T-PM-Po58 THE EFFECT OF TEMPERATURE ON TUBULIN OLIGOMER, Janice S. Barton and G. Hossein Riazi, Department of Chemistry Texas Woman's University, Denton, Texas 76204

For an oligomeric form of tubulin to participate in microtubule assembly it must be present at assembly temperatures (20-35°C).To ascertain if oligomer dissociated to dimer with increased temperature, we compared chromatographic experiments conducted with and without 10μM podophyllotoxin in the presence of 25μM GTP at 4°and 31°C. Mocrotubule protein, obtained from bovine brain by two cycles of assembly/disassembly, was chromatographed through 8% agarose using 50mM pipes, pH 6.5, 0.3mM $MgSO_4$, and 25μM GTP. Two protein zones were observed at 4°and 31°C, and both the void volume, containing oligomer, and the dimer zones were unchanged in area by temperature, indicating that oligomer was not dissociated at polymerizing temperatures. The void volume and dimer peaks exhibited little evidence of the presence of microtubules at either temperature in 25μM GTP when observed by electron microscopy. However, the oligomer did polymerize when it was made 1mM in GTP. The possibility of oligomer dissociation to dimer was also investigated by carefully examining the first 120 seconds of assembly turbidimetrically. Assembly was initiated by temperature jump and by dilution of a small aliquot of microtubule protein at 4°C with buffer at 35°C. No significant decrease in absorbance was found; at 350nm the absorbance decrease was less than 2% of the initial value using a Cary 15 spectrophotometer and an absorbance span of 0.1 unit. These experiments do not support an obligatory dissociation of tubulin oligomer prior to assembly into microtubules. These results are based on work supported by NSF Grant No. PCM78-20083 and a Faculty Research Grant from Texas Woman's University.

T-PM-Po59 THE INTERACTION OF TUBULIN WITH SINGLE RING ANALOGS OF COLCHICINE. Jose M. Andreu and Serge N. Timasheff, Graduate Department of Biochemistry, Brandeis University, Waltham Massachusetts, 02254.

Simple analogs of the tropolone and trimethoxyphenyl moieties of colchicine have been used as probes for the colchicine binding site of purified calf brain tubulin. [^{3}H]-tropolone methyl ether was found to bind to one site per tubulin molecule with an equilibrium constant of $2.2\pm0.2 \times 10^3 M^{-1}$ at 0°C, the interaction having $\Delta H^\circ_{app} = -8.3\pm1.0$ Kcal mol^{-1} and $\Delta S^\circ_{app} = -15.2\pm3.6$ e.u. The binding of tropolone methyl ether and colchicine were mutually inhibited. Both tropolone and its methyl ether inhibited tubulin polymerization into microtubules in vitro. [^{3}H]-N-acetyl-mescaline bound to tubulin with $K \sim 4 \times 10^2 M^{-1}$ at 37°C. This interaction was inhibited by colchicine and at lower temperatures was below the sensitivity of the measuring method employed. [^{14}C]-mescaline interacted with higher affinity site(s) not related to the colchicine site. Both mescaline and N-acetyl-mescaline inhibited partially the microtubule assembly at 10^{-3}M concentrations. No linkage was observed between the binding of tropolone methyl ether and N-acetyl-mescaline. The relatively weak interactions of both separate parts can account quantitatively for the much tighter binding of colchicine to tubulin within a model proposed in which the entropic advantage of colchicine as a bifunctional ligand is considered. (Supported by grants from the National Institutes of Health, CA 16707 and GM 14603 and a fellowship from the U.S.A.-Spain Joint Committee for Scientific and Technological Cooperation (J.M.A.)).

T-PM-Po60 FORMATION OF A MICROTUBULE-NEUROFILAMENT COMPLEX IN VITRO. M.S. Runge and R.C. Williams, Jr. Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

Microtubule protein and purified neurofilaments were mixed together at 4° at concentrations of 2 to 6 mg/ml in buffers (0.1 M Pipes, 1 mM $MgSO_4$, 2 mM EGTA, 2 mM DTE, 0.5 mM GTP, pH 6.9) that contained or lacked 1 mM ATP. Each mixture was placed in a falling ball viscometer and incubated at 36° for 20 min. Its apparent viscosity was then measured by timing a single fall of the ball. The apparent viscosity of the mixture in the presence of ATP was found to be about 20 times as large as the apparent viscosity of the mixture in the absence of ATP. The apparent viscosity of the mixture in the absence of ATP was approximately equal to the sum of the separately measured viscosities of a neurofilament preparation and polymerized microtubule protein. When phosphocellulose-purified tubulin was employed in place of microtubule protein, the change in apparent viscosity was nearly 200-fold. The size of the viscosity change increased with the concentrations of neurofilaments and of microtubule protein. It was reversible by cooling to 0° for 90 sec. No change in viscosity was seen when pre-polymerized microtubules were mixed with neurofilaments, or when UTP, CTP, or GTP were substituted for ATP. Sedimentation velocity experiments were also carried out on mixtures of tubulin and neurofilaments that were prepared in the cold and then incubated 20 min at 35° in the ultracentrifuge cell prior to acceleration of the rotor to speeds between 7,000 and 14,000 rpm. A rapidly sedimenting component (greater than 2,000 S) was found to be present in the ATP-containing mixture and absent in the mixture made without ATP. This component comprised about 30% of the total material in the mixture. We interpret these results to mean that an ATP-dependent complex forms between microtubules and neurofilaments in vitro. (Supported by Grant GM 25638 of the National Institutes of Health.)

T-PM-Po61 QUANTITATION OF G- AND F-ACTIN IN CHINESE HAMSTER OVARY (CHO) CELLS. C.S. Heacock, B.W. Bernstein, J.R. Bamburg, Department of Biochemistry, and W.C. Dewey, Department of Radiation Biology, Colorado State University, Fort Collins, Colorado 80523

A model system in which to study the in vivo regulation of microfilament assembly is the cell cycle of CHO cells. Changes in cell shape which occur following mitosis are accompanied by a disappearance of the contractile ring and the formation of stress fibers. CHO cells may be obtained in greater than 98% synchrony by mitotic shake-off. Quantitation of total actin throughout the cell cycle by SDS-PAGE of whole cell homogenates showed that about 4% of the total protein is actin and that the actin level remains relatively constant during the cell cycle. A technique was developed to quantitate G-, F-, and total actin in a small number of cells. Briefly, CHO cells in monolayer are washed, frozen in a Triton X-100/glycerol buffer, scraped from the plate, centrifuged at 170,000Xg, and the supernatant assayed for G-actin using a modification of the DNase I inhibition assay of Blikstad et. al., Cell 15, 935 (1978). The cell pellet is resuspended in an actin depolymerizing buffer (2mM Tris, 0.5mM ATP, 0.2mM $CaCl_2$, 0.5mM Dithioerythritol, pH 8.0) and aliquots are assayed directly using the DNase inhibition assay. The total actin levels obtained by adding the actin levels in the supernatant and pellet fractions are similar to the values determined in whole cell lysates using either the DNase assay or quantitative SDS-PAGE. ^{3}H-Actin was prepared in vitro to greater than 10^5 DPM/ug by reductive alkylation. When added to the CHO cell monolayer before lysis, the labeled actin was shown to partition in the expected fraction, i.e. F-actin was in the pellet and G-actin was in the supernatant. Actin recovery was about 75% in each fraction. About 4% of the total protein in asynchronous CHO cells is actin and 55% of this actin is in the filamentous state. (Supported by grants NS10429, CA18334 and a BRS grant from NIH).

T-PM-Po62 MOLECULAR PACKING IN DRY ELASTOIDIN: A COLLAGEN PHASE CHANGE. R.J. Chandross. Department of Anatomy, University of North Carolina, Chapel Hill, NC 27514

Elastoidin is the substance comprising the clear, glassy spicules (ceratotrichia) which form the fin rays of sharks. It is known to consist of collagen and at least one other proteinaceous component, both of which show clearly in the electron density profiles of wet elastoidin, as determined by small angle X-ray diffraction. When the spicules are permitted to dry, the diffraction pattern changes drastically both in regard to intensity distribution and lattice spacing. In addition, the small angle streak becomes non-meridional, with various workers reporting cross-armed patterns, or even "one-armed" patterns. We find that the reason for this apparently anomalous behaviour is that the spicules, at least when dry are radially anisotropic, with decidedly different diffraction patterns obtained from different orientations of the fiber. In general, when the edge of the ovoid spicule is perpendicular to the beam, we get a clearly defined, symmetrical, two-armed diffraction pattern, while a face-on view gives a rather weak, and often diffuse, meridional pattern. The implication of these experiments is that water forms an integral part of the wet structure, forcing the collagen molecules to orient themselves parallel to the fiber axis, and arranged in layers similar to those of an onion. When the water is permitted to evaporate, the structure collapses and a phase change occurs in which collagen interactions may occur across the original layers, rather than within them.

T-PM-Po63 WATER RELAXATION TIMES FOR HUMAN NORMAL AND CANCEROUS COLON CELL LINES AND CLONES. Paula T. Beall[1], Carlton F. Hazlewood[1], and Lynne P. Rutzky[2], Depts. Physiology and Pediatrics Baylor College of Medicine[1], and Dept. Surgery, Univ. Texas Med. Sch[2],Houston, TX 77030.

Cells from established lines of human cancer of the colon, LS 180, LS 174, HT-29,SW 480, and SW 1345, demonstrated nuclear magnetic proton relaxation times of 460 to 982 ms for T_1 and 83 to 176 ms for T_2. Clones derived from single cells of line LS 174 demonstrated T_1 values of 663 ms (clone 3-5) and 716 ms (clone 6-6) in comparison with the parent line at 744 ms. Differences in T_1 and T_2 values for water protons in these lines did not correlate with hydration levels. Among the parent line LS 174 and its clones T_1 values could be correlated with rate of population doubling and especially with the tumor producing capacity of the line. Normal adult colon cells grown in early passage were wetter and had higher T_1 values than established cancer cells (T_1 = 1091 ms). Human fetal colon cells in early passage were also wetter and had higher T_1 values (1082 ms) than established colon lines. These results suggest that differences seen in other studies for NMR relaxation time values in cancer of the colon biopsies are due mostly to intracellular differences, which are not simple differences in hydration. The study also proves that primary and early passage cells are not good controls for established cell lines, as we have shown previously for primary cultures and established cell lines of mouse mammary tissue. Differences in water properties among similar cell lines may be due to the relationship of water to complex macromolecular matrices in the cellular cytoplasm. This work was supported in part by the Office of Naval Research contracts N00014-76-C-0100 and N00014-78-C-0068 , and National Institutes of Health grants CA 21624, GM 20154, CA 22370, and grant(American Cancer Society)76-22.

T-PM-Po64 X-RAY TAXONOMY OF TENDONS. B. Brodsky and E. Eikenberry, Dept. of Biochemistry, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854

X-ray diffraction has been used to study collagen fibril structure in connective tissues. Rat tail tendon, the most studied tissue, gives a well-oriented x-ray pattern with a series of meridional Bragg reflections (parallel to the fiber axis) and several sharp equatorial maxima (perpendicular to the fiber axis). The meridional reflections are derived from a D period stagger of molecules by 670 Å, and their intensities are related to the electron density distribution within one D unit. The existence of equatorial maxima indicate the presence of an ordered lateral packing of molecules in the fibril.

Biochemical investigations show a considerable variation in the components of different connective tissues, which could be the basis for specific fibril structures. Tendons of different animals all have type I collagen as their major constituent. Though highly homologous, the type I collagens from different species contain amino acid substitutions in about 5% of the positions in the α1 chain and 10% in the α2 chain. X-ray patterns of different tendons in the rat were compared and found to be indistinguishable. The patterns from tendons of different mammals showed small differences in the meridional intensities (14th-18th orders) and varying degrees of lateral order. Avian and mammalian tendon patterns were found to differ in several respects. The 7th meridional order was stronger, and the 2nd, 4th, and 6th orders weaker in avian tendons, suggesting a smaller gap:overlap ratio than found in mammalian tendons. The intensities of the higher meridional orders (29th-52nd) were also different, which could be directly caused by the amino acid sequence differences. The lateral spacing between molecules was greater for avian than for mammalian tendons, a feature which may be necessary for calcification. Thus, x-ray patterns of tendons, and hence their collagen fibril structures, appear to be phylogenetically correlated.

T-PM-Po65 EFFECT OF TEMPERATURE AND DIVALENT CATIONS ON PLATELET ADHESION. C. Richard Zobel and J.R. LaFountain, Departments of Biophysical Sciences and Biology, State University of New York, Buffalo, N.Y., 14214.

Human platelets have been isolated from blood collected into 0.38% Na_3-citrate by centrifugation over 30% BSA and resuspension in media (120 mM NaCl, 4.3 mM KCl, 1 mM $MgCl_2$, 3mM glucose, 10 mM HEPES, pH 7.4) containing either 5 mM EGTA or 2 mM Ca^{2+}. Following incubation of platelet suspensions at 0C and 37 C for 30 min., cells were allowed to settle and adhere to glass coverslips. Platelets in Ca^{2+} media spread significantly more rapdily than those in EGTA. However, about 50% of the cells in Ca2+ media rounded-up partially withdrawing from the substrate either during or subsequent to spreading. Cells in EGTA did not appear to spread completely but assumed intermediate or dendritic forms. They did not show any tendency toward withdrawal or rounding. Low temperature strongly suppressed spreading of cells in either media but did not otherwise appear to cause noticeable alterations in cell morphology. Lysis of suspended cells with 1% Triton X-100 occurred instantly at 37 C in EGTA and more slowly for cells at 0° C or in Ca^{2+}. Centrifugation of lysates (30 min., 12,000xg) followed by SDS-PAGE analysis showed that the insoluble residue from EGTA-treated cells contained components with mol. wts. of 250,000, 240,000, 95,000, 70,000, 43,000, and 32,000d. Residues from Ca^{2+}-treated cells contained primarily 200,000, and 43,000d components. Supernatant constituents were essentially the same for EGTA- or Ca^{2+}-treated cells with the exception of missing 250,000 and 240,000 components in Ca^{2+}-treated cells and the concomitant appearance of a band at 190,000d. This result suggests specific Ca^{2+}-activated cleavage of the 250,000-240,000d components to a 190,000d mol. wt. species. Lysis and sedimentation of cells in 0.75M G-HCl revealed the presence of a small amount of insoluble material with Mr=43,000d.

T-PM-Po66 LYMPHOCYTE MEMBRANE PROTEINS AND THEIR INTERACTION WITH CYTOSKELETAL ELEMENTS. P.G. Lerch*and W. Lesslauer, Th. Kocher Institute and Dept. of Biochemistry, University of Bern, 3012 Bern, Switzerland

Surface membrane proteins and membrane-cytoskeleton interactions in human peripheral blood lymphocytes were studied by lactoperoxydase-catalysed iodination of cells and by actin affinity chromatography on Sepharose-DNAase I in detergents with phytohemagglutinin [pha]-treated and control cells. Lymphocytes were isolated with Ficoll density gradients. Membrane proteins were analysed by O'Farrell's two-dimensional gel electrophoresis of detergent lysates of cells. Labeled surface components were detected by autoradiography of gels. Actin is the major spot in gels stained for protein. With viable cells no radioactivity was incorporated into actin. In lymphocytes incubated with pha, the mitogen was highly selectively labeled. After a pha-pulse, the typical labeling pattern of surface proteins returned slowly, although pha remained a major spot in the autoradiogramm for a prolonged period of time. Actin in detergent lysates of cells could be completely adsorbed on Sepharose-DNAase I and eluted with guanidin-HCl. It was observed that with lymphocytes treated with a short pha pulse and surface labeled after prolonged incubation, pha was retained together with actin on Sepharose-DNAase I. In conclusion it appears that pha remains for a long time at the cell surface and becomes linked with actin. No evidence for a bridging protein between actin and pha could as yet be obtained, which might represent the pha-receptor.

T-PM-Po67 CONTROL OF CELL FORM:CONTROL OF CELL LENGTH IN STENTOR POLYMORPHUS. V. K-H. Chen*(Intr. by R.A. Spangler),Dept. of Biophysical Sciences,State Univ. of New York at Buffalo, Buffalo,NY 14214

Stentor polymorphus generates rapid and extreme changes in cell body length changes in nature. Free-swimming Stentor is conical in shape(1mm long) It contracts to 20% initial length in response to a intense stimulus.This all-or none contraction is preceeded by a hyperpolarizing action potential Graded shortening results from weak mechanical(not electrical)stimuli. Prior to feeding,attached Stentor will elongate to 200-400% initial length. Study of the mechanisms controlling cell length requires that methods be developed to intervene at various stages of control.I found that 2X-(nitrogen substituted)benzimidazoles(10^{-9}-10^{-6}M)activated elongation with no loss of viability or loss of sensitivity to various stimuli.Final length was proportional to benzimidazole concentration.After all-or-none contraction cell relax to drug determined length. Cells treated with benimidazole I found could be preserved at different stages of cell shortening by addition of Mg^{+} (1-5mM). Length of the preserved cells was proportional to Mg^{+}added. If the treated cell was exposed to Mg^{+}+EGTA or EGTA alone rapid shortening to 20% initial length. My results are consistant the hypothesis that benzimidazole acts upon a tubulin component of the km fibers to regulate the degree of elongation and that Mg^{+} acts upon force generating component of the km fibers of the myonemes.

T-PM-Po68 A NEW ESTIMATE FOR THE CYTOPLASMIC ACTIN CONTENT OF PORCINE BRAIN. Jerry P. Weir* and Dixie W. Frederiksen. Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232

Actin was purified from porcine brain by a procedure involving anion-exchange chromatography, polymerization-depolymerization, and gel filtration chromatography. The extent of purification at each step of the isolation procedure was analyzed by densitometry of Coomassie Blue stained gels after electrophoresis in SDS-polyacrylamide. Both brain and skeletal muscle actins migrate on these gels with apparent molecular weights of 48,000. The yield of actin for the preparative procedure determined by this method was approximately 3%. About 10% of the protein in the initial extract appeared to be actin. The purification at each step of the isolation procedure was also determined by an isotope-dilution technique. Purified brain actin was radioactively labeled with tritiated N-ethylmaleimide. The labeled actin retained the ability to polymerize and to activate the Mg-ATPase of skeletal muscle myosin. The incorporation of [^{3}H]N-ethylamleimide into actin was 0.4 mole/mole. Tritiated actin was added to the initial extract of brain. Recovered radioactivity at each subsequent purification step indicated the relative purification of the cytoplasmic actin. The final yield of the preparative procedure as determined by this method was about 12%. Only about 3% of the total protein in the initial extract appeared to be actin. The cytoplasmic actin content of nonmuscle cells is probably overestimated when determined by staining of gels after SDS-polyacrylamide gel electrophoresis.

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T-PM-Po69 IMMUNOFLUORESCENT LOCALIZATION OF CREATINE KINASE DURING THE MITOTIC CYCLE. Stephen J. Koons, Barry S. Eckert* and C. Richard Zobel. Departments of Biophysical Sciences and Anatomical Sciences*, State University of New York, Buffalo, NY 14214.

We have prepared rabbit antibody against myofibrillar (M-line) creatine kinase (CK) from chicken breast muscle. By examining large numbers of mitotic PtK_1 cells, we have been able to study the localization of CK throughout the mitotic cycle. In interphase cells affinity purified antibody decorates a network of cytoplasmic fibers that we have previously identified as intermediate filaments. During prophase the fibers appear to radiate from several points, later coiling about the nucleus in a manner similar to that of intermediate filaments. During metaphase and anaphase the staining appears in the mitotic spindle, with no staining of cytoplasmic fibers other than occasional staining of fibers radiating from the poles. In early telophase cytoplasmic fibers reappear, though the most intense fluorescence spans between the chromosomes. After the nuclei have formed, fluorescence is evident throughout the cytoplasm, with intense staining radiating from the midbody. 2 hour incubation of the cells in 5 or 10 mM epoxycreatine, a specific CK inhibitor, does not alter interphase CK staining, nor does it alter microfilament structure, as determined by anti-actin immunofluorescence. Rounded cells in 10 mM epoxycreatine display no spindle staining, having instead a central stellar region of diffuse fluorescence. Condensed chromosomes array radially about this aster-like structure. Phase-contrast microscopy reveals no spindles in these cells, and we have not observed subsequent chromosome separation. Some cells treated with 5 mM epoxycreatine display the altered mitotic structure, while others have normal spindles. The disruption of mitotic structures suggests that CK may be required for energy supply for mitosis. Epoxycreatine was the generous gift of Dr. George Kenyon.

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T-PM-Po70 MOBILIZATION OF CELLULAR CALCIUM IS REQUIRED FOR MACROPHAGES TO SPREAD John A. Trotter and Ray M. Quintana, Department of Anatomy, University of New Mexico, School of Medicine, Albuquerque, NM 87131

Spreading of macrophages on artificial substrata is an energy-requiring process, analagous to phagocytosis, which uses the locomotor apparatus of the cells. Peritoneal macrophages of normal BALB-C/Bailey-J mice spread rapidly and reproducibly in the presence of 50μg/ml of Subtilisin (Rabinovitch and DeStefano, 1974, Exp. Cell Res. 88, 153). This experimental model has been used to examine the role of Ca^{2+} in the process of spreading. We have found that the rate of spreading is unaffected by concentrations of extracellular calcium up to 4mM, or by EGTA up to 5mM. In contrast, the rate of spreading is decreased, in a dose-dependent manner, by the calcium antagonist TMB-8 [8(N,N-diethylamino)-octyl 3,4,5-trimethoxybenzoate-HCl] ; the dose required for 50% inhibition (ID_{50}) is approximately $2x10^{-4}$M. Normal spreading behavior resumes when the TMB-8 is removed from the cells. Inhibition by TMB-8 is relieved by the inclusion of $CaCl_2$ in the incubation medium: the extent of relief is directly proportional to the concentration of $CaCl_2$ up to 8mM; and inversely proportional to the level of inhibition in the absence of added $CaCl_2$. Verapamil and $LaCl_3$, which are thought to block the entry of Ca^{2+} across the plasmalemma, also inhibit macrophage spreading, in the absence of extracellular calcium, with an ID_{50} of approximately $2.5x10^{-4}$M. These results indicate that: 1) mobilization of cellular calcium is required for macrophages to spread; and 2) that the site from which the calcium is mobilized may be a compartment of the plasmalemma which is distal to the calcium channel(s) but the calcium of which is not readily accessible to EGTA. Supported by a grant from the American Lung Association.

T-PM-Po71 THREE-DIMENSIONAL RECONSTRUCTION OF THE FLAGELLAR HOOK FROM CAULOBACTER CRESCENTUS. T. Wagenknecht, D.J. DeRosier, L. Shapiro,† and A. Weissborn,† The Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254; and †Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, NY 10461.

The bacterial hook, a tubular protein polymer, is the component of the bacterial flagellum that connects the filament to the basal body. The function of the hook in motility is unknown. Usually, isolated hooks assume a curved shape in which the subunits can only be approximately helically related. We have analyzed electron micrographs of negatively stained hooks obtained from a mutant that produces unusually straight hooks in which the protein subunits are exactly helically arranged. Analysis of optical and computed diffraction patterns shows that the helical lattice is characterized by a single-start right-handed helix of 22Å pitch and by intersecting families of 5-, 6- and 11-start helices, the approximate selection rule being $\ell=7n+39m$. These parameters are very similar to those of the flagellar filaments from several bacterial genera and these similarities have implications for how the hook and filament are joined together. The data from eight computed diffraction patterns were combined and used to produce an averaged three-dimensional map of the hook. In the map the subunits appear as curved rods (~25Å x 90Å) which at outer radii are in close contact along the 6-start helices, forming sheets which are separated from one another by deep continuous grooves; these features appear to be important in allowing the hook to bend with a minimal amount of structural reorganization. A stain-filled channel about 25Å in diameter running along the hook axis may be used for transporting flagellin subunits during in vivo filament assembly.

T-PM-Po72 LYSOLECITHINASE, PHOSPHODIESTERS AND THEIR INTERACTION IN REPRODUCTIVE SYSTEMS. Hedda J. Ribolow, C. Tyler Burt, Mitchell Martin, Department of Chemistry, Reed College, Portland, Oregon 97202.

We have previously reported the presence of phosphodiesters in human and chicken semen. We now extend our studies to salmon where we also see a phosphodiester present. Since one of these phosphodiesters, glycerophosphorylcholine (GPC) is formed during the reaction catalyzed by the enzyme lysolecithinase, we have measured its activity in semen, seminal plasma and sperm from several different species. These values are 5.4, 4.0, and 12.5 nmoles lysophosphatidylcholine hydrolyzed per hour per 10^6 cells or the equivalent volume of plasma for human semen, seminal plasma and sperm respectively, and 0.88, 0.92 and 0.75 nmoles per hour per 10^6 cells or equivalent volume of plasma in the fractions from salmon semen, seminal plasma and sperm respectively. When we tested for product inhibition with 1 mM of GPC all the fractions were inhibited by about 30% except for human sperm which was inhibited twice as much. These investigations are being extended to other primates and to humans with impaired fertility. Since lysolecithin has been implicated in such diverse functions as cell lysis and membrane fusion, its control by GPC (a chemical of substantial concentration but unknown function) raises interesting questions in regard to in vivo regulation and membrane function. This is particularly significant in light of our previous report that GPC levels are decreased in human samples having decreased sperm motility. Supported by NIH 7R01 AM 27566-01 and NIH 2507 RR07168.

T-PM-Po73 EFFECT OF PROTEINS ON CYTOPLASMIC STREAMING IN PERFUSED CHARA CELLS. Eugene A. Nothnagel and W. W. Webb, Field of Applied Physics, Cornell University, Ithaca, NY 14853.

The mechanism of force generation driving cytoplasmic streaming in Chara is thought to involve interaction between subcortical actin bundles and presumptive endoplasmic myosin. We have shown (E. A. Nothnagel, L. S. Barak, J. W. Sanger and W. W. Webb, J. Cell Biol., in press.) that phallotoxins perfused into Chara cells do not inhibit streaming but do reverse the inhibitory effect of cytochalasin B (CB). Based on these and other observations it was hypothesized that CB may release actin from the subcortical bundles into the endoplasm where it competitively inhibits the assumed force generating interaction between the subcortical actin bundles and endoplasmic myosin. To test whether endoplasmic actin can inhibit streaming, rabbit actin has been perfused into Chara cells. Strong inhibition of streaming is observed with actin concentrations as low as 0.1 mg/ml. The effects of perfusion with several actin-binding proteins have also been determined. Perfusion with unconjugated heavy meromyosin does not alter streaming whereas perfusion with fluorophore-conjugated heavy meromyosin strongly inhibits streaming. Deoxyribonuclease I (DNase I), an inhibitor of amoeboid movement and fast axonal transport, does not inhibit streaming in Chara. Fluorophore-conjugated DNase I binds to stress cables and microfilaments in mammalian cells but does not bind to Chara actin bundles, thus suggesting that the ineffectiveness of DNase I as an inhibitor of streaming arises from an unusual inability to bind to Chara actin bundles. Perfusion with various control proteins including tubulin, ovalbumin, bovine serum albumin and immunoglobulin either slightly stabilizes or does not alter streaming. These results support the hypothesis that streaming is generated by interaction between subcortical actin bundles and endoplasmic myosin and that this force generation does not require cycling of actin between polymerized and depolymerized forms.

T-PM-Po74 EFFECT OF Mg^{2+} ON ACTIN-ACTIVATED Mg^{2+}-ATPase ACTIVITY OF ACANTHAMOEBA MYOSIN II. Jimmy H. Collins and Edward D. Korn, NHLBI, NIH, Bethesda, MD 20205.

The actin-activated Mg^{2+}-ATPase activity of Acanthamoeba myosin II is independently regulated by Ca^{2+} and phosphorylation (Collins and Korn (1980) JBC 255,8011). Myosin II is fully actin-activated in the presence of micromolar Ca^{2+} when two sites on each heavy chain are unphosphorylated. Myosin II isolated by the procedure of Collins and Korn (ibid) is only 65% phosphorylated (1.3 mol P/mol heavy chain) and is, therefore, partially actin-activated (about 25-fold, to a specific activity of 0.18 $\mu mol \cdot min^{-1} \cdot mg^{-1}$). Actin-activation and Ca^{2+}-sensitivity of this preparation of myosin II are also regulated by Mg^{2+}. Actomyosin II ATPase is inactive at concentrations of free Mg^{2+} lower than about 3 mM and fully active at Mg^{2+} concentrations greater than 4 mM. It is stimulated by micromolar Ca^{2+} between about 3-4 mM Mg^{2+} but is not affected by Ca^{2+} at either lower or higher Mg^{2+} concentrations. The specific activity of the actomyosin II Mg^{2+}-ATPase also increases with increasing concentrations of myosin II when the free Mg^{2+} concentration is in the range of 3-4 mM but is independent of the myosin II concentration at lower or higher concentrations of Mg^{2+}, suggesting that myosin-myosin interactions occur in the Mg^{2+} concentration range in which the transition from inactive to active myosin occurs. However, these effects of Mg^{2+} apparently are not related to myosin filament formation which, as detected by sedimentability and light scattering, occurs only at higher Mg^{2+} concentrations (6-7 mM). Also, both sucrose and EGTA differentially affect the Mg^{2+}-dependent changes in enzymatic activity and myosin sedimentability. Further work with an active, soluble fragment of myosin II, which we are now attempting to prepare, may clarify the interrelationships between the Mg^{2+} and Ca^{2+} requirements and enzymatic activity and filament formation.

T-PM-Po75 IMMUNOLOGICAL CHARACTERIZATION AND INTRACELLULAR LOCALIZATION OF MYOSIN LIGHT CHAIN KINASE. Primal deLanerolle, Robert S. Adelstein, NHLBI, NIH, Bethesda, Md. 20205, James R. Feramisco*, and Keith Burridge*. Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 11724

Myosin phosphorylation is thought to regulate contractile activity in non-muscle cells. This regulatory effect appears to be mediated through the Ca^{++}-calmodulin dependent enzyme, myosin light chain kinase (MLCK). We have raised an antibody (Ab) to MLCK and used this Ab to determine the intracellular distribution of MLCK in non-muscle cells. MLCK purified from turkey gizzard smooth muscle was used to immunize rabbits and the MLCK Ab was purified by chromatography on a MLCK-Sepharose 4B affinity column. The purified Ab cross-reacts with purified smooth muscle MLCK but does not cross-react with a variety of proteins including actin, myosin, calmodulin or the catalytic subunit of cAMP-dependent protein kinase. The purified Ab inhibits the catalytic activity of purified MLCK whereas pre-immune IgG has no effect on kinase activity. Immunofluorescent studies with non-muscle cells, using the purified MLCK Ab, have demonstrated that the most prominent localization of MLCK is along stress fibers of cultured fibroblasts. These experiments have also shown the following:
(1) MLCK is distributed periodically along stress fibers in gerbil fibroma cells.
(2) The stress fiber staining pattern is abolished when the Ab is incubated with purified MLCK prior to staining. (3) The staining pattern is unaffected by preincubating the Ab with the smooth muscle proteins actin, myosin, tropomyosin or α-actinin. (4) MLCK has the same periodicity as myosin and tropomyosin, but is antiperiodic to α-actinin in gerbil fibroma cells. The localization of MLCK on stress fibers, a structure known to contain actin and myosin, is consistent with the role of MLCK in regulating actin-myosin interactions in non-muscle cells.

T-PM-Po76 NBD-ACTIN AS A PROBE FOR CYTOCHALASIN ACTION. T.P. Walsh* and A. Weber (intr. by M. Das). Dept. of Biochemistry and Biophysics, Univ. of Pennsylvania, Philadelphia, PA.

Many of the cytochalasin (C) effects have been studied by viscosimetry which has the disadvantages of requiring relatively high protein concentraions, of shearing filaments and of being sensitive to length and filament interactions. Using the fluorescence increase of NBD-actin associated with polymerization and 0.1 μM CD we measured an increase in critical concentration from 0.15 to 0.4 μM (0.1 MKCl-2mm $MgCl_2$), an inhibition of binding of 0.08-0.4 μM NBD actin to non-fluorescent nuclei and a slowing of binding at higher concentrations of NBD actin (confirming Moosecker and Pollard's electron microscopic data). We did not find that CD accelerated depolymerization: at 0.05 μM total actin the rate was unaffected by CD. By comparison DNase I increased the rate of depolymerization above that of native actin. We confirm Pollard's finding with pure actin that CD up to 20 μM did not accelerate nucleation. The lag period was the same in the presence and absence of CD.

At high concentrations CD seemed to bind to G-actin as suggested by the lowering of fluorescence of G-actin when CD concentrations were raised above μM CD; this effect was not saturated by 100 μM CD and was enhanced by Mg ions.

T-PM-Po77 2-nm FILAMENTS INTERCONNECT PIGMENT GRANULES IN FISH MELANOPHORES. D.B. Murphy and W.A. Grasser, III. Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, MD 21205. (Introduced by D.P. Kiehart)

The movement of pigment granules in fish melanophores is thought to depend on cytoplasmic microtubules, but the molecular basis of the movement has not yet been resolved. To examine the ultrastructure of the cell cytoplasm in more detail, melanophores of black tetras were isolated from the dermis by collagenase digestion, cultured in vitro on carbon-coated grids, lysed by hypotonic shock, and negatively stained with 1% aqueous uranyl acetate for examination by EM. Lysis in water, dilute medium or medium containing 0.05% Triton X-100 gave similar results: (1) A few granules were clearly dissociated from the mass of pigment, but most remained closely associated in clumps. (2) When sufficiently dispersed, the granules in the aggregates were observed to be joined by interconnectives of varying length and width that formed a network in which any one granule was associated with several other adjacent granules. In some cases the connectives looked like membrane. (3) Occasionally granules were observed to be associated with 2-nm filaments (mean diameter 1.6 nm) which were often organized into bundles of a few to several dozen filaments. The 2-nm filaments were distinct from membrane folds and from actin filaments which had a mean diameter of 7.2 nm in preparations of negatively stained blood platelets. Unlike actin filaments, the 2-nm filaments were not decorated after incubation in preparations of myosin subfragment-1. It is not yet known if the 2-nm filaments are associated with microtubules or membranes or if they are somehow involved in the intracellular migration of pigment. These are among the first observations of organized bundles of 2-nm filaments in vertebrate cells.

T-PM-Po78 PERTURBATION OF EQUILIBRIUM BY GLUTARALDEHYDE FIXATION OF RIBOSOMES, Crossin, M., Tai, M. and Kegeles, G., University of Connecticut, Biological Sciences Group, Biochemistry and Biophysics Section, Storrs, CT 06268.

The fixation of E. coli MRE600 ribosomes and ribosomal subunits by glutaraldehyde has been studied using equilibrium and stopped flow light scattering techniques. Rates of fixation were determined by analysis of stopped flow experiments in which ribosomes in a Mg^{++} buffered solution are flowed against a solution of differing free Mg^{++} concentration containing 0% to 4.0% glutaraldehyde. The rate of formation or dissociation of 70s particles was determined from the change of 90° light scattering intensity. Curves were analyzed using a Runge-Kutta integration procedure and fit to four simultaneous reactions:

$30s + 50s \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} 70s$, $30s \overset{k_3}{\longleftrightarrow} 30s$ (fixed), $50s \overset{k_5}{\longrightarrow} 50s$ (fixed), and $70s \overset{k_7}{\longrightarrow} 70s$ (fixed).

A satisfactory fit was obtained by assuming equal rates of fixation for ribosomes and subunits ($k_3=k_5=k_7$). This rate, however, is not fast enough to freeze the equilibrium at glutaraldehyde concentrations below 0.25%. The concentration-independent dissociation predominates and the final degree of association is decreased via LeChatelier's principle. The perturbation is increased with decreasing glutaraldehyde concentrations. While large concentrations (>0.25%) are capable of freezing the subunit⟷ribosome equilibrium, further self-aggregation of the particles complicates analysis at these concentrations. (Ribosomes were generously provided by Dr. A. Wahba and computer program by Dr. A. Wishnia.)

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T-PM-Po79 SEDIMENTATION EQUILIBRIUM MEASUREMENTS OF INTERMEDIATE SIZE TOBACCO MOSAIC VIRUS (TMV) PROTEIN POLYMERS. John J. Correia, Steven J. Shire, David A. Yphantis and Todd M. Schuster, Biol. Sci. Group, Biochem. and Biophys. Sect., Univ. of Conn., Storrs, CT 06268.

Short column sedimentation equilibrium methods (1) have been applied for the first time to TMV protein (0.1 M ionic strength orthophosphate) at pH 6.5 and 7.0 in order to obtain molecular weight data. Previous sedimentation velocity experiments (2) at pH 6.5 and 20°C have led to the conclusion that the major boundary with an $s^{o}_{20,w}$ value of 24.4 ± 0.1S (> 85% by weight, the actual amount depending on the polymerization heating rate) consists of a distribution of polymers which are mainly three turn 48 to 51 subunit aggregates. The directly measured Z average molecular weight of 873000 ± 18000, together with sedimentation velocity compositional data is entirely consistent with this assignment of three turn aggregate which has been predicted to exist by Caspar (3). Molecular weights have also been determined under two conditions where a large mass fraction of the protein sediments with an $s^{o}_{20,w}$ value of 20.3 ± 0.2S. At pH 6.5 at 6 to 8°C this boundary is metastable (2) and comprises 40 to 50% by weight and at pH 7.0 at 20°C at equilibrium 70% of the protein sediments at 20.3S. The major conclusion from this study is that the molecular weight distribution of the protein aggregates in the 20.3S boundary is very similar under both conditions. This result is important for the interpretation of previous kinetic measurements of metastable TMV protein reassembly with RNA at pH 6.5 and 6.5°C, especially in regard to the role of aggregates within the 20.3S boundary in the elongation phase of assembly.

(1) Fed. Proc. 39, 1604 (abst. #19)(1980); (2) J. Mol. Biol. 127, 487 (1979); (3) Adv. in Protein Chem. 18, 37 (1963); (4) Proc. Natl. Acad. Sci. USA 76, 2745 (1979).
Supported by NIH research grant AI 11573 (TMS) and NSF grant PCM 7621847 (DAY).

T-PM-Po80 DIFFERENTIAL SCANNING CALORIMETRY OF ASPARTATE TRANSAMINASES. A. Iriarte, A. Relimpio, J. Chlebowski and M. Martinez-Carrion. Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Differential scanning calorimetry (DSC) has been applied to study thermally induced denaturation of mitochondrial and cytoplasmic isozymes of the dimeric pyridoxal enzyme aspartate aminotransferase. The consequences of binding of coenzyme and substrate derivatives to both the apo and holo forms were investigated and are interpreted in terms of the stabilization of the native form of these enzymes.

Binding of pyridoxal phosphate coenzyme increases the free energy differences between the native and denatured states of the apo protein. The stabilization produced by coenzyme binding to the apoprotein appears to be primarily due to the Schiff's base and phosphoryl moieties of the coenzyme. Holoenzyme stability depends on the chemical nature of the catalytically significant group occupying the C-4' position of the bound coenzyme. The stabilization afforded by binding of the aldehyde form which exists as an internal Schiff's base with the protein Lys 258 residue diminished after chemical reduction or when the aldehyde is replaced by an amine (pyridoxamine form).

Substrates and their analogues (aspartate, α-ketoglutarate, erythro-hydroxyaspartate and α-methyl-aspartate) perturb the stability of both the holo and apo forms of the enzymes. These results confirm findings derived from nuclear magnetic resonance studies on the ability of apoenzyme to bind substrates (Martinez-Carrion, et al (1973) J. Biol. Chem. 248, 2153).

DSC also appears as a sensitive method for the study of the dependence of enzyme-ligand complex stabilization on the stereochemistry of the ligand. (Supported by NIH Grant HL-22265)

T-PM-Po81 DETERMINATION OF THE STRUCTURE OF THE METAL CLUSTERS IN METALLOTHIONEIN BY CADMIUM-113 NUCLEAR MAGNETIC RESONANCE. James D. Otvos, Richard W. Briggs, and Ian M. Armitage, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

^{113}Cd NMR has been used to determine the nature of the multiple metal-binding sites in the two major isoproteins of metallothionein (MT) from rabbit liver and kidney and from crab hepatopancreas. MT is a small (6000 Dalton) metal-binding protein lacking aromatic amino acids, but rich in lysine, serine, and cysteine, the latter comprising about one-third of the 60 residues. It has been postulated to play a role in zinc regulation/metabolism and metal detoxification. MT-1 and MT-2 isolated from $^{113}Cd^{2+}$ injected crabs contains no metals other than Cd^{2+}. ^{113}Cd NMR spectra of the two crab isoproteins are unique and both exhibit 6 resonances in the 610-670 ppm region, characteristic of sulfur ligation. Extensive ^{113}Cd-^{113}Cd spin-spin coupling is observed, which results from metal clusters in which neighboring cadmiums are bridged by Cys sulfurs. Homodecoupling and spin echo experiments demonstrate the presence of two three metal clusters. Native MT from liver and kidney of rabbits injected with $^{113}Cd^{2+}$ contains an appreciable amount of Zn, about 2-3 g-atoms of the total of 7. Cd/Zn ratios seem relatively constant in liver, but vary with injection period in kidney. As a result of this metal heterogeneity, the ^{113}Cd spectra are complex but can be shown to arise from a 4-metal and a 3-metal cluster with Zn exhibiting a preferential affinity for the binding sites in the smaller cluster. Preparation of the homogeneous ^{113}Cd-MT by *in vitro* metal exchange has been accomplished; the ^{113}Cd spectrum from this species is considerably simpler than the one from the ^{113}Cd,Zn-protein. As reflected by ^{113}Cd NMR, the metal clusters of ^{113}Cd,Zn-MT-1 and ^{113}Cd,Zn-MT-2 differ, while those of the isoproteins containing only ^{113}Cd are virtually identical. (Supported by NIH Grants AM 18778 and ES 01674 and NSF Grant PCM 77-18941).

T-PM-Po82 AFFINITY CHROMATOGRAPHY OF BACTERIAL LUCIFERASES, T. F. Holzman and T. O. Baldwin, Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

Bacterial luciferase is a heterodimeric ($\alpha\beta$) flavin hydroxylase which catalyzes the oxidation by O_2 of $FMNH_2$ and a long chain aldehyde to yield FMN, the carboxylic acid, and blue-green light. Previous studies have shown that the reaction catalyzed by luciferase can be inhibited by compounds bearing structural resemblance to natural substrates [2-(2,3-dichloro-6-phenylphenoxy)ethylamine, DPEA; 2-(2,3-dichloro-6-phenylphenoxy)-N,N-diethylamine, DPDA; 2-N,N-diethylaminoethyl 2,2-diphenyl-n-pentanoate, SKF-525A]. We have found that a series of n,n-diphenyl alkyl acids and amines are also capable of inhibiting the bioluminescence reaction *in vitro*. We have examined the action of one of these compounds, 2,2-diphenylpropyl amine (DØPA), on the bioluminescence reaction *in vitro*. DØPA had little if any effect on the binding of $FMNH_2$ to luciferase, but rather appeared to be a competitive inhibitor of aldehyde binding ($K_I \sim 1 \times 10^{-4}$ M). A new affinity chromatographic resin has been synthesized for isolation of bacterial luciferases based upon the properties of DØPA. The affinity ligand, DØPA, was coupled to an insoluble support through a bisoxirane spacer arm. The resulting resin had high affinity, high specificity, and high capacity for binding bacterial luciferase. Titration of the affinity resin with mutant luciferases having either reduced $FMNH_2$ binding and normal aldehyde binding or normal $FMNH_2$ binding and reduced aldehyde binding, indicated the aldehyde binding mutant had a reduced affinity for the affinity resin. The mechanism of the luciferase interaction with the affinity resin appears to involve phosphate-mediated binding at the luciferase aldehyde binding site. The resin is useful for fast, high yield purification of luciferases from three species of luminous marine bacteria, *Beneckea harveyi*, *Photobacterium fischeri*, and *Photobacterium phosphoreum*. (Supported by NSF PCM 77-27219, and NIH AG-00884.)

T-PM-Po83 INHIBITOR STABILIZATION OF CARBONIC ANHYDRASE AS PROBED BY THE POSITRON ANNIHILATION METHOD. D.H. Ewert*, G. Graf, Dept. of Biochemistry and J.C. Glass*, Dept. of Physics, North Dakota State University, Fargo, ND 58105.

The positron annihilation lifetime technique is sensitive to electron density variations in proteins. The long-lived ortho-positronium is subject to various quenching mechanisms dependent on the physical state of the environment. In light of the free volume model, lifetimes and intensities reflect the number and size of sites in which the positron resides and annihilates. This technique allows for the direct observation of conformational differences in the catalytically active and the inhibited enzyme carbonic anhydrase (carbonate hydro-lyase; EC 4.2.1.1). Here we report that upon inhibition with acetazolamide(5-acetamido-1,3,4-thiodiazole-2-sulfonamide) the positron annihilation parameters clearly show a structural stability over the temperature range 0°-50°C, *vs.* the temperature dependence of the uninhibited enzyme reported from our laboratory (Handel, E.D., *et al.*, Biophys. J., in press; 1980). The stabilization of the tertiary structure cannot presently be observed by conventional biochemical techniques. To evaluate the positron annihilation parameters of the protein in aqueous suspension, the temperature profile of the pure water phase is also reported. This work is supported by the North Dakota Agricultural Experiment Station, Fargo, ND 58105.

T-PM-Po84 A FLUORESCENCE STUDY OF THERMALLY-INDUCED CONFORMATIONAL CHANGES IN YEAST HEXOKINASE. Paul Horowitz and Zygmunt Wasylewski, The University of Texas Health Science Center at San Antonio, Texas 78284.

Fluorescence studies as a function of temperature have been performed on the enzyme hexokinase (EC 2.7.1.1) from yeast. Observations of both the intrinsic protein fluorescence and the fluorescence of the noncovalently bound apolar probe 2-(p-toluidinyl) napthalene-6-sulfonic acid (TNS) indicate that significant thermal structural transitions occur in the protein in the physiological range of temperature (0°-40°C) and that there are different temperature-dependent forms of the enzyme. Thermal transitions between these forms are affected by the binding of the substrates D-glucose and ATP-Mg and also depend on the monomer-dimer interaction which can occur in hexokinase. The results are consistent with a model in which catalysis by hexokinase is linked to a change in the interaction between the domains of which this protein is composed. (Supported by NIH Grant GM25177 and Welch Grant AQ-723.)

T-PM-Po85 THE DEPENDENCE OF THE RATE OF TOBACCO MOSAIC VIRUS (TMV) ASSEMBLY ON PROTEIN COMPOSITION. Mary L. Adams, Steven J. Shire and Todd M. Schuster, Biological Sciences Group Biochem. and Biophys. Sect. University of Connecticut, Storrs, CT 06268.

The relative amounts of 4S and ~20S aggregates in a solution of TMV protein (TMVP) are very pH and temperature dependent (1, 2). We have investigated using light scattering the kinetics of TMV reassembly as a function of pH and temperature (pH 6.7, 6.8, 7.0 and 7.2 at 18, 20 and 24°C) and have attempted to correlate the results with TMVP association state as measured by sedimentation velocity analysis. The results indicate that the kinetics are biphasic and very sensitive to small changes in pH and temperature. This sensitivity can usually be ascribed to large differences in protein composition. For example, the initial rate doubles at pH 7.2 when the temperature is increased from 18 to 20°C and the 19.2S concentration increases from 8 to 36 wt.%, whereas at pH 6.7 little change occurs in protein composition and there is only an 11% increase in initial rate over the same 2°C range. Composition differences can also be obtained at pH 7.0 at 20°C upon protein dilution due to the slow depolymerization of the ~20S boundary (3). Kinetic progress curves of virus assembly using TMVP prepared 2 ways - by equilibration at reaction concentration (≤ 5 mg/ml) or by equilibration at ~15 mg/ml and dilution just prior to reassembly - were biphasic in all cases. The differences in protein composition due to sample handling manifest themselves in the basic shape of the curves, especially in the initial rates of the reaction. These differences are interpreted in terms of a proposed subunit elongation model. In particular, the size distribution within the so-called "20S" boundary may be important to both phases of the assembly reaction.

(1) Durham, J. Mol. Biol. 67, 289 (1972); (2) Schuster, et al., Biophys. J. 32, 313 (1980); (3) Butler, Phil. Trans. R. Soc. Lond. B. 276, 151 (1976). Supported by NIH grant AI 11573.

T-PM-Po86 THE SURFACE POTENTIAL OF UNIFORMLY CHARGED SPHERICAL MACROIONS IN AQUEOUS SOLUTION. GENERALIZATION OF THE DEBYE-HÜCKEL (DH) THEORY FOR SOLUTIONS OF FINITE CONCENTRATION WITH APPLICATION TO THE ELECTROPHORETIC MOBILITY OF BIOLOGICAL MACROMOLECULES. Eugene N. Serrallach* Naval Blood Res. Lab., Boston Univ. School of Medicine, Boston, MA 02118; Robert Schor, Dept. of Physics and Institute of Materials Science, Univ. of Connecticut, Storrs, CT 06268.

The interpretation of the electrophoretic mobility measurements on the basis of the DH theory underestimate the surface potential ψ_0 and therefore the derived charge z on the surface of the macromolecules when z is compared with the charge calculated from titration experiments. For example, the lack of quantitative agreement for Albumin, Ovalbumin, Lysozyme, β-Lactoglobulin (1) can be up to -40% for the total range of pH's on either side of the isoelectric point. In order to account for the finite concentration and size of the charged macroions we have generalized the usual 3 regions DH model to include IV regions. Region I represents the central macroion of radius R, region II the water layer of thickness a, region III the double layer from R+a to 2(R+a) containing only co-and counterions. The new feature of the model is the introduction of region IV from 2(R+a) to infinity, which contains not only the double layer, but the macroions as well, and thus introduces an additional electric screening of the central macroion by the surrounding macroions. On the basis of the linearized Poisson-Boltzmann equation, we have derived expression for the electrostatic potential around the central macroion in each of the IV regions. ψ_0 in this new model is dependent upon the macroion concentration, which is now included in a generalized ionic strength. In the limit of zero macroion concentration the expression for ψ_0 reduces to the one predicted by the DH theory. For the case of 5g% albumin concentration at the minimum salt point with 10 charges, ψ_0 is 28% lower than predicted by the usual DH theory. Consequently, without having to assume ion binding to the surface of the macroion (which may exist) this generalized theory improves the quantitative agreement of these two methods of measuring z to within about 10%. (1) Electrophoresis, Ed. M. Bier, Ac. Press 1959.

T-PM-Po87 PHOSPHORESCENCE AND OPTICALLY DETECTED MAGNETIC RESONANCE STUDY OF HUMAN SERUM ALBUMIN* Henry C. Brenner and Kenneth L. Bell, Department of Chemistry, New York University, New York, NY 10003

The phosphorescence and optically detected magnetic resonance (ODMR) of the lone tryptophan in the protein human serum albumin (HSA) are studied. This residue shows a red-shifted phosphorescence and D - E triplet zero field splitting frequency, which suggests that it is buried in a hydrophobic region of the protein, based on trends in triplet state properties of tryptophan residues in peptides and proteins which have recently been observed by other groups. This conclusion is in agreement with the results of previous fluorescence quenching studies of HSA using hydrophobic quenchers. The ODMR linewidth is, however, substantially larger than the linewidths usually obtained from buried tryptophans, and this linewidth decreases when the protein is denatured with urea. These latter observations are qualitatively rationalized on the basis of the known loosely-organized structure of the native protein, which leads to a particularly large heterogeneity in ODMR transition frequencies. Phosphorescence wavelengths, decay times and zero field splittings are also studied as a function of binding of halides and acetate to HSA. The tryptophan triplet lifetime is shortened when iodide and bromide bind, apparently due to the external heavy atom effect; chloride and acetate have no effect on the phosphorescence lifetime. ODMR and phosphorescence measurements suggest that the degree of solvent exposure of the tryptophan does not change appreciably upon the binding of these anions.

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T-PM-Po88 AGGREGATION OF RNA POLYMERASE, S. L. Shaner, R. R. Burgess, and M. T. Record, Jr. (Intr. by J. Anderegg), Dept. of Chemistry, University of Wisconsin, Madison, WI 53706.

The [NaCl] dependence of the aggregation of E. coli RNA polymerase has been studied by sedimentation velocity in the presence and absence of 10 mM $MgCl_2$ (pH 7.9). At high salt (≥0.2 M NaCl), with or without $MgCl_2$, the core and holoenzymes exist in their protomeric forms with sedimentation coefficients of 12.0±0.1 S and 13.5±0.4 S, respectively. The molecular weight of the 12S core species determined from the Svedberg equation using a diffusion coefficient obtained by quasi-elastic light scattering (Piatt, D. M., et al., in preparation) is in good agreement with the accepted value for the core protomer. At low salt, core and holo form different aggregates. The dimerization of holo appears to go to completion in the presence of $MgCl_2$ at the protein concentrations routinely used (0.3 mg/ml), but not in its absence. Molecular weights obtained for the low salt core species indicate that core aggregates to a tetramer in the absence of $MgCl_2$ and to a hexamer in its presence. The detailed effects of pH, [$MgCl_2$], and effector molecules on the aggregation equilibria are currently under investigation.

T-PM-Po89 PROTEIN HYDROGEN EXCHANGE RATES ARE NOT CORRELATED TO THERMAL STABILITY IN UREA. Clare Woodward, Bruce Hilton and Kathleen Trudeau*. Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

Hydrogen isotope exchange kinetics of backbone protons measure the internal motions of proteins. Exchange rates of single, assigned, peptide amide NH protons have been reported for bovine pancreatic trypsin inhibitor (BPTI). We have interpreted the complex pH and temperature dependence of these exchange rates as arising from changes in mechanism between two processes that differ in temperature dependence (Hilton and Woodward, Biochemistry 18, 5834, 1979; Woodward and Hilton, Biophys. J. 32, 561, 1980). One process, characterized by E^*_{app} in the range 20-35 kcal/mol, involves motions of the folded state that allow exchange of interior protons. The second process, characterized by E^*_{app} ~65 kcal/mol, corresponds to major, cooperative unfolding. Our model explains all of the unusual features of the pH and temperature dependence of the kinetics of the slowest exchanging protons and differs fundamentally from the interpretation of the same data by Wagner and Wuthrich (J. Mol. Biol. 134, 75, 1979). We expect that exchange rates having high E^*_{app} will be correlated with thermal stability, while exchange rates having low E^*_{app} in general, will not. It follows that exchange rates with E^*_{app} in the range 20-35 kcal/mol will not be accelerated by denaturants. As a test of this, we have measured the effect of 8 M urea on BPTI thermal stability and on the tritium-hydrogen exchange rates at pH 6.5 and temperatures <35°. Although 8 M urea decreases the midpoint of thermal unfolding, T_m, by >15°, it does not accelerate low E^*_{app} tritium-hydrogen exchange rates. We conclude that rates for exchange from the folded conformation are not correlated, in general, with the thermal unfolding temperature in denaturants. In both KCl and 8 M urea, BPTI thermal stability decreases between pH 4.5-2, apparently from the titration of one or both aspartate carboxyl groups.

T-PM-Po90 THE CONTRIBUTION OF ELECTROSTATIC AND HYDROPHOBIC FREE ENERGIES TO THE ACID STABILITY OF NATIVE MYOGLOBINS. Margaret A. Flanagan, Stephen H. Friend, and Frank R.N. Gurd, Department of Chemistry and Medical Sciences Program, Indiana University, Bloomington, IN 47405.

The calculated electrostatic and hydrophobic free energies of interaction, ΔG_{el} and ΔG_H, when taken together successfully predict the magnitude and direction of the experimentally determined acid stabilities of nine cetacean myoglobins. The relative free energy favoring the native structure of each species was determined at pH 4.0 from the acid denaturation profiles obtained at an ionic strength of 0.01 M. The range in ΔG_{obs} was 0.15 to 1.55 kcal/mol relative to sperm whale myoglobin. The differences in ΔG_{el} calculated using the Tanford-Kirkwood electrostatic theory as modified by Shire, ranged from 0.22 to 0.52 kcal/mol. Differences in ΔG_H were computed in terms of -24 cal/mol per A^2 of additional buried surface area. The computed differences in ΔG_H ranged from -0.26 to 2.80 kcal/mol and were assumed to be independent of pH and ionic strength. The sum of ΔG_{el} and ΔG_H for each species gave a range from 0.07 to 3.30 kcal/mol and gave the best agreement between calculated and observed trends. The amount of deviation between predicted and observed values does not correlate individually with the total number of sequence differences, the extent of charge site substitutions, or the amount of additional buried surface area. The computed results show that for free energy differences among a homologous series of proteins, ΔG_H and ΔG_{el} are of the same order of magnitude and hence can be competitive forces. Although the results do not rule out significant contributions from other categories of free energy change, their net effects appear to be small. (Supported by PHS Grants HL-05556 and HL-14680.)

T-PM-Po91 STRUCTURAL FLUCTUATIONS IN LYSOZYME AS REVEALED BY THE THERMODYNAMICS OF ISOTOPIC EXCHANGE. R.B. Gregory*, D. Knox*, A.J. Percy*, and A. Rosenberg. Department of Chemistry and Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN. 55455.

The hydrogen-tritium exchange of lysozyme has been measured as a function of temperature. Complete outexchange curves were constructed by methods described previously (Knox and Rosenberg, 1980, Biopolymers 19, 1049). The data was collected at constant pOH in contrast to the usual constant pH method, thereby eliminating contributions to the thermodynamic parameters due to the ionization of water. The average rate constants <K> were obtained by numerical differentiation of the outexchange curves. The analysis of the temperature dependence of ln<K> provided the values $\langle\Delta G^{\ddagger}\rangle$ $\langle\Delta H^{\ddagger}\rangle$ $\langle\Delta S^{\ddagger}\rangle$ and $\langle\Delta Cp^{\ddagger}\rangle$ as functions of the number of hydrogens remaining unexchanged. The fastest hydrogens exchanged with $\langle\Delta H^{\ddagger}\rangle$ values of between 12 and 20 Kcal/mole, while the slowest hydrogens exchanged with a high $\langle\Delta H^{\ddagger}\rangle$ equal to 40 Kcal/mole. The high $\langle\Delta H^{\ddagger}\rangle$ was accompanied by a high $\langle\Delta Cp^{\ddagger}\rangle$ value characteristic of a thermal unfolding pathway for exchange. The most interesting feature of the data is that the increase in $\langle\Delta H^{\ddagger}\rangle$ is compensated by corresponding changes in $\langle\Delta S^{\ddagger}\rangle$. The compensation plot, however, reveals two distinct apparent compensation temperatures, which reflect the operation of two qualitatively different mechanisms of exchange. A similar analysis has been performed for the hydrogen-tritium exchange of lysozyme in the presence of glycerol. The results are also compared with similar studies on model polypeptides.

T-PM-Po92 KINETICS OF MYOSIN SELF-ASSOCIATION
Herbert R. Halvorson and William R. Johnston* Henry Ford Hospital, Detroit MI 48202

Myosin was isolated from rabbit skeletal muscle and purified by ion-exchange chromatography. A repetitive pressure-jump instrument was used to study the relaxation kinetics of self-association (pH 8.3, ionic strength 0.16 M), using scattered light as the observable. Although the perturbation was sufficiently small to ensure the validity of the relaxation approximation, the complexity of this system gives rise to distinctly non-exponential responses. Accordingly, the kinetic behavior was studied in terms of the mean relaxation rate. A striking observation was that in the vicinity of 2 mg/mL total protein concentration the relaxation rate decreased dramatically with increasing protein concentration (a 10-fold increase in concentration reduced the rate to 1/80 its previous value). Of 12 alternative models considered to explain this phenomenon, one is in reasonable accord with the data. It suggests the existence of a rapid nucleation process that is thermodynamically unfavorable. Further, the step-wise equilibrium constants for subsequent growth pass through a maximum. The implications of this model to the mechanism of myosin self-association will be discussed, as will a comparison of kinetic and equilibrium results.

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T-PM-Po93 BOVINE SERUM ALBUMIN CONFORMATION CHANGES UPON ADSORPTION TO A SOLID SURFACE: A TOTAL INTERNAL REFLECTION FLUORESCENCE ENERGY TRANSFER STUDY. Thomas P. Burghardt and Daniel Axelrod, Biophysics Research Division and Dept. of Physics, University of Michigan, Ann Arbor, Michigan 48109.

A change in the conformation of bovine serum albumin (BSA) upon adsorption to a quartz surface has been detected by a novel fluorescence technique. The technique combines total internal reflection fluorescence excitation of surface adsorbed BSA with a fluorescence spectroscopic examination of energy transfer between two different fluorophores that are both covalently bound to each BSA molecule. The donor is dichlorotriazinyl aminofluorescein and the acceptor is tetramethylrhodamine. For studies of surface adsorbed BSA, we constructed a chamber in which the excitation light of a standard spectrofluorimeter totally internally reflects from a surface at which adsorbed BSA is in equilibrium with a solution. The shallow evanescent wave thereby created excites fluorescence from only those BSA molecules in close proximity to the surface. Spectral examination suggests significantly less resonance energy transfer from the donor to the acceptor in surface adsorbed BSA relative to that in native solubilized BSA. The spectroscopic change between native and adsorbed states of fluorescence labeled BSA cannot be explained by changes in extinction coefficients or quantum efficiencies of the donor or acceptor due simply to the proximity of each fluorophore to the surface. These results support the conclusion that the BSA spreads upon irreversible adsorption to quartz. (Supported by PHS HL 24039 and NS14565.)

T-PM-Po94 LOCALIZATION OF STRUCTURAL TIGHTENING IN LIGAND BINDING: RIBONUCLEASE-S + 2'CMP. Joseph J. Rosa and Frederic M. Richards, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511, USA.

The effects on the conformational dynamics of ribonuclease-S of the binding of 2'CMP have been monitored by a medium resolution hydrogen exchange technique. The method permits measurement of the average rates of exchange for known regions of a protein (J. Mol. Biol. 133, 399 (1979)). Previously we showed that S-peptide binding to S-protein produced effects propagated through virtually the entire structure of S-protein (J. Mol. Biol., in press). Rates of hydrogen exchange from regions of S-protein representative of most of the structure were dramatically slowed by complex formation. Sites notable for their lack of protection were found in the C-terminal peptide (121-241) and peptide 10 (34-46 plus 92-96). In contrast to these otherwise widespread effects, the termolecular complex between 2'CMP and RNase-S at pH 5.35 and 21° produced relatively small changes in rates of exchange from S-protein. Interestingly, the only observed region significantly perturbed was the C-terminus, residues 121-124. Assuming a heat capacity change of -10 to -15 cal K^{-1} per 100 daltons accompanies rigid (native) structure formation, the immobilization of this tetrapeptide segment itself may largely account for 1/3 to 1/2 the loss of 150 cal K^{-1} mol^{-1} in heat capacity observed by Eftink and Biltonen (Biophys. Disc., 1980) in the binding of 3'CMP to ribonuclease. The residual effect may be found in peptide segments not so far directly monitored in this study. The present results suggest that the structural alterations are restricted to only a small part of the whole peptide chain for this particular ligand interaction.
[This work was supported by NIH grant #GM-22778.]

T-PM-Po95 PROTEIN STRUCTURAL STABILITY AND pH: ELECTROSTATIC EFFECTS IN RIBONUCLEASE. James B. Matthew and Frederic M. Richards, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA.

The solvent accessibility modified Tanford-Kirkwood discrete charge model for electrostatic effects in proteins is a useful tool in understanding pH dependent processes (Biochem. 18: 1919 (1979)). The interaction energies of sites are retained to the degree that the protein structure limits access of the solvent to the charge loci. Effective dielectric constants of 50 or more account for observed pK shifts even for protein ion pairs which lack intervening solvent. This effect is attributed to shielding by solvent dipoles and counterions. The model is now applied to both ribonuclease A and S. Overall titration, individual site behavior and the pH dependent free energy of denaturation are correctly predicted. The electrostatic contribution of S-peptide to the stability of RNase-S is calculated. Repulsive interactions at low pH predict the compensatory dislocation of S-peptide. The attractive interactions of the S-peptide charge array at neutral pH are examined for docking specificity. Finally, the pH dependence of the hydrogen exchange rates of amide protons is examined. Proximity to solvent, electrostatic component of protein stability, and the protein charge array are important factors which may hinder (or enhance) catalytic ion migration or affect the stability of the charged intermediate. The rank order of amide exchange rates for either the S-peptide helix amides (J. Mol. Biol. 133: 399 (1979)) in RNase-S or β-sheet amide protons of BPTI can be predicted on the basis of proximity of solvent and the protein potential at the amide. The pH dependent behavior of protein HX rates is shown to follow the protein's overall electrostatic stabilization, modulated at each amide by the variance of the formal charge array with pH.
[Supported by NIH grant GM-22778 and grant IF32 CA-06633.]

T-PM-Po96 **INDEPENDENCE OF TERTIARY AND QUATERNARY STRUCTURAL TRANSITIONS IN HUMAN HEMOGLOBIN.** D. W. Pettigrew, P. H. Romeo, and G. K. Ackers, intr., by M. C. Farmer, Dept. of Biology, The Johns Hopkins University, Baltimore, Md. 21218.

Equilibrium constants are presented for dimer-tetramer assembly of 16 human hemoglobins in their liganded (oxy) and unliganded states. These results provide, for each hemoglobin, a determination of the effect of oxygenation upon stability of the $\alpha_1\beta_2$ intersubunit contact, where the major structure change occurs upon oxygenation. The studies include both chemically-modified normal hemoglobin and naturally occuring variants. Equilibrium constants were determined by several independent methods, yielding results in very good agreement. Results of these determinations show that the oxygenation-sensitive dimer-tetramer equilibrium may be drastically altered by structural perturbations in the $\alpha_1\beta_2$ interface. The difference in free energy for assembly of liganded versus unliganded tetramers is reduced from 6.3 kcal/mol (normal hemoglobin) to about 1 kcal/mol by modification of single amino acid residues in this region. Thus, energetic effects of structure perturbations in the $\alpha_1\beta_2$ contact are not additive, indicating that the quaternary structure of normal hemoglobin reflects a delicate balance of interactions. By contrast, the energy of oxygenation-linked dimer-tetramer assembly is not significantly altered for hemoglobins in which the structure modification is outside the $\alpha_1\beta_2$ contact. These structure modifications may produce alterations in energy of tertiary change for dimer oxygenation. Since these alterations are not reflected in dimer-tetramer assembly, the tertiary energy for tetramer oxygenation in a given hemoglobin is the same as for dimer oxygenation. These results argue against the role of tertiary effects (including events at the heme site) as a source of the cooperative energy in human hemoglobin. Supported by NSF Grant PCM 78-97582 and NIH Fellowship GM 07119-0151.

T-PM-Po97 TIME RESOLVED UV SPECTROSCOPY OF PROTEIN AGGREGATION AND CONFORMATION: TOBACCO MOSAIC VIRUS PROTEIN (TMVP). Martin Potschka, Biological Sciences Group, Biochemistry and Biophysics Section, University of Connecticut, Storrs, CT 06268.

The UV-absorbancy of aromatic amino acids overlaps in the narrow range between 250-310nm, has few characteristic features and small difference amplitudes. In most cases a monomer has more than one chromophore and not all subunits in an aggregate need to be equivalent. Kinetic analysis of the complex extinction changes upon temperature perturbation separates individual spectral events and both kinetic and spectroscopic information is obtained. Experimental technique and limitations of the procedures of turbidity - and other corrections will be discussed. The approach is not limited to UV absorbancy. Selected applications for the study of the structure and mechanism of TMVP aggregation will be discussed using the three catagories of difference spectra analyzed so far: (1) Polymerization independent temperature response within less than 1 μ sec. The spectrum may relate to increased segment flexibility at elevated temperature, which is common for proteins.
(2) Tryptophan blue shift upon formation of "20S" and other medium sized aggregates.
(3) Events at higher concentration at alkaline pH tentatively identified as "8S" formation are probably dominated by Tyrosine. To obtain some of the spectral information, computerized spectra manipulation was required. Some results are: (a) In course of "20S" formation at least one Tryptophan is either exposed to solvent or a hydrogen bond is broken.
(b) Judged by UV spectroscopy, "20S" and "23S" are much closer related to each other than to the oligomeric A-protein. (c) Two environmentally different Tryptophans or one chromophore with different fine structure are identified. This study was done under supervision of T. M. Schuster. His help is gratefully acknowledged.
Supported by NIH grant AI 11573 (TMS).

T-PM-Po98 STABLE INTERMEDIATES IN THE FOLDING OF THE α SUBUNIT OF TRYPTOPHAN SYNTHASE. C. R. Matthews and M. M. Crisanti. Chem. Dept., Penn. State Univ., University Park, PA 16802

The urea induced unfolding of the α subunit of tryptophan synthase from E. coli was monitored by optical spectroscopy and by urea gradient gel electrophoresis. Three independent lines of evidence support the conclusion that one or more stable intermediates are present in this process:

(i) Satisfactory fits of the equilibrium unfolding transitions obtained from difference spectroscopy at 286 nm and circular dichroism spectroscopy at 222 nm require a model which involves a stable intermediate in addition to the native and unfolded forms.

(ii) Kinetic studies of the change in extinction coefficient at 286 nm show that while the unfolding is well described by a single exponential change, the refolding kinetics are complex. The nature of the dependence of the refolding kinetics on the initial concentration of urea support the conclusion that at least one stable intermediate exists.

(iii) The patterns obtained from urea gradient gel electrophoresis experiments on the α subunit show that at least one and possible two stable intermediates are involved; the intermediates have markedly different degrees of compactness. A kinetic model for the folding of the α subunit, consistent with all of these results, can be formulated.

T-PM-Po99 DYNAMIC COMPRESSIBILITY OF GLOBULAR PROTEINS. B. Gavish, E. Gratton, and C. Hardy, (SPON: H. Frauenfelder). University of Illinois, Urbana, IL 61801

The adiabatic compressibility of metmyoglobin, apomyoglobin, and lysozyme was determined through sound velocity and density measurements on solutions of the proteins. An acoustic resonator was used to find sound velocity in the solutions to an accuracy of 0.01%. A digital picnometer measured the density to 0.01%. The sound velocity was measured in the frequency range of 0.5 to 3.5 MHz. There was no dispersion, to within the accuracy of our measurements, for all of the proteins studied. The compressibility β_p was found to be $\beta_p = (10\% \pm 2\%)\ \beta_{water}$ for metmyoglobin, $\beta_p = (9\% \pm 2\%)\ \beta_{water}$ for apomyoglobin, and $\beta_p = (10\% \pm 2\%)\ \beta_{water}$ for lysozyme, where $\beta_{water} = 45.5 \times 10^{-6}$ bar^{-1}.

It is interesting to note that these values for protein compressibility are roughly a factor of 20 lower than those for liquid hydrocarbons. This cannot be explained by the presence of the hydration layer, which has been shown to give a negative contribution to the compressibility of amino acids in solution. This negative contribution, when volume averaged with the contribution of the protein's interior to yield the observed values for the total compressibility, requires a compressibility for the interior which is still a factor of 10 less than that of the liquid hydrocarbons.

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T-PM-Po100 THE PATH OF THE POLYPEPTIDE OF BACTERIORHODOPSIN: ELECTRON DIFFRACTION STUDIES OF PURPLE MEMBRANE. B.A. Wallace[+*] and R. Henderson[#]. [+]Dept. of Biochemistry, Columbia University, N.Y. 10032 and [+#] MRC Lab. of Molecular Biology, Cambridge, England.

A model for the folding of bacteriorhodopsin has been proposed (Engelman, Henderson McLachlan & Wallace, PNAS 77, 2023 (1980). It represents the most probable way of fitting 7 helical regions in the sequence into 7 helices in the density map. To test this model, electron diffraction studies of specifically modified purple membranes were undertaken. Purple membranes were digested to quantitatively remove either 10 or 21 C-terminal amino acids. Difference Fouriers of native vs. either of the digested membrane samples indicate the amino acids removed are almost entirely disordered and free to take up many positions and that the removal of these residues does not affect the crystal packing. Low resolution X-ray and electron diffraction difference maps indicate the general location of the C-terminus. In addition the location of a single lysine residue which was labeled with tetrakis (acetoxymercuri) methane was determined. A preliminary difference Fourier map of bromine-labeled retinal has been calculated. These three results locate specific portions of the molecule, severely limit the number of models possible, and are consistent with the model previously proposed. B.A.W. was supported by a Jane Coffin Childs Grant.

T-PM-Po101 STRUCTURAL STUDIES OF CYTOCHROME c BINDING TO REACTION CENTER-LIPID MEMBRANES. J.M. Pachence, P.L. Dutton, and J.K. Blasie, University of Pennsylvania, Phila., PA 19104.

Reaction center (RC) protein was isolated from photosynthetic bacterium Rps. sphaeroides (R26 mutant) using the detergent LDAO. A technique to remove the LDAO in the presence of pure egg phosphatidylcholine (EPC) was used to prepare unilamellar protein-lipid vesicles. Partially dehydrated, oriented membrane multilayers were formed from dispersions of EPC/RC membrane vesicles (molar ratio of lipid/protein = 100:1) and varying amounts of horse heart cytochrome c (added to the suspension of preformed vesicles). In the EPC/RC/cyto c membrane multilayers, cyto c was shown to be kinetically coupled to reaction centers. More than 90% of the reaction centers in these membranes was available to cytochrome c for both vesicle dispersions and membrane multilayers. Lamellar X-ray diffraction data were obtained from a number of membrane multilayers consisting of cyto c/RC ratios ranging from 0 to 3.3. The periodicity was $\sim$128 Å with no cyto c, $\sim$140 Å when the cyto c/RC = 0.8, and $\sim$155 Å when the cyto c/RC = 1.6 (relative humidity of 90% at 6°C). When the cyto c/RC was greater than 2.5, the lamellar X-ray diffraction yielded reflections on at least two lattices, of approximately 150 Å and 175 Å, and having a higher degree of lattice disorder than that from multilayers having a lower cyto c/RC. Electron density profiles were calculated for the lamellar X-ray diffraction data having a well-defined single lattice. The systematic changes observed in the electron density profiles versus cyto c/RC ratio indicate the position of bound cyto c in these profiles and suggests that there are two distinct cyto c binding sites per reaction center. Supported by NIH grant GM 27309.

T-PM-Po102 IDENTITY OF THE OPTICAL CROSS SECTION FOR OXYGEN FORMATION AND FLUORESCENCE YIELD INCREASE AND EVIDENCE FOR TOTAL ANNIHILATION IN THE OXYGEN FORMING SYSTEM. A. Ley and D. Mauzerall The Rockefeller University, New York, NY 10021.

Both the yield of O_2 formation and the increase in fluorescence yield have been measured simultaneously in Chlorella as a function of the energy of a short (0.4-0.7 μs) pulse of 596nm light from a dye laser. The O_2 was measured on a Pt polarograph and the fluorescence yield was measured by a weak (<1% O_2 formation) $\sim$1 μs blue probe flash fired 30 μs following the main laser pulse. The normalized yields of both O_2 and fluorescence increase were identical, proving that the same photochemical reaction in the photosynthetic unit determines both parameters. At super saturating laser energies a reversible and quantitatively identical decrease in both yields was observed. The reversiblity of the decline in yields allows us to preclude irreversible photochemical damage as the trival explanation of the effects, although such damage will occur at still higher energies. Traps are increasingly likely to be missed as the photon flux increases. We interpret this effect as evidence for total annihilation in the antenna of the oxygen photosystem, wherein two excitations interact to produce heat and/or triplets, etc., and their combined energies are totally lost to effective photochemistry. The odd-even character of the event predicts that one half of the traps will remain open and this is consistent with the data. A rough fit of the data is obtained at about 2000 hits per unit (not traps). The lifetime of this state is thus calculated to be about 80 ps, consistent with the lifetime of the excitation in the antenna before encountering a trap.

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T-PM-Po103 CHLOROPHYLL-PROTEIN AGGREGATES AND 3,3',5,5'-TETRAMETHYLBENZIDINE-STAINED PEPTIDES IDENTIFIED ON ELECTROPHORETIC PATTERNS OF ANACYSTIS NIDULANS THYLAKOID MEMBRANES. James A. Guikema and Louis A. Sherman, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

Thylakoid membranes of Anacystis nidulans R2 were solubilized using lithium dodecyl sulfate (LiDS) and the protein content of these membranes were assessed by polyacrylamide gradient electrophoresis. This assessment revealed: (1) 6 chlorophyll-containing bands ranging in apparent molecular weights from 360-34 Kd and (2) a series of peptide bands which possessed peroxidase activity -- probably owing to the association of these peptides with heme. Peroxidase activity was identified by staining gels with tetramethylbenzidine (TMBZ) + H_2O_2, a method introduced by Thomas et al. (Analyt. Biochem. (1976) 75, 168-176) to detect cytochrome P-450 on acrylamide gels. Three major TMBZ-staining peptides were identified in the 29, 17.5, and 14.5 Kd regions of the electrophoretic profiles. Increasing from 4C to 70C the temperature at which membranes were solubilized prior to electrophoresis yielded the following: (1) Heating in the presence of dithiothreitol caused the loss of peroxidase activity from each of the 3 bands; (2) In the absence of DTT, the peroxidase activities of the 17.5 and 14.5 Kd bands were stable to increased solubilization temperature; (3) Heating resulted in decreased TMBZ activity at 29 Kd, correlated with the appearance of a minor TMBZ-staining band at 34 Kd. In addition, heating caused a loss of protein at 29 Kd with corresponding increases in protein at 28 Kd and at 34 Kd. Our results with Anacystis, coupled with TMBZ-staining electrophoretic profiles of maize mutants which lack spectral activities of certain photosynthetic cytochromes, suggest that the TMBZ-staining peptides correspond to membrane-bound cytochromes. Supported by NIH grants GM 21827 and GM 07704.

T-PM-Po104 MÖSSBAUER STUDIES OF REACTION CENTERS FROM R. SPHEROIDES. B. Boso and P. G. Debrunner, Physics Department, University of Illinois, Urbana, IL 61801 and M. Y. Okamura and G. Feher, Physics Department, University of California, San Diego, CA 92037

We report new Mössbauer results on six different preparations of reaction centers (RCs) isolated from a ^{57}Fe-enriched culture of the photosynthetic bacterium R. Spheroides R-26. The samples differed in ubiquinone (Q) content (Q = 0.1, 1.02, 2.06 per RC), detergent (LDAO or Triton), oxidation state (native or dithionite-reduced in Triton), or the presence of the inhibitor o-phenanthroline. In zero magnetic field, all spectra display a single quadrupole doublet and an isomer shift consistent with a high-spin ferrous state of the iron atom. All samples have splittings in the range $\Delta = 2.22 \pm 0.07$ mm/s at 15 K, and the isomer shifts are, within error, the same, $\delta = 1.175 \pm 0.01$ mm/s. A high degree of homogeneity of the samples is indicated by the ratio of the areas of the two quadrupole lines, which differs from unity by at most 5%. The lack in sensitivity of the electric hyperfine interaction to the presence of quinone(s) or inhibitor rules out binding of either one in the first coordination sphere of the iron, in agreement with EXAFS data (P. M. Eisenberger, et al., Federation. Proc. 39, 1802 (1980)).

High field ($H \leq 4T$) variable temperature ($4.2\ K \leq T \leq 175\ K$) Mössbauer measurements on a non-reduced sample are compatible with the spin Hamiltonian parameters deduced from susceptibility measurements (W. F. Butler, et al., Biophys. J. 32, December 1980). The 4.1T spectra show a broad unresolved doublet at 4.2 K, which separates and narrows to a minimum width between 15 K and 30 K, and at high temperature is indicative of a positive V_{zz} value. Our simulations also indicate that the iron atom occupies a site whose symmetry is lower than orthorhombic.

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T-PM-Po105 SPECTROPHOTOMETRIC AND VOLTAGE CLAMP CHARACTERIZATION OF MONOLAYERS OF BACTERIAL PHOTOSYNTHETIC REACTION CENTERS. David M. Tiede, Paul Mueller and P. Leslie Dutton, Eastern Pennsylvania Psychiatric Institute, Philadelphia, PA. 19129, and Dept. Biochem. and Biophys., Univ. of Pennsylvania, Philadelphia, PA. 19104.

Monolayers of isolated reaction centers (RCs) of Rps. sphaeroides R-26 spread on an air-aqueous interface and collapsed to a surface density of about 1 RC/2200 Å^2 have been transferred to glass slides for optical analysis by raising the slide vertically from below the monolayer through the air-aqueous interface. Optical spectra of the monolayer reveal characteristic RC absorptions, while the linear dichroism spectra are qualitatively analogous to those seen for RCs in the natural chromatophore membrane but are distorted by optical interference effects. Submerging the slide in water quenches the interference effects and permits quantitative determination of the monolayer orientation by linear dichroism analysis. The dichroic ratios (A_V/A_h; $\theta = 60^0$) of the 860nm, 800nm and 760nm absorptions of 1.8, 1.3, 0.7 ± 0.05 are indistinguishable from those seen in the natural membrane, indicating that the RC orientation on the air-aqueous interface is similiar to that in the chromatophore membrane. The measurement of light-induced capacitative charging and discharging currents in monolayers deposited on platinum coated slides further demonstrates the retention of photochemical activity, asymmetry of the monolayer, and that the bacteriochlorophyll dimer is located near to the aqueous phase. Slides repeatedly transferred downwards through arachidate monolayers and upwards through RC monolayers show linear increases in RC absorptions, with little alteration in dichroic ratios, demonstrating the possibility of constructing well-ordered, asymmetric multilayers.

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T-PM-Po106 A RAPID PROCEDURE FOR THE PREPARATION OF PHOTOSYNTHETIC REACTION CENTRES FROM RHODOPSEUDOMONAS SPHAEROIDES R-26. M. Kendall-Tobias and M. Seibert. Solar Energy Research Institute*, Golden, CO 80401

A rapid purification prodedure has been developed for the isolation of reaction centres from R. sphaeroides strain R-26. Chromatophores formed by french pressure cell disruption of whole cells are suspended in 200 mM NaCl, 0.5% LDAO (Ammonyx-LO), and 10 mM Tris-Cl, pH 8 buffer. After centrifugation at 200,000 x g, the supernatant is adjusted to 1% LDAO and 0.28 g/ml ammonium sulphate. A low speed centrifugation produces a levitate which is resuspended in buffer containing 0.05% LDAO and 0.13 g/ml ammonium sulphate. After centrifugation about 5 g Celite (Fisher) is added to the supernatant and the ammonium sulphate concentration raised to 0.26 g/ml. The slurry is poured into a small column and washed with 0.25 g/ml ammonium sulphate, 0.05% LDAO. Elution at low salt concentration produces reaction centres with a yield of over 60%. Starting with frozen cells the procedure takes 13 hrs to produce reaction centres. The ratio of the optical densities at 280 nm and 800 nm is <1.4. Approximately two ubiquinone molecules are present per reaction centre and very little phospholipid is detectable.

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T-PM-Po107 OXIDATION-REDUCTION TITRATION OF LOW POTENTIAL INTERMEDIATES IN PHOTOSYSTEMS 1 AND 2. Joseph T. Warden, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N.Y. 12181.

The nature of primary and secondary electron transport in Photosystems 1 and 2 of green plant photosynthesis has been examined by potentiometric analysis in conjunction with flash-photolysis electron spin resonance (esr) studies at room and cryogenic temperatures. To access the low potentials required for reduction of the secondary electron acceptors in Photosystem 1 ($E_h \geq -550$ mV), the versatile reducing agent aminoiminomethane sulfinic acid has been utilized. Redox titrations of the spin polarization in PS1, combined with esr analysis of the resonances associated with the secondary acceptor $A_2(X)$, assign a midpoint potential for this component of ~ -710 ± 20 mV. The tertiary electron acceptors, iron-sulfur centers A and B, exhibit midpoint potentials of -550 ± 10 mV and -595 ± 10 mV respectively.

Examples of representative titrations and methodology as well as preliminary studies in Photosystem 2 will be presented. (Supported by USDA Grant #5901-0410-80073 and by NIH Grant #1R01 GM 26 133-01).

T-PM-Po108 THE ASSOCIATION OF REACTION CENTERS WITH THE UBIQUINONE-CYTOCHROME c_2 OXIDO-REDUCTASE IN THE CHROMATOPHORE MEMBRANE. D.P. O'Keefe, K. Matsuura, and P.L. Dutton, Dept. of Biochemistry and Biophysics, Univ. of Pennsylvania, Phila., PA. 19104

There is an excess (30-40%) of reaction center (RC) over the content of ubiquinone-cytochrome c_2 (Q-c_2) oxidoreductase in chromatophores from Rps. sphaeroides. Unless the Q-c_2 is freely mobile among the reaction centers, or binds in a functional 1:2 complex with the RC, this excess could lead to a heterogeneity in the reactions of the secondary quinone (Q_{II}) of the RC. The observation that $Q_{II}^{\cdot-}$ oscillations can be observed in only ~30% of the RC population suggests that a heterogenous population of RC does exist: ~70% in a relatively stable 1:1 association of RC and Q-c_2 (RC:Q-c_2 pair) and ~30% as lone RC (where $Q_{II}^{\cdot-}$ oscillations occur). Partial solubilization of the membrane with detergent leads to an increase in the amount of $Q_{II}^{\cdot-}$ oscillations, and decrease in cytochrome b reduction, suggesting that the RC:Q-c_2 association is disturbed, and more lone RC are present. The ratio of H_{II}^+ (antimycin sensitive) binding to slow cyclic electron transfer ($t_{1/2}$~25ms) at high E_h is >1.0. H_{II}^+ binding under these conditions takes place at a rate which is comparable to an electrogenic step which involves completion of the electron transfer cycle by <50% of the available electrons. A possible interpretation of this data is that electron transfer between RC:Q-c_2 pairs can take place by the dismutation of $2Q_7^-$ in neighboring RC:Q-c_2 pairs to form one Q_7H_2 and one Q_7. These results indicate that the RC:Q-c_2 association is relatively stable on a timescale of seconds, and that reducing equivalents can exchange out of the strict confines of a single RC:Q-c_2 pair at steps involving Q_{II} of the RC, or Q_7 of the Q-c_2 oxidoreductase. Supported by NIH grant GM 27309.

T-PM-Po109 PHOTOCHEMICAL AND PHOTOPHYSICAL PROCESSES INDUCED BY HIGH-INTENSITY LIGHT: A STUDY OF THE REACTION CENTER CHLOROPHYLL (P700) OF PHOTOSYSTEM I IN SPINACH CHLOROPLASTS. Mark S. Crowder and Alan Bearden, Department of Biophysics and Medical Physics, University of California, Berkeley, Ca. 94720.

The yield of irreversible $P700^+$ of spinach chloroplasts at low temperature measured by Electron Paramagnetic Resonance spectroscopy has been determined with different types of high-intensity laser excitation. The types include a single laser flash (10^{17}photons/cm^2 in 500nsec pulse) and a dual laser flash where the flashes are delayed in time from each other. The amount of irreversible $P700^+$ produced by a single saturating laser flash is approximately 40-50% of the total P700 population. This is contrary to results of Mathis and Conjeaud who monitored P700 photooxidation optically.(1) When dual laser-flashes are used, the yield of irreversible $P700^+$ depends upon the time delay between the two high-intensity flashes. If the time between the laser flashes is one microsecond, the amount of irreversible $P700^+$ formed is only slightly (<5%) above that produced by a single laser flash. As the time delay is incresed between the flashes, the amount of irreversible $P700^+$ formed also increases. The relationship between the time delay and the amount of $P700^+$ produced is temperature dependent and can be represented by a sum of two exponentials. The time constant of one of these exponentials (15 μsec at 25K) agrees well with the lifetime of long-lived quenchers in the antenna system of Photosystem I.(2) The origin of the longer exponential dependence (375 μsec at 25K) is discussed in terms of both the photochemical and photophysical processes associated with the photooxidation of P700.

1) Mathis,P. and H. Conjeaud. 1979. Photochim. Photobiol. 29:833-837.
2) Breton,J. et. al., 1979. Biochim. Biophys. Acta. 548:616-635.
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