptophanyl residue (*trp-115*) within the 18.5 kD species isolated from bovine white matter. Bimolecular rates of fluorescence quenching approach the diffusion limitation and decrease with increasing viscosity of the bulk medium. Anisotropy of MBP decays rapidly, with a single correlation time of ≈1 nsec, and shows a near linear dependence on viscosity. The presence of a second component, with a subnanosecond correlation time, becomes evident under conditions of high viscosity ($\eta/\eta_0 \simeq 10$). The observed correlation times of MBP are much faster than predicted for a rigid spherical protein of 18.5 kD ($\phi_{predicted} \approx 7$ nsec) and are of the same magnitude as correlation times seen for small peptides such as ACTH or glucagon. These results indicate that *trp-115* is highly accessible to the bulk aqueous medium, and belongs to a segment of the protein that is highly mobile. This conformational mobility, coupled with the high net positive charge characteristic of MBP, permits the protein to adapt to changes in its immediate cytoplasmic environment.

W-Pos43

## PEPTIDE CLEAVAGE OF ADENYLATE KINASE BY UV LIGHT AND VANADATE.

Kristina M. Hatlelid and Christine R. Cremo, Chem. Dept. Colorado College, Colorado Springs, CO 80903.

Adenylate kinase was specifically oxidized at the active site by irradiation with UV light above 300 nm in the presence of vanadate. The oxidized enzyme was inactivated and cleaved near either the N or C terminus, producing two fragments of approximately 18.5 and 2.0 kDa. Loss of enzymatic activity preceded cleavage, suggesting at least two steps in the oxidation. $P^1,P^5$-di(adenosine-5')pentaphosphate, a potent inhibitor of adenylate kinase, completely protected against both inactivation and cleavage. The natural substrates, $Mg^{2+}$ATP and AMP also protected against cleavage and inactivation, with ATP being more effective than AMP in accordance with their equilibrium binding constants. These results indicated that the binding and cleavage by vanadate are specific to the active site. $^{51}$V-NMR studies showed that the oligomer, tetravanadate, not mono or divanadate, is the species that binds to the enzyme to effect cleavage. Tetravanadate, as a tetraphosphate analogue, may be binding to the polyphosphate binding site on the bisubstrate enzyme. The exact site of cleavage is under investigation, and should provide direct chemical evidence to place specific amino acids at the polyphosphate binding site. This work may provide insights into a generalized mechanism for cleavage of peptides by vanadate (Cremo and Wilcott, abstract this meetings).

W-Pos44

BINDING OF HYDROPHOBIC LIGANDS TO PEANUT AGGLUTININ, E. J. Zaluzec, M. Yung, P. Archer, S. F. Pavkovic, and K. W. Olsen, Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Road, Chicago, IL 60626.

The hydrophobic binding of 1,8-anilinonaphthalenesulfonic acid (ANS), 2,6-toluidinylnaphthalenesulfonic (TNS) and $N^6$-benzylaminopurine (BAP) to the plant lectin peanut agglutinin (PNA) was studied by fluorometric titrations, giving association constants of 24.4 X $10^3$, 9.1 X $10^3$ and 0.21 X $10^3$ $M^{-1}$, respectively. The binding of dansylgalactosamine (DnsGalN) was also studied by fluorescence. BAP caused a small increase in the binding affinity of DnsGalN. Crystals of a PNA-lactose complex with unit cell dimensions of a=130 Å, b=127 Å, and c=78 Å, in the orthorhombic space group $P2_12_12$ have been grown in the absence of a hydrophobic ligand. Crystals of PNA grown under similar conditions but in the presence of BAP were different in both morphology and space group. The new space group was P2 with a=67 Å, b=35 Å, c=65 Å and $\beta$=69°. Analysis of the volume/dalton indicated that the lactose-PNA crystals contain a tetramer per asymmetric unit, while the BAP-PNA crystals have only a monomer per asymmetric unit. Thus, even using the crystallographic diad to generate a dimer, the binding of BAP appears to have dissociated the protein, which may have significant functional consequences. (Supported by a Biomedical Research Support Grant from NIH.)

W-Pos45

**SOLUTION STRUCTURE AND INTERACTIONS OF A PEPTIDE FRAGMENT OF POL I STUDIED BY 2D NMR, CD, AND FLUORESCENCE SPECTROSCOPY.** **G.P. Mullen, J.B. Vaughn, Jr., P. Shenbagamurthi, and A.S. Mildvan. Johns Hopkins Medical School, Baltimore, MD 21205.**

**Transferred nuclear Overhauser effects (NOE's) from protons of DNA Polymerase I to those of bound dNTP substrates indicate hydrophobic (Ile,Leu) and an aromatic amino acid (Tyr) at the substrate binding site (Biochem. 25, 5131, 1986). Peptide I, a synthetic 50-residue peptide, based on residues 728 to 777 of the Pol I sequence, containing the conserved sequence L-I-Y-G, shows tight binding of dNTP substrates, the fluorescent substrate analog 2′,3′-trinitrophenyl-ATP, and duplex DNA, providing direct evidence that the active site for polymerization lies in this region, near Leu-764, Ile-765, and Tyr-766 (J. Biol. Chem., in press). While the X-ray structure of this region of the enzyme shows 48% helix, CD of peptide I reveals 29% helix and the sequence specific NOESY analysis indicates that 28% of the residues have helical character. 2D NMR studies at 600 MHz reveal that while the N-helix is largely preserved in peptide I in solution, the O-helix is partially unfolded into two helical turns and an extended loop. Two of the three $\beta$ turns found in the crystalline state, from residues 746 to 749, and from residues 772 to 775, were preserved as Type I $\beta$ turns in solution. The overall shape of the folded peptide is globular with the L-I-Y-G sequence exposed and accessible.**

W-Pos46

CALCULATIONS OF pKa's OF CHARGED AMINO ACIDS IN PROTEINS
An-Suei Yang and B. Honig, Dept. of Biochemistry, Columbia University, New York, NY 10032

The accurate calculation of the pKa's of amino acid sidechains is an essential step in the understanding of their roles in the activity of enzymes and in the stabilization of protein structures. pKa's depend on the hydration energy of the titratable group and on the electrostatic interactions of the group with the polarizable electrons, permanent dipoles and real charges on the protein. The Finite Difference Poisson-Boltzmann method is used to calculate these terms in several proteins. The extent of protonation of each titratable sidechain at various pH values is then calculated with either the Tanford-Roxby (self-consistant interation) scheme or from statistical mechanical considerations. The pKa's of asp-3, asp-50, glu-7, and glu-49 in BPTI were calculated from the midpoints of their simulated titration curves as 3.4, 2.6, 5.4, 4.2. The experimental values are 3.0, 3.4, 3.7, 3.8, respectively. Using the same method, the pKa's of the charged sidechains in the active sites of several serine proteases are also calculated and compared well with the experimental data.

W-Pos47

ELECTROSTATIC STUDIES OF CALCIUM BINDING PROTEINS. R.V. Sampogna, M.R. Gunner, B.H. Honig. Dept. of Biochemistry and Molecular Biophysics, Columbia University, NY

Proteins exhibiting $Ca^{+2}$ ion binding affinities with $pK_D$ values ranging from 1.5 to 9 have been investigated in an attempt to correlate electrostatic interactions with this wide range of $pK_D$s. Both the electrostatic potential at the binding site and the cost of desolvating the $Ca^{+2}$ ion were calculated using DelPhi, a program which solves the linearized Poisson-Boltzmann equation. Both factors correlated with the respective $pK_D$ values, establishing a relationship between the $Ca^{+2}$ ion affinity and the electrostatic interactions at each site. We also have studied the pattern of electrostatic potential in intestinal calcium binding protein and carp parvalbumin. In the absence of $Ca^{+2}$ ions or when monovalent ions are bound, these proteins have an overall dipolar character. When $Ca^{+2}$ ions are bound, however, there are dramatic changes eliminating the dipolar pattern. The results from these investigations will be discussed in terms of the nature of the $Ca^{+2}$ ion ligands, the extent to which the binding sites are buried and factors such as conformational change and cooperativity which play a role in $Ca^{+2}$ ion binding.

W-Pos48

SITE-DIRECTED MUTAGENESIS OF A CALMODULIN-DEPENDENT PROTEIN KINASE: EFFECTS OF CHIMERIC CALMODULIN BINDING SEGMENTS ON CALMODULIN RECOGNITION AND ACTIVITY.
M. O. Shoemaker, W. Lau, T. J. Lukas and D. M. Watterson, Dept. of Pharmacology, Vanderbilt Univ., and Howard Hughes Medical Institute, Nashville, TN 37232

A calmodulin-dependent protein kinase was engineered from the cDNA sequence of fibroblast myosin light chain kinase (MLCK). The parent construct, rMLCK-1 produces a 70Kd protein which has the substrate specificity, calmodulin (CaM) sensitivity, and kinetic properties of tissue isolated enzyme. In previous studies, (Lukas et al. Biochemistry 25, 1458, 1986) much of the CaM-binding of the MLCK was found within a 20 residue peptide termed RS20. Chimeric enzymes which contain the "RS20-like" sequences from the skeletal muscle MLCK isozyme and from CaM-dependent protein kinase-II (CaMPKII) were constructed. When tested for CaM activation by wild-type and mutant CaMs, the construct containing the skeletal muscle RS20 analog gave CaM recognition very similar to the rMLCK-1. In contrast, the construct based upon CaMPKII exhibited CaM sensitivity and selectivity similar to CaMPKII, demonstrating that the RS20 region is essential for CaM recognition by MLCK.

W-Pos49

POINT MUTATIONS IN CALMODULIN THAT EXHIBIT ALTERED ACTIVATION OF MYOSIN LIGHT CHAIN KINASE.
T. J. Lukas, T. A. Craig, A. P. Kwiatkowski and D.M. Watterson. Dept. of Pharmacology, Vanderbilt Univ., and HHMI, Nashville, TN 37232.

The activation of myosin light chain kinase (MLCK) by calmodulin (CaM) is adversely affected by charge perturbation mutations in two acidic clusters of CaM (Weber et al. Proteins 6:1,70-85,1989). These clusters are located in $\alpha$-helices that contribute to the asymmetric charge potential surface in CaM. The most important amino acids in these clusters have been identified as Glu-84 and Glu-120. The primary effect of Glu→Lys mutations at residues 84 and 120 was to increase the Km of MLCK for peptide substrate, demonstrating that one role of CaM in the CaM:kinase complex is to enhance the interaction of enzyme and substrate. Molecular modeling of CaM revealed that Glu-84 and 120 flank a recessed hydrophobic surface. In models of mutants where Glu-82,83, or 84 were deleted, the relative position of Glu-84 was occupied by another acidic residue. Changing Asp-78 to Asn in the 82-84 deletion mutant altered MLCK activation, confirming the model. These results reconcile the differences in activity of substitution and deletion mutants of CaM.

W-Pos50

PEPTIDE INDUCED CONFORMATIONAL CHANGES IN FLUORESCENT CALMODULIN MUTANTS.
Chabbert M., Lukas T.J.*, Watterson D.M.* and Prendergast F.G., Mayo Foundation, Rochester, MN 55905 and *Vanderbilt University and Howard Hughes Medical Institute, Nashville, TN 37232.

Calmodulin mutants, each with a single tryptophan residue at various positions, were used to study peptide induced conformational changes in calcium saturated calmodulin. The tryptophan residues in calmodulin were set either at the seventh position of each calcium binding loop ( residues 26, 62, 99 and 135) or in the central helix (residue 81). The peptides were based on the calmodulin binding domains of myosin light chain kinase and protein kinase II. Both peptides bound to each calmodulin mutant with a 1:1 stoichiometry. Upon peptide binding, changes in fluorescence properties were similar between Trp-26 (loop I) and Trp-135 (loop IV) and between Trp-62 (loop II) and Trp-99 (loop III). This result supports the inference that both lobes of calmodulin influence peptide binding. The two peptides induced similar changes in fluorescence for tryptophans in the calcium binding loops, but different results for tryptophan in the central helix (Trp-81).

W-Pos51

MATURATION OF ALKALINE PHOSPHATASE
J.F. Chlebowski, L.A. Randall, R. Tyler-Cross, C.H. Roberts, S. Olafsdottir and N. D. Ulbrandt. Department of Biochemistry and Molecular Biophysics, VA Comm. Univ., Richmond, VA, 23298-0614

The mature alkaline phosphatase is a metalloenzyme dimer localized in the periplasmic space of the E. coli. Investigation of the metal free or apo form of the mature enzyme and variants bearing an amino-terminal deletion indicate that the apoprotein monomer can adopt a conformation incapable of assembly and metal ion binding. In vivo the enzyme appears in the periplasm in two distinct forms. One of these, the immature form, converts to the mature form in vitro, suggesting that it is an intermediate in the formation of the mature enzyme. In comparison to the mature dimer the immature enzyme is an acidic protein of reduced catalytic potency but enhanced stability to thermal or enzymatic alteration. The properties of the immature enzyme derive from the tenacious association of an adenylated polypeptide which is released in the course of maturation. The properties of the immature protein suggest that the intermediacy of this species provides a means of maintaining the translocated enzyme in a form competent for assembly.

W-Pos52

## FLUORESCENCE DETECTION OF SOLVENT DIPOLAR RELAXATIONS IN VISCOUS SOLVENTS AND IN PROTEINS.

J. Fishkin, E. Gratton & B. Feddersen. *UIUC, LFD, 1110 W. Green St., Urbana, IL 61801*

Dipolar relaxation processes have been considered to be a factor affecting fluorescence decay in proteins. We have introduced a new method to analyze steady-state spectra of proteins using the concept of generalized polarization. An analog of the Perrin equation is used to determine the rate of dipolar relaxation. Data have been collected for NATA and tryptophan in glycerol-water solutions and for several proteins using tryptophan fluorescence or external labeling. The results have been tested by direct time-resolved measurements using differential phase measurements in our Gigahertz phase fluorometer.

(Supported by PHS-1-P41-RR03155.)

W-Pos53

## FLUORESCENCE ANISOTROPY DECAY: ORIENTATIONAL EXCHANGE APPROACH IN PROTEINS

E. Gratton & D.W. Piston*. *UIUC, LFD, 1110 W. Green St., Urbana, IL 61801. *Current address: Cornell Univ., Ithaca, NY 14853.*

Fluorescence depolarization is a powerful technique in resolving dynamics of molecular systems. Models for analyzing data from depolarization due to rotational motion have been largely based on the rotational diffusion equation. We have derived, in a compartmental formalism, the general result for excitation and emission dipoles not necessarily coincident with any of the principal rotational axes of the fluorophore based on Weber's 90° exchange model and find it, in general, to be different from that of the diffusion equation approach. However, this derivation allows us to define the limits of validity of the 90° exchanges to model rotational diffusion. This compartmental formalism can be used to easily combine rotational motions with discrete position jumps or other level kinetics. It is shown that for the case of single tryptophan protein anisotropy data, this formalism can be used to combine the overall rotational diffusion of the protein with the internal movement of the tryptophan residue. (Supported by PHS-P41-RR03155.)

W-Pos54

## ROLE OF HIS244 IN THE FUNCTION AND STABILITY OF TISSUE PLASMINOGEN ACTIVATOR KRINGLE-2 DOMAIN.

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Microcalorimetric methods have been used to compare the ligand binding and thermal denaturation behavior of wild-type kringle-2 domain (residues 174-263 of t-PA expressed in E. coli) and a mutant having His244 replaced by Tyr. This site was examined because modeling studies [Tulinsky, et al., (1988)] suggested that the His244 side chain could play an important role in ligand binding by forming an ion-pair with the carboxylate of the ligand, L-lysine. Thermodynamic parameters for binding of L-lysine at 25°C and pH8 determined using titration calorimetry are: Wild-type kringle-2 - n=1.3±0.3; $K_a$=15,000±3000$M^{-1}$; $\Delta H$=-5500±600 cal/mole; $T\Delta S$=230 cal /mole; H244Y kringle-2 - n=1.7; $K_a$=5,000$M^{-1}$; $\Delta H$=-4400 cal/mole; $T\Delta S$=700 cal/mole. A mutant having His244 replaced with Phe also binds lysine indicating that the side chain of residue 244 does not form an electrostatic interaction with the ligand carboxylate. The 3-fold reduced lysine affinity of the H244Y protein is related to a change in the chain length specificity of the lysine binding site. Thermal denaturation studies show that the H244Y mutant is also more stable ($\Delta Tm$=17°C at pH3) than the wild-type protein.

W-Pos55

## COMPUTATIONAL INVESTIGATION OF HELIX-HELIX INTERACTIONS IN MEMBRANES USING SURFACE REPRESENTATIONS.

Jesus E. Rame, Christopher Dempsey, and Donald M. Engelman; Dept. of Molecular Biophysics and Biochemistry, Yale University

Theoretical and experimental evidence suggests that transbilayer helices are a major motif in integral membrane protein structure. We present a series of calculations that attempt to provide a quantitative approach for describing helix-helix and helix-lipid interactions in membrane systems. Contact and molecular surface area and volume calculations were done on two-helix and multi-helix systems from the reaction center of *Rhodopseudomonas viridis* and for soluble proteins containing interacting helices. These calculations are aimed at characterizing the surface roughness of helix systems by determining an index of relative surface texture, the fractal dimension, *D*, over the protein surface (Lewis and Rees, Science 230, 1163, 1985). Surface maps affording the variation in fractal dimension across the protein surface are being generated to describe the protein-protein and protein-lipid interaction within membranes. The extension of this work to the association of transmembrane helix systems currently under experimental investigation will be described.

W-Pos56

USE OF RAPID SCAN SECOND DERIVATIVE ABSORPTION SPECTROSCOPY TO RESOLVE FOLDING INTERMEDIATES IN PROTEINS. Mark T. Fisher , Lab. of Biochemistry, National Heart, Lung, & Blood Institute, NIH, Bethesda, MD 20892.

Equilibrium and kinetic analysis of folding/unfolding or ligand-protein interactions using rapid scan second derivative absorption spectroscopy enables one to resolve changes in polarity surrounding tryptophan, tyrosine, and phenylalanine residues. Phosphoglycerate kinase, carbonic anhydrase, and glutamine synthetase possess intermediates in unfolding/refolding and/or ligand binding reactions. The primary sequence of carbonic anhydrase has an even distribution of aromatic residues throughout the structure of this protein. Changes in polarity of these residues in this protein as a result of unfolding/refolding reactions reveal identical fractional changes for all three aromatic residues. In contrast, phosphoglycerate kinase (yeast and horse) and glutamine synthetase (*E. coli*) possess an asymmetric distribution of tryptophan and tyrosine residues which reside in separate domains of these proteins. These latter two proteins exhibit easily resolved differences in tryptophan and tyrosine residue solvation in response to unfolding/refolding and ligand binding reactions. Second derivative uv spectroscopy can be used to observe intermediates during protein folding and specifically identify fast and slow steps in unfolding/refolding and/or ligand binding reactions.

W-Pos57

COMPARISON OF TRYPSIN STRUCTURE AT 120 K AND 295 K. Thomas Earnest and Robert Stroud Dept. of Biochemistry and Biophysics, University of California School of Medicine San Francisco, CA 94143.

The structure of rat trypsin was solved from data collected to 1.59Å at 120 K using the room temperature structure as an starting model. Unit cell dimensions decreased by 1% for this cubic crystal form (I23). Several cycles of refinement (PROLSQ) gave a model with R=17.2%.This structure was compared to the structure at 295 K (R=14.3% to 2.3A).The overall B factor, based on Wilson plots, is reduced from 26.4 $Å^2$ to 9.61 $Å^2$ for the 120K data.This decrease in the fall off of average intensity, along with increased crystal lifetime, allows for an increase in the attainable resolution. Analysis of the individual, isotropic B factors along with temperature induced structure changes allows for investigation of protein flexibility and dynamics.Least squares overlap of the C-alpha's gives an RMS difference of .178Å between the two structures.The protein exhibits anisotropic expansion in going from 120 K to 295 K. The surface area of the protein also shows an increase of about 1% at 295 K.Also several more solvent molecules can be localized at 120 K.

W-Pos58

KINETICS OF OXYGEN BINDING TO R-STATE NATIVE AND αα-CROSS-LINKED HUMAN HEMOGLOBIN

K.D. Vandegriff, R.J. Rohlfs[Y], and R.M. Winslow, from the Blood Research Division, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129 and the [Y]Department of Biochemistry, Rice University, Houston, TX 77251.

From analysis of oxygen equilibrium curves, cross-linking human hemoglobin (HbA) between the α chains by bis(3,5-dibromosalicyl) fumarate (αα-Hb) causes a significant reduction in K4. To resolve the transient events for K4 at pH 7.4 and 25°C, the kinetics of $O_2$ binding to the R-state hemoglobins have been measured. Kinetic traces were fit to the equation $\Delta A/\Delta A_T = 0.5[\exp(-k_\alpha t) + \exp(-k_\beta t)]$ to determine rates for the individual subunits. Time courses from laser photolysis of oxyhemoglobin show that k'4 is decreased by ~80% for the α chains and by ~20% for the β chains in αα-Hb compared to HbA. Oxygen dissociation rate constants (k4) from rapid mixing experiments are ~2-fold higher for both α and β chains in αα-Hb. The resulting, calculated equilibrium dissociation constants are 0.3 μM for both subunits in HbA and 2.5 μM and 0.6 μM for the α and β chains in αα-Hb, respectively. These results suggest that in native R-state human hemoglobin, the subunits have about the same oxygen affinity, which is reduced ~2-fold for the β chains and ~8-fold for the α chains by cross-linking between αLys(99) residues.

W-Pos59

A CIRCULAR DICHROIC STUDY OF BAND 3 IN LUBROL PX. M.M. Batenjany, P.L. Pingerelli, H. Mizukami. Division of Regulatory Biology and Biophysics, Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

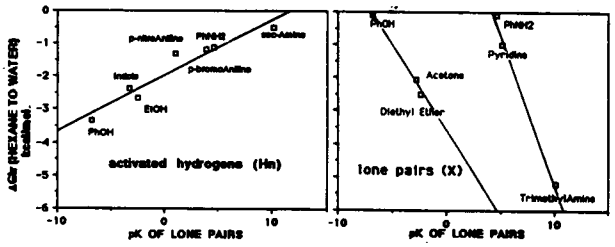
Human erythrocyte band 3 is now considered a ubiquitous anion exchanger. It consists of a membrane domain essential for anion transport and an intracellular domain that binds to many cytoplasmic proteins and the cytoskeleton. However, this dual nature has made the study of this protein difficult and its *in situ* structure remains elusive. In order to mimic the environment this protein may encounter in the membrane, we have studied structural changes of band 3 in the nonionic detergent Lubrol Px. The Circular dichroism (CD) of band 3 in the the presence of varying concentrations of Lubrol Px ($C_{12}E_9$) was used to investigate changes in its secondary structure. In 0.1% Lubrol PX in 5P8, the far UV CD (184 nm to 250 nm) of band 3 shows 30-35% α-helix, 28-30% ß-sheet, and 22-24% random coil. Decreasing concentrations of Lubrol Px (.1%-.01%) resulted in significant changes in the secondary structure estimations of band 3 showing an increase in α-helix (43-69%), and decrease in random coil (51-71%) content. Additionally, the study of a sample with aggregated band 3, demonstrated an increased amount of ß-sheet, suggestive of the mode of packing or aggregation. A correlation of these secondary structure changes with the ability of band 3 to bind some stilbene inhibitors and pyridoxal phosphate (PLP) and their effect on changes in the secondary structures of band 3, with varying concentrations of Lubrol Px is discussed. (supported by a grant from The Graduate School, WSU).

**W-Pos60**

## LONE PAIR BASICITY OF $R\ddot{X}H_n$ SOLUTES AND THE STRENGTH OF $\ddot{X}$-WATER AND $H_n$-WATER HYDROGEN BOND INTERACTION

J. M. KESKE, J. M. BRUCE, AND P. L. DUTTON, (Intro. by Daniel Safer), Dept. of Biochemistry and Biophysics, Univ. of Pennsylvania, Philadelphia, PA.

Multiple solvent partition coefficients for solutes of the $R\ddot{X}H_n$ type have been used to distinguish contributions from solute cavity, dipole moment, and hydrogen bonds to the observed transfer free energy ($\Delta G_{tr}$) of a molecule out of water into hexane. Individual contribution of lone pairs and activated hydrogens of $\ddot{X}H_n$ to the $\Delta G_{tr}$ have been resolved and estimated. The resolved interaction strengths are likely to arise from the hydrogen bonds ($X\ddot{:}$ --HOH) and ($H_0$--$OH_2$). Thus, we find with decreasing pK on $\ddot{X}$ (i.e. $\ddot{X}H_n/\ddot{X}H_{n+1}$) that hydrogen bond strengths with water decrease for the $\ddot{X}$ lone pairs (right figure; cf. Arnett JACS **96** 3875) and increase for $H_n$ (left figure).




These values may be relevant to the energetics of water exclusion during protein folding and to estimating the H-bond strengths of residues and cofactors within proteins. PHS GM 27309 and NSF DMB 88 77240

**W-Pos62**

## HEXAMERS OF SUBUNIT II OF LIMULUS HEMOCYANIN (A 48-MER) HAVE THE SAME QUATERNARY STRUCTURE AS WHOLE PANULIRUS HEMOCYANIN MOLECULES.

By Karen A. Magnus, Case Western Reserve University, Eaton E. Lattman, Johns Hopkins University, Wim G.J. Hol and Anne Volbeda, University of Groningen.

Hemocyanins are large multisubunit proteins that transport oxygen in various arthropods and mollusks. Molecular oxygen is reversibly bound by each subunit at an active site of two coppers directly ligated by protein side chains. Hexamers of 75,000 m.w. subunits are the fundamental structural units of arthropod hemocyanins. Using x-ray diffraction data from 8.5 to 6 Å from oxygenated crystals of subunit II phased by molecular replacement, we have shown that a hexamer of subunit II of Limulus hemocyanin is isomorphous hexameric Panulirus hemocyanin. These crystals diffract to about 2.7 Å and work on the higher resolution structure of the protein in both oxygenated and deoxygenated forms is in progress.

Supported by NIH grant AM-36358 and RR-5378.

**W-Pos61**

## STRUCTURAL STABILITY OF THE NUCLEOCAPSID PROTEIN OF AVIAN MYELOBLASTOSIS VIRUS (AMV).

Craig A. Gelfand and Joyce E. Jentoft. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

The nucleocapsid protein (NC) of AMV interacts with single stranded RNA and is essential in recognition and packaging of genomic viral RNA during viral assembly. We have studied the structural stability of NC in solution. The intrinsic fluorescence quantum yield of the single tryptophan residue (Trp 80) of NC is 80% reduced from that of N-acetyltryptophanamide, suggesting that some regular structure exists around Trp 80. Intrinsic fluorescence temperature dependence varies with guanidine concentration. Fluorescence quenching by acrylamide shows 80% accessibility of acrylamide onto Trp 80 under non-denaturing conditions, while showing 100% accessibility in 6M Guanidine HCl. The effects of quenching as a function of guanidine concentration will be reported. In contrast, no significant changes in the CD spectrum were induced even in 6 M Guanidine HCl. The structural stability of the NC protein will be incorporated into an overview of the NC structure. Supported by NIH Grant GM36948.

**W-Pos63**

## RECOGNITION SITES FOR KINASE DEPENDENT PHOSPHORYLATION IN LENS SPECIFIC AND RECRUITED-CRYSTALLINS.

P.N. Farnsworth and B. Groth-Vasselli. UMDNJ-Medical School, Newark, New Jersey.

In the lens, the supramolecular organization of its major proteins, the crystallins, provides the basis for transparency. This study presents evidence for a ubiquitous distribution of tyrosine and/or serine kinase dependent phosphorylation sequences in lens specific and recruited-enzyme crystallins. The $\beta\gamma$ superfamily of crystallins contain sequences for both serine and tyrosine phosphorylation. The serine phosphorylation sites in the carboxy terminus of $\gamma$-crystallin, actin and profilin, an actin binding protein, suggest that phosphorylation could be important in the modulation of cell structure. Computer-assisted molecular modeling of the local environment of the putative tyrosine phosphorylation site of bovine $\gamma$ II and the proven phosphorylation site of lactate dehydrogenase shows that the composition and the three-dimensional structure of these sites are similar. We suggest that phosphorylation may modulate the lens macromolecular order. (Supported by NIH).

W-Pos64

## MONOMER/DIMER (M/D) TRANSITION OF ENZYME I OF THE *ESCHERICHIA COLI* PHOSPHOTRANSFERASE SYSTEM (PTS); KINETIC STUDIES.

F. Chauvin, S. Roseman, and L. Brand. Dept. of Biology, The Johns Hopkins Univ., Baltimore, MD 21218

PTS catalyzed sugar transport requires sequential phosphotransfer from PEP *via* PTS proteins to the sugar. Enzyme I (EI) is the first protein in the chain. Apparently only EI dimer accepts phosphate from PEP, and the M/D transition may be the rate-limiting step in sugar transport. Previous studies have given $K'_a$ for 2M = D, but not $k_1$ or $k_{-1}$.

We are using fluorescence anisotropy (FA) to determine $K'_a$, $k_1$, and $k_{-1}$. Pyrene maleimide is coupled to the EI C terminal Cys (Han, *et al.*, JBC, in the press); labeled EI is fully active. M and D give different FA values, allowing kinetic studies of the M/D transition. $K'_a = 3 \times 10^8\ M^{-1}$ (2 mM PEP, 5 mM $Mg^{2+}$, pH 6.5, 6° C), in agreement with equilibrium studies. M and D interconvert slowly in each direction; $t_{1/2}$, about 30 min at 6° C, 15 min at 23° C. Catalytic activity correlates with [D]. The effects of ligands on M/D will be presented. NSF Grant DIR 87 21059

W-Pos65

## THE 1989 MUTATION DATA MATRIX

G.Y. Srinivasaro, D.G. George, and W.C. Barker. National Biomedical Research Foundation, Washington, D.C.

The Dayhoff Mutation Data Matrix (MDM) has been found to be the most sensitive scoring matrix available for detecting distantly related proteins. It is routinely employed in a number of sequence comparison and database searching methods and has been used as the basis of simulation studies to evaluate sequence alignment and tree-building methods. It has been analyzed to answer questions such as the role of Markovian processes in evolution and the effect of the physicochemical and structural properties of amino acids on their evolutionary acceptance rates. MDM is an empirical matrix based on amino acid exchange frequencies observed among closely related sequences. It was last compiled in 1978; since that time, the amount of data available to recompile the matrix has increased nearly tenfold. Moreover, there are now enough data to compile amino acid exchange matrices specific to different classes of proteins. We are recompiling MDM and will report on current progress. This work was partially supported by grants RR01821 and GM37273 from the NIH.

W-Pos66

## AGE RELATED-CHANGES IN THE STRUCTURE OF BOVINE ARTICULAR CARTILAGE.

Volpi M., Katz P.E.
BioStructure & Function
U. of Connecticut Health Center
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Bovine articular cartilage was analyzed using low angle x-ray diffraction and an area detector. A mirror-monochromator system was used to bring the x-ray beam to a point focus. Our results show that in weight-bearing areas of the femur head, the spacing of the equatorial pattern changes from the surface to the subchondral bone, suggesting a compression of the collagen fibers by the proteoglycans. Nevertheless, in animals of different age, these changes show significant differences implying a gradual adaptation of the cartilage to gravity-related stresses. In particular, in cartilage of approximately the same depth, the equatorial spacing goes from 1.59 to 1.63 nm in the 2-year old cow, and from 1.58 to 1.78 nm in the 14-year old cow. The absence of this gradient, in a sample derived from the same femur but from a non-weight bearing area of the 14-year old animal, indicates that this adaptation does not involve the whole surface of the cartilage, but instead, that it is a local adaptation to stress.

W-Pos67

## Structural Studies of cAMP-dependent Protein Kinase Catalyic Subunit

Mitchell, R.^, T.R. Sosnick*, D. Glass^, D. Walsh^ and J.Trewhella.*
^ Dept. of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616; *Life Sciences Division, Los Alamos National Laborary, Los Alamos, NM 87545

Circular Dichroism studies of the cAMP-dependent Protein Kinase catalytic subunit indicate there is a significant loss of $\alpha$-helix and a dramatic increase in ß-sheet structure on binding to a substrate peptide (1). We have completed small-angle X-ray scattering experiments to evaluate the potential changes in overall shape of the bovine cAMP-dependent Protein Kinase catalyic subunit upon binding to ATP and the active portion of the heat-stable inhibitor protein specific to the enzyme. The X-ray measurements indicate a contraction of the enzyme upon binding to either ATP/Mg with or without the peptide. Both Guinier and vector pair-distribution function analysis indicate a significant decrease in the radius of gyration (from approx. 28 Å to 23 Å) upon binding, the predominant effect being a reduction in the number of long vectors within the structure.

(1) Reed et al., (1985) Biochemistry 24:2967

W-Pos68

**Calmodulin Structure Studied Using Neutron Scattering: Peptide Interactions and Measurement of Distances Between Calcium-Binding Sites**, Henderson, S., Rokop, S., Seeger, P.A., *Blumenthal, D.K., Hobart, D., Palmer, P., #Crespi, H., and Trewhella, J; Los Alamos National Lab., *University of Texas at Tyler, #Argonne National Lab.

Calmodulin (CaM) complexed with two peptides derived from the catalytic subunit of phosphorylase kinase has been studied using neutron scattering and contrast variation. Information has been obtained on the relationship between the peptides and CaM. This complex is significantly different from CaM complexed with the myosin light chain kinase peptide (MLCK1)(1).

X-ray scattering studies of CaM suggest that its solution structure is different from the crystal structure (2). The solution intramolecular distances between the calcium binding sites would further test this. Pu(III) ions have the same ionic radius as Ca(II), and we have shown that these both bind and activate CaM. Resonant (anomalous) neutron scattering from (240)Pu/CaM should determine the separation distances between the bound ions. Such a measurement has been performed, and the results will be discussed, along with difficulties encountered in the incorporation of plutonium into CaM.

(1) Heidorn et al., Biochemistry 28:6757, 1989
(2) Heidorn & Trewhella, Biochemistry 27:909, 1988

W-Pos69

**STRUCTURE AND FUNCTION STUDIES ON THE DnaA PROTEIN OF E.coli.** H. I Eberle and W-S Zhu. Dept. of Biophysics, Univ. Rochester School of Med. Rochester, NY 14642.

The DnaA protein of Escherichia coli is a 52.5 kD protein whose function is essential for the initiation of DNA replication from the chromosomal origin (ori C). It has been demonstrated by others that DnaA binds to special repeated sequences at ori C, it has at least one ATP binding domain, and has DnaA subunit-subunit interactions, as well as possible interactions with subunits of other proteins involved in replication. In order to identify structural and functional relationships of DnaA, we have examined intragenic suppressor mutations that restore function to dna A temperature sensitive mutants. Several suppressor mutations have been identified and their possible structural relationships to the functional domains, especially the ATP binding site, will be discussed.

W-Pos70

**PICOSECOND TIME-RESOLVED FLUORESCENCE SPECTROSCOPY ON THE MILLISECOND TIME-SCALE: CORRELATION OF ULTRAFAST MOTIONS AND SOLUTION STRUCTURE WITH SLOWER BIOLOGICAL STATE TRANSITIONS IN PROTEINS.** Joseph M. Beechem, Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.
Recent developments in data analysis, laser light sources, and micro-channel plate photomultiplier technology have caused a radical change in the manner in which time-resolved fluorescence studies can be performed. Utilizing these recent developments, a special time-resolved fluorometer is being constructed which integrates a high repetition rate Nd:YAG laser, 3-syringe stepper-motor controlled stopped-flow sample compartment, 1/4 meter polychromator, 10-linear multi-anode microchannel plate photomultiplier, high frequency amplifiers/constant fraction discriminators/TAC's, with a set of ultra-high speed analog-to-digital converters. With this apparatus it is possible to collect multiple emission wavelength picosecond time-resolved fluorescence decay data in approximately 10 milliseconds. Application of this technology towards understanding protein folding pathways will be described. Changes in intrinsic tryptophan fluorescence and energy-transfer studies are emphasized. In the energy-transfer studies, multiple decay curves of both donor and acceptor are simultaneously obtained during protein folding. Global analysis of both donor and acceptor decay (Beechem & Haas, Biophys. J. 55:1225-1236, 1989) allows reconstruction of the millisecond changes in intra-molecular distances and dynamics. Support: JMB is a Lucille P. Markey scholar in biomedical science.

W-Pos71

**RAMAN STUDIES OF ELONGATION FACTOR Tu.** Danny Manor[1], Gezhi Weng[1], Hua Deng[1], Francis Jurnak[2] and Robert Callender[1]. Physics Dept. City College of New York, New York NY 10031(1) and Biochemistry Dept. University of California at Riverside, Riverside CA 92521(2)
Elongation Factor Tu (EF-Tu) is a key protein in the elongation steps of bacterial protein synthesis. The protein binds either GDP (EF-Tu•GDP) or GTP (EF-Tu•GTP). The two forms differ drastically in both their biological activity and their conformation. We used classical Raman spectroscopy to investigate the specific protein-nucleotide interactions that are involved in these complexes. Raman spectra of the free and bound nucleotides reveal specific protein-nucleotide binding interactions. Comparison of the protein spectra in the two conformations reveal changes in the apoprotein between the two states. The above spectra will be presented and their relevance to the mechanism of EF-Tu action will be discussed

W-Pos72

CONFORMATIONAL STATES OF P22 COAT PROTEIN IN RELATION TO SELF ASSEMBLY. Peter Prevelige, Dennis Thomas, Jonathan King, (MIT Cambridge, MA.) George Thomas, Stacy Towse (Univ. Missouri, Kansas City, MO.)

The in vivo self-assembly of properly dimensioned bacteriophage capsids requires the transient presence of a scaffolding protein which functions as a molecular chaperone. In the absence of scaffolding protein a variety of morphological forms of polymerized coat protein are observed. These forms have features suggestive of regular repeated bonding interactions and likely result from inappropriate placement of the hexameric and pentameric clusters.

Purified coat and scaffolding proteins together copolymerized to form properly dimensioned capsid precursor shells. Purified coat protein alone will polymerize into forms similar to those produced in vivo in the absence of scaffolding protein.

We have employed Raman spectroscopy to examine the difference in the coat protein conformation between properly dimensioned shells and the abberant polymers produced in vitro. The observed spectral differences can be accounted for by the conversion of 10 out of 430 residues of the coat protein from an alpha helical conformation to a beta sheet conformation.

W-Pos73

CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDIES OF AN ANTI-4-HYDROXY-3-NITROPHENACETIC ACID (NP) FAB FRAGMENT NP COMPLEX

Stacieann Yuhasz, Xavier Ysern, Mette Strand, and L. Mario Amzel, Dept. of Biophysics and Dept.of Pharmacology, The Johns Hopkins University, School of Medicine, Baltimore, Md 21205.

The anti-NP monoclonal antibody, 88C6/12, is one of a series of monoclonal antibodies designed for investigating antibody specificity through the use of a series of structurally related haptens, which include 4-hydroxy-3-nitrophenylacetic acid, 4-hydroxy-3-nitrophenacetyl-ε-aminocaproic acid, 4-hydroxy-3-iodo-5-nitro-phenylacetic acid, and 4-hydroxy-3-iodo-5-nitrophenacetyl-ε-aminocaproic acid. Interestingly, the binding affinities of 88C6/12 for the iodinated haptens (which were not used for immunization) are approximately fifteen times larger than those of the immunizing hapten. We have obtained crystals of the Fab fragment with 32% PEG 3400 in the presence 40-400 mM NP, space group $P2_12_12_1$ with cell dimensions a= 81.2Å, b=86.9Å, c=131.1Å. There are two molecules of the complex per asymmetric unit related by a local two-fold axis parallel to the crystallographic b axis located at x=0.218 and z=0.25.

W-Pos74

Crystallization and X-ray Diffraction Data of an Anti-Hormone Fab and of the Hormone-Fab Complex

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Mab-131 is a monoclonal antibody that binds with high affinity($K_a$=7.4 x$10^9 M^{-1}$) to an 8-residue peptide hormone,Angiotensin II, the major effector of the renin/angiotensin system. Mab-131 is also a member of a well characterized idiotypic antibody network since it was raised as an anti-anti-idiotype of a Mab raised against AII. The crystals of the apo and complexed form of the Fab, which diffract to 3.5Å, are tetragonal, space group $P4_1$(or $P4_3$) with cell dimensions a=b=78.6Å,c=125.2Å, and two Fabs in the asymmetric unit. Using a different buffer, a second crystal form has been grown which diffracts to 3.0Å, is space group $P4_1$(or $P4_3$), and has cell dimensions a=b=109.6Å, c=125.2Å. Knowledge of the three-dimensional structure of this Fab and of the hormone-Fab complex will give insight into two problems:1) the recognition of flexible peptides with high affinity by proteins, and 2) the nature of conservation of antibody combining sites in idiotypic networks.

W-Pos75

STATISTICAL DISTRIBUTION OF HYDROPHOBIC RESIDUES IN PROTEINS: IMPLICATIONS FOR PROTEIN FOLDING.

S.H. White & R.E. Jacobs, Dept. Physiology & Biophysics, Univ. Calif. Irvine, CA 92717

The amino acid sequence of any protein reveals that hydrophobic residues appear to occur in clusters along the length of the chain. In this discussion we consider the statistical distribution of hydrophobic amino acid residues along the length of protein chains. For this purpose we use a binary hydrophobicity scale which assigns hydrophobic residues a value of unity and non-hydrophobes a value of zero. The resulting binary sequences are tested for randomness using several standard tests. For the majority of the more than 5,000 sequences examined, the distribution of hydrophobic residues along the sequences cannot be distinguished from that expected for a random distribution. This suggests that (1) functional proteins may have originated from random sequences, (2) the folding of proteins into compact structures may be much more permissive than previously thought, and (3) the clusters of hydrophobic residues along chains which are revealed by hydrophobicity plots are a natural consequence of a random distribution and can be conveniently described by binomial statistics. Supported by grants from the NSF & NIH.

W-Pos76

A CHROMATOGRAPHIC DETERMINATION OF AMINO ACID SIDECHAIN INTERACTION FREE ENERGIES. Thomas C. Pochapsky and Quinton Gopen, Chemistry Dept., Brandeis University, Waltham MA 02254

An HPLC stationary phase (SP) which mimics the leucine sidechain was prepared by treating microparticulate silica with isobutanyltrimethoxysilane. This SP was used for the chromatographic separation of a series of N-acetyl-α-amino acid N'-methylamide analytes using water as a mobile phase. Both hydrophobicity and steric factors are shown to be important for determining retention. Differences in chromatographic retention are related to the differences in free energies of adsorption onto the SP for this series of analytes. The utility of these free energy values for describing the solution interactions between amino acid sidechains will be discussed, including their applicability to describing the initial stages of protein folding.

W-Pos77

**FLUORESCENCE STUDIES WITH BACTEROIDS MALATE DEHYDROGENASE, TWO-TRYPTOPHAN CONTAINING PROTEIN.** **by Camillo A. Ghiron, James K. Waters, and David Emerich, Department of Biochemistry, University of Missouri, Columbia, MO 65201 and Maurice R. Eftink Department of Chemistry, University of Mississippi, University, MS 38677.**

**A number of fluorescence studies, both of trp residues and bound NADH, have been reported for porcine MDH. The large number of trp residues (6) complicates the interpretation of some studies. To circumvent this we have performed studies with a two tryptophan (per subunit) MDH from Rhizobium japonicum 3I1B-143 bacteroids. We have performed phase/modulation fluorescence lifetime measurements, as a function of temperature and added quencher KI, in order to resolve the 1.3 ns (blue) and 6.6 ns (red) contributions for the two classes of trp residues. Anisotropy decay studies have also been performed. The binding of NADH dynamically quenches the fluorescence of both trp residues, but, unlike mammalian cytoplasmic and mitochondrial MDH, there is not a large enhancement in fluorescence of bound NADH upon forming a ternary complex with either tartronic acid or D-malonate.** **This research was supported by NSF Grant DMB 88-06113 to MRE.**

W-Pos78

CORRELATIONS BETWEEN SECONDARY STRUCTURE AND $^1$H NMR CHEMICAL SHIFTS. Lisa M. Smith. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

Is there secondary structural information contained in the $^1$H NMR chemical shifts of proteins? In addressing this question a critical evaluation was performed of five proteins for which both the high resolution crystal structure and the complete assignments of the 2-D $^1$H NMR spectra are known. This analysis was limited to the aliphatic region of the spectra from 0.80 to 4.00 ppm, excluding ring current shifted methyl resonances. Eleven regions of the spectra had correlations of better than 50% between elements of secondary structure and the chemical shifts. Unique amino acid side chains in a defined secondary structure comprised more than 40% of the resonances in four of these eleven regions. The database is being expanded to include several additional proteins in order to test the validity of these correlations. The results of this study imply that structural information can be abstracted from even one-dimensional NMR spectra in the unique regions thus far defined. (Supported by NIH grant GM 36948.)

W-Pos79

**Does the Complexing Protein of Adenosine-Deaminase have a Regulatory Role?**

Dahlia Yaron, Karen Singer, Nurith Porat and Abraham H. Parola*. Department of Chemistry Ben-Gurion University of the Negev, Beer-Sheva, Israel 84 150

The enigmatic role of Adenosine-Deaminase Complexing Protein (ADCP) has led to speculations regarding its modulatory role.We now provide first evidence on ADCP's modulatory effect, particularly at low, physiological substrate concentrations. We were intruiged by ADCP's inhibitory effect at low substrate concentration and accelerating effect at high (>500μM) substrate concentration.Thus, at adenosine concentrations between 1-50μM, ADCP (from calf kidney) was found (by radio-activity assay) to inhibit the activity of small subunit adenosine deaminase (ss-ADA) by 42+2%. Furthermore, while free ss-ADA shows Michaelis-Menten kinetics at 1-1000μM adenosine, when bound to ADCP it shows a bi-phasic activity curve; A plateau at 100-400μM, followed by an increase to another plateau at 600-1000μM adenosine.The latter corresponds to the accelerating effect of ADCP already reported by us.

W-Pos80

DIHYDROFOLATE REDUCTASE (DHFR) FROM E. COLI CATALYZES THE OXIDATION OF NADPH IN THE ABSENCE OF FOLATES, F. Y. Huang, L.-E. Khaw,* Q.-X. Yang, L. Gelbaum,* and T.-h. Huang, School of Physics and *School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332.

DHFR from E. coli was found to catalyze the oxygen-mediated oxidation of NADPH. The maximum activity, as determined from the initial rate of change of proton NMR resonances and spectrophotometric methods, is about four orders of magnitude slower than the normal reductase activity for dihydrofolate and is pH dependent. It varies from ∿ 15 mU/mg at pH = 5.8 or lower to ∿ 1.5 mU/mg at pH = 8.0. The midpoint occurs at pH 6.5. The activation energy was determined to be 20 kcal/mole at pH = 6.8. Trimethoprim and methotrexate total inhibit this activity whereas folate and tetrahydrofolate do not. The implication of this finding will be discussed.

W-Pos81

Fluorescene Investigations of Monoamine Oxidase, the key Enzyme for Biogenic Amines' Degradation. HENRY M. ZEIDAN and SHARUNDA BUCHANAN, Department of Chemistry, Clark Atlanta University, Atlanta, GA 30314.

The fluorescence probes 1-anilino-napthalene-8-sulfonate (ANS), and N-(1-pyrene) maleimide (NPM) were used to probe the active site of the Monoamine Oxidase (MAO). The fluorescence emission of ANS was markedly increased in the presence of ANS. The substrate produced a specific and reversible decrease in the fluorescence emission spectrum. The association constant of ANS binding was determined. The topochemistry of the active site was investigated by using dual fluorescence probe methodology in which fluorescence lables, NPM covalently bound to the essential sulfhydryl group and 1-anilino-napthalene-8-sulfonate and ANS, were used. The spectra data suggested that ANS binding site was within 50° A apart from the essential cystine residue. This work was supported by NIH, RCMI Grant #1G12-RR03062 and NIH Grant #RR 08247 to HZ.

W-Pos82

TYROSINE AS THE NEGATIVE SUBSITE FOR LIGAND BINDING TO THE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR)?

S. F. A. Pearce, Paula Preston-Hurlburt and Edward Hawrot, Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

Structural comparisons of nicotinic agonists and antagonists have long predicted a negative subsite on the receptor to interact with the positively charged nitrogen moiety common to all nicotinic agents.We have used intrinsic fluorescence spectroscopic analyses together with binding studies of selectively modified peptide fragments of the nAChR to suggest that one or two invariant tyrosine residues at postions 190 and 198 on the α-subunit provide the critical negative subsite required for ligand binding. Intrinsic fluorescence studies of peptide fragment α 181-198 (18mer), which is known to bind to α-Bungarotoxin (BGTX) in solid phase assays, ($K_d$ = 0.1 μM), show a marked enhancement of fluorescence on binding. Intrinsic fluorescence of tyrosine in the 18mer was red-shifted indicating the formation of an excited state tyrosinate. This result suggests that hydrogen-bonding of the tyrosine phenolic hydroxyl may be involved in ligand recognition. When the peptide was modified at position 190 by replacing the Tyr by Phe the binding affinity was reduced by 50 fold to 5 μM. If instead Tyr from position 198 was removed, this reduced the binding affinity of the peptide to BGTX by 30-fold to 3 μM. Further modification of this peptide by replacing the Tyr with Phe at position 190 showed no detectable BGTX binding activity ($K_d$ >> 20 μM). In both peptides where the tyrosine at position 190 has been replaced by Phe there is no enhancement accompanying the lower affinity binding to BGTX. Although a number of residues contribute to BGTX-binding, our data suggests that the electronegativity of the tyrosine side chain plays a critical role in the binding site. Supported by the AHA, NIH grant GM32629 and training grant CA-09085.

W-Pos83

SIMULATION OF BINDING AND CATALYSIS IN PROTEINS. Fredy Sussman and Harel Weinstein
Dept. of Physiology and Biophysics, Mount Sinai School of Medicine, N.Y., N.Y. 10029.

We present two applications of recent advances in computer simulations of the energetics of binding and catalysis aimed at a detailed understanding of these processes at the molecular level. In the first, we have studied specificity and structure-function relations in the mechanisms of ion binding in Ca-binding proteins. An important question regarding the function of these proteins is their higher affinity for Ca2+ over other divalent ions such as Mg2+, especially since the intracellular concentration of Mg2+ is often higher than that of Ca2+. We have determined the relative free energies of complexation of Ca and Mg ions in complexes with cyclo-(l-Pro-Gly)$_3$, a small synthetic peptide that binds divalent ions with an affinity comparable to those of naturally occurring proteins. We show that the ion selectivity resides in the difference in the solvation energies of the competing ions in water. In parallel simulations on the known Ca-binding protein calbindin, we have investigated the effect of Ca2+ binding on the structure and dynamics of the motif common to many Ca-binding proteins, the E-F hand. Results indicate that the structure in solution resembles that observed in the crystals of this and other members of this family of proteins, and that removal of the Ca2+ does not lead to unfolding of the EF-hand.

In the second application, computer simulations have been used to study the molecular basis of enzyme catalysis in serine proteases. In this case, the simulations helped us determine why the replacement of the Ser nucleophile by a Cys reduces the catalytic power of the enzyme by more than 3 orders of magnitude. Supported in part by NIH grant GM-41373.

**W-Pos84**

BILE SALT-INDUCED SWELLING OF CARBONIC ANHYDRASES. D.E. Milov, R.B. Shireman, and P.W. Chun, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610.

Bile salts are potent inhibitors of bovine carbonic anhydrase (BCA) and human carbonic anhydrases I (HCA-I) and II (HCA-II). To further characterize the binding of bile salts to carbonic anhydrase, rate constants for the $CO_2$ hydration reaction in the presence of deoxycholate, cholate, glycocholate and taurocholate were determined using stop-flow experiments and scanning molecular sieve chromatography. Values for $K_m$ for BCA, HCA-I and HCA-II were found to be 8.7, 7.7 and 6.7 mM, respectively. The $K_I$ values for the various bile salts tested ranged from 0.1 to 1 mM for BCA, 2.2 to 16 mM for HCA-I, and 0.09 to 0.7 mM for HCA-II.

Bile salt binding to carbonic anhydrases results in an increase in partition radius, molecular volume and surface area. Such bile salt induced conformational swelling may be important to the regulation of enzymatic activity. Bile salt binding to carbonic anhydrase seems to regulate bicarbonate ion availability and bile salt-carrier protein interaction along the enterohepatic circulation. (Supported by NSF Grant DMB 83-12101-02).

**W-Pos85**

NOVEL FEATURES OF THE MITOCHONDRIAL CHANNEL, VDAC, REVEALED IN FREEZE-FRACTURED AND FREEZE-DRIED MEMBRANE CRYSTALS.

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The mitochondrial outer membrane of Neurospora crassa contains a channel-forming protein called VDAC which forms crystalline arrays. Negative stain (NS), freeze-drying (FD), and freeze-fracture (FF) techniques have been used to examine the structure of these arrays. The respective unit cell parameters are a=12.5 nm, b=11.4 nm; $\Theta$=109°; a=12.3 nm, b=11.2 nm, $\Theta$=108°; a=13.8 nm, b=13.4 nm, $\Theta$=117°. The larger unit cell dimensions of the FF specimens reflect their greater lipid:protein ratio (the other preparations were treated with phospholipase $A_2$). Nevertheless, essentially the same morphological unit seems to be present in both the FD and the FF replicas, suggesting that they are simply more closely packed in the lipid-depleted vesicles. In computer-filtered images of negatively stained crystals, substructure is discernible in the stain-excluding regions that separate the six-fold clusters. These stain-excluding regions appear to correlate with the features that are predominantly contrasted in the shadowed specimens, and presumably represent outcrops of protein. These new morphological findings will be discussed in terms of how the VDAC subunits may be arrange in the membrane, and in the molecular disposition of the aqueous channels. (Supported in part by ONR grant #N00014-85-K-0651).

**W-Pos86**

STRUCTURE / FUNCTION STUDIES OF H-RAS p21 EFFECTOR MUTANTS. Sharon Campbell-Burk and Drew E. Van Dyk, E. I. du Pont de Nemours and Company, Wilmington, DE; Channing Der, LaJolla Cancer Research Foundation, LaJolla, CA; David Jameson, University of Hawaii, Oahu, Hawaii.

Mammalian ras proteins are critical in cellular growth control, although their precise role is still unknown. Certain point mutations in the coding sequence of ras genes give rise to oncogenes whose protein products possess transforming properties. We have generated several ras mutants with single amino acid substitutions in the 'putative' effector or target recognition domain of H-ras p21. This domain has been identified as being critical for biological activity and is believed to be the site of interaction with a protein (termed GAP) capable of stimulating the intrinsic GTPase activity of ras proteins. Single tryptophan containing proteins were made to aid in biophysical analysis of these proteins. H-ras mutants with transforming and non-transforming properties were generated by site directed mutagenesis, and then characterized using isotope-edited NMR techniques as well as steady state and time resolved fluorescence spectroscopy. The biochemical and biological properties of these H-ras mutants were also characterized. Our results support a dual role for GAP as both a GTPase activating protein and downstream regulator of ras proteins.

**W-Pos87**

**SELECTIVE MODIFICATION OF THE AMINO ACID RESIDUES OF THE A1 HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN AND ITS PROTEOLYTIC PRODUCT, UP1.** **Jose R. Casas-Finet[1], Richard L. Karpel[1] and Samuel H. Wilson[2]. [1]Dept. of Chemistry & Biochemistry, UMBC, Baltimore, MD. [2]Dept. of Biochemistry, National Cancer Institute, NIH, Bethesda, MD.**

**Both mammalian A1 protein (320 aa) and its N-terminal fragment UP1 (196 aa) bind to single-stranded polynucleotides with high affinity. Electrostatic interactions of charged residues (Lys,Arg) and hydrophobic interactions of aromatic residues (Trp, Tyr, Phe) are likely to be involved. This hypothesis was tested by selective modification of these residues. Cys structural role was also investigated. Reductive methylation of UP1 lysines resulted in smaller polyd(A-T) $T_m$ depression and poly(A) $\theta_{264}$ reduction induced upon binding, relative to unmodified UP1. Photochemical modification by trichloroethanol of the single Trp residue of UP1 or A1 did not alter their affinity for polyethenoadenylic acid. Ligand binding induced quenching of UP1 and A1 Tyr fluorescence, suggesting that Tyr residues in both UP1 and A1 C-terminal domain are involved in stacking; Trp did not exhibit significant quenching of its emission. Nitration of Tyr residues in UP1 by tetranitromethane reduced its affinity for single stranded nucleic acids. Alkylation of the 2 Cys residues of A1 or UP1 by N-ethylmaleimide did not affect binding affinity. Modification of Cys with the polarity-sensitive probe 6-acryloyl-2-(dimethylamino)naphtalene indicated that the dye fluorophores are distant from the polynucleotide lattice in the complex.**

W-Pos88

INTERACTION BETWEEN A PROTEASE, SUBTILISIN, AND A PROTEASE INHIBITOR, STREPTOMYCES SUBTILISIN INHIBITOR (SSI), STUDIED BY SDS-PAGE. Tsutomu Arakawa, Kelly Lazenby, Carl G. Kolvenbach, Thomas P. Horan and Linda O. Narhi, Amgen Inc., Amgen Center, Thousand Oaks, CA 91320.

Interaction between subtilisin and SSI was examined by SDS-PAGE. When a subtilisin/SSI mixture was incubated and then mixed with SDS at room temperature, it showed an abnormal migration upon SDS-PAGE. From this, it was concluded that subtilisin/complex is stable in SDS and migrates abnormally as a complex in SDS-PAGE.

The denaturation of the subtilisin/SSI complex was examined by heating it at different temperatures and after rapid cooling, subjecting it to SDS-PAGE. The band corresponding to the complex gradually disappeared as the temperature was increased from 70° to 85°C; from this, the transition temperature was determined to be about 78°C. This temperature is higher than the transition temperature of the subtilisin alone, indicating stabilization of the subtilisin by SSI.

W-Pos89

CONFORMATIONAL STUDIES ON A SYNTHETIC LEADER PEPTIDE FROM PREPRO BOVINE SERUM ALBUMIN

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Leader or signal peptides, a class of peptides which functions in protein secretion, lack sequence homology but are characterized by hydrophobic and hydrophilic patterns in primary structure. In an effort to test the hypothesis that these typical primary structural features allow leader peptides to fold into common secondary structures, we have been studying the solution conformation of a synthetic leader peptide (LP): MKW(CHO)VTFISLLLLFSSAYSRGVFRR($NH_2$). Size exclusion chromatography studies indicate that preproBSA LP undergoes monomer-oligomer equilibrium in 1% acetic acid. CD studies in acetic acid show that this micellization is accompanied by major changes in the backbone conformation; prepro BSA LP assumes a predominantly aperiodic structure below the critical micelle concentration (cmc) and is predominantly β-sheet above cmc ($>10^{-4}$ M). Addition of trifluoroethanol easily shifts the backbone conformation to α-helix. Helical structure is also favored in aqueous mixtures of acetonitrile and methanol. Using these various solvent systems, we are now employing 2D $^1H$ NMR methods to identify secondary structure on the level of specific residue assignments.

W-Pos90

NUCLEAR MAGNETIC RESONANCE STUDIES OF A SINGLE PROTOTYPICAL ZINC FINGER DOMAIN

B. T. Amann, V. J. Kilfoil, B. A. Krizek, and J. M. Berg (intro. by B. J. Geffney), Department of Chemistry, The Johns Hopkins University, 34th & Charles St., Baltimore, MD 21218

Since the discovery of multiple zinc binding domains in *Xenopus* transcription factor IIIA in 1985, a "zinc finger" DNA-binding motif with the repeated sequence (Tyr,Phe)-X-Cys-$X_{2,4}$-Cys-$X_3$-Phe-$X_5$-Leu-$X_2$-His-$X_3$-His-$X_n$ where X represents an unconserved amino acid has been observed in over 20 other proteins. A 26 amino acid single "zinc finger" peptide composed of the conserved residues and the most frequently occurring residues at the non-conserved positions has been synthesized. This peptide binds both $Zn^{2+}$ and $Co^{2+}$ as shown by NMR and UV absorbance studies. The spectrophotometric studies suggest the peptide binds to a zinc ion in a tetrahedral site formed by the conserved cysteine and histidine residues. Two dimensional NMR studies of the zinc bound peptide reveal evidence of an alpha-helix and an anti-parallel beta sheet. In addition, pH studies indicate an unwinding of the helix as the histidine residues are protonated. The NMR of the $Co^{2+}$ bound peptide is paramagnetically shifted and contains long distance structural information which should further elucidate the folded structure of the nucleic acid binding motif.

W-Pos91

METAL BINDING STUDIES OF A RETROVIRAL NUCLEIC ACID BINDING PROTEIN AND ITS METAL BINDING CORE

L.M. Green and J.M. Berg, Department of Chemistry, The Johns Hopkins University, 34th & Charles Streets, Baltimore, MD, 21218

Retroviral gag gene-encoded nucleic acid binding proteins contain either one or two sequences of the form $CysXaa_2CysXaa_4HisXaa_4Cys$. It has been proposed that these sequences may form metal-binding domains in analogy with the "zinc finger" domains first observed in transcription factor IIIA. An 18 amino acid peptide derived from the core nucleic acid binding protein of Rauscher murine leukemia virus had been synthesized, for which we reported a $Co^{2+}$ dissociation constant for the peptide-metal complex of 1 micromolar, and preferential binding of $Zn^{2+}$ over $Co^{2+}$. The absorption spectra of the peptide-$Co^{2+}$ complex is highly suggestive of a tetrahedral coordination, involving three cysteinates and one histidine. The entire core nucleic acid binding protein from Rauscher murine leukemia virus has since been synthesized. The absorption spectra, metal ion affinities and other properties of the peptide are compared to those of the protein from which it was derived. In addition, peptide sequence variants corresponding to reported protein mutations have been synthesized, and their metal-binding properties will be correlated to biological activity.

W-Pos92

**A HISTIDINE TO CYSTEINE VARIANT OF A ZINC FINGER PEPTIDE**

**D. L. Merkle and J. M. Berg (Intro. by Warner E. Love) The Johns Hopkins University, Department of Chemistry, 34th and Charles Streets Baltimore, MD 21218**

**Unknown before 1984, the zinc finger protein conformation is now recognized as an ubiquitous protein folding motif. A peptide of the sequence Pro-Tyr-Lys-Cys-Pro-Glu-Cys-Gly-Lys-Ser-Phe-Ser-Gln-Lys-Ser-Asp-Leu-Val-Lys-His-Gln-Arg-Thr-Cys-Thr-Gly was synthesized in which the coordinating amino acids were typical of the metal-binding domains of retroviral nucleic acid-binding proteins, and the spacings and conserved residues were those of a consensus peptide derived from detailed analysis of 131 finger sequences. The titration with $Co^{+2}$ of reduced, HPLC-purified peptide was monitored spectrophotometrically, as was the back-titration with $Zn^{+2}$ of reduced, HPLC-purified peptide in the presence of excess $Co^{+2}$. Resultant data allowed the calculation of dissociation constants for the binding of $Co^{+2}$ ($K_d^{Co}$ - 4 x $10^{-6}$ M) and $Zn^{+2}$ ($K_d^{Zn}$ - 0.5 x $10^{-9}$ M) to the peptide. The spectrum of the peptide-$Co^{+2}$ complex resembled that of a retroviral peptide with the ligands Cys-Cys-His-Cys. The relationships among spectrophotometric properties and dissociation constant values for a more normal Cys-Cys-His-His zinc finger consensus peptide, a Cys-Cys-His-His zinc finger peptide derived from TFIIIA, a Cys-Cys-His-Cys retroviral peptide, and the peptide described above will be discussed.**

W-Pos94

THE ROLE OF POSITION 49 IN THE FOLDING OF THE α SUBUNIT OF TRYPTOPHAN SYNTHASE FROM *ESCHERICHIA COLI*, B. A. Chrunyk and C. R. Matthews, Chemistry Dept., The Pennsylvania State University, University Park, PA 16802.

The advent of recombinant DNA techniques has greatly expanded the possibilities for solution of the protein folding problem. Since sequence determines structure[1], alteration of the primary sequence of amino acids by site directed mutagenesis can provide information as to which residues are key to the folding process. This approach has been taken with the α subunit of tryptophan synthase, a small (28,700 MW) monomeric protein whose folding is well characterized. In this study, a key residue for stability and activity, Glu 49, was replaced by Gln. Comparison of the results obtained from both equilibrium and kinetic studies with previous results from the wild type and Met 49 mutant protein [2], indicate that position 49 is critical to the structure of a folding intermediate and that the degree of the perturbation caused by replacements is modulated by the nature of the residue involved. This work supported by General Medical Sciences grant GM 23303.

[1] Anfinsen, C. B. *Science 181*:223 (1973).
[2] Beasty et al., *Biochemistry 25*: 2965 (1986)

W-Pos93

RECOMBINANT DHFR FUSION DIMER

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Dihydrofolate reductase (DHFR) is a small monomeric protein of MW 18KDa whose folding has been extensively studied.

We have successfully fused the DHFR structural gene of E. coli to form a single polypeptide chain composed of two DHFR sequences. The purified recombinant protein shows twice the enzyme activity (per molecule) as that of wild type monomeric enzyme. Inhibitor binding experiments using Methotrexate or trimethoprim show that two molecules of each of the inhibitors bind to one molecule of the "DHFR fusion dimer " suggesting that the dimer has two active DHFR domains.

To determine whether the covalently linked DHFRs act as independent folding units, the urea induced unfolding of the dimer was investigated. The equilibrium unfolding transition observed by UV different spectroscopy at 293 nm is described by a three-state model, differing from the wild type DHFR which obeys a two-state model. This is suggestive that the two domains are interacting with one another. The kinetic analysis of the fusion dimer is currently under way.

W-Pos95

**REPULSIVE RESTRAINTS FOR HYDROGEN BONDING IN LEAST-SQUARES REFINEMENT OF PROTEIN CRYSTALS:**
A Neutron Diffraction Study of Myoglobin Crystals

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Center for Structural Biology, Department of Biology, Brookhaven National Laboratory, Upton, New York 11973

A set of empirical rules have been established that describe stereochemical restraints on hydrogen bonding within proteins and their associated solvent which can be included in the refinement of x-ray or neutron structures of protein crystals. The parameters which define the geometry of hydrogen bonding, i.e., the correlation between distances and angles, are based on the results of an analysis of hydrogen bonding in crystal structures of myoglobin derivatives analyzed by neutron diffraction[1,2].

[1] Schoenborn,B.P. (1988) *J. Mol. Biol.* **201**, 741-749
[2] Cheng,X. and Schoenborn,B.P. (1989) *Acta Cryst.* in press
[a] Department of Physics, State University of New York at Stony Brook, Stony Brook, New York 11794

W-Pos96

EFFECT OF A GLYCINE TO ALANINE SUBSTITUTION ON TRIPLE HELICAL STRUCTURE. Cynthia G. Long and Barbara Brodsky, Biochemistry Dept., UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

A required feature of collagen triple-helical structure is the presence of glycine as every third residue, resulting in a Gly-X-Y pattern. In the fibril forming collagens, a single substitution of a glycine can be lethal. In contrast, a number of other genetic collagen types contain multiple interruptions in the Gly-X-Y pattern. A database has been established containing sequence information on reported interruptions in collagen triple helical domains. Classification of interruptions and searches for patterns in the surrounding amino acids are in progress. A $(Gly\text{-}Pro\text{-}Hyp)_{10}$ computer model and energy minimization are being used to determine the local effect on the triple helical structure of a glycine to alanine substitution in one, two and three chains. In addition a synthetic peptide $[(Pro\text{-}Hyp\text{-}Gly)_5\text{-}(Pro\text{-}Hyp\text{-}Ala)\text{-}(Pro\text{-}Hyp\text{-}Gly)_4]$ has been synthesized and its kinetic and thermodynamic parameters will be compared with those of $(Pro\text{-}Hyp\text{-}Gly)_{10}$.

W-Pos97

INVESTIGATIONS OF THE SOLUTION CONFORMATION OF PEPTIDE ANALOGS OF THE ADHESIVE POLYPHENOLIC PROTEIN FROM M. edulis. MARK W. TRUMBORE and LEO G. HERBETTE, Biomolecular Structure Analysis Center, UConn. Health Center, Farmington, CT 06032. Previous work by Williams et. al. (Arch. Biochem. Biophy. 269:415,1989) has shown that the secondary structure of the native adhesive polyphenolic protein from the Common Blue Mussel Mytilus edulis has little or no alpha helix or beta sheet. We here report studies of the solution conformation of peptide analogs of the native adhesive as obtained by small angle x-ray scattering and size-exclusion chromatography. Our studies have shown that the peptide analogs studied form well defined aggregates in solution with radii of gyration on the order of ≈25Å. The aggregates were stable to denaturation by both guanidine and urea. Conformational models of the peptide analogs were constucted based upon molecular mechanics calculations and solution scattering and chromatography data. This analysis indicated that the peptide aggregates have a rod-like conformation in solution. This work was supported by the State of CT Dept. of Higher Education Cooperative High Technology Program with Biopolymers Inc. of Farmington, CT.

W-Pos98

ON THE USE OF PLS FOR THE DETERMINATION OF THE CONFORMATION OF PROTEINS IN AQUEOUS SOLUTION FROM THEIR INFRARED SPECTRA. M. Pézolet and F. Dousseau, CERSIM, Département de chimie, Université Laval, Québec, Canada.

Infrared spectra of $H_2O$ solutions of severals proteins of known X-ray structures have been recorded and corrected for the spectral contribution of water in the amide I and II regions using an algorithm developed in our laboratory (Appl. Spectrosc. 43, 538 (1989)). This calibration set of spectra have been analysed using the partial least-squares (PLS) multivariate statistical method (Anal. Chem. 60, 1193 (1988)). The results show that the best agreement between the secondary structure determined by X-ray crystallography and predicted by infrared spectroscopy is obtained when both the amide I and II bands are used to generate the calibration set, and by assuming that the secondary structure of proteins is composed of only four structures: extended and disordered α-helices, β-sheet and unordered conformation. The average absolute standard deviation on the prediction of the conformation of the proteins in the calibration set is 4.8% for the α-helices, 3.7% for the β-sheet and 5.1% for the unordered conformation, while the regression coefficients are 0.98, 0.98 and 0.74, respectively. These results will be compared to those obtained from $^2H_2O$ solutions.

W-Pos99

CHANGES IN THE CONFORMATION OF SPINACH FERREDOXIN UNDER DIFFERENT PH CONDITIONS. Kieleczawa, J., France, L.L., Hind, G. and Sutherland, J.C., Biology Dept., Brookhaven National Lab. We have monitored changes in the conformation of spinach ferredoxin under differet pH conditions (pH 5 - 11), using steady-state and time-resolved fluorescence techniques. Fluorescence quenchers (acrylamide, $I^-$, $Cs^+$) were used to study their accessibility to the single tryptophan residue in spinach ferredoxin. Hydrophobicity measurements were conducted by introducing micelles containing brominated Brij-96. An increase in $\lambda_{max}$ (emission), an increase in protein hydrophobicity, an increase in the fluorescence lifetime (in the absence of quenchers), and an increase in dynamic fluorescence quenching by acrylamide, $I^-$, and $Cs^+$, were observed with increasing pH. These results indicate that as the solvent pH increases, the tertiary structure of ferredoxin changes, so that the tryptophan shifts from a moderately buried position to a more solvent-exposed position.

W-Pos100

SPECTROSCOPIC ANALYSIS OF SINGLE TRYPTOPHAN MUTANTS OF CYTOCHROME $B_5$ NONPOLAR PEPTIDE. J.F. Hedstrom and F.G. Prendergast, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905.

The nonpolar binding segment of cytochrome $b_5$ (with three tryptophans at positions 108, 109 and 112) has served as a model for peptide insertion into membranes. Previous fluorescence resonance energy transfer data have been interpreted to mean that only trp(109) is fluorescent and that this residue is buried in the apolar region of the bilayer. Investigation of single tryptophan analogues of cyt $b_5$ nonpolar peptide (with phe for trp substitutions) greatly simplifies the experimental problem *a priori*, since the origin of the tryptophan fluorescence can be assigned unambiguously. Comparisons of the quantum yield, CD, fluorescence emission and lifetime data for each of the analogues to the data from the native peptide yields information on the structural conservation among these peptides and the microenvironment of each tryptophan. These data provide the basis for interpreting spectroscopic results for native cyt $b_5$ in lipid vesicles. Supported by GM 34847 (to FGP) and GM 13232-01 (to JH).

W-Pos101

FLUORESCENCE PROPERTIES OF α-CYCLODEXTRIN: 5-METHYL INDOLE COMPLEX. COMPARISONS WITH PROTEIN FLUORESCENCE. Sedarous, S.S., Madden, B., Bajzer, Z., Axelsen, P.H., and Prendergast, F.G., Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905.

The fluorescence intensity decay of 5-methyl indole (5-MeI) complexed with α-cyclodextrin has been examined. Complexation results in (i) a dramatic blue shift in emission, (ii) the appearance of well resolved fine structure in the emission and excitation spectra, (iii) a four-fold increase in quantum yield (compared to 5-MeI in water) with a commensurate increase in lifetime (from 3.0 to 10.7 ns) and (iv) a complete inaccessibility to acrylamide or iodine, but continued accessibility to oxygen. Apparently the complex shows 2:1 cyclodextrin:indole stoichiometry. Validity of data analysis procedures was assessed by studying samples bearing free and bound 5-MeI. Despite similarities in tryptophan environment in apo-azurin and the cavity of the cyclodextrin, the trp fluorescence of apo-azurin was much less structured, with shorter lifetimes and lower quantum yields, implying protein matrix effects on nonradiative decay. Supported by GM 34847.

W-Pos102

NEAR-UV CIRCULAR DICHROISM OF SINGLE TRYPTOPHAN PROTEINS. P.J. Fisher, K.E. Nollet and F.G. Prendergast, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905.

Near-UV CD bands of tryptophan and tyrosine have been identified and shown to be environmentally sensitive. Previously published studies have been concerned largely with deconvolution of spectra obtained from proteins with multiple absorbing groups. Examination of single tryptophan proteins has enabled us to ascribe changes in characteristic CD bands to changes in the environment of the fluorophores, including solvent accessibility and proximity of other aromatic groups. For example, the binding of metals to both whiting parvalbumin and eel TnC causes tryptophan CD band sharpening and shifting indicative of a decrease in environmental polarity, consistent with fluorescence data. In addition, changes in peak intensity may be attributed to changes in distance between fluorophores. Near-UV CD data on other single tryptophan proteins support the inferences drawn for these two calcium binding proteins. Molecular graphics depictions are being used in an attempt to rationalize the basis for the near UV CD properties. Supported by GM 34847.

W-Pos103

PROTEIN SECONDARY STRUCTURES IN WATER DETERMINED FROM SECOND DERIVATIVE AMIDE I INFRARED SPECTRA. Aichun Dong, Ping Huang, and Winslow S. Caughey, Department of Biochemistry, Colorado State Univ., Fort Collins, CO 80523.

Infrared spectra for 13 proteins which vary widely in secondary structure have been measured in aqueous solution at 20°. Criteria were developed which optimize the subtraction of spectra in the amide I region due to liquid and gaseous water. Second derivative amide I spectra gave highly consistent frequencies for bands due to the specific secondary structures: α-helix, β-sheet, unordered, and turns. α-Helix and unordered structures could each be assigned to only one band whereas multiple bands were associated with both β-sheets and turns. Relative amounts of each type of secondary structure determined from second derivative band areas correspond closely to the amounts determined crystallographically. These findings demonstrate the similarity of solution and crystal structures and the utility of second derivative amide I infrared spectra for the qualitative and quantitative measurement of polypeptide secondary structure.
(Supported by U.S. Public Health Service Grant HL-15980).

W-Pos104

RAMAN AND *AB-INITIO* STUDIES OF SHIFTS IN THE AMIDE III FREQUENCIES OF ALA-X PEPTIDES
Robert W. Williams†, James L. Weaver†, and Alfred H. Lowrey‡. †Department of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd, Bethesda, MD 20814-4799. ‡Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C., 20375-5000. Raman spectra of aqueous solutions of alanine-X peptides, where X is an uncharged amino acid, show that the amide III band shifts systematically to lower frequencies as the sidechain of the X amino acid becomes larger. The range of this shift is about 20 $cm^{-1}$(1275 $cm^{-1}$ for ala-gly, 1251 $cm^{-1}$ for ala-trp) with a correlation coefficient of 0.93 with the mass of the X amino acid side chain for 10 peptides. This shift may result from changes in: the average conformational preference of the peptide, vibrational coupling of the amide III modes with the X amino acid sidechain, or vibrational force constants. Computational techniques have been evaluated for the purpose of interpreting these shifts. Optimized structures and frequencies have been calculated for ala-X peptides using GAUSSIAN86 with the 4-31G basis, MOPAC, CHARMm, and normal-mode methods based on empirical force fields. 1) Frequencies calculated from structures optimized by MOPAC and CHARMm do not show a systematic shift in the amide III frequency. 2) Frequencies calculated from scaled GAUSSIAN86 forces correlate well with the experimental observations. 3) Changes in the optimized peptide conformations from $\beta$-sheet to $\alpha$-helix produce large changes in both calculated amide I and amide III frequencies. 4) Changes in the dihedral angle $\phi$ produce a change in the calculated amide III frequency consistent with the experimental observations.

W-Pos106

PHYSICAL PROPERTIES OF THE RETROVIRAL MATRIX PROTEIN.
Joyce E. Jentoft, Craig A. Gelfand, John D. Cameron and Qi Wang. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

The matrix (MA) protein forms the inner core of the virion in retroviruses and interacts with the viral membrane. We have initiated a characterization of the physical properties of the MA protein from avian myeloblastosis virus. The size of the MA protein in solution at pH 5.0 at an ionic strength of 0.3 M was determined using high resolution gel filtration; it is a mixture of hexamers (115 kDa), trimers (59 kDa), and dimers (33.5 kDa). The secondary structure of the MA at pH 7 in 40 mM MES buffer was deduced to be 12% alpha helix, 34% beta sheet, 15% beta turn and 38% random coil, based on analysis of the 185 to 250 nm region of the circular dichrosim spectrum. The intrinsic fluorescence and polarization anisotropy was characterized for the MA protein, as was its interaction with the extrinsic fluorophore, bis-ANS, which showed a large enhancement of its fluorescence emission upon binding to the MA protein (Supported by NIH grant GM36948).

W-Pos105

EVIDENCE FOR MULTIPLE CONFORMATIONS OF THE AMV NUCLEOCAPSID PROTEIN.
Josephine Secnik and Joyce E. Jentoft. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106.

The nucleocapsid (NC) protein of avian myeloblastosis virus (AMV) is a small basic protein that binds to viral RNA. We utilized the extrinsic fluorescent probe bis-ANS to probe the properties of NC. Bis-ANS binds to the NC protein with a 4-fold enhancement of the intrinsic fluorescence of the bis-ANS, and energy is transferred from NC to the bis-ANS. A continuous variation plot showed that 3 bis-ANS bound per NC molecule. This emission intensity is very sensitive to pH, added salt, and added nucleic acid. Bis-ANS binding to NC is maximum in the pH range 5-7. The energy transfer is efficiently quenched by added salt, with a $K_{0.5}$ of 30 mM for NaCl at pH 5.5, indicating that salt interferes with bis-ANS binding. Based on these studies, we propose the presence of at least two solution conformations for the AMV NC. The fluorescence of the bis-ANS in the NC/bis-ANS complex is quenched by added nucleic acid, suggesting that nucleic acid binding to the NC protein can be monitored via the fluorescence of the bis-ANS complex. (Supported by NIH grant GM36948).

W-Pos107

Modulation of Keratin Structure by Phosphorylation.
P. L. Yeagle†, J. Frye¶, and B. S. Eckert§, Departments of †Biochemistry and of §Anatomical Sciences, University at Buffalo School of Medicine, Buffalo, NY 14214 and ¶Department of Chemistry, Colorado State University, Fort Collins, CO 80523.
Aggregation of keratin intermediate filaments appears to be accompanied by keratin dephosphorylation. Natural abundance cross polarization (CP) MAS $^{13}C$ NMR was used to examine the effect of phosphorylation on keratin structure. Using short contact times, cAMP-dependent phosphorylation was shown to increase the number of residues in the motionally restricted portions of the protein. CD spectra were characteristic of $\alpha$ helix for this keratin. Phosphorylation of the keratin by cAMP-dependent protein kinase resulted in a CD spectrum with the same shape but of greater apparent intensity. This may have been the result of an increase in the $\alpha$ helical content of the protein. When the keratin was phosphorylated using cold ATP, magic angle spinning (MAS) $^{31}P$ nuclear magnetic resonance revealed two resonances arising from the phosphorylation sites on the keratin. The more shielded resonance was shown to arise from cAMP-dependent protein kinase phosphorylation. Static $^{31}P$ NMR measurements suggested that at least two classes of cAMP-dependent sites existed. NSF DCB8800611

W-Pos108

## HIGH RESOLUTION CALORIMETRY OF BOVINE LENS CRYSTALLINS

A.C. Sen[1], B. Chakrabarti[1,2] and M.T. Walsh[3]
[1]Eye Research Inst., [2] Dept. of Ophthalmology, Harvard Med. Sch., [3]Dept. of Biophysics, Boston Univ. Sch. of Med., Boston, MA Crystallins (CR's) are a mixture of highly concentrated eye lens proteins. Alteration in their chemical and physical properties leads to cataract formation. We report the first study of the thermal behavior of two purified subfractions of CR's: $\alpha_L$-an 800kD multisubunit aggregate and γII-a 20 kD monomer using high resolution calorimetry with comparison to our previous far UV circular dichroism (CD) data. $\alpha_L$ exhibits a high enthalpy (ΔH) endothermic transition at $T_m$ of ~60° with a peak of lower ΔH at 45°. CD has shown $\alpha_L$ to be mostly β-sheet, with α-helix increasing from 4 to 12% above 60° and no denaturation or precipitation up to 100°. The endotherm and secondary structural change at 60° suggest dissociation of the high molecular weight $\alpha_L$ 'aggregate' into subunits, perhaps preceded by a pre-dissociation with a low ΔH at 45°. γII exhibits an endothermic transition at $T_m$ of 70° and an exothermic peak at 73° and precipitation. CD has shown γII to undergo a native-to-denatured transition at $T_{1/2}$ of 70° with irreversible aggregation at ~73°. The calorimetric data support our previous model that γII undergoes a two-state reversible unfolding prior to irreversible denaturation.

W-Pos109

## THE FOLDING OF DHFR BY GDN•HCL AND GDN•SCN

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The folding of dihydrofolate reductase (DHFR) from E. coli has previously been examined using urea as the denaturant (Touchette et al., Biochem, (1986) 25, 5445.). To determine whether the folding mechanism depends on denaturant, we have now examined the equilibrium and kinetic folding properties of DHFR using GdnHCl and GdnSCN. The midpoints of the equilibrium transition curves were found to be 1.6 M for GdnHCl, 0.8 M for GdnSCN, and 3.1 M for urea, at pH 7.8, 15°C. While the slow ($\tau$>10 sec) unfolding kinetics in urea are well described by two phases, only a single phase is apparent in unfolding with GdnHCl. The difference may be due to the difference in ionic strength between the two denaturants. This work is supported by NSF grant DMB-8705673.

W-Pos110

## HYDROXYL HYDROGEN CONFORMATIONS DETERMINED BY NEUTRON $D_2O$-$H_2O$ DIFFERENCE MAPS.

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Neutron diffraction maps have been used to assign the rotor conformations of the hydroxyl hydrogens in trypsin. Most hydroxyl groups are rotationally ordered with their highest populated conformation near the low energy staggered orientation. For the low energy conformers of Ser and Thr, the trans (-180°) position is most highly populated. In trypsin only 1 out of 24 serines was found in the +60° conformer. Serine hydroxyls preferentially act as H-bond acceptors and rarely as H-bond donors alone. Threonines were more likely than serines to participate in two H-bonds; tyrosines prefer to H-bond as donors alone. In H-bonding situations where there was incompatibility between the energies defining the barrier to rotation and the local electrostatics, the electrostatic criteria dominated. Overall, the findings support a model of hydrogen bonding where there exists strong inherent complementarity between the low energy hydroxyl orientations and the local electrostatic environment.

W-Pos111

## STRUCTURE AND STABILITY OF A SERUM RETINOL BINDING PROTEIN COMPLEX WITH RETINOIC ACID.

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Previously (1) we have studied the thermodynamic stability of serum retinol binding protein (N) containing retinol (R), and have suggested the following three-stage model for its thermal unfolding and refolding involving stable intermediates I and I/R:

$$N \rightleftharpoons I/R \rightleftharpoons I + R \rightleftharpoons U + R$$

By introducing retinoic acid to the unfolded form U, instead of retinol, we have generated a new retinoid-protein complex (N'). Using primarily CD, we have shown that N' has very similar secondary and tertiary structures to that of N. However, the thermodynamic values obtained from the fit of the DSC curve for the unfolding process showed that N' was less stable than N, even though it has been reported that both retinoids have similar binding constants to the protein. These data support the proposed mechanism by showing that the first two steps of the mechanism are sensitive to retinol.

1. Waterhous et al. Biophys. J. (1989) 55, 414a.

W-Pos112

CIRCULAR DICHROISM AND FLUORESCENCE OF PLANT CALMODULIN. G. Sanyal*+, C. R. Middaugh*, F. M. Thompson+ and D. Puett!. *Merck Sharp & Dohme Res. Labs., West Point, PA, +Hamilton College, Clinton, NY and !University of Miami, Miami, FL.

We have used circular dichroism (CD) and fluorescence to study secondary structure and tyrosine (Y) environment of wheat germ calmodulin (CaM). The fluorescence quantum yield (Φ) of the single Y (Y-138) is increased upon chelation of apo-wheat CaM with $Ca^{2+}$. The Φ for the $Ca^{2+}$-bound form is sensitive to varying pH from 5 to 7. The CD spectra for both forms are, however, insensitive to pH in the same pH range, suggesting no alteration in the secondary structure. Thus the Y-138 possibly senses a local perturbation in its environment upon changes in pH, such as ionization of a nearby residue or a subtle change in its orientation and exposure to solvent. Binding of $Ca^{2+}$ to apo-wheat CaM causes a large increase in helicity. The thermal stability of wheat CaM secondary structure has been measured by CD and compared with that of bovine testes CaM.

(NSF Grant DMB 8805082 and PRF Grant 18076-GB4 to G. S. are gratefully acknowledged).

W-Pos113

## MULTI-FREQUENCY CALORIMETRY OF CYTOCHROME C.

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Multi-frequency calorimetry is a new technique for measuring the power spectrum of the enthalpy fluctuations associated with structural transitions in biological macromolecules. As such, it permits resolution of the magnitude and time course for the energetic events which comprise a transition. In conjunction with other techniques, the kinetics and energetics of specific structural events can be determined.

A multi-frequency calorimeter has been used to study the folding-unfolding transition of cytochrome c, at pH 4. Under these conditions the transition enthalpy is 80 kcal/mol. At the midpoint of the transition, 66°C, the data indicate a relaxation process having a characteristic time of 380 ± 75 ms, and involving about 85% of the excess heat capacity of the transition. (Supported by NIH grants GM-37911 and RR-04328.)

W-Pos114

## A CALORIMETRIC STUDY OF GUANIDINE HCl INDUCED PROTEIN DESTABILIZATION IN DIPHTHERIA TOXIN.

G. Ramsay and E. Freire, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

The thermodynamic structure of diphtheria toxin (DT) has been further investigated by differential scanning calorimetry (DSC) and guanidine hydrochloride (GuHCl) destabilization. Equations which describe the linked functions for the thermal and solute stability of DT have been developed. At present the best model which describes the excess Cp surface is the denaturant binding model and two independent, two-state transitions, with the results of the fitted parameters being ΔH1=205 kcal/mol, Tm1 = 54.0 °C, ΔCp1 = 5230 cal/mol*K, Δn1 = 79 sites, ΔH2 = 92.2 kcal/mol, Tm2 = 54.8°C, ΔCp2 = 2960 cal/mol*K, and Δn2 = 60 sites, and k"a = 0.50 M-1. These parameters show that in the GuHCl concentration range of 0.5 to 1.0 M there exists an intermediate state in which the low enthalpy domain (assigned to the A fragment) is denatured, while the high enthalpy domain (assigned to the B fragment) still exhibits structure. This intermediate state is possibly significant to the translocation of the toxin into the cytoplasm. (Supported by NIH grants GM-37911 and RR-04328.)

W-Pos115

## THE THERMAL UNFOLDING OF INDIVIDUAL SUBUNITS IN YEAST CYTOCHROME C OXIDASE.

P.E. Morin, D. Diggs, E. Freire Department of Biology and Biocalorimetry Center The Johns Hopkins University Baltimore, Maryland 21218

Using the methods of Differential Solubility Thermal Gel Analysis (DSTGA) and Differential Scanning Calorimetry (DSC) we have studied the thermal unfolding of yeast cytochrome c oxidase reconstituted into phospholipid vesicles in active form. The thermal unfolding of the enzyme complex is an irreversible process found to be kinetically controlled as demonstrated by experiments performed at different scanning rates. Individual subunit unfolding profiles determined by DGSTA are well represented in terms of a kinetic two-state transition model. Activation energies for the subunits are on the order of 40 kcal/mole and have been found to depend upon the phospholipid composition as well as the chemical and physical environment of the system. Supported by NIH grant GM-37911.

W-Pos116

**THE DISSOCIATION OF THREE HYDROPHILIC SUBUNITS FROM YEAST CYTOCHROME C OXIDASE.** D. Montgomery & E. Freire, Department of Biology and The Biocalorimetry Center, The Johns Hopkins University, Baltimore, MD 21218

Yeast cytochrome c oxidase is composed of nine subunits, the three largest ones are synthesized in the mitochondria and the remaining ones are imported from the cytoplasm. Our previous studies suggested different layers of interactions that define three major cooperative structures in the holoenzyme. The subunit components of these structures appear to be: a) I and II; b) III; and, c) IV-VIII. To confirm these assignments and study the assembly of the enzyme, we have started a research program to dissociate the various subunits and then perform stability and reassembly experiments. Subunits IV,V, and, VI have been dissociated from the enzyme by hydrophobic interaction chromatography with a GuHCl gradient. After removal of the GuHCl, these hydrophilic subunits show secondary structure and undergo a reversible thermally induced unfolding transition centered at 33° C as demonstrated by CD and DSC. The remaining hydrophobic subunits undergo a thermally induced unfolding transition centered at 45° C. These studies show that these two groups of subunits mutually stabilize each other in the holoenzyme, Tm=55° C. (Supported by NIH grants GM-37911 and RR-04328).

W-Pos118

Phospholipid Induced conformational change in profilin.

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The membrane lipid component, phosphatidylinositol 4,5-biphosphate ( PIP2 ) induces conformational changes in profilin as determined by the shift of the aromatic resonances and a change in the chemical shift dispersion of the aromatic residues in the high resolution $^1H$ NMR spectrum of profilin. This conformational change is observed at physiological salt concentrations and is inhibited in presence of divalent cations like $Mg^{2+}$ and $Ca^{2+}$. The narrower resonance lines in the one dimensional spectra and the sharper cross-peaks in the two dimensional spectra indicate that the protein conformation in presence of PIP2 is demonstrating a faster correlation time than in the native state i.e in absence of the lipid. Increasing the lipid:protein ratio shifts the resonances in favour of the new conformation. Furthermore circular dichorism spectra indicate changes in the secondary structural elements of the protein. This suggests a mechanism by which PIP2, an intermediate in the PI-cycle, may cause the dissociation of the profilactin complex and the subsequent polymerisation of the actin under agonist induced activation of cells.

W-Pos117

DISULFIDE FORMATION IN CYSTEINE-RICH PEPTIDES. Rumin Zhang & Grayson Snyder (Dept. of Biological Sciences, SUNY, Buffalo, NY 14260)

We are interested in factors contributing to formation of specific disulfides in cysteine-rich proteins. For species as large as 60 residues, burying of hydrophobic sidechains and formation of hydrogen-bonded secondary structures assist in stabilizing particular disulfide combinations. For small proteins having fewer than 20 residues such as some conotoxin and enterotoxin species, formation of a specific set of disulfides occurs in spite of the absence of alpha-helix, beta-sheet, or a buried interior. The mechanism of folding of these toxins is unknown. We will report on investigations of thermodynaic and kinetic factors affecting disulfide formation in synthetic peptide analogs of these toxins. One finding is that sidechains responsible for the biological functioning of the toxins can be replaced by alanines without diminishing specificity of disulfide formation in these 4-cysteine and 6-cysteine molecules. The presence and relative positioning of a few key residues are implicated as necessary and sufficient for the folding.

W-Pos119

GLOBAL ANALYSIS OF LIFETIMES OF DANSYLATED PHOSPHAGEN KINASES. Steven H. Grossman, University of South Florida, Tampa, FL 33620

Lifetimes of doubly-dansylated dimeric creatine kinase (CK) and the homologous singly-dansylated monomeric arginine kinase (AK) were determined using phase/modulation methods and global analysis of multiple data sets. The most suitable decay law for CK is a bimodal Lorentzian distribution with centers at 3.71 and 12.63 ns, corresponding relative intensities of 0.327 and 0.673 and distributions of 5.95 and 3.09 ns, respectively. The dead-end complex (creatine/MgADP) broadens the short lifetime and narrows the long lifetime distributions. Central lifetimes are unaffected but the intensity ratio now approaches unity. Arginine kinase exhibits a bimodal distribution with centers at 5.52 and 17.55 ns, relative intensities of 0.344 and 0.656, and distributions of 4.41 and 4.68 ns, respectively. Denaturation significantly changes parameters but the bimodal distribution persists. A model is developed which includes the occurrence of two conformers for the monomeric and dimeric phosphagen kinases and conformational changes associated with substrate binding. (Supported by NIH Grant NS-23396)

W-Pos120

KINETICS OF HETERODIMER FORMATION IN TROPOMYOSIN COILED-COILS. Sumio Ozeki, Marilyn Emerson Holtzer, and Alfred Holtzer, Dept. of Chem., Washington University, St. Louis, MO 63130. Mixtures of tropomyosin ($\alpha\alpha$) and sulfhydryl-blocked tropomyosin ($\alpha'\alpha'$) coiled coils at 38°C are assayed for formation of $\alpha\alpha'$ heterodimers as a function of time. Under these conditions, dimers are the principal species and the equilibrium fraction (h) of heterodimer, is 0.5. The three most likely mechanisms are: 1) dissociation-reassociation; 2) dissociation-displacement; 3) double displacement. For all, kinetic analysis shows $h = 0.5\ (1-e^{-\kappa t})$, wherein $\kappa = kC^n$, C being the loading protein concentration. In mechanism 1), n = 0 and k is the rate constant for dissociation into chains. In mechanism 2), n = 0.5 and $k = 2k_2\sqrt{K}$, wherein $k_2$ is the rate constant for displacement and K is the dissociation equilibrium constant. In mechanism 3), n = 1 and k is the rate constant for double displacement. Experiment shows n = 0, indicating that heterodimer formation is largely by dissociation-reassociation. The dissociation rate constant at 38°C is $k_1 = 8 \times 10^{-5}$ $sec^{-1}$.

[Supported by NIH and MDA.]

W-Pos121

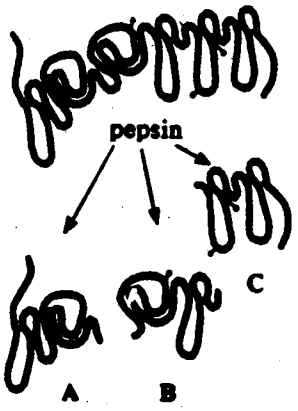
THE IONIC STRENGTH DEPENDENCE OF COILED-COIL UNFOLDING EQUILIBRIA. Jian-Ming Mo, Marilyn Emerson Holtzer, and Alfred Holtzer, Dept. of Chem., Washington University, St. Louis, MO 63130. Non-polymerizable tropomyosin (NPTM) unfolds almost exactly like the parent, but does not aggregate at low ionic strength (I). CD for NPTM shows that reduction of I is markedly destabilizing. Fraction helix (f) or $T_{\frac{1}{2}}$ vs I show a regime change at $I \approx 0.1$ M. At 40°C, f vs I data cover a wide range of f. CD spectra vs T or I show an isodichroic point at 203 nm, suggesting a local two-state model. At given T, such a local coil $\rightleftharpoons$ helix equilibrium suggests: $\ln[f/(1-f)] = A_1 + A_2 I^n$; wherein $A_1$, $A_2$, and n are constants. For low I, well-known limiting theories for charge-charge, dipole-dipole, and apolar-apolar (salting out) interactions give, respectively, n = 0.5, 1.0, and 1.0. Our experiments show n = 1.0, suggesting that I stabilizes either by reducing helix dipole-dipole repulsion or by enhancing helix-helix hydrophobic interactions.

[Supported by NIH and MDA.]

W-Pos122

**Stability and Interactions of the Finger & Kringle-like Modules in Gelatin-Binding Fragments of Fibronectin.** S.Litvinovich, D.Strickland, L.Medved, & K.Ingham Am Red Cross, Rockville, MD & Inst Biochem, Kiev, UkSSR

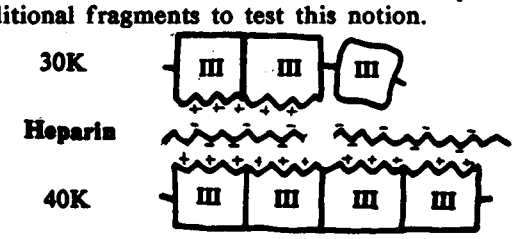
The 42kDa gelatin-binding "domain" of fibronectin exhibits a single reversible melting transition near 60° with $\Delta H_{cal}/\Delta H_{vH} = 1.6$ suggesting a complex subdomain structure involving interactions between its constituent modules. To further elucidate the folding properties, 3 pepsin-generated subfragments were purified. Fragments A & C are independently folded with $T_d$ close to that of the parent and $\Delta H_{cal}/\Delta H_{vH}$ close to unity. Fragment B, obtained in low yield, is also independently folded as determined by fluorescence measurements as f(T, GdmCl). However, B is much less stable than A & C, suggesting a strong interaction with one or both neighbors in the parent. A 30Kda fragment containing A & B was as stable as 42kDa with a melting profile that deconvoluted into two independent transitions. We conclude that 42K is composed of at least three independently-folded subdomains, one of which is destabilized when isolated from its neighbors.




W-Pos123

**Differential Effects of Heparin on Thermal Stability of 30 and 40K Hep-2 Fragments of Fibronectin: Evidence for Two Heparin Binding Sites in 40K.** F. Schwarz[+], D. Atha[#], & K. Ingham[*], [+]Ctr. Adv. Res. Biotech, [#]Nat. Inst. Stds. Tech., & [*]Am Red Cross, Rockville[+*] and Gaithersburg[#], MD.

Homogeneous 30 and 40 kDa heparin-binding fragments, derived from the C-terminal region of the A and B chains of fibronectin, are identical except that 40K contains a 4th type III homology unit, C-terminal to the 3 that it shares with 30K. In the calorimeter at pH 7.4, both fragments exhibit sharp irreversible endotherms near 70° with $\Delta H_{cal} \simeq$ 500 kJ/mol and $\Delta H_{cal}/\Delta H_{vH}$ ratios near 0.5. In the presence of 10 mg/ml heparin, the 40K peak remains sharp and is elevated by 4°. In contrast, the 30K peak becomes broader and is only partially stabilized. Fluorescence measurements at 20-fold lower protein concentrations and 5-10 fold faster heating rate produced a similar pattern. One interpretation is that the additional type III unit in 40K interacts with one of the other 3 units to create additional binding site(s) for heparin. This other unit would then become stabilized by heparin in 40K but not in 30K. Current efforts are aimed at producing additional fragments to test this notion.



W-Pos124

## SPECTRAL CHARACTERIZATION OF ENVIRONMENTALLY-SENSITIVE FLUORESCENT CONJUGATES OF INTERLEUKIN 1-β

D.E. Epps, A.W. Yem, and M.R. Deibel, The Upjohn Company, Kalamazoo, Michigan

Interleukin 1-β (IL-1β) was labelled with acrylodan (ACR) at Cys8 and another yet to be identified residue as described in the companion poster. Cys8 lies in the barrel region of the protein. The fluorescence emission spectra of ACR-Cys8 IL-1β was blue-shifted with respect to the model compound ACR-Cys, and polarized emission and excitation spectra indicate that there was no variance of the polarization ratio as a function of wavelength. Static polarization measurements and Perrin plots revealed that ACR-Cys8 is somewhat restricted in its ability to rotate in its environment within the barrel region. Further information concerning the environment of ACR-Cys8 was obtained from fluorescence lifetime, quenching and anisotropy decay measurements. This fluorescent conjugate may be useful for studying the interaction of IL-1β with its receptor.

W-Pos125

## PREPARATION, RESOLUTION, AND BIOCHEMICAL PROPERTIES OF ACRYLODAN-LABELED RECOMBINANT HUMAN INTERLEUKIN-1β

A.W. Yem, D.E. Epps, W.R. Mathews, and M.R. Deibel, Jr. (Intro. by T.K. Sawyer), The Upjohn Company, Kalamazoo, Michigan

Interleukin-1 beta contains 2 cysteines at residues 8 and 71. Using acrylodan, an environmentally sensitive fluorescent probe, rIL-1B was modified at a reagent:protein molar ratio of 3:1. One of two fluorescent products was resolved from unmodified protein using DEAE SP5PW HPLC, digested with Endo Lys C, and the peptides resolved by C18 HPLC. Only one peptide exhibited fluorescence, and when sequenced indicated that only cys8 was modified with acrylodan. The intact fluorescent protein, analyzed by bioactivity, was shown to behave similarly to unmodified rIL-1B, indicating that cys8 is not essential for its biological function. Spectral and fluorescence properties of this molecule are presented in the companion poster. A second modified protein is currently being investigated. Data suggest that the fluorescent proteins may be suitable for both receptor binding studies and structure/function analysis.

W-Pos126

## CALORIMETRIC ANALYSIS OF THE HISTONE H2A-H2B DIMER: RESPONSES TO CHANGES IN IONIC STRENGTH AND pH.

*Andreas D. Baxevanis, Ernesto Freire, and Evangelos N. Moudrianakis, Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218 USA.*

The histone H2A-H2B dimer was analyzed by differential scanning calorimetry over the ionic strength and pH range where the interactions of the H2A-H2B dimer with the $(H3\text{-}H4)_2$ tetramer are particularly sensitive. Decreases in the $T_m$, calorimetric enthalpy, and $\Delta H_{van't\ Hoff}/\Delta H_{cal}$ of the dimer are observed as the ionic strength of the solution is decreased, especially below 0.6 M NaCl. The calorimetric parameters of the dimer as a function of pH in 2 M NaCl do not significantly change between pH 6 and 9 but do change markedly outside this range. The transition is completely reversible at neutral pH for NaCl concentrations up to 0.3 M; the degree of reversibility decreases as the NaCl concentration is increased. The transition is not reversible in 2 M NaCl below pH 5.5 or above pH 9.5. At all pH and ionic strength values, $\Delta H_{VH}/\Delta H_{cal} > 1$, indicating that one cooperative transition is taking place at melting. The changes in the calorimetric parameters as a function of both pH and ionic strength closely follow changes in net secondary structure and the ability of the H2A-H2B dimer to pair with the $(H3\text{-}H4)_2$ tetramer to form a functional core octamer, changes which may be a prelude to assembly.

(Supported by NIH grants RR-04328 amd GM-33495)

W-Pos127

## TIME-RESOLVED FLUORESCENCE STUDIES OF THE NATIVE AND UNFOLDED STATES OF STAPHYLOCOCCAL NUCLEASE MUTANTS. UNFOLDING BY TEMPERATURE, PRESSURE AND UREA.

by Maurice R. Eftink, Department of Chemistry, University of Mississippi, University, MS 38677 and Camillo A. Ghiron, Department of Biochemistry, University of Missouri, Columbia, MO 65201.

Fluorescence studies with two mutants of nuclease A, having reduced thermal stability, will be reported. These mutants are nuclease PA56 (pro→ala), and nuclease con A (contains a 6 residue splice from conconavalin A). These mutants show $T_m$ of 30 and 32°C, respectively, in comparison to $T_m$ = 51°C for the wild type. The mutants are also denatured by relatively low urea concentration and low pressure (<1 kbar). Here we report fluorescence intensity decay and anisotropy decay data for the folded and unfolded forms of these mutants. We compare time resolved data for the unfolded proteins produced by increased temperature, pressure and urea concentration. Whereas the anisotropy decay of the folded state of these mutants is dominated by a 10-12 nsec rotational correlation time, the motion of the unfolded states is dominated by a 1-2 nsec rotational correlation time.

This research was supported by NSF grant DMB 88-06113.

W-Pos128

FLUORESCENCE STUDIES WITH POTATO CARBOXYPEPTIDASE INHIBITOR. by Camillo A. Ghiron, Department of Biochemistry, University of Missouri, Columbia, MO 65201, Maurice R. Eftink, Department of Chemistry, University of Mississippi, University, MS 38677, James Longworth, Department of Physics, Illinois Institute of Technology, Chicago, IL 60616, and Clarence Ryan, Department of Agricultural Chemistry, Washington State University, Pullman, WA 99163.

Potato carboxypeptidase inhibitor (CPI) is a 39-residue globular protein whose X-ray structure is known. The protein's two tryptophan residues (W22 and W28) appear to be on the surface in the crystal structure. The fluorescence spectrum of CPI has an maximum at 344 nm. Acrylamide solute quenching yields an upward curving Stern-Volmer plot with $K_{sv} \simeq 9\ M^{-1}$. KI quenching yields a linear plot with $K_{sv} \simeq 5.5\ M^{-1}$. These studies indicate that emission occurs from a solvent exposed residue(s). Fluorescence lifetime measurements were fitted to a double exponential with $\tau_1 = 0.9$ ns, $f_1 = 0.22$, and $\tau_2 = 3.9$ ns. Anisotropy decay data were described by a single rotational correlation time of 1.2 ns. This is somewhat small for the global rotation of a protein of this size (in comparison to globular proteins of similar size, such as epidermal growth factor). Whether one or both trp residue is fluorescent, the rotational motion of this residue(s) appears to be relatively independent of that the whole protein. This research was supported by NSF grant DMB 88-06113 to MRE.

W-Pos129

CONFORMATIONAL ADJUSTMENT OF A PARVALBUMIN MOLECULE UPON Ca/Mg EXCHANGE. A STUDY BY X-RAY CRYSTALLOGRAPHY AND 2D $^1$H NMR.

J.Parello*, J-P.Declercq+, B.Tinant+ J.Rambaud* and A.Cavé# (Intro. by B.Zimm)

We provide for the first time a precise description of the conformational events which occur in parvalbumin (PA) upon Ca/Mg exchange. Two crystal structures, i.e. a fully Ca-loaded form of pike PA (pI 4.10) and a half Mg-loaded form, are compared at a resolution of 1.65 and 1.75 Å, respectively. Substitution of $Ca^{2+}$ by $Mg^{2+}$ is accompanied by a general rearrangement of the co-ordination sphere, i.e. a reduction of the metal-oxygen distances by 0.2-0.3 Å and of the co-ordination number (from 7 to 6), as well as the occurrence of defined conformational variations for selected side-chains. 2D $^1$H NMR shows that not only those resonances from residues directly involved in metal co-ordination have their chemical shifts affected, but also resonances from internal residues in the hydrophobic core. This suggests a participation of the whole protein structure in the cation binding process.

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W-Pos130

STRUCTURAL MODELING BASED ON MULTIPLE SEQUENCE ALIGNMENT: APPLICATION TO THE SERINE PROTEASES. D J States, NCBI/NLM, Bethesda, MD. Intro. by JA Ferretti. For many proteins, experimental structure determination has not been possible but primary sequence data identifies several homologs and structural data is available for one of these related proteins. An algorithm is presented for structural modeling based on such information, and the reliability of this automated procedure is assessed by application to the serine proteases. A sequence alignment metric based on information content has been derived and applied to align a set 31 serine protease primary sequences including bovine trypsin and porcine pancreatic elastase (PPE). The information density evaluated at each site along the peptide sequence, and the correlation of individual sequences with the aligned profile, were used to define structural constraints. A three-dimensional model for PPE was built using trypsin as a reference. Where the sequences agreed, the initial model coordinates were assigned to the homologous trypsin coordinates. These sites were used as a reference to place the remaining coordinates using stereotypic geometries. The structure was then allowed to relax by empirical potential energy minimization subject to information content derived constraints. The model deviated from the crystallographic PPE structure by less than 0.5 Å for 60 of 240 residues including all members of the catalytic triad, and by less than 1.0 Å for 127 of 240 residues. Errors were clustered and associated with surface loops and insertion/deletion sites with several residues deviating from the X-ray structure by more the 5 Å. Energy minimization improved agreement with the crystallographic structure. Despite 63% sequence divergence, trypsin and its structure provided a reasonable basis for modeling PPE, yielding a correct description of the active site geometry and many features of the protein core.

W-Pos131

SYNTHETIC STABILIZATION OF ALPHA HELICES: STRUCTURES OF CYCLIC ANALOGS OF GROWTH HORMONE RELEASING FACTOR (GRF) DETERMINED BY 2-D NMR AND MOLECULAR DYNAMICS.

David Fry, Vincent Madison, David Greeley, Edgar Heimer, and Arthur Felix. Hoffmann-La Roche Inc, Nutley, NJ 07110

Solution structures were determined for [Ala15]-GRF(1-29)-$NH_2$; two monocyclic analogs: cyclo$^{8-12}$[Asp8,Ala15]-GRF(1-29)-$NH_2$ and cyclo$^{21-25}$[Ala15]-GRF(1-29)-$NH_2$; and a dicyclic analog: dicyclo$^{8-12;21-25}$[Asp8, Ala15]-GRF(1-29)-$NH_2$. Assignments were made using 2-D NMR. Distance constraints derived from NOEs were applied in the program CHARMM to produce final structures.

In 75%$CD_3OH$/25%$H_2O$ pH 6, all four analogs were fully α-helical. The linear peptide had a greater tendency to kink skightly, near res. 16 and 25.

In 90%$H_2O$/10%$D_2O$ pH 3, the linear analog was α-helical at res. 8-14 and 21-26. In the cyclo$^{8-12}$ analog, the N-term. helical region was extended to res. 7-17. In the cyclo$^{21-25}$ analog, the C-term. helical region was extended to res. 18-28, but res. 8-14 were disordered. The dicyclic analog had the largest N-term. α-helical region at res. 7-19; but the shortest C-term. helix.

An $asp_i$-$lys_{i+4}$ or $lys_i$-$asp_{i+4}$ lactam can locally stabilize α-helical structure. Stabilization extends in a C-term. direction. The effects of two lactams are not additive

W-Pos132

## KINETICS OF RECA PROTEIN FILAMENT ASSEMBLY.

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Dynamic and static light scattering has been employed to analyze the self-assembly reaction kinetics of RecA protein from *Escherichia coli*. At 20°C in solutions of [$MgCl_2$] from 5 to 7 mM the filamentation reaction was slow enough to permit reliable measurements of the radius of gyration ($R_G$) and molecular weight ($M_w$) of the growing filaments as a function of time. Two distinct linear kinetic phases were observed. During the first kinetic phase the change in $R_G$ with $M_w$ ($dR_G/dM_w$) was independent of [$MgCl_2$] between ~5-7 mM and RecA monomer concentrations from 0.5 to 2.5 $\mu$M. During the second phase ($dR_G/dM_w$) decreased linearly with increasing [$MgCl_2$] and [RecA]. Because the filamentation reaction was highly temperature dependent the Arrhenius activation enthalpy of each phase of RecA filamentation could be determined. Results of self-assembly reaction kinetics and DNA binding experiments conducted under *in vitro* strand exchange conditions (65 mM NaCl, 10 mM $MgCl_2$, pH = 7.5) will be presented.

W-Pos133

## STRUCTURAL AND THERMODYNAMIC DOMAINS OF ENZYME I OF THE *SALMONELLA TYPHIMURIUM* PHOSPHOTRANSFERASE SYSTEM (PTS)

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Enzyme I (EI) is the first in a sequence of phosphotransferase proteins of the PTS, a system that regulates a variety of cell processes (e.g. sugar transport). EI is therefore a likely target for controlling multiple functions. To develop an understanding of the enzyme's thermodynamic structure, the thermal stability of EI has been studied by high sensitivity differential scanning calorimetry (DSC). At pH 7.5, EI unfolds by a two step mechanism indicating that the enzyme consists of two distinct cooperative regions. Cleavage of EI (63.5 kD) by trypsin yields a 29-kD fragment containing the N-terminus and the active-site His. The thermal stability of the fragment is similar to the high temperature transition observed for the intact protein and most likely represents a structural domain. Preliminary data suggest the fragment is functional in one of the EI activities; it accepts a phosphoryl group from phospho-HPr but cannot be phosphorylated by phospho-EI or PEP. The thermal and pH stability of EI and the N-terminal fragment suggest a model for EI structure.

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W-Pos134

## BENT CONFORMATIONS OF IMMUNOGLOBULIN E IN SOLUTION AND BOUND TO CELL MEMBRANE RECEPTOR

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Fluorescence resonance energy transfer measurements have been used to investigate the conformations of IgE before and after binding to receptor on plasma membrane vesicles derived from rat basophilic leukemia cells. Anti-dansyl IgE with genetically engineered cysteine residues placed near the C-terminal end of Fc portion was labeled specifically with fluorescein maleimide. Resonance energy transfer measured between donor fluorescein at these C-terminal sites and acceptor eosin-dansyl in the antigen binding sites indicates the distance between the spectroscopic probes was 65 Å, implying that the IgE molecule has a bent conformation in solution. When IgE binds to its receptor on membrane, there is no significant change in the energy transfer distance, so the bent conformation is likely being preserved. The distance measured between fluorescein at the C-terminus of receptor bound IgE and acceptor probes at the membrane surface was found to be ~53 Å. These results, together with our earlier structural mapping data provide a picture of a bent IgE in solution binding to receptor on the membrane surface and maintaining this conformation: the Fab arms extend outward, the interface of the Cε2 and Cε3 domains in the Fc segment interacts with the receptor, and the Cε4 domain bends away from the membrane.

W-Pos135

## STUDY OF CONFORMATIONS OF RGD CONTAINING PEPTIDES BY MONTE CARLO AND NMR METHODS.

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Many proteins that mediate cell adhesion such as fibronectin, fibrinogen, vitronectin, von Willebrand factor, osteopontin, laminin and collagens contain the amino acid sequence RGD. Peptides that include this sequence have been shown to inhibit the binding of cell adhesion proteins to their receptors and to have dramatic effects on developmental processes involving cellular recognition. To evaluate the stable conformations accessible to RGD containing peptides, we analyzed tri-, tetra- and penta-peptides using molecular mechanics and Monte Carlo methods, and studied the solution conformations of the pentapeptide GRGDS using 1D and 2D NMR at 250 MHz. The Monte Carlo method was used for a) identifying the low energy conformations of the peptides and b) evaluating their thermodynamic properties. In the case of GRGDG, the four stable conformations computed include three with reverse turns and one open structure, consistent with 1D and 2D NOE data on GRGDS, which indicate a type II reverse turn in the RGDS position. Attempts to crystallize GRGDS are being carried out. Preliminary results will be presented.

W-Pos136

THE MOBILITY OF A SURFACE POLYPEPTIDE LOOP AND THE RATE-LIMITING STEP IN LACTATE DEHYDROGENASE CATALYSIS. K.W. Hart, A.R. Clarke and J. J. Holbrook. Molecular Recognition Centre, University of Bristol, Bristol BS8 1TD, U.K.

Using SDM we made a lactate dehydrogenase from *B. stearothermophilus* with only one tryptophan residue at position 106 : the solvent-most tip of a polypeptide loop which extends 0.8nm out into the solvent. This loop is made to close over the active site by mixing, in a stopped flow fluorimeter, the enzyme at 0.01mM active sites with 10mM oxalate and 1 mM $NAD^+$, pH 6. Rate of loop closure, monitored from the fluorescence of the single tryptophan residue at 25° is $450s^{-1}$ and equals $k_{cat}$. Rate of loop closure increases with a linear Arrhenius plot (5° to 60°). $k_{cat}$ with NADH and NADD are the same at 5° to 30°. Above 30° $k_{cat}$ with NADD becomes slower than $k_{cat}$ with NADH: at 50° the difference is 2.5-fold. Above 30° rate monitored from tryptophan on the loop is faster than $k_{cat}$. We conclude below 30° the enzyme rate is limited by the dynamics of polypeptide surface loop closure. Above 30° the bond-breaking step is rate-limiting.

W-Pos137

PRO→ALA-35 CYTOCHROME C2 SHOWS DYNAMIC NOT STRUCTURAL DIFFERENCES. Paul R. Gooley & Neil E. MacKenzie, (intro. by M. A. Cusanovich) Dept. Pharm. Sci., Uni. of Arizona, Tucson Az., 85721. Comparative analysis of short and long range NOEs show that the time average conformation of the wild type and mutant Pro→Ala-35 *Rhodobacter capsulatus* cytochrome $c_2$ are indistinguishable. The ring resonances of Phe-51 and Tyr-53, proximal residues to residue 35, show the flip rates for these rings increase in P35A. NH exchange studies show that the exchange rates of the NH proton of Gly-34 and the $N_\pi H$ of His-17 increase by $\approx 10^2$ in P35A suggesting that their respective hydrogen bonds to the CO of Cys-16 and Ala-35 are destabilized in this protein. However, $^3J_{\alpha NH}$, $^1H$ and $^{15}N$ chemical shift data argue that these bonds are intact. Indeed the $^{15}N$ chemical shifts of the ring nitrogens of His-17 indicate that it's hydrogen bond may be slightly shorter in P35A. These data are compatible if the substitution of a Pro for an Ala sidechain creates a cavity which reduces steric hinderance and increases solvent accessibility. This reasoning would account for the observed increased NH exchange rates despite chemical shift evidence for unchanged or slightly stonger H-bonding.