M-AM-Sym-1 THE PHOTOSYNTHETIC REACTION CENTER FROM RHODOPSEUDOMONAS VIRIDIS. J. Deisenhofer[1] and H. Michel[2], [1]Howard Hughes Medical Institute/University of Texas Southwestern Medical Ctr. 5323 Harry Hines Blvd., Dallas Texas 75235-9050, [2]Max-Planck-Institut fuer Biophysik, Heinrich-Hoffmann-Str. 7, D-6000 Frankfurt 71, Federal Republic of Germany.

The photosynthetic reaction center (RC)from the purple bacterium Rhodopseudomonas viridis was one of the first membrane proteins for which well ordered 3-D crystals were obtained (1). The X-ray structure analysis at 3.0Å resolution of these crystals (2,3,4) allowed the construction of an atomic model including the RC's four protein subunits, and the major pigment cofactors. Crystallographic refinement at 2.3Å resolution (5) further improved the model, and led to the discovery of additional cofactors, and of localizaed solvent molecules.

The RC is an elongated complex whose surface is hydrophobic in the middle, and polar at both ends. The central membrane spanning polypeptide chains are folded into 11 helices, connected by chains containing shorter helices or peptides without regular secondary structure. The trans-membrane helices vary in length between 21 and 28 amino acid residues.

The cofactors located in the membrane spanning region of the RC form two approximately symmetric pathways for electron transfer across the membrane. However, under most circumstances, only one of these pathways is used. This functional asymmetry in the presence of structural symmetry is one of the most surprising properties of the RC complex. Light driven electron transfer originates at a pair of closely associated bacteriochlorophylls; the heme groups of the cytochrome reduce the photo-oxidized special pair.

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M-AM-Sym-2 ULTRAFAST SPECTROSCOPIC STUDIES OF ELECTRON TRANSFER IN MUTANT AND WILD-TYPE PHOTOSYNTHETIC REACTION CENTERS. Dewey Holten and Christine Kirmaier, Department of Chemistry, Washington University, St. Louis, MO 63130; Edward J. Bylina and Douglas C. Youvan, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 01239.

The conversion of light energy into chemical potential by photosynthetic organisms occurs in pigment-protein complexes called reaction centers (RCs). This primary photochemistry involves a series of electron transfer reactions that separate charge across a membrane with a quantum yield of essentially unity. In bacterial RCs electron transfer occurs from the excited primary electron donor P^* to BPh_L in 3 ps, followed by movement of an electron from BPh_L to Q_A in 200 ps. (P is a dimer of bacteriochlorophyll (BChl) molecules, BPh_L is the bacteriopheophytin associated with the L polypeptide, and Q_A is the primary quinone.) Subpicosecond time- resolved absorption spectroscopy has been used to explore the primary photochemistry in *Rhodobacter capsulatus* RCs. In wild-type RCs, we have investigated the possible role of the accessory pigment $BChl_L$, a molecule that is situated somewhat between P and BPh_L. RCs containing site-directed mutations at two residues also have been investigated. We discuss the effects on the spectroscopy and electron transfer dynamics of changing a glutamic acid residue hydrogen bonded to BPh_L to leucine and glutamine. Changing a histidine residue coordinated to the central magnesium atom of one of the BChls of P to leucine yields a reaction center containing a BChl-BPh heterodimer. The primary photochemistry in this mutant gives valuable insights into how the electronic properties of the primary donor influence the initial stage of charge separation in wild-type RCs.

M-AM-Sym-3 **MECHANISM OF CHARGE SEPARATION IN PHOTOSYNTHETIC REACTION CENTERS: ELECTRIC FIELD EFFECTS.** S.G. Boxer and D.J. Lockhart, DepT. of Chemistry, Stanford Univ., Stanford, CA 94305

The effects of electric fields on the absorption and fluorescence spectra of bacterial photosynthetic reaction centers have been used to characterize the nature of the initially excited state and its decay into the first product of electron transfer. The Stark effect on the absorption spectrum of the special pair Q_y transition has a second derivative lineshape, with little contribution from a zeroth or first derivative [1,2]. The fluorescence Stark effect has primarily a zeroth derivative lineshape, with some first derivative and little second derivative contribution [3]. The anisotropy of the fluorescence Stark effect demonstrates that the state whose formation competes with fluorescence is not the P^+B^- state [4]. The mechanism of electron transfer $^1PBH \rightarrow P^+BH^-$ is generally believed to involve participation of states such as P^+B^- (superexchange). The second derivative lineshape of the Stark effect for the Q_y absorption band of P suggests a substantial change in dipole moment upon with photoexcitation (relative to a monomer), but not a field-dependent mixing of states. A model for superexchange in which ^1P is mixed with P^+B^- leads to the prediction that the absorption Stark effect could have a substantial first derivative contribution. This is not observed, so limits can be placed on the magnitude of this mixing. The temperature and angle dependence of the fluorescence Stark effect also provide information on the mechanism, as does electric field modulation of the reaction rate. Models to explain the small first derivative contribution to the fluorescence Stark effect will also be presented. The combination of steady-state and time-resolved electric field effects provides information on the nature of the states which participate in electron transfer and their interactions [5]. [1]Biochem.26,664(1987);[2]PNAS85,107(1988);[3]Chem. Phys. Lett144,243(1988);[4]J.Chem.Phys.,89,1408(1988);[5]J.Phys.Chem.,in press. Support NSFDMB8607799

M-AM-Sym-4 LONG-RANGE ELECTRON TRANSFER IN PROTEINS. H. B. Gray, Arthur Amos Noyes Laboratory, California Institute of Technology, Pasadena, California 91125.

Electron transfer through proteins is the fundamental process that is responsible for energy storage and conversion in living cells. For many years it was believed that these electron transfers would have to occur between atoms in contact at short range, but recent experiments have shown that an electron can jump across fifteen to twenty atoms in a large protein molecule in less than one-millionth of a second. Work on four ruthenium-modified myoglobins and on cytochrome c has shown that protein electron-transfer rates decrease exponentially with the donor-acceptor edge-to-edge distance. Theoreticians have begun to explore the possible atom-to-atom pathways for electron transfer over these long distances. Evidence that solvent and peptide-chain reorganization can control protein electron transfer has been obtained in experiments involving systematic variations in donor-acceptor energetics. It appears that high reorganization energies are associated with changes in the water structure in and around the protein. Many oxidation-reduction enzymes probably are activated by conformational changes in which water is eliminated from the medium that separates the electron donor and acceptor.

M-AM-Min-1 MULTIMODE LIGHT MICROSCOPY OF LIVING CELLS. D. Lansing Taylor, Dept. of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213.

Cellular functions involve the temporal and spatial interplay of ions, metabolites, macromolecules and organelles. In the last few years, a variety of quantitative light microscopic methods have emerged as powerful tools for defining the structural, chemical and molecular dynamics in living cells. We have constructed light microscope imaging workstations that have the capability of acquiring data from cells using a variety of distinct modes of microscopy. The ability to combine data from different modes of microscopy allows correlations of many structural, chemical and molecular changes in cells during specific cell functions. Video-enhanced contrast microscopy has made it possible to detect structures and movements well below the limit of resolution of the light microscope. In particular, fluorescence methods have permitted chemical and molecular measurements. The distribution of specifically labeled analogs of proteins and other macromolecules have been determined in cells using low light imaging techniques and the local mobilities of these analogs have been analyzed by Fluorescence Redistribution after Photobleaching. Ratio imaging has become an important method for determining ionic changes within cells, detecting resonance energy transfer, and quantifying the relative distributions of labeled macromolecules in living cells. Up to five distinct structural and chemical parameters have been investigated in the same cell using multiple parameter imaging. The three-dimensional organization of cells has been explored using confocal microscopy and through focus deconvolution methods. Multimode light microscopy of cell migration and growth factor stimulation of quiescent cells will be presented.

M-AM-Min-2 DIRECT 3-D MICROSCOPY IN CONFOCAL LIGHT MICROSCOPY AND SEM. J. Pawley (Intr. by W. Chiu), Integrated Microscopy Resource, Univ. of Wisconsin, 1675 Observatory Dr., Madison, WI 53706.

Two recent improvements in microscopical instrumentation have greatly increased the amount and quality of 3-D data on the micro-morphology of biological samples. The confocal light microscope produces 3-D data sets, in which intensity is directly related to fluorescent label or refractive index, by optically sectioning the bulk sample and utilizing the confocal principle to mechanically exclude the out-of-focus data which others remove by massive computer processing. (Paddy et al EMSA 1988 46, 34). Although such data can readily be processed into stereo-pairs for viewing, more sophisticated manipulations, such as rotating stereo displays with overlaid operator-driven graphics or flexible 3-D thresholding in which the threshold would be elastic for volume elements near points above threshold, is beyond the capabilities of the most current equipment. Given the demonstrated ability of fluorescent makers to label biologically significant structures, inadequacies in the systems for conveying 3-D intensity data to the scientist constitute an important limitation on the usefullness of the technique.

The new generation of high resolution SEM's couple a cold field-emission gun to a low aberration final lens and operate with a low beam voltages to produce high-contrast surface images of virtually any biological structure that can be seen in a TEM thin section. Stereo images can be simply made by tilting the specimen in the microscope or by electromagnetically deflecting the electron beam as it approaches the specimen (Pawley, EMSA 1988, 46, 206). It remains to be seen if the sophisticated cross-correlation algorithms that are used to automatically derive topographical data from aerial photographs can be used to obtain quantitative 3-D information from such images.

M-AM-Min-3 THREE-DIMENSIONAL LIGHT AND ELECTRON MICROSCOPY OF CHROMOSOMES. D.A. Agard, Y. Hiraoka, A. Belmont, M. Paddy, J.W. Sedat. HHMI Structural Biology Unit, University of California at San Francisco, San Francisco, CA 94143-0448.

We have been using three-dimensional light and electron microscopy methods to explore the spatial organization of chromosomes within the nucleus and to understand their detailed fine structure. 3-D light microscopy of fixed and living nuclei has revealed many new features of chromosome dynamical behavior during the mitotic cell cycle. Of central interest are the direct observations of sister and homologous chromatid pairing, a spatial wave of condensation and heterochromatic organization. 3-D tomographic EM studies show a hierarchy of chromosome structural organization beyond the 300 angstrom fiber in interphase and metaphase chromosomes, and show that mitotic chromosomes are not radial-loop structures.

M-AM-Min-4 3-DIMENSIONAL STRUCTURE ANALYSIS OF VIRUS PARTICLES BY CRYO-ELECTRON MICROSCOPY AND IMAGE ANALYSIS. W. Chiu, Baylor College of Medicine, One Baylor Plaza, Houston, TX. 77030.

Cryo-electron microscopy has been developed to a stage that one can record low dose images of individual virus particles embedded in a thin layer of vitreous ice without stain and fixative. A 3-dimensional structure of the virus can be reconstructed by computer processing from images of virus particles at various orientations. Jeng and co-workers (J. Mol. Biol., In press) have used this technique to show the alpha helices of the coat protein and the RNA in tobacco mosaic viruses. These structural features agree with those from X-ray diffraction analysis. This technique has been used in many laboratories to study large animal viruses such as rotavirus and Semliki-forest virus which are not easily amenable for structural study by X-ray diffraction (see reviews by Prasad et al., Micron Microsc. Acta 18, 327, 1987; Dubochet et al., Quat. Rev. Biophys. 21, 129, 1988). The structural data from this type of analysis allows one to determine the spatial distribution of multiple shells of viral coat proteins and the structural variations of the virion in different stages of morphogenesis and functional states. (This research has been supported by NIH RR02250.)

M-AM-Min-5 Electron Microscopy of Bacteriorhodopsin
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Recent advances in cryo-electron microscopy and computerised image processing are allowing the determination of the high resolution structure of bacteriorhodopsin. In previous work, the structure of bacteriorhodopsin at about 6 A resolution has been determined independently in three different crystal forms (1,2,3). In present work, bacteriorhodopsin in the naturally occurring purple membrane of Halobacterium halobium has been studied to higher resolution. The 2.8 A projection structure has been determined (4) by recording micrographs at 4 K and carrying out image processing of the entire area of the micrograph. We have been fortunate to find an excellent collaboration with Fritz Zemlin and Erich Beckmann at the Fritz Haber Institute in Berlin, who have taken all the micrograhs.

The collection and processing of high resolution three-dimensional data from tilted specimens is now underway. Many very good 22-degree tilts have been obtained and some 45-degree tilts. It is hoped to calculate a map with this limited tilt data before the meeting.

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(2) Leifer & Henderson (1983) J. Mol. Biol. 163, 451-466.
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M-AM-A1 EFFECT OF LIPID SURFACE CHARGE ON K^+ CHANNEL CONDUCTION: AN ELECTROSTATIC PARADOX.
Jacques Neyton, Rod MacKinnon, and Christopher Miller (Intr. by J. Lisman).
Graduate Dept. of Biochemistry, Brandeis Univ., Waltham, MA.

It has been well established that negatively charged lipids increase the conductance of Ca^{2+}-activated K^+ channels reconstituted into planar lipid bilayers (Moczydlowski et al., J. Membr. Biol. 83:273 [1985]). A natural interpretation of this effect was that the electrostatic potential established by the charged lipids raises the local concentration of K^+ ions near the entryway to the pore. We sought to test this idea by using ionic pore-blockers as probes of the local potential set up by lipid charge. High-conductance Ca^{2+}-activated K^+ channels were incorporated into planar bilayers formed from neutral (PE/PC) or charged (PE/PC/PS) lipid mixtures. In 100 mM symmetrical KCl, the single-channel conductance was 50% higher in PS-containing membranes than in neutral membranes. However, identical blocking parameters were found in the two types of membranes for a variety of positively charged blockers. The apparent affinities of block by TEA (internal and external), by internal trimethylbenzylammonium, internal Ba^{2+}, and external charybdotoxin were not affected by the lipid charge, nor were the on-rates for Ba^{2+} and charybdotoxin. Since these are all known to block the conduction pathway, these results are incompatible with the idea that the channel mouth senses the electrostatic potential due to lipid headgroup charge.

M-AM-A2 CHARACTERIZATION AND REGULATION OF THE OUTWARD CURRENT THROUGH INWARD-RECTIFYING K-CHANNELS (IK1) IN CARDIAC CELL-ATTACHED PATCH-CLAMP EXPERIMENTS. M. Mazzanti and L. J. DeFelice, Department of Anatomy and Cell Biology, Emory University, Atlanta, GA 30322.

The resting K permeability of cardiac cells is regulated by K channels displaying inward rectification (IK1). While the inward current through the single channel is well characterized, many attempts have failed to observe an outward current in cell-attached experiments. By perfusing the internal side of the membrane with a divalent cation-free solution, Matsuda et al. (Nature 325:156-159, 1987) and Vandenberg (PNAS 84:2560-2564, 1987) demonstrated the dependence of rectification on Mg^{++}, and Mazzanti & DiFrancesco (Pflugers Arch. in press, 1988) demonstrated the dependence on Ca^{++}. Using a double-electrode technique (Mazzanti & DeFelice, Biophys. J. 52:95-100, 1987) in spontaneously beating single embryonic chick ventricle cells, we observed an outward current through IK1 channels during the plateau phase of the spontaneous action potential. We filled the cell-attached pipette with a solution containing in mM: 60 KCl, 70 NaCl, 1.5 $CaCl_2$, 0.5 $MgCl_2$. To focus attention on whether Ca^{++} or Mg^{++} is responsible for the rectification, we studied the effects of divalent cations in the external solution. While the absence of Mg^{++} did not change channel behavior, the removal of Ca^{++} ions from the patch-pipette solution resulted in an increase of the outward current. We conclude that, although Mg^{++} is responsible for the macroscopic background rectification, Ca^{++} modulates the outward current through IK1 channels. (Supported by NIH grant HL27385)

M-AM-A3 BLOCK OF THE HIGH-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNEL: COMPETITION BETWEEN CHARYBDOTOXIN AND TETRAETHYLAMMONIUM. Christopher Miller, Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254

Single high-conductance Ca^{2+}-activated K^+ channels from rat skeletal muscle plasma membranes were incorporated into planar lipid bilayers. The actions of two inhibitors of this channel, tetraethylammonium (TEA) and charybdotoxin (CTX), were examined. Although both TEA and CTX inhibit the channel from the external solution, they operate on very different time-scales, and so their separate effects can be easily distinguished. TEA is a fast, open-channel blocker, while CTX induces long-lived nonconducting intervals of about 15 seconds mean duration. The effect of TEA on the kinetics of CTX block was studied in order to ascertain whether these two blockers interact with the channel independently of each other. Addition of TEA lowers both the observed channel conductance and the on-rate of CTX in a precisely parallel fashion. On the other hand, TEA does not affect the CTX off-rate. The probability distributions of both CTX-blocked and unblocked times are single-exponential, regardless of TEA concentration. These results demonstrate that TEA and CTX block this channel in a mutually exclusive way. Occupancy of the channel by either blocker precludes occupancy by the other. Since TEA is known to block the externally facing region of the K^+-conduction pathway, these results suggest that the site of CTX block is located in the outer mouth of the pore.

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M-AM-A4 Ca^{2+}-ACTIVATED K CHANNELS IN B AND T LYMPHOCYTES. L.C. Schlichter and M.P. Mahaut-Smith. Department of Physiology, University of Toronto, Ontario, Canada M5S 1A8.

Evidence for Ca^{2+}-activated K^+ channels in lymphocytes comes from fluorescent dye and tracer flux studies; however they have not been observed electrophysiologically. We have now found these channels in human tonsillar B lymphocytes and rat thymocytes in single-channel recordings from cell-attached and excised patches. In cell-attached recordings, intracellular Ca^{2+} was raised by two methods; ionomycin or addition of Ca^{2+} to Ca^{2+}-free medium. With the second method Indo-1 showed that $[Ca^{2+}]_i$ rose from ~90 to 260 nM. Both techniques activated channels of two conductances; 25 and 8 pS (slope conductance at 0 mV applied, with 140 mM K^+ in the pipette). Both channels were selective for K^+: In the presence of ionomycin, the 25 pS channel reversed (E_{rev}) at an applied potential (Vp) of -85 mV with KCl and -78 mV with Kaspartate in the pipette. With 140 mM KCl in the bath, E_{rev} was 0mV with either KCl or Kaspartate in the pipette. This channel showed little voltage-dependence of opening between Vp +30 to -30mV, but could be activated by large depolarizations (Vp -140mV). Inward rectification was seen in symmetrical K^+ solutions. The same channel was seen in excised, inside-out membrane patches, where the threshold for activation was 200-300 nM Ca^{2+}, similar to that in cell-attached patches. Wash-out was often observed: fewer excised patches were active and channel openings were more flickery. Open and closed-time histograms showed at least two open and two closed states in cell-attached recordings; however, in most excised patches there appeared to be only one open state. Hence these K^+ channels are activated by internal $[Ca^{2+}]$ in the physiological range (i.e. similar to mitogenic stimulation) and may play a role in cell activation. Supported by MRC and NCIC (Canada).

M-AM-A5 **K CHANNEL BLOCK BY INTERNAL CALCIUM AND STRONTIUM.** **Clay M. Armstrong and Y. Palti. Dept. of Physiology, University of Pennsylvania, Philadelphia, Pa.; Technion, Haifa, Israel; and MBL, Woods Hole, Mass.**

We used voltage clamped, internally perfused squid giant axons, and cells from the giant fiber lobe (GFL) of squid in whole cell configuration. Results in the two preparations were qualitatively similar. Further, Ca^{2+} and Sr^{2+} were similar in their effects. With 10 to 20 mM Sr^{2+} inside, a curious kinetic pattern of block becomes apparent at V_m above +50 mV: I_K activates, 'inactivates' partially, and then grows larger again as the block is relieved. Above +50 mV 'inactivation' is more prominent, and there is no relief of block, so steady-state current is quite small. Below +30 mV block is inappreciable. On stepping from 30 mV (no block) to 130 mV, current jumps instantaneously by ~2X because of the increased driving-force, then decays with a time constant of less than a millisecond to a low value as Ca^{2+} or Sr^{2+} enter and block. Channels that are blocked at 130 mV by internal Ca^{2+} reopen at 30 mV with a time course that is similar to the late phase of the normal opening kinetics at this voltage. The presence of 10 mM Rb^+ externally completely eliminates the blocking effect of internal calcium in GFL neurons. The results show that internal Ca^{2+} and Sr^{2+} can enter K channels over a high energy barrier to occupy a site near the outer end of the channel. The results suggest that the affinity of the site for Ca^{2+} and Sr^{2+} decreases over the first few milliseconds following depolarization. Apparently occupation of the site by Rb^+ prevents block. Supported by NIH Grant. No. NS12543.

M-AM-A6 VOLTAGE AND CALCIUM ACTIVATION OF A CHANNEL IN PARAMECIUM CAN BE REMOVED BY THERMOLYSIN TREATMENT. A. Kubalski, B. Martinac, Y. Saimi, and C. Kung, Lab. of Molecular Biology, University of Wisconsin, Madison, WI 53706.

Ca^{++}-activated potassium channel from Paramecium membrane vesicles was discovered and investigated using patch-clamp method. The single channel conductance was 70 ± 9 pS in symmetrical 100 mK K^+ solution. The channel was more active upon hyperpolarization than upon depolarization. The channel activity was also increased in the presence of Ca^{++} at concentrations above 10^{-6}M. The Hill coefficient (n = 2.29) indicated that more than two Ca^{++} bindings are necessary for full channel activation. Under sustained exposure to high Ca^{++} concentration (10^{-4}M) the channel activity was irreversibly reduced. We believe that this current regulates the resting potential and contributes to the macroscopic Ca^{++}-activated K^+ current upon hyperpolarization (Richard, Saimi, and Kung, J. Membrane Biol. 91:173-181, 1986).

Effects of trypsin, thermolysin (both from Sigma) and pronase (Calbiochem) on gating properties of the channel were studied. We found that 100-200 µg/ml proteases did not affect the single channel conductance, but they significantly changed the channel activity. After exposure to thermolysin channel remained fully open and Ca^{++} - and voltage- dependence was abolished. The effects of trypsin and pronase were not as drastic - but they all were irreversible.

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M-AM-A7 Ca^{2+} BLOCK IN THE LARGE Ca^{2+} ACTIVATED K^+ CHANNEL: AN ESTIMATION OF THE SURFACE CHARGE OF THE INTERNAL VESTIBULE. A. Villarroel and G. Eisenman Department of Physiology and Brain Research Institute, UCLA School of Medicine Los Angeles CA 90024-1751.

The large conductance Ca^{2+} activated K^+ channel has been postulated to have a negative charge in its vestibules. Ca^{2+} is known to block the channel. The surface charge should increase the local Ca^{2+} concentration producing a distortion of the blockade, particularly at low ionic strength. We therefore studied the reduction of current due to Ca^{2+} blockade by added internal Ca^{+2} over a wide range of low K^+ concentrations in order to estimate the surface charge. Voltage ramps of single channel currents in symmetrical K^+ concentrations from 5 to 100 mM were measured. We found that Ca^{2+} produces a blockade whose voltage dependence ($z\delta=0.38$) over this range agrees with the value found previously for divalent cations at 150 mM K (Oberhauser et al, J.Gen.Physiol, 92:67, 1988). However, as anticipated in presence of surface charge, the concentration dependence of the blockade does not follow the rectangular hyperbola expected from the classical Woodhull model. In particular, the blocking effect of Ca^{2+} is disproportionately large at low Ca^{2+} concentration. The data can be fitted by using the conventional binding model by also assuming that negative surface charge produces an increase in local Ca^{+2} concentration, according to Gouy-Chapman theory. The best fit is obtained with 1 negative charge per 314 $Å^2$. This value is higher than the one found in our K^+ permeation studies (1 charge per 5480 $Å^2$); but this apparent discrepancy could be due to deficiency in planar approximation or might indicate a different location of the Ca^{2+} site and the K^+ site. Because we do not use our previous K^+ permeation model, the present results constitute an independent estimation of the internal surface charge of the channel.

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M-AM-A8 INWARD RECTIFIER CHANNELS IN ENDOTHELIAL CELLS ARE NOT GATED BY INTERNAL MAGNESIUM. by M.R. Silver & T.E. DeCoursey, Depts. of Medicine & Physiology, Rush Medical Center, Chicago, IL 60612.

Outward currents through inward rectifier (IR) channels in cardiac myocytes are blocked by internal magnesium ($[Mg^{2+}]_i$) at μM concentrations (Vandenberg, 1987, *PNAS* 84:2560; Matsuda *et al*, 1987, *Nature 325*:156). We examined the effects of $[Mg^{2+}]_i$ on IR channels in bovine pulmonary artery endothelial cells. These channels resemble IR channels in other preparations: high K–selectivity, voltage–dependent block by external Cs^+, Ba^{2+} and Na^+, and activation at more positive potentials as $[K^+]_o$ is increased. To test whether $[Mg^{2+}]_i$ is responsible for IR channel gating, we compared macroscopic currents at "low" $[Mg^{2+}]_i$ {KF or $KMeSO_4$, no added Mg, 10 *mM* EDTA or 5 *mM* ATP} and "high" $[Mg^{2+}]_i$ {$KMeSO_4$, 2 *mM* $MgCl_2$, 10 *mM* EGTA, free Mg^{2+} 1.9 *mM*}. Only subtle differences were detected in whole–cell currents with high or low $[Mg^{2+}]_i$. IR activation was quantified by a variable prepulse followed by a test pulse to a potential at which activation kinetics were resolvable. The amplitude of the instantaneous current during the test pulse was taken to reflect activation during the prepulse. Measured in this way, the steady–state voltage–dependence of IR gating was well–fitted by a Boltzmann distribution: $I_o(V_{pre})=[I_{o,max}-I_{o,min}]/\{1+\exp[(V_{pre}-V_{0.5})/k]\}+I_{o,min}$. The midpoint of the activation curve, $V_{0.5}$, at all $[K^+]_o$ from 4.5 to 160 *mM* was about 10 *mV* negative to the K reversal potential both with low $[Mg^{2+}]_i$ (-9 ± 7 *mV*, mean ± SD, n=18) and with high $[Mg^{2+}]_i$ (-9 ± 5 *mV*, n=17); the slope was steeper with low $[Mg^{2+}]_i$: k was 4.0 ± 0.7 *mV* (n=18) *vs.* 5.7 ± 1.9 *mV* (n=17), respectively ($p<0.005$). Both transient and maintained outward IR currents were present with high or low $[Mg^{2+}]_i$. We conclude that inward rectification in endothelial cells is due mainly to an intrinsic gating mechanism which is only subtly affected by $[Mg^{2+}]_i$. *Supported by the Parker B. Francis Foundation (MS) and NIH grants SK04–HL01928 & R01–HL37500 (TD).*

M-AM-A9 THREE K CHANNELS IN HUMAN PANCREATIC B–CELLS IDENTICAL TO THOSE IN RAT B–CELLS. Atwater, I., Li, M.X., Carroll, P., Li, M.Y., Jia, M. and Rojas, E. LCBG, NIDDK, NIH, Bethesda, MD.

Several K channels have been described in rat and mouse B-cells, three have been well characterized in several laboratories, and two are known to be sensitive to glucose in cell-attached patches of membrane. This study compares the properties of the K channels in B-cells from the pancreas of a normal human transplant donar (NDRI) with those obtained from rat pancreases. Islets were hand picked after collagenase digestion; B-cells were isolated using EDTA and dispase and cultured in CMRL 1066 medium (Gibco) with 5.6 mM glucose for 1 to 15 days. Standard patch clamp methods were used. K(Ca) channels had a conductance of 95 pS for outward currents (135 mM Na in pipet; 140 K in bath) and the open probability was reduced by addition of 20 mM glucose. K(ATP) channels showed rectification and had a conductance of 55 pS for inward currents (140 K in pipet) and 18 pS for outward currents (135 Na in pipet) and were closed by 20 mM glucose in cell attached patches and by ATP in excised patches. The small 8 or 9 pS K channel was occasionally seen, but did not appear to be sensitive to glucose. These results indicate that K channels in human pancreatic B-cells are identical to those in rat pancreatic B-cells and indicate that the rat islet model may be used to study normal human islet behaviour. Furthermore, these results confirm the involvement of two K channels in the process of sensing in pancreatic B-cells.

M-AM-A10 EFFECTS OF TEMPERATURE ON K+ CURRENTS IN HUMAN T - LYMPHOCYTES P.A. Pahapill and L.C. Schlichter. Dept. of Physiology, University of Toronto, Toronto, Canada. M5S 1A8.

Voltage-activated K channels have been implicated in lymphocyte function, including killing and mitogenesis. Cell function is optimal at 37°C whereas studies of ion channels in lymphocytes have been conducted at room temperature. We have now examined $I_k(V)$ in whole-cell recordings from 5 to 42°C. Increasing temperature progressively increased the rates of channel activation and inactivation as well as maximum K conductance of the cell. At 20°C both activation and inactivation rates had similar temperature sensitivities, wheras at 37°C inactivation rate was much more temperature sensitive than activation rate. In contrast, at 5°C inactivation rate was much less temperature sensitive than activation rate. At all temperatures channel kinetics were more temperature sensitive than peak conductance. Arrhenius plots for all three parameters were non-linear but with no evidence of a phase transition. Increasing temperature shifted the steady-state activation curve in the hyperpolarizing direction and the steady-state inactivation curve in the depolarizing direction. This resulted in a greater overlap of the curves with a large increase in activatable K channels at 37°C vs. room temperature. K currents were well described by Hodgkin - Huxley n^4j kinetics at all temperatures. The effects of temperature changes were fully reversible with no signs of hysteresis over a six-hour experimental period. Hence, channel function is qualitatively and quantitatively different at physiological temperatures and caution should be exercised in extrapolating results from studies conducted at room temperature. (Supported by MRC and NCIC (Canada)).

M-AM-A11 PROTEIN-ATP-POTASSIUM CO-COMPARTMENTS OF THE LIVING CELLS AS REVEALED FROM DETERGENT INDUCED RELEASE KINETICS STUDIES.

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The effect of non-ionic detergents on living cells is biphasic. First, the detergent molecules disrupt the lipoprotein membranes. Second, the mobilizable intracellular constituents like proteins, ATP, and inorganic elements are released. If proteins, ATP, and K^+ are free within the cytoplasm, they will immediately equilibrate with the external environment following membrane disruption. Our findings show that, with specific membrane disruptive detergents, a significant delay (minutes) occurs before the almost simultaneous release of K^+, ATP and proteins is observed. The detergent induced disruptions of cell membranes were verified by electron microscopy, and thin layer chromatography for mobilized membrane lipids. Supported by NSF 8505920 and MTA 30.070/32/88 joint grant and OTKA 83.

M-AM-B1 ADP BINDING TO RIGOR CROSSBRIDGES PRODUCES NO MAJOR CHANGE IN THEIR CONFORMATION. O.Obiorah and M.Irving. Dept. of Biophysics, King's College London, London WC2B 5RL.

ADP binding to crossbridges in rigor muscle fibres changes the orientation of fluorescent probes attached to the reactive sulphydryl (SH-1) of the myosin heavy chain (Burghardt et al., P.N.A.S. 80, 7515, 1983). However spin probes attached to the same site indicate little or no angular change (Thomas et al., Biophys.J. 47, 380a, 1985). We used parallel measurements of muscle fibre birefringence (BF) and of the equatorial X-ray diffraction pattern (Peckham & Irving, Biophys.J. 53, 24a, 1988) to look for any large scale changes in crossbridge conformation associated with ADP binding to rabbit psoas muscle fibres in rigor.

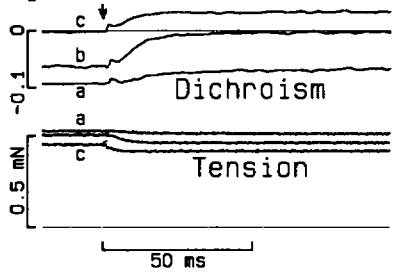
When fibres were transferred from rigor solution to a similar solution containing 2.5mM MgADP (plus 10mM glucose, 0.1mg/ml hexokinase and 0.1mM di-adenosine pentaphosphate to minimise contaminant ATP) there was no significant change in BF; the paired difference was -0.02 ($\pm$ 0.02) x 10^{-3}, mean $\pm$ S.E. of mean, n=6. In contrast, addition of 1mM MgATP to rigor fibres in the absence of calcium produced a BF increase of 0.24 ($\pm$ 0.06) x 10^{-3}, in agreement with previous results. The X-ray equatorial intensity ratio, I_{11}/I_{10}, was 3.64 ($\pm$0.43) in rigor, 3.54 ($\pm$0.40) in the MgADP-containing solution, and 0.57 ($\pm$0.08) in the presence of MgATP.

These results show that MgADP binding to rigor crossbridges results in no large scale change in either their orientation with respect to the fibre axis or their proximity to the thin filaments. The MgADP-dependent change in orientation of fluorescent probes on SH-1 is therefore more likely to be due to a local structural change in the vicinity of the probe.

Supported by the M.R.C., the Wellcome Trust and the Royal Society.

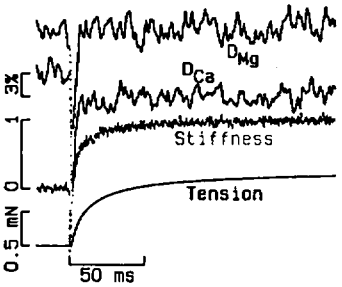
M-AM-B2 **DICHROIC ABSORPTION AND RIGOR FORCE TRANSIENTS INITIATED BY PHOTOLYSIS OF CAGED ADP IN FLUORESCENT-LABELED MUSCLE FIBERS.** Jonathan W. Tanner, Douglas P. Vallette, David D. Thomas+, and Yale E. Goldman. Dept. of Physiology, University of Pennsylvania, Philadelphia, PA 19104 and Dept. of Biochemistry+, University of Minnesota, Minneapolis, MN 55455.

We have used laser flash photolysis of caged ADP to study the relationship between force and molecular orientation of fluorescent-labeled myosin heads in single glycerol-extracted fibers from rabbit psoas muscle. Iodoacetamidotramethyl rhodamine covalently labeled the reactive thiol (SH-1) of the myosin head. A 546 nm excitation beam, incident perpendicular to the fiber, was modulated at 84 kHz between parallel and perpendicular polarizations. Dichroic absorption was detected from the fluorescence emission above 590 nm using a lock-in amplifier. As reported by Borejdo et al. (J. Mol. Biol. 158:391, 1982), dichroism was negative in rigor and positive at 1-2.5 mM ADP, indicating that for the AM.ADP state the absorption dipoles of the probes are aligned more parallel to the fiber axis than in rigor. Tension decreases by 15.4 $\pm$ 0.3 % (mean $\pm$ s.e.m.) on addition of ADP to a rigor fiber and partly recovers on removal of ADP. In the steady state, tension reduction depends on [ADP] hyperbolically with half-saturation value 45.6 $\pm$ 8.0 μM. In the figure the tension traces a, b & c, recorded during photolysis trials liberating 17, 85 and 410 μM ADP successively within the same fiber, show that ADP alters tension and dichroism on the millisecond time-scale. Dichroism was somewhat slower than tension, reflecting either an extra kinetic step or strain dependence of the observed rates. Both transients were faster at higher [ADP]. The results indicate that molecular motions of rhodamine on SH-1 are related to cross-bridge structural changes leading to force generation. Supported by the MDA and NIH grants AR26846, AR32961 & RR04300



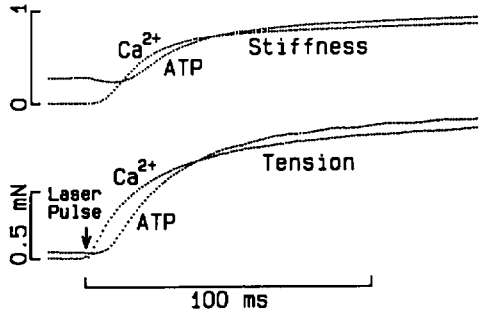
M-AM-B3 STRUCTURAL CHANGES IN TNC DURING ACTIVATION OF SKINNED PSOAS FIBERS BY LASER PHOTOLYSIS OF CAGED CA T. Allen, R.J. Barsotti, A.M. Gordon, J.H. Kaplan, and Y.E. Goldman (Intr. by B. Hille) Depts of Physiology, U. of WA, Seattle, WA 98195 & U. of PA, Phila., PA 19104 & Graduate Hospital, Phila., PA 19146

We measured changes in linear dichroism of fluorescently labeled troponin-C (TnC) and development of stiffness and tension of single muscle fibers following laser photolysis of DM-nitrophen, a new caged Ca^{2+} (Kaplan and Ellis-Davies, PNAS 85:6581, 1988). TnC, labeled with rhodamine at Cys-98, was exchanged for endogenous TnC in glycerinated fibers from rabbit psoas muscle. Absorption dichroism was observed using a lock-in amplifier to detect synchronous modulation of total fluorescence when the polarization of the fluorescence excitation was modulated at 84 kHz. Absorption dichroism of rhodamine-TnC decreases when Ca^{2+} exchanges for Mg^{2+} at the Ca-Mg-binding sites, when Ca^{2+} binds at the Ca-specific sites, and when myosin attaches to actin. In the figure, tension and stiffness (2 kHz) half-times are 10.9 and 5.8 ms respectively when a fiber is switched from relaxation to full activation by photolysis of caged Ca^{2+} at 22°C. Simultaneously measured dichroism (D_{Ca}) shows an initial optical artifact, but has already attained its lower, steady level 10 ms after the laser pulse. Similar D_{Ca} signals are obtained with fibers in rigor or stretched beyond filament overlap. Dichroism measured during photolysis of DM-nitrophen complexed with Mg in the absence of Ca (D_{Mg} in figure) shows the optical artifact, but no steady deflection. The results suggest that the D_{Ca} signal reflects molecular events determining changes of the orientation distribution of TnC when Ca^{2+} binds. The structural changes responsible for the decrease in dichroism are completed before all the cross-bridges are attached. (GM07270, NS08384, HL31962, AM26846, HL30315, HL15835 to Pa. Mus. In., and MDA)



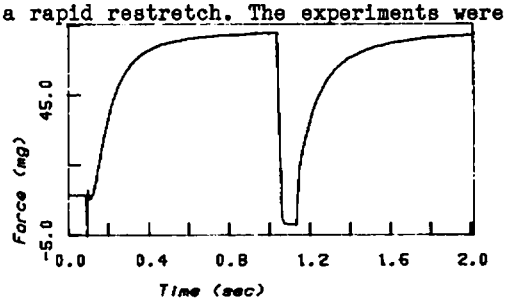
M-AM-B4 KINETICS OF SKELETAL MUSCLE FIBER ACTIVATION BY PHOTOLYSIS OF DM-NITROPHEN (CAGED Ca^{2+}) AND CAGED ATP. R.J. Barsotti, G. Ellis-Davies, J.H. Kaplan & Y.E. Goldman, Dept. of Physiol., Univ. of Penna. & Rockus Res. Inst., Graduate Hosp. Phila., PA 19104

We compared the kinetics of activation of contraction following laser-pulse photolysis of DM-nitrophen (DMNP, caged Ca^{2+}, Kaplan and Ellis-Davies, PNAS 85:6581, 1988) to that following the photolysis of caged ATP. Prior to the laser pulse, single glycerol-extracted fibers from rabbit psoas muscle were either relaxed in 100 mM TES, 2 mM DMNP, 1.2 mM Mg^{2+}, 1.0 mM total Ca^{2+}, 3 mM ATP, 10 mM glutathione, 25 mM HDTA and 20 mM CP, pH 7.1, 200 mM ionic strength, 21°C or in rigor in a similar solution but 10 mM caged ATP and CaEGTA (pCa 4.5) replaced ATP and DMNP. Rigor fibers were released to reduce tension to nearly the relaxed level before photolysis. A single focused 347 nm, 80-120 mJ laser pulse released Ca^{2+} by photolysis of DMNP. Tension then increased within 1 ms and reached maximum force with a $t_{1/2}$ of 22 ms. On photorelease of 1 mM ATP from caged ATP in the same fiber, tension increased 3-5 ms later but thereafter increased at a rate slightly higher than that with caged Ca^{2+}. Stiffness is higher in rigor before the laser pulse, so the extra lag following caged ATP photolysis is not due to end compliance. Rather, following liberation of ATP, binding, cross-bridge detachment, ATP hydrolysis and reattachment probably precede force generation. The results indicate that the cross-bridges in relaxed muscle are "primed" to attach and produce force quickly after the regulatory system is switched on by Ca^{2+}. Supported by NIH grants HL40953, HL30315, GM 39500 and AM26846.



M-AM-B5 THE RATE OF FORCE RECOVERY AFTER RAPID SHORTENING AND RESTRETCH IS EQUAL TO THAT AFTER PHOTOLYSIS OF CAGED ATP AT 10°C IN RABBIT PSOAS MUSCLE.
K. Burton, M. Irving, & J. Sleep. MRC Cell Biophysics Unit, 26-29 Drury Lane, London WC2B 5RL.

The rate of force development, k_{cage}, after a rapid release of ATP from caged ATP has been interpreted as a measure of the rate of the force generating step in the contractile cycle and at 20°C the reported rate is 80-100 s^{-1} (Goldman et al, J. Physiol 354, 605). The rate of force recovery after a rapid restretch following a period of unloaded shortening, k_{recov}, has also been interpreted in this way and at 20°C is 20 s^{-1} (Brenner & Eisenberg, PNAS 83, 3542). We have made a direct comparison of the two rates by first releasing ATP in a rigor fibre and then, 1 or 2 seconds later, imposing a period of unloaded shortening followed by a rapid restretch. The experiments were carried out at 10°C to minimise loss of sarcomeric order which results in a reduction in the rate of force recovery. Under our conditions (I_1 = 170 mM, pH 7, sarcomere length 2.3 um), k_{cage} was 8 s^{-1} and this was equal within experimental error to k_{recov}. These rates were also similar to k_{recov} measured during steady state activation at 5 mM ATP within 1 minute of the laser flash and to the reported results of Brenner & Eisenberg at 10°C. Our results suggest that k_{cage} and k_{recov} are controlled by the same fundamental process in the crossbridge cycle.



M-AM-B6 ORIENTATION OF SPIN-LABELLED LIGHT CHAIN-2 EXCHANGED ONTO MYOSIN CROSSBRIDGES IN MUSCLE FIBERS. B.D. Hambly, K. Franks, and R. Cooke. Dept. of Biochemistry and CVRI, University of California, San Francisco, CA 94143.

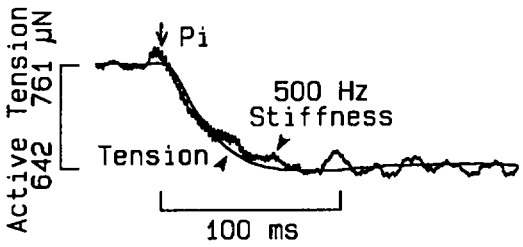
A lysine residue of purified rabbit skeletal muscle light chain-2 (LC2) has been selectively labelled with the spin label 3(5-fluoro-2,4-dinitro-anilino)-PROXYL (FDNA-SL). The FDNA-SL LC2 has been separated from unlabelled LC2 by cation exchange HPLC and exchanged into glycerinated rabbit psoas muscle fibers. Electron paramagnetic resonance (EPR) spectra obtained from FDNA-SL LC2 exchanged fibers show: (1) In rigor the probe is oriented (74° to the muscle axis); (2) In relaxation the probe appears to be completely disordered; and (3) In contraction the probe is also completely disordered, indistinguishable from relaxation. No probes with rigor orientation are seen during contraction, in contrast to spin label probes at the SH-1 and ATP sites, where a proportion of probes show a rigor orientation during contraction. Tension generated by exchanged fibers is close to control values. The spectrum of rigor fibers is unchanged by the addition of ADP or pyrophosphate. The spectrum of rigor fibers is also unchanged by the application of forces that approximate active tension. LC2 is believed to bind to the half of the crossbridge that lies closest to the thick filament, hence these EPR spectra are expected to be reporting on the orientation of this region of the crossbridge. Our failure to resolve any discrete orientation during contraction is consistent with a high degree of disorder in this region during contraction. Supported by a grant from the USPHS, AM30868 and from the NH&MRC of Australia.

M-AM-B7 CONDITIONS WHICH SIMULATE MUSCLE FATIGUE HAVE LITTLE EFFECT ON THE EPR SPECTRA OF PROBES IN ACTIVE SKINNED MUSCLE FIBERS. A.J. Baker, N. Naber and R. Cooke. Dept. of Biochemistry and CVRI, University of California, San Francisco, CA 94143.

Inorganic phosphate (Pi) and hydrogen ions (H+) are products of muscle metabolism whose accumulation during fatigue are thought to be important in determining the decline in muscle force. There is a loss of muscle force (more than 50%) in skinned fibers when Pi is elevated to 54 mM and pH is decreased to 6.0. Previous work has shown that the spectra of EPR probes, which are reacted at the SH-1 position of the myosin head, have two components in active fibers: 80% are disordered resembling those seen in relaxed fibers and approximately 20% are ordered similar to those seen in rigor. Ordering of EPR probes has been interpreted to represent ordering of cross-bridges during the attachment phase of the cycle. The present experiments have examined whether the fraction of the ordered probes in contracting muscle is influenced by the conditions which model fatigue and produce a loss in force. Under conditions where force declines by over 50%, we find that the fraction of ordered probes (19.3 ± 3% n=6) remains unchanged (19.8 ± 3% n=6). The drop in force upon addition of phosphate is thought to cause a redistribution of cross-bridges into states earlier in the powerstroke that precede phosphate release. The mechanism of H+ action is unknown. The present results show that the orientation of the probes is insensitive to the changes in cross-bridge states that lead to lower tensions. Supported by a grant from the USPHS, AM30868.

M-AM-B8 **TENSION AND STIFFNESS DECLINE FOLLOWING PHOTOLYSIS OF CAGED PHOSPHATE WITHIN ACTIVELY CONTRACTING GLYCEROL-EXTRACTED FIBERS OF RABBIT PSOAS MUSCLE.** J.A Dantzig & Y.E. Goldman, Department of Physiology University of Pennsylvania, Philadelphia, PA 19104.

The decline of tension on the millisecond time-scale following photolysis of caged P_i in actively contracting skinned muscle fibers (Dantzig et al. Biophys. J. 51:3a, 1987) and other results suggest that P_i binding to an AM˜.ADP cross-bridge state can reverse the reaction step leading to the force-generating intermediate. If reversal of the power stroke leads to states which are weakly binding or detached, caged P_i photolysis should cause a decrease of stiffness as well. Sinusoidal ~1 μm length changes at 500 Hz or 2 kHz were applied to fibers (lengths 1.9-2.1 mm) contracting in the presence of 10 mM caged P_i. A 347 nm laser pulse released 0.8-1.2 mM P_i from caged P_i leading to a mean tension reduction of 6.70±0.24% (s.e.m, n=34) at 20 °C and 10.59± 0.98% (n=13) at 10 °C. Stiffness (measured as the magnitude of the sinusoidal component of tension) was obtained using a lock-in amplifier or a computer program. The latter method gave a sensitive indication of the phase angle which did not change significantly ($\Delta\phi$=0.38±0.19°, n=11). Stiffness declined simultaneous with tension (fig.: 10 °C normalized traces), but less in magnitude relative to values before the laser pulse (77.6±7.3% (s.e.m., n=7) at 500 Hz and 87.9±9.3% (n=7) at 2 kHz). After 100 ms tension declined further for 1-3 s, but stiffness was constant. The results indicate that P_i binding reverses the cross-bridge cycle toward states partly detached or toward attached states making small contributions to stiffness. Support: MDA and NIH grant HL15835 to the Pennylvania Muscle Institute.



M-AM-B9 INSECT CROSSBRIDGES, RELAXED BY SPIN-LABELED NUCLEOTIDE, SHOW WELL-ORDERED 90-DEGREE STATE BY X-RAY DIFFRACTION AND EM, BUT SPECTRA OF EPR PROBES REPORT DISORDER. M.K. Reedy and R. Cooke. Dept. of Cell Biology, Duke University, Durham NC, 27710 and Dept. of Biochemistry/Biophysics and CVRI, University of California, San Francisco, CA 94143.

We have examined muscle fibers relaxed by spin-labelled ATP and Vanadate using X-ray diffraction, electron microscopy and EPR spectra. By X-ray diffraction and electron microscopy, myosin crossbridges in glycerinated Lethocerus insect flight muscle (Leth-IFM) are well-ordered, showing bridges at a uniform 90° angle to the filament axis in relaxed fibers and predominantly at a 45° angle in rigor. EPR of spin label (SL) probes on myosin heads report uniform orientation only in attached rigor bridges, while indicating orientational disorder among myosin heads not tightly actin-bound. We have now shown that the X-ray/EM and EPR approaches each give their usual result when applied to Leth-IFM, and particularly to relaxed Leth-IFM. We obtained excellent relaxation (of Mg-ATP quality) by mechanical, EPR, X-ray and EM criteria using vanadate trapping of 2'SL-3'deoxyATP at 3°C (Crowder and Cooke, Biophys J. 51:323-333). Thus the spin label nucleotide orientation is highly disordered under conditions in which EM and X-ray diffraction both show ordered bridges. Several explanations of this data exist. 1. a rigid myosin head could possess considerable motional freedom in the ordered array about the thick filament, or 2. the nucleotide site could be on a mobile, probably distal domain of myosin while a more proximal region is well ordered on the thick filament backbone. Supported by grants from USPHS AM30868 (RC) and AM14317 (MKR).

M-AM-B10 DYNAMIC TENSION AND X-RAY MEASUREMENTS ON ACTIVATION OF RABBIT PSOAS MUSCLE USING CAGED-CALCIUM. K.J.V.Poole, *J.H.Kaplan, $Y.Maeda, G.Rapp and R.S.Goody, (Intr. by L.Castellani) Max Planck Inst. Med. Research, Heidelberg, FRG,*Dept. of Chem., Univ. Philadelphia, Penn.,USA, $ EMBL, DESY, Hamburg, FRG.

We have succeeded in fully activating small bundles(0.2x0.4mm) of psoas fibres from giant belgian rabbits at different temperatures by photolysis of the calcium chelator DM-Nitrophen. Changes of the inner equatorial reflections were measured with 5ms time resolution using a rapid counting linear detector on the EMBL beamline at the Hamburg synchrotron, DESY. Relaxed fibres were bathed in 2mM DM-Nitrophen solution containing 1.5mM $CaCl_2$, 20mM MOPS, 3mM MgAc, 3.2 ATP, 70mM KPr, 20mM CP, 1mg/ml CPK, pH 6.8. The cell was lowered to expose the fibres within a temperature and humidity controlled chamber where light from a uv flashlamp initiated the Ca release. At 25°C tension rose with a half life of 15-20ms after < 2ms delay. The equatorial 10 & 11 reflections changed with a half life of ca. 10ms, reaching a 11/10 ratio ca. 80% of that in rigor. At 10-12°C the extents of tension and equatorial changes were reduced. Tension rose with a half life of ca. 40-50ms after a 3-4 ms delay, but the rate of equatorial change is barely affected (half life 10-15ms). The 25°C data are very similar to those obtained from tetanically stimulated living frog muscle (Kress et. al. J.Mol.Biol. 188, 1986), but at lower temperature the rabbit activations here are almost 2x slower. Activations from rigor at 25°C, achieved using caged ATP in the presence of Ca ions, appear as fast as those using caged Ca, after 5ms delay. Equatorials show a very rapid step change to active levels. At 10-12°C tension production is 3-4x slower and follows a 25ms delay. Reducing the Mg concentration below that of the released ATP does not affect these transients.

M-AM-B11

TIME COURSE OF SPACING CHANGE OF 143Å MERIDIONAL CROSSBRIDGE RELECTION DURING RAPID SHORTENING.
H.E. Huxley, R.M. Simmons* and A.R. Faruqi**. Rosenstiel Center, Brandeis University, *MRC Cell Biophysics Unit, London and **MRC Laboratory of Molecular Biology, Cambridge, England.

The axial spacing of the approximately 143Å meridional reflection in frog sartorius muscle is known to increase by about 1% during contraction but the detailed behavior of this spacing change had not been investigated for technical reasons. Using the SERC Daresbury Storage Ring as a high intensity X-ray source, a six metre specimen-to-detector focusing camera designed by Dr. Joan Bordas, and a high-speed multiwire detector, we have made time-resolved measurements of this spacing in muscle undergoing active shortening at different speeds, and also during active stretch. During steady shortening at Po/2, when there is a substantial decrease in the intensity of the 143Å reflection, there is only an approximate 0.1% decrease in spacing as compared to the value during isometric contraction, and during active stretch by tensions up to 50% greater than Po, no increase in spacing was recorded. Thus the effect does not resemble an elastic stretch of the thick filaments when under load. During rapid shortening however (at Po/10), the spacing changed more than halfway towards its value in relaxed muscle (i.e. it decreased by about 0.6%) and then returned to the characteristic contracting value as tension redeveloped after the shortening period. Although the intensity decrease at the beginning of shortening was very rapid, the spacing change took 20-30 msec to develop fully, a time course similar to the changes seen in the equatorial reflections during rapid shortening. This suggests that the axial cross-bridge spacing may be modulated by the radial position of the myosin heads, and that after detachment their position may change with a rate constant comparable to that of ATP cleavage.

M-AM-B12 **A RATCHET DIFFUSION MODEL FOR DIRECTED MOTION IN MUSCLE.**
Scott Braxton & Ralph G. Yount, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164

It is widely accepted that muscle contraction occurs by the relative sliding of actin and myosin filaments. This "sliding filament" theory accounts for many of the properties of muscle; however, the molecular basis for this relative sliding has remained elusive. The most common belief is that at some stage in the ATPase cycle while actin and myosin are interacting, there is a conformational change in the S1 head or in the S1-S2 hinge which forces the actin filament to slide past the myosin filament. The best estimates indicate each ATP hydrolysis yields 5 to 60 nm of movement. At present, there are no data that document a conformational change in S1 which would generate movement of this magnitude.

Brownian motion of F-actin is dramatic when observed in the *in vitro* systems developed by Spudich, Yanagida and their co-workers. Observations of this type suggested to one of us (S.B.) that the thermal energy which exists at physiological temperatures might be sufficient to drive muscle contraction. A model was developed in which the random diffusional movements of actin filaments and myosin heads are captured by a ratchet-like mechanism to impart an overall large-scale directed motion with a high velocity. The theory requires the cooperative interaction of adjacent crossbridges in different conformations (controlled by ATP and its hydrolysis products) to permit directed movement. The hydrolysis of ATP is necessary only to provide directionality to the relative movement of actin filaments past myosin filaments. This model removes the burden of "force generator" from the head of myosin and gives it the role of an anchor or ratchet pin, resisting regressive movement of the actin filament, yet capable of allowing the actin filament to move forward by being passed to another anchor downstream. Simple calculations show that the speed of diffusion of actin filaments is about 100 times faster than a V_{max} contraction over the distance between ratchet pins, i.e., adjacent myosin heads. This model accounts for the need for a close approximation of heads for movement in the *in vitro* systems along with several other phenomena of muscle. Supported by NIH (DK05195).

M-AM-C1 cDNA CLONING OF HUMAN SKELETAL MUSCLE CALSEQUESTRIN

Terrence L. Scott and Ghazala Ali, Dept. of Muscle Research, Boston Biomedical Research Institute and Dept. of Biol. Chem. and Mol. Pharm., Harvard Medical School, Boston, MA (Intr. by D.R. Sanadi)

The complete amino acid sequence of calsequestrin has been deduced from cDNA clones derived from human fetal skeletal muscle. The human sequence was compared to those obtained for rabbit skeletal and cardiac calsequestrins (Fliegel, et al., PNAS, 84, 1167, 1987; Scott, et al., J. Biol Chem., 263, 8958, 1988). The human sequence obtained has characteristics similar to those of rabbit fast-twitch calsequestrin. The cDNA sequence has a high degree of similarity to that of the rabbit, with the major discrepancies occurring in the 5' and 3' untranslated regions. The human protein shares significant amino acid homology with that of the rabbit, with most of the differences occurring at the COOH terminus. There is a large number of discrepancies in the pre-calsequestrin domain. The human transcript has a second open reading frame after the first as does the rabbit cardiac form, but it does not terminate in an in-frame stop codon. Supported by grants from NIH (r0132247) and an Established Investigatorship of the American Heart Association to TLS.

M-AM-C2 THE COMPLETE SEQUENCE OF HUMAN SARCOPLASMIC RETICULUM Ca-ATPASE DEDUCED FROM cDNA CLONING

Terrence L. Scott and Ghazala Ali, Dept. of Muscle Research, Boston Biomedical Research Institute, and Dept. of Biol. Chem. and Mol. Pharm., Harvard Medical School, Boston,MA

The complete amino acid sequence of the human fast-twitch form of the sarcoplasmic reticulum Ca-ATPase has been determined from cDNA clones of fetal muscle. The human enzyme has significant amino acid homology with the rabbit ATPase, but with several important differences. There are substantial discrepancies in the 5' and 3' untranslated regions between the two species. In addition, a major portion of the cDNA corresponding to the human slow/cardiac form of the ATPase has been sequenced. Comparisons of the human slow vs. fast twitch forms and between the human and rabbit ATPases will be examined. Supported by grants from NIH (RO132247) and an Established Investigatorship of the American Heart Association to TLS.

M-AM-C3 INTERACTIONS BETWEEN CALSEQUESTRIN AND THE JUNCTIONAL FACE MEMBRANE OF SARCOPLASMIC RETICULUM - I Michel Ronjat[a,b], and Noriaki Ikemoto[a,c] a, Dept. Muscle. Res., Boston Biomed. Res. Inst., Boston, Mass. 02114; b, CEN-G, Grenoble, France; c, Dept. Neurol., Harvard Med. Sch.

Treatment of heavy SR vesicles with a low concentration (conc.) of cholate (0.1:1 cholate:SR protein) in the presence of 0.4 M NaCl led to a selective extraction of calsequestrin (CQ); after removal of the detergent the vesicles were capable of active Ca^{2+} pumping. However, depletion of 50% CQ resulted in 80% inhibition of caffeine-induced Ca^{2+} release compared with the control that was treated in the same way without NaCl. Upon reassociation of CQ with the vesicles by dilution of NaCl, a normal amount of CQ and Ca^{2+} release activity were restored. The fact that a partial depletion of CQ by high NaCl produces almost complete inhibition of Ca^{2+} release suggests that a portion of CQ whose solubility is dependent upon [NaCl] plays an important role in Ca^{2+} release. The following results suggest that the NaCl-sensitive portion of CQ is associated with the junctional face membrane (jfm). Although a large portion of CQ is solubilized at high conc. of detergent, about 30% of the total CQ remains insoluble and is recovered together with the membrane segment derived from the jfm. The jfm-attached CQ dissociated upon treatment of the jfm-CQ complex with high conc. of salt (> 0.4 M NaCl), even at very low detergent conc. Upon dilution of salt CQ was reassociated with the jfm. These results suggest that the jfm-linked CQ plays an essential role in the Ca^{2+} release mechanism. (Supported by grants from NIH and MDA).

M-AM-C4 INTERACTIONS BETWEEN CALSEQUESTRIN AND THE JUNCTIONAL FACE MEMBRANE OF SARCOPLASMIC RETICULUM -II Noriaki Ikemoto[a,b], Michel Ronjat[a,c], and László G Mészáros[d] a, Dept. Muscle Res., Boston Biomed. Res. Inst., Boston, Mass. 02114; b Dept. Neurol., Harvard Med. Sch.; c, CEN-G, Grenoble , France; d, Semmelweis Univ. Med. Sch., Budapest, Hungary.

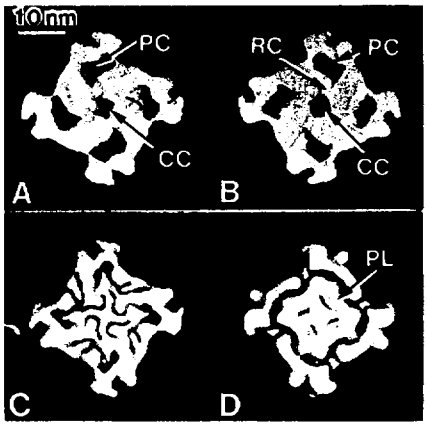
A close correlation between the kinetic properties of Ca^{2+} release and the extent of Ca^{2+}-binding to calsequestrin (CQ) suggested that CQ regulates Ca^{2+} release (Ikemoto and Koshita, Biophys. J. 53, 421a, 1988). An SR membrane segment consisting of the junctional face membrane (jfm) and attached CQ (designated as jfm-CQ complex) was prepared, whose 400 kDa and 32 kDa jfm proteins (but not CQ) were labeled with N-(7-dimethylamino-4-methyl-3-coumarinyl) maleimide (DACM). The DACM fluorescence increased with Ca^{2+} ($C_{1/2}$=0.3 mM ; = $C_{1/2}$ for the changes of CQ tryptophan and circular dichroism). Upon detachment of CQ from the jfm by salt treatement, the DACM fluorescence change was abolished, indicating that the Ca^{2+}-induced conformational changes of CQ are transmitted to the jfm proteins. We propose that this mechanism plays a chief role in the regulation of the release function by CQ. On the other hand, upon addition of caffeine (or quercetin, polylysine) to the jfm-CQ complex a portion of the CQ-bound Ca was dissociated as determined by stopped-flow spectrophotometry with tetramethylmurexide. No Ca^{2+} dissociation took place when CQ was detached from the jfm, indicating that the Ca^{2+} dissociation from CQ was triggered via the jfm proteins. In the intact SR vesicles, this would lead to the production of a very high $[Ca^{2+}]_i/[Ca^{2+}]_o$ gradient. (Supported by grants from NIH and MDA).

M-AM-C5 EVIDENCE THAT SUBUNIT-SUBUNIT INTERACTIONS IN THE Ca^{2+} PUMP OF SARCOPLASMIC RETICULUM (SR) ARE NOT OBLIGATORY FOR ACTIVE Ca^{2+} TRANSPORT. Jeffrey Froehlich, Eung Kim and Phillip Heller. National Institute on Aging, National Institutes of Health, Baltimore, MD 21224.

Although physicochemical studies (radiation inactivation, fluorescence energy transfer) favor an oligomeric structure for the SR Ca^{2+} pump, the minimum functional subunit composition is unknown. To resolve this question, several laboratories have tried labelling the enzyme with fluorescein isothiocyanate (FITC) which prevents ATP from binding. A linear relationship between residual CaATPase activity and bound FITC has been reported, indicative of a functional monomer. We have extended these studies to include kinetic measurements of $^{45}Ca^{2+}$ uptake and inorganic phosphate (P_i) production in the presteady state and steady state in order to test the efficiency of energy coupling following modification with FITC. The effect of FITC labelling on the pattern of dephosphorylation of the CaATPase by ADP was also examined. At 40% inhibition, FITC had no effect on energy coupling or ADP-induced dephosphorylation. Between 70 and 95% inhibition, the proportion of phosphoenzyme in the ADP-sensitive (E_1P) state decreased, consistent with a decrease in conformational stability due to weakened subunit-subunit interactions (Froehlich and Heller (1985) Biochemistry, 24:126-136). However, energy coupling remained intact under these conditions. From these results we conclude that: (1) the kinetic effects resulting from FITC modification are consistent with the alteration or elimination of subunit-subunit interactions; (2) loss of these interactions does not impair the efficiency of energy coupling between Ca^{2+} transport and ATP hydrolysis.

M-AM-C6 THREE-DIMENSIONAL ARCHITECTURE OF THE CALCIUM RELEASE CHANNEL FROM SKELETAL MUSCLE. T. Wagenknecht, R. Grassucci, J. Frank, A. Saito*, M. Inui*, and S. Fleischer*. Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany, NY 12201; and *Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

Three-dimensional image analysis has been applied to electron micrographs of negatively stained, non-crystalline calcium release channels/foot structures (CRC's) purified from rabbit skeletal muscle. The reconstructed CRC (central cutaway views, Figs. A,B) shows a central stain-filled cavity (CC) connected to four symmetrically related peripheral cavities (PC) by radial channels (RC). We also show surface representations of the CRC viewed from the top (Fig. C; cytoplasmic face) and bottom (D; sarcoplasmic reticulum face, i.e. the junctional face membrane of the terminal cisternae). The bottom of the CRC exhibits a square-shaped projecting platform (PL) part of which inserts into the membrane of the sarcoplasmic reticulum. The 3-D model suggests that calcium ions enter the central cavity via the platform and then traverse the radial channels to the peripheral cavities from which the cytoplasm would be readily accessible. [Supported by NIH GM29169 (J.F.), DK14632 and Muscular Dystrophy Association (S.F.)].



M-AM-C7 LOCALIZATION OF THE CA-BINDING DOMAIN ON THE CA-ATPASE OF THE SARCOPLASMIC RETICULUM. F. Norman Briggs and Karen Kaechele. Department of Physiology, Virginia Commonwealth University, Richmond, Va. 23298.

Optimal conditions for identifying high affinity calcium binding sites with N-cyclohexyl-N'-(4-dimethylamino-alpha-napthyl)carbodiimide (NCD-4) have been developed along with a partial identification of the location of those sites. The optimal conditions for labeling the calcium specific sites (sites blocked by 30 μM Ca) were 0.25 mg/ml sarcoplasmic reticulum and 10 μM NCD-4. The labeled sarcoplasmic reticulum was solubilized in Li-dodecyl sulfate and subjected to HPLC gel filtration chromatography. Monomer and dimer forms of the enzyme were labeled to about the same level by NCD-4. Calcium (30 μM) strongly blocked the labeling of the monomer but had little effect on the dimer. Addition of 30 μM Ca reduced NCD-4 incorporation (nmol NCD-4/nmol enzyme) in the A1 peptide from 0.50 to 0.17, in the A2 peptide from 0.34 to 0.15, and in the B peptide from 0.20 to 0.00. The data suggest that helices from all of the major peptides contribute to high affinity calcium binding.

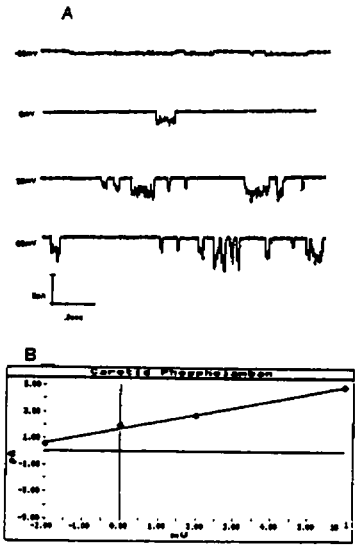
M-AM-C8 PHOTOOXIDATION OF SR VESICLES RESULTS IN RAPID CALCIUM RELEASE. Janice Stuart* and Jonathan J. Abramson#. Portland State University. Dept. of Physics# and Chemistry*, P.O. Box 751, Portland, Oregon 97207.

Treatment of SR vesicles with the photooxidizing dye Rose Bengal increases the permeability of the vesicles. Vesicles actively loaded with calcium, using acetyl phosphate as pump substrate, rapidly release accumulated calcium when irradiated in the presence of 3 uM Rose Bengal. Ruthenium red, ATP or caffeine in the reaction buffer inhibits the release induced by Rose Bengal. Magnesium increases an initial lag phase preceding calcium release, however, the maximal calcium efflux rate is magnesium independent. At a Rose Bengal concentration of 1-3 uM the Ca^{2+}-Mg^{2+} ATPase activity is greater than 70% of maximum after 1 minute of exposure, yet all of the calcium has exited from the vesicles in less than 30 seconds. Unfractionated SR is 70% more sensitive to Rose Bengal than longitudinal SR.

Rose Bengal is known to oxidize several amino acids, one of which is histidine. The modification of SR vesicles by Rose Bengal can be reduced in the presence of large excess of histidine. When cysteine or lysine are added to the reaction buffer vesicle modification by Rose Bengal is unaffected. Treatment of SR vesicles with ethoxyformic anhydride, another histidine modifying reagent, also increases calcium efflux. This work is supported by AHA Grant 87 915. J. J. Abramson is an established investigator of AHA.

M-AM-C9 SMOOTH MUSCLE PHOSPHOLAMBAN FORMS ION CHANNELS IN LIPID BILAYERS. Richard J. Kovacs, Larry R. Jones, and Adam D. Wegener, Dept. of Medicine and the Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN

Phospholamban (PLB) isolated from sarcoplasmic reticulum of heart has been studied extensively and functions both to modulate the "Ca-pump" and to form Ca specific channels, as demonstrated in artificial bilayers. The protein has been identified in vascular smooth muscle, where its function is unknown. PLB was purified from the media of porcine carotid arteries using highly specific monoclonal antibodies to cardiac PLB. PLB purified from carotid artery was incorporated into lipid bilayers on the tips of patch pipettes. PLB formed channels permeable to divalent cations, and selective for Ca^{++} over K^{+}. Panel A shows current tracings from an experiment using 10 mM Ca^{++} in the pipette and 1 mM Ca^{++} in the bath. Channel openings are downward deflections from the baseline. The current voltage relationship for the channel is shown in panel B. Conductance of smooth muscle PLB was comparable to cardiac PLB. We conclude that PLB isolated from vascular smooth muscle is capable of forming ion channels in lipid bilayers.



M-AM-C10 **SOME PROPERTIES OF PHOSPHOLAMBAN SYNTHESIZED IN VITRO FROM SYNTHETIC RNA.**

Bruce T. Scott, Richard J. Kovacs, and Larry R. Jones, Dept. of Medicine and the Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN.

The integral sarcoplasmic reticulum protein phospholamban has been implicated in the regulation of calcium metabolism in the heart. To study the biochemical mechanisms of phospholamban synthesis, quaternary structure formation, and insertion into membranes, cDNA clones were isolated from a canine cardiac lambda gt10 library and used for in vitro synthetic reactions. A 600 base pair fragment of clone 5 $\emptyset$, which contained the entire coding region, was subcloned to plasmid vector pGEM-3Z and used for making in vitro RNA transcripts. Phospholamban monomers (apparent Mr 5,000 on SDS-PAGE) were translated with high efficiency from these RNA transcripts in a rabbit reticulocyte lysate system. Translated monomers spontaneously form pentamers (Mr 25,000) and, to a lesser degree, higher order structures in the absence of membranes. Monomeric phospholamban associations were completely disrupted by boiling in SDS prior to PAGE. Preliminary experiments in which synthetic phospholamban was inserted into planar lipid bilayers revealed cation selective ion conductions similar to that seen with native phospholamban isolated from cardiac tissue. Our results suggest that phospholamban monomers synthesized in vitro contain all the necessary information required for self-association and formation of cation selective channels.

M-AM-C11 **PROTECTIVE EFFECT OF CHOLESTEROL AND CANAVALINE A ON THE THERMAL INACTIVATION OF THE Mg^{2+} ATPase FROM TRANSVERSE TUBULAR MEMBRANE**

ALICIA ORTEGA AND JAMES R. LEPOCK, DEPARTMENT OF BIOLOGY, UNIVERSITY OF WATERLOO, WATERLOO, ONTARIO, CANADA, N2L 3G1.

Transverse tubular (TT) of skeletal muscle contains a very active glycoprotein, Mg^{2+} -ATPase, which comprises a high porcentage of the integral membrane protein. Considerable evidence shows that denaturation of membrane proteins ocurr during hyperthermia and that the increase levels of membrane cholesterol protect the membrane from thermal inactivation. Since the TT membrane is a system with natural high contents of cholesterol, we use it to study the relation of choleterol to thermal inactivation of Mg^{2+} -ATPase activity and ATP dependant calcium uptake. The effect observed on Mg^{2+}-ATPase activity caused by extracting 35 % of the total cholesterol from TT membranes, shows an earlier loss of its activity compared to the TT membranes not depleted, being reversible when cholesterol levels are reincorporated. On the other hand concanavaline A at 100 ugr/mg protein produces significant effects of the protecion from thermal inactivation which for the Mg^{2+}-ATPase activity occurrs in the range of 56 to 60 °C. In the cholesterol depleted membranes addition of Con A does not results in protection of the Mg^{2+} -ATPase during the exposure of high temperatures.

M-AM-C12 **COMPETITIVE BINDING TO THE CA-ATPASE OF SKELETAL SARCOPLASMIC RETICULUM USING CA^{2+} AND TB^{3+}** **C. D. Sprowl and D. D. Thomas, Dept. of Biochemistry, Univ. of Minnesota Medical School, Mpls., MN** 55455 We have investigated the specificity of Tb^{3+} binding to the high-affinity Ca^{2+}-binding sites (HA-Ca-BS) of the Ca-ATPase. Previous evidence for specific binding of lanthanides (Ln^{3+}) to the HA-Ca-BS has come from indirect measurements such as inhibition of activity or reversal of a Ca^{2+}-induced affect. However, these results may be due to allosteric affects of Ln^{3+} bound to sites other than the HA-Ca-BS. Here we report the results of an equilibrium dialysis study in which we observed directly the competition between Ca^{2+} and Tb^{3+} for the two HA-Ca-BS. $^{45}Ca^{2+}$ was used and Tb^{3+} was detected via dipicolinic-acid-sensitized emission. Ca^{2+} binding to DOC purified SR at pH=6.5 in the absence of Tb^{3+} appears to be sequential, saturated at ~2 mol/mol Ca-ATPase with ^1K_D=9.02E-6 M and ^2K_D=1.22E-6 M, and n_{Hill}=1.45. Bound Ca^{2+} was displaced by adding uM Tb^{3+} to the dialysis buffer. If the two ligands were in direct competition, then one mole of bound Tb^{3+} should displace one mole of bound Ca^{2+}. However, the ratio of displacement was ~4:1 rather than 1:1. In the converse experiment, 10uM Ca^{2+} had no affect on the level of bound Tb^{3+}. This allowed us to characterize Tb^{3+} binding to DOCSR. We observed saturated Tb^{3+} binding at 8 mol/mol Ca-ATPase, ^1K_D=24.07E-6 M and ^2K_D=0.86E-6 M for the two classes of Tb^{3+}-binding sites, and n_{Hill}=1.49. Ca^{2+} binding in the presence of 10uM constant free Tb^{3+} exhibited saturation at 2 mol/mol Ca-ATPase, with decreased affinity for Ca^{2+}, and increased cooperativity (n_{Hill}=2.03). We conclude that Tb^{3+} is bound to sites other than the HA-Ca-BS. Tb^{3+} exerts an allosteric rather than a direct competitive affect on Ca^{2+} binding. In this system, as in others, Tb^{3+}, and possibly other Ln^{3+}, is not a Ca^{2+} analogue.

M-AM-D1 INTERFACIAL WATER AND THE MACROMOLECULAR RESPONSE. Robert G. Bryant, Biophysics Department, University of Rochester Medical Center, Rochester, NY 14642.

The mysteries of water in biology have had a considerable and controversial history. Though many crucial questions remain, there is now at hand a large body of both structural and dynamical information obtained from both experiment and theory that dispels a number of working hypotheses about the behavior of water at surfaces of proteins, nucleic acids, saccharides, and membranes. New types of spectroscopy including multinuclear magnetic resonance in solids has provided both structural and dynamic information that permit rather precise estimation of the time scales for structural interchanges of water in interfacial and interstitial environments. A number of these experiments will be presented, and the case of proteins and carbohydrates will be examined specifically with respect to the structural and dynamic response of the macromolecule to hydration. In addition to the very rapid motion of water at surfaces demonstrated, these experiments provide a basis for identifying two major classes of macromolecular behavior: one group of molecules that tightens their structure with little change in local dynamics on hydration, and one group that becomes dramatically more structurally labile with the addition of very little water.

M-AM-D2 MOLECULAR DYNAMICS STUDY OF ION PERMEATION IN THE GRAMICIDIN A CHANNEL. Benoit Roux and Martin Karplus, Department of Chemistry, Harvard University, Cambridge, MA 02138

Ab initio calculations are used to parameterize the interaction of ions in the Gramicidin A channel. Molecular dynamics combined with the "window sampling" method is used to calculate the free energy profile of sodium inside a periodic Gramicidin-like helix in the presence of water and to evaluate the sensitivity of the selectivity to the ion size. A dynamics of the fully solvated GA dimer in a model membrane is reported. The dynamics of the solvated pore entrance is characterized, and its relevance to ions transport is discussed.

M-AM-D3 CONFORMATIONAL SUBSTATES IN THE POLYPEPTIDE BACKBONE OF THE GRAMICIDIN A TRANSMEMBRANE CHANNEL. L.K. Nicholson, P.V. LoGrasso, M.T. Brenneman and T.A. Cross. Dept. of Chemistry and The Institute of Molecular Biophysics, Florida State University, Tallahassee, Fl. 32306.

A method for determining the orientational dispersion of the peptide linkages in the gramicidin channel backbone is demonstrated. Solid state ^{15}N NMR has been employed to investigate the structure and dynamics of specific sites along the polypeptide backbone of the channel. Spectra obtained from uniformly aligned DMPC bilayers containing single site ^{15}N labeled gramicidin show site dependent broadening of the individual resonances when the sample is in the gel phase. This data provides strong evidence for conformational substates in which the chemical shift frequencies reflect a range of orientations for a specific site, while the overall channel structure is only slightly perturbed. In and above the phase transition the ^{15}N NMR spectrum is motionally averaged by both the global rotation of the channel about the bilayer normal and by rapid transitions between conformational substates. As the temperature is lowered below the phase transition, peptide linkages are trapped in local potential energy minima corresponding to conformational substates. Simulations of the low temperature spectra are presented, based on an motional model of peptide linkage rotation about the C_α- C_α axis and on a gaussian probability distribution among substates. These simulations show the dependence of the spectra on the orientation of the chemical shift tensor relative to the peptide linkage reference frame, and on the mean orientation of both the C_α- C_α axis and the peptide linkage plane relative to the magnetic field.

M-AM-D4 CONFORMATIONAL DYNAMICS OF APOMYOGLOBIN DETECTED BY TRYPTOPHANYL PHOSPHORESCENCE

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~C.N.R. Istituto di Biofisica - Via S. Lorenzo 26, 56100 Pisa, Italy.

A protein molecule, in a particular conformational state, can assume a very large number of substates having the same coarse overall structure but differing in small structural details. Four hierarchically organized levels of substates, characterized by a specific energy and interconversion time, have been recognized in myoglobin. We have observed that the tryptophanyl triplet state lifetime of apomyoglobin in a rigid glass at low temperature ,100°K, is poorly sensitive to the microenvironmental differences existing among substates, i.e., the decay is represented as single exponential. The temperature dependence of the phosphorescence decay of the native protein reveals a biphasic behaviour. The decay, monoexponential below 210°K, becomes multiexponential between 210°and 260°K and, finally, it becomes monoexponential above 260°K. The presence of increasing amounts of guanidine hydrochloride makes the protein more susceptible to the thermal effect. The results are explained in terms of the hierarchical substate organization of myoglobin molecule.

M-AM-D5 QUANTUM MECHANICAL ANALYSIS OF ELECTRON AFFINITIES OF SUBSTITUTED QINONES AS MODELS FOR QUINONE REDUCTASES. Howard H. Robinson and Scott D. Kahn, Dept. of Physiology and Biophysics and Dept. of Chemistry, University of Illinois at Urbana/Champaign, Urbana, IL 61801

We are investigating the physical forces underlying the microscopic chemical properties of selected quinone oxido-reductase reaction mechanisms of biological energy conversion pathways using non-empirical quantum mechanical methods. The effects of solvation and immediate environment (i.e., local dielectric) upon the oxidation/reduction properties of quinones are being explored to provide a better understanding of these factors on charge storage and charge transfer mechanisms involving protein-bound quinones. Stabilization of semiquinone intermediate structures are key elements in understanding the mechanism of enzymatic quinone reduction and hydroquinone oxidation by energy conversion proteins. There are strong electrical interactions between the radical anion species and the protein groups forming the catalytic site. To examine these microscopic chemical interactions in detail, we have first examined the electron affinities of a series of substituted quinones *in vacuo* and compared these results of these calculations with experimental *in vacuo* electron affinities (Heinis,T., et al., J.Am.Chem.Soc. 1988, 110, 400-407). The results of *ab initio* quantum mechanical calculations at the 3-21G basis-set level reproduce the general trend of the experimental electron affinities. Supplementing the 3-21G basis set with diffuse functions greatly improves the agreement between the experimental data and the *ab initio* calculations. We are greatly encouraged by the ability of the theoretical calculations to account for the *in vacuo* experimental data. To address the interaction of solvent molecules with these substituted quinones, we are computationally investigating the microscopic chemical interactions of just a few solvent molecules, such as dimethylformamide, in the first solvation shell of the quinone or the quinone radical-anion. The differential interactions of the solvent with the quinone or quinone radical anion relates the *in vacuo* quinone electron affinities to the quinone one-electron redox potentials in the solvent. By extension, the differential interaction of the quinone and quinone radical anion with the environment of the reductase catalytic site can be analysed in detail.

M-AM-D6 ACTIVE SITE REGION IN ELASTASE AND INHIBITION. Shankar Subramaniam, Malcolm E. Davis and J. Andrew McCammon, Department of Chemistry, University of Houston, Houston, Texas 77204-5641.

Elastase, which is a serine protease that cleaves peptides with small hydrophobic residues like alanine, has a number of physiological functions. The active site in elastase comprises the typical catalytic triad of serine, histidine and aspartate and the atypical valine and threonine in the mouth of the pocket. Inhibitors to elastase are mostly mechanism-based and compete with the amide carbonyl for the nucleophilic attack of the hydroxyl group of serine. In this study, we model the initial complex of an isocoumarin inhibitor to elastase using the crystal structures of the native enzyme and the inhibitor-bound enzyme. Molecular graphics and molecular dynamics are used to explore favorable configurations of the inhibitor in the active site region. The electrostatic environment in the active site region is studied using the Poisson-Boltzmann method.

Supported in part by grants from the National Science Foundation and HNS Supercomputers Inc.

M-AM-D7 CONFORMATION AND DYNAMICS OF A 2–AMINOPURINE SUBSTITUTED DNA DECAMER DUPLEX. A. Gräslund[1], Th. Nordlund[2,3], S. Andersson[2], L. Nilsson[2], L.W. McLaughlin[4] and R. Rigler[2]. [1]Dept. of Medical Biophysics, University of Umeå, S–901 87 Umeå, Sweden, [2]Dept. of Medical Biophysics, Karolinska Institutet, Box 60400, S–104 01 Stockholm, Sweden, [3]Dept. of Biophysics, Dept. of Physics & Astronomy, Laboratory for Laser Energetics, University of Rochester, Rochester, NY 14642, USA, and [4]Dept. of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167, USA.

500 MHz ^{1}H NMR and time–resolved fluorescence studies as well as molecular dynamics simulations have been carried out on self–complementary oligodeoxyribonucleotides 5'–CTGAATTCAG or 5'–CTGA(2AP)TTCAG containing the Eco RI endonuclease recognition site GAATTC with adenine or a fluorescent base analog 2–aminopurine (2AP) in the center. Previous studies have shown that both decamer duplexes are recognized and cleaved by Eco RI endonuclease. For CTGAATTCAG, most proton resonances were assigned through two–dimensional techniques (NOESY and double quantum filtered COSY). – NOESY spectra and molecular dynamics simulations show that the 2AP–substituted duplex is in an overall B–like conformation, like its non–substituted counterpart. The NMR results also indicate that in the 2AP–substituted duplex, the major changes in conformation and/or motion relative to the non–substituted one, occur in the GA(2AP) part of the molecule. – Molecular dynamics simulations show that large (several Å) transient displacements of the 2AP base relative to its neighbours occur along an axis perpendicular to the helix axis. These stacking–unstacking motions are accompanied by a distribution of fluorescence lifetimes of the 2AP base. – Fluorescence anisotropy decay measurements on decamer duplexes with one or both strands containing 2AP show a rapid internal mobility of 2AP, with a rotational correlation time τ_c = 40 – 400 ps in the temperature range 50 – 6^{0}C. These studies demonstrate that 2AP is a potentially very useful and sensitive probe for DNA interactions and conformations, considering both steady–state and dynamic properties.

M-AM-D8 DYNAMICS OF TERTIARY AND QUATERNARY CONFORMATIONAL CHANGES IN TROUT I HEMOGLOBIN. James Hofrichter, Eric R. Henry, Lionel P. Murray, William A. Eaton (NIDDK, NIH, Bethesda, MD); Massimo Coletta and Maurizio Brunori (University of Rome, Rome, Italy)

Time-resolved absorption spectroscopy has been used to study the dynamics of Trout I hemoglobin subsequent to the photodissociation of carbon monoxide. The results show a low-amplitude geminate rebinding phase (~8%) and associated conformational relaxation occurring at 40-50 ns at room temperature. When all of the ligands are photodissociated, geminate rebinding is followed by a conformational relaxation which occurs at about 15 μs. This relaxation has been identified as a quaternary R->T transition. The apparent activation energy for this relaxation is 7.4 kcal/mole. Ligand rebinding to fully photolyzed samples is at least biphasic, with the majority of the ligands rebinding in a slow process having an apparent bimolecular rate constant of 1.5 x 10^5 $M^{-1}s^{-1}$ at 20^{o}C and an activation energy of about 16 Kcal/mol. At low degrees of photolysis, ligand rebinding remains biphasic, but both relaxation rates increase significantly, with the majority of the rebinding ocurring with a rate of 3 x 10^6x $M^{-1}s^{-1}$. Evidence for rapid intersubunit communication is obtained from the absorption spectra of the early photoproducts. The spectra of the immediate (~10 ns) photoproduct and the spectral change associated with the geminate rebinding relaxation (40-50 ns) show clear evidence of changes in the spectrum of liganded as well as of deoxy hemes. We interpret these data as evidence that intersubunit communication in Trout I hemoglobin can take place much more rapidly than in human hemoglobin, presumably by direct propogation of tertiary relaxations from one subunit to another as well as by the change in quaternary conformation.

M-AM-D9 THE ACRYLAMIDE QUENCHING OF TRYPTOPHAN FLUORESCENCE IN RNASE T1 AND COD PARVALBUMIN: EFFECT OF VISCOSITY IN MIXED COSOLVENT SYSTEMS. Kingman Ng, Maria Punyiczki and Andreas Rosenberg, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, USA. Bela Somogyi, Department of Biophysics, Medical University of Debrecen, Debrecen, Hungary.

Fluorescence quenching studies have been shown to yield useful information on the structural dynamics of the protein matrix. However, it is still limited by inadequate kinetic models in describing the quenching reactions. On the other hand, it is also not clear how the internal movements within proteins are involved, as usually probed by the external bulk viscosity. We have, in an effort to address these questions, measured the viscosity dependence of acrylamide quenching in Ribonuclease T1 and Cod Parvalbumin at 25°C using differing contents of glycerol and ethylene glycol. Our studies show that the probability of quenching by acrylamide molecules is not related to the trajectories of the free quencher molecules in bulk solution. Consequently the customary gating or penetration models are not useful. In order to reconcile these findings with the clearly present dynamic quenching mechanism we have adopted a new two phase model for the process of quenching in protein matrix (Somogyi et al. 1988). Our data show that the large differences between the effects of two cosolvents at isoviscous conditions are related to the cosolvent effects on the activities of the quencher and the protein molecules.

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M-AM-D10 COMPUTING THE ELECTRIC FIELD OF SOLVATED MACROMOLECULES by R.J. Zauhar and R.S. Morgan, the Biotechnology Institute and the Department of Molecular & Cell Biology, Penn State University, University Park, PA 16802.

Calculations of the electric field of proteins in solutions have typically involved the methods and assumptions of classical electrostatics, where the solvent is represented as a continuum of high dielectric constant enclosing a macromolecular interior of relatively low polarizability. Specific computational procedures employed have ranged from the simple application of Coulomb's law with a fixed or distance-dependent dielectric constant (Getzoff, et al., Nature 306, 287-290)to the solution of Poisson's equation in three dimensions by the method of finite-differences (Warwicker & Watson, J. Mol. Biol. 157, 671-679). We have proposed that the electric field of macromolecules may be more accurately and efficiently computed by applying the method of polarization charge. In this approach, a two-dimensional distribution of surface charge on the molecular boundary is calculated which reproduces all of the polarization effects in the protein/solvent system, providing an alternative to the direct solution of Poisson's equation in three dimensions. A simple version of the method has been used in a trial calculation for lysozyme (J. Mol. Biol. 186, 815-820), and a more detailed and accurate procedure has been successfully applied to molecules as large as a hexapeptide (J. Comp. Chem. 9, p.171). Recently, an algorithm has been developed (R. Zauhar, in preparation) that permits the macromolecular surface to be decomposed into curvilinear finite-elements, and opens the way for a more general application of polarization-charge techniques to the problem of computing the electric field of large molecules. Results will be presented for several small proteins, as well as some organic molecules of biological interest.

M-AM-D11 ELECTROSTATIC ENERGY AND FORCES INCLUDING POLARIZATION.
Malcolm E. Davis and J. Andrew McCammon
Dept. of Chem.; Univ. of Houston; Houston, Texas 77204-5641

Electrostatic interactions are vital to many molecular interactions of biological importance and induced polarizations play a significant role in these interactions. Typical methods treat ligands as test charges on a precalculated potential, which ignores induced polarization effects. We present methods for calculating electrostatic forces and energies, including all reaction fields arising from induced polarizations. Using the finite difference Poisson-Boltzmann equation, self-consistent electrostatic potential calculations are made for irregular geometries. Comparisons are made between these methods and "test charge" methods both for simple systems amenable to analytic solutions as well as more complicated protein systems.

M-AM-D12 COMPUTER-GENERATED RANDOM CONFORMERS OF POLYALANINE AS A MODEL FOR THE UNFOLDED STATE. Eaton E. Lattman and L. Mario Amzel, Department of Biophysics, Johns Hopkins Medical School, Baltimore, MD 21205, USA.

The unfolded state of a protein molecule is often visualized in terms of the random-walk model of classical polymer theory. The $\phi-\psi$ angles at each Cα in the chain are distributed over time in the allowed portion of the energy diagram according to the Boltzmann distribution, but values of these angles for any two residues are uncorrelated. Computer simulation would seem ideal for exploring models of this type. However, there are to our knowledge no efficient algorithms for generating the large number of random conformers that are required to represent an ensemble of random walks. Here we propose a method based on successive doubling, in which two polyalanine n-mers in allowed conformations are joined to make a putative 2n-mer. The starting point is a recent molecular dynamics simulation of di-alanine in water.[1] The method seems capable of producing a large library of polyalanine conformers that obey the conventional distribution rules. A valuable result will be determination of the function g(n), the fraction of conformation space that is energetically and sterically accessible to an n-mer. This function is important for understanding what the actual conformational restrictions are during folding. Other parameters, such as the radius of gyration and variance, can also be calculated and compared with experiment. With a major investment in computer time this method can be extended to sequences of real proteins. This work is supported by NIH grant GM-36358, and by ONR grant N0001487-K-0344.

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M-AM-E1 OSMOTIC PRESSURE OF BOVINE LENS CRYSTALLINS. A. D. Magid, T. J. McIntosh, and S. A. Simon, Departments of Cell Biology Neurobiology, and Anesthesiology, Duke University Medical Center, Durham, N. C. 27710.

The lens of the vertebrate eye possesses a radial gradient in refractive index which corrects for spherical aberration and thus improves visual acuity. This refractive index gradient is caused by a corresponding gradient in cytoplasmic protein (crystallin) concentration within the successive layers of lens cells, such that the crystallin concentration in the lens nucleus (about 400mg/ml) is considerably higher than that in the lens cortex (about 250mg/ml). Why doesn't water move to dissipate this protein concentration gradient? As an initial approach to this problem we have measured the osmotic pressure of crystallins isolated from three concentric zones of cow lenses -- cortical, intermediate, and nuclear. A secondary osmometric method was used (Prouty *et al.*, J.Mol.Biol. 184:517). In this method, a protein solution is dialysed to equilibrium against a dextran solution of known osmotic pressure, and its concentration then measured by refractometry. The specific refractive increments of proteins isolated from cortex and nucleus were very similar, 0.188 ± 0.003 ml/g (mean ± SEM, N = 8). SDS-PAGE showed only negligible degradation of protein samples during the several days needed to achieve equilibrium. The π-C isotherms differed substantially for the three regions such that, for the same pressure, the required concentrations are nuclear > intermediate > cortical. To obtain information on the Donnan pressure (due to protein charge), crystallin concentrations were measured at a fixed osmotic pressure as a function of pH (I=0.15M). The C-pH curves were parallel and peak at about pH 5 for both cortical and nuclear crystallins. This implies that the differences in the π-C isotherms largely reflect differences in osmotic coefficients. These results support this hypothesis: the osmotic coefficients of the various crystallins differ, and that the proteins in the younger, peripheral cells provide a greater pressure per unit concentration than the older, more interior cells.

M-AM-E2 HIGH SPEED AFFINITY CHROMATOGRAHY: MEASUREMENT OF ANTIBODY-RECEPTOR CAPTURE KINETICS. Richard Hammen, Carol Cook, Holly Thompson, David Pang, (Intr. by Walt Hill), ChromatoChem, Inc. Missoula, Montana, USA.

Affinity isolations of recombinant proteins or antibodies (Ab) from clarified cell culture broths or serum requires the processing of large volumes of fluid with low concentrations of protein. We describe kinetic experiments with wide pore silica immunoaffinity columns which measure the second order rate constant for binding Abs to Protein A, G, and antigen receptors. A HiPAC™ Protein G affinity column extracted >95% of a IgG1 MAb with an in-column residence time of 5.5 seconds. The cycle times for the column were less than 9 min and the processing capacity was > 120 g of IgG1/liter column/8 hr. The Protein G-IgG binding rate constant was > 4.5*10^4 l/sec/mol, while a capture kinetic experiment with a Protein A column showed that a residence time > 18-27 sec to recover > 95% of the IgG1 from a hybridoma harvest fluid. The cycle times for the column were 13 min and the processing capacity was 34 g of IgG1/liter column/8 hr shift. The Protein A-IgG1 binding rate constant was > 8290 l/sec/mol.

The capture kinetics of the silica immunoaffinity columns were measured with immobilized antigens (BSA, and bovine IgG) and immobilized Ab raised against these antigens. For example, BSA was coupled to a 10 X 0.46 cm HiPAC aldehyde column and the column was used to affinity purify rabbit Ab. The Ab was coupled in another column. The second order rate constant for binding BSA to the Ab was measured by injecting the antigen into the Ab column at various flow rates from 1-10 column vol/min. The bound BSA was quantified by eluting with 2% HOAc and measuring the chromatographic peak area. The Ab-antigen binding rate constant was 1.1*10^5 l/sec/mol.

M-AM-E3 MULTI-FREQUENCY CALORIMETRY: THEORY AND IMPLEMENTATION. O. L. Mayorga, J. L. Lacomba, W. W. van Osdol, and E. Freire. Johns Hopkins University, Baltimore, MD 21218.

A multi-frequency calorimeter has been designed to measure the magnitude and time regime of the enthalpy fluctuations associated with structural or conformational transitions in biological macromolecules. The heat capacity is proportional to the mean square amplitude of the enthalpy fluctuations in a system. Biological macromolecules undergo a variety of thermally induced transitions, during which the heat capacity of these systems displays maxima, indicating enhanced enthalpy fluctuations. Linear response theory and the fluctuation-dissipation theorem provide the framework within which to investigate the frequency spectrum of equilibrium fluctuations in thermodynamic state variables. The multi-frequency calorimeter has been designed in accordance with this theory. Relaxation times for enthalpy fluctuations are obtained by analyzing the frequency dependence of the amplitude and the phase shift of the response of the system to weak, oscillatory temperature perturbations. In the limit of zero perturbation frequency, thermodynamic parameters may also be estimated.

This instrument has been used to study the gel-liquid crystalline transition of dimyristoylphosphatidylcholine large unilamellar vesicles. The data indicate two relaxation processes, with time constants of 80 ± 20 ms, and 3.8 ± 0.4 s, respectively, at the midpoint of the transition. Approximately 80% of the relaxation amplitude is associated with the fast relaxation process, 20% with the slow process. Thus, this instrument allows one to dissect the enthalpy of a process into components occuring on different time scales.

We are currently using the multi-frequency calorimeter to examine the folding/unfolding transition of small, globular proteins. (Supported by NIH Grants GM-37911 and NS-24520.)

M-AM-E4 APPROACH TO DISTANCE MEASUREMENTS IN LARGE BIOLOGICAL SYSTEMS: ROTATIONAL RESONANCE ENHANCED EXCHANGE OF NUCLEAR SPIN MAGNETIZATION, D.P. Raleigh, F. Creuzet, M.H. Levitt, and R.G. Griffin, Francis Bitter National Magnet Lab.,MIT, Camb.,MA 02139

In the last few years NMR methods for determining distances and therefore the solution structure of small proteins and nucleic acids (MW<15 kD) have been developed, and these are now in routine use in a number of laboratories. However, there are many systems — large soluble proteins and membrane proteins —to which these techniques are not applicable. We report here a possible NMR approach to distance measurements in these latter systems. The technique, **rotational resonance (R2)**, is based on introduction of isotopic labels and magic angle spinning (MAS). In MAS experiments, on samples containing dilute homonuclear dipolar coupled spin pairs, R2 occurs when the spinning speed is adjusted so that the condition $\omega_\Delta^{iso} = n\omega_r$ is satisfied. Here ω_Δ^{iso} is the difference in isotropic chemical shifts, ω_r is the spinning speed, and n is an integer. Under these conditions the normally sharp resonance lines broaden and split. In addition, a rapid oscillatory exchange of Zeeman-order between the dipolar coupled spins is observed. The time dependence of the exchange and the spectral lineshapes agree quantitatively with numerical simulations. The method has been employed to measure through-space ^{13}C-^{13}C dipolar couplings of ≈60 Hz, corresponding to distances of ≈500 pm in polycrystalline solids.

M-AM-E5 QUANTITATIVE EPR OF S > 1/2 METALLOPROTEINS. Betty Jean Gaffney, Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

Use of expressions from quantum mechanics for *frequency-swept* spectroscopy in analysis of *field-swept* electron paramagnetic resonance (EPR) spectra is not direct if the object is to count spins in samples characterized by highly anisotropic interactions. Computer simulations of EPR line shapes for high-spin iron in lipoxygenase and transferrin have been applied to determining the pK of an interaction in lipoxygenase and the equilibrium constants for anion binding to transferrin. In these cases, three, or more, EPR spectra overlap. In the case of lipoxygenase, prominent signals at g ~ 6.1 are seen at low pH and at g ~ 7.5 for high pH. Analysis of pH titration results requires use of line shapes that are broadened by a distribution in E/D and summation of spectra from lower and middle Kramers doublets of one species. For cases of bidentate anions binding to transferrin, as many as eight overlapping signals may need to be considered.

M-AM-E6 ^{17}O AND ^{1}H NMR TRANSVERSE RELAXATION STUDIES OF MYOSIN A AND MYOSIN B HYDRATION, ACTIVITY AND SALT BINDING AS A FUNCTION OF pH/pD AND CONCENTRATION. A.Mora-Gutierrez*, I.C.Baianu* and P.J. Bechtel**, University of Illinois at Urbana, *Dept. of Food Science, Physical Chemistry and NMR Laboratories, and **Dept.of Animal Sciences, Muscle Biology Lab., 580 Bevier Hall, 905 S.Goodwin Avenue, Urbana, Illinois 61801.

Nonlinear dependences of ^{17}O and ^{1}H NMR transverse relaxation rates of myosin A and B on protein and salt concentrations are observed related to their non-ideal behaviors in solution. Our previous work (1,2) has shown that such concentration dependences can be quantitatively fitted only by taking into account both the protein and salt activities. Two charge-interaction models involving ion-binding to myosin were employed in conjunction with microcomputer , nonlinear regression analyses to fit the dependence of the NMR relaxation data on concentration of myosin A or B, in the absence or presence of added salt. Our results indicate that long-range, charge-charge (repulsive) interactions dominate entirely the myosin activity and its effects on myosin hydration and ion binding to myosin. Ion specific effects are also observed with NaCl and KCl. Myosin A and B have minimal hydration at their isoelectric points (pI's), whereas water "binding" increases on either side of the pI's, as seen from the increase in the NMR transverse relaxation rates away from the pI's. The molecular dynamics of the hydration water in myosin solutions, as determined by NMR relaxation techniques, is consistent with a dual-motion, anysotropic hydration model, with fast exchange between bulk and hydration water populations (3).

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M-AM-E7 THE PATHWAY DEPENDENCE OF SKELETAL MUSCLE TROPOMYOSIN ASSEMBLY. IDENTIFICATION OF THE REGIONS THAT DIRECT HETERO- AND HOMODIMER FORMATION. Fred Schachat and H. Richard Brown, Department of Cell Biology, Duke University Medical School, Durham, North Carolina and Department of Anatomy, SUNY, Buffalo, NY.

Skeletal muscle tropomyosin assembles from α and β subunits to form a two-stranded α-helical coiled-coil protein. Both the homology of the α and β subunits and the stereotypical nature of the coiled-coil interaction have led to the assumption that the subunits will assort independently, in accord with the binomial distribution, when dimers form. However, this is not the case *in vivo* or during renaturation. *In vivo* the heterodimer is the overwhelmingly preferred species, whereas most denaturation-renaturation protocols result in preferential formation of the homodimer. Using a variety of renaturation protocols we have identified regions of the subunits that are responsible for the differences in dimer preference. Heterodimer formation is directed by a sequence of 30 to 40 amino acids in the N-terminal third of the subunits, while homodimer formation appears to be the result of interactions involving sequences around the cysteine at position 190. Homodimer formation appear to be the outcome of an equilibrium-controlled process, while heterodimer formation is kinetically-directed. A model based on a conformational free-energy scale for coiled-coil proteins will be presentedto explain the homodimer preference at equilibrium.

M-AM-E8 AN ANALOG OF aprA-SUBTILISIN WHICH EXHIBITS ENHANCED STABILITY AT LOWER CALCIUM CONCENTRATIONS. Linda Owers Narhi, Mark M. Zukowski and Tsutomu Arakawa, Amgen Inc., 1900 Oak Terrace Lane, Thousand Oaks, CA 91320.

The subtilisins are bacterial serine proteases which are unusually resistant to denaturation by urea, guanidine-HCl, and detergents. They contain a single, high affinity calcium binding site, and often a low affinity site. While calcium is not needed for activity, it greatly enhances the stability of these proteases. We have previously shown that aprA-subtilisin is resistant to denaturation by SDS (1). In this report, an analog of aprA-subtilisin was constructed to further enhance the stability of the protein in solution. A single alteration in the presumed calcium binding site of the aprA-subtilisin was introduced to an enzyme with two other changes designed to stabilize the backbone. The stability of the native and analog proteins was assessed using UV spectroscopy, protease activity and SDS-PAGE. The latter technique is based on our observation that subtilisin migrates normally in the gel only when the protein is unfolded prior to SDS-PAGE. When subjected to thermal transition analysis, the analog showed a higher melting point than the native enzyme at the same calcium concentration. It also appeared to reach maximum stability at lower calcium concentrations and exhibited greater stability to detergent-induced denaturation. Following incubation with SDS, a larger proportion of the analog remained correctly folded. It also retained more of its original activity than the native enzyme. The activation energy for detergent-induced denaturation of the analog was greatly increased over that of the aprA-subtilisin. Thus the three amino acid substitutions resulted in an enzyme with an increased affinity for calcium and greater stability than the native protease.

M-AM-E9 MODELLING SITE-SPECIFIC ION BINDING TO MACROMOLECULES: ENERGETICS OF Cl^-, Na^+ AND H^+ BINDING TO HEMOGLOBIN. Bertrand García-Moreno E. and Gary K. Ackers, Department of Biology, The Johns Hopkins University, Baltimore MD 21218

An algorithm has been developed for the identification of sites on the ion-accessible surfaces of macromolecules where the ions of simple salts might bind. Binding sites are identified as extrema in the magnitude of the surface electrostatic potential calculated at a resolution of 0.20 A. The dielectric model used in the calculation of the electrostatic potential is consistent with the one employed in the solvent accessiblity-modified Tanford-Kirkwood algorithm (SA-TK). It takes into account the differential screening effects due to the high dielectric of the solvent, the low dielectric of the interior of macromolecules, screening effects by bulk ions (bulk ionic strength), and charge attenuation by territorial counterions (local ionic strength). The energetics of ion binding are quantitated with a form of the SA-TK algorithm that includes a thermodynamic formalism which explicitly handles the linkage between the binding of protons and the binding of other ions. The site-bound ions are represented in the calculation as dissociatable point charges.

The availability of separate crystallographic structures of the oxygenated and the deoxygenated forms of hemoglobin has allowed the calculation of the ion binding behavior of ligated and unligated forms of dimers and tetramers. Markedly distinct ion binding characteristics are calculated for the individual structures, predicting pH-linked, differential effects of ion binding on the oxygenation-linked structural transitions between these forms of the molecule, as well as on the assembly reactions of tetramers from dimers. Theoretical modelling of the site-specific ion binding properties of hemoglobin will further our understanding of the atomic mechanisms underlying the experimentally observed effects of pH and ion concentration on its functional energetics.

M-AM-E10 1 N^6-ETHENO (ERYTHRO-9- (-2-HYDROXY-3- NONYL)) ADENINE, ε-EHNA: A FLUORESCENT INHIBITOR FOR ADENOSINEDEAMINASE.
Valeria R. Caiolfa, David Gill and Abraham H. Parola, Department of Chemistry Ben-Gurion University of the Negev, Beer-Sheva, Israel 84 150

Adenosine deaminase is an established malignancy marker. The observed variation of its activity in various maliginancies is not yet understood.

We have attempted to label ADA with a new fluorescent probe. The adenine analog EHNA, a tight reversible inhibitor (Ki=1.6 10^{-9}M) of ADA was synthesized and modified into the fluorescent ε-EHNA. ε-EHNA was found to be still a competitive inhibitor of ADA with Ki=5-6 10^{-6}M. It retains the spectroscopic characteristics of known etheno nucleotide derivatives: 1) Ph dependent UV absorption and fluorescence emission 2) pk and 3) limiting anisotropy r_o. Fluorescence lifetime of ε-EHNA was determined by both phase-modulation and single photon correlation spectroscopies. Binding of ε-EHNA to ADA was followed by steady state fluorescence polorization and active site binding was verified by competition with EHNA, pentostatin and adenosine, resulting in complete loss of polorization. ε-EHNA seems to bind loosely to ADA active site as evidenced by the relative low polarization value P=0.025. This confirms the suggested shallow structure of ADA active site.

M-AM-E11 THE EFFECT OF CHARGE ON THE DIFFUSION OF BOVINE SERUM ALBUMIN
Kenneth S. Schmitz, Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110

Bovine serum albumin (BSA) is a rigid, almost spherical, polyelectrolyte whose charge can easily be adjusted by variation of the pH of the solution. Because of its relatively small size in relation to visible light used in dynamic light scattering experiments, one does not need to consider internal relaxation modes in the interpretation of dynamic light scattering (DLS) data. DLS and osmometry data obtained in our laboratory are presented along with a review of literature results of fluorescence recovery after photobleaching (FRAP), viscometry, and other DLS studies on BSA as a function of pH, added salt concentration, and BSA concentration. The composite picture which emerges from this comparison is that: (A) electrolyte dissipation effect significantly influence the value of the friction factor of the "isolated" BSA particle; (B) direct polyion-polyion interactions govern the osmostic susceptibility measurements; and (C) fragile, shear rate dependent, long range correlated "structures" exist below well defined "transition" conditions which depend upon the ionic strength and BSA concentration. These data are discussed in terms of direct polyion-polyion interactions, small ion-polyion interactions, hydrodynamic interactions, and Brownian dynamics effects.

M-AM-E12 SOLVENT-PROTEIN INTERACTION, PROTEIN STABILITY AND PROTEIN DYNAMICS.
Shi-long Yuan, Song-pin Han, Lucy L.-Y. Lee and James C. Lee. Department of Biochemistry, St. Louis University, School of Medicine, St. Louis, MO 63104.

Organic solvents have been utilized extensively by bacteria, plants and animals as osmolytes. Empirically, polyhydric alcohols and sugars are introduced both in the cell or in solvent medium in order to stabilize biological macromolecules. These solvents include glycerol, sucrose, methylamines such as trimethylamine -N- oxide (TMAO) and polyethyleneglycols (PEG). In an effort to elucidate the common factor(s) exhibited by these solvents in stabilizing proteins the preferential solvent interactions of PEG, sucrose and TMAO with bovine serum albumin (BSA) at pH 7.0 and 9.0 were monitored. Results show that under all experimental conditions BSA is preferentially hydrated i.e. the organic solvents are excluded from the protein domain. However, at pH 9.0 BSA is more preferentially hydrated than at pH 7.0. Protein stability was monitored by exposing BSA to guanidine hydrochloride. Results show that BSA unfolds in higher concentrations of denaturant in the presence of stabilizing solvents. The effects of organic solvents on the rate of tritium exchange from BSA to solvent were monitored. Results show that the exchange rate is decreased in the presence of stabilizing solvents. Control experiments conducted in the presence of proteins denaturant yielded higher rate of exchange. Additional control experiments are conducted to study the effect of these solvents on the kinetics of exchange for freely exposed amides. Thus, at present it may be concluded that stabilizing solvents are excluded from the protein domain and resulted in dempening of the dynamic motions of the protein. As a consequence, BSA is more resistant to unfolding by denaturant.

M-AM-F1 NMR STUDY OF INTERACTIONS OF SHORT-CHAIN SATURATED DIACYLGLYCEROLS WITH DIPALMITOYLPHOSPHADITYLCHOLINE BILAYERS. Hilde De Boeck and Raphael Zidovetzki, Department of Biology, University of California, Riverside, CA 92521.

The perturbations of dipalmitoylphosphatidylcholine (DPPC) bilayers by five saturated diacylglycerols (DAG) (diC_n with n=6,8,10,12 and 14) was studied by using a combination of ^{31}P- and ^{2}H-NMR. Two of the DAGs, dilaurin (diC_{12}) and dimyristin (diC_{14}), induce immobilization of the DPPC molecules at 40°C, with the amount of the immobilized lipids decreasing upon increase in temperature. At temperatures where no immobilized phase is present, both diC_{12} and diC_{14} cause an increase of the order parameters of DPPC. The three DAGs with shorter chains, dihexanoin (diC_6), dioctanoin (diC_8) and dicaprin (diC_{10}) do not induce immobilization of DPPC molecules. All three affect the order parameters of the DPPC side chains, but to a different extent and in a different way. In the presence of 25mol% of dicaprin there is a large increase in the quadrupole splittings of all resolved peaks along the acyl chains of perdeuterated DPPC. The same concentration of dioctanoin induces a small but significant increase of the quadrupole splittings in the plateau-region of the spectrum only. In the presence of 25mol% of dihexanoin on the other hand, a decrease in the quadrupole splittings is observed; this effect however is not uniform along the acyl chains; in the plateau region the values of the quadrupole splittings are the same as in the absence of dihexanoin. The observed difference in effects between the shorter and longer chain DAGs is important in view of the fact that the shorter chain saturated DAGs (up to diC_{10}) are known to be protein kinase C activators, while the longer chain DAGs are not, which may be related to the extent and type of perturbation induced by a DAG to the bilayer structure.

M-AM-F2 ^{13}C AND ^{31}P NMR STUDIES ON HYDROLYSIS OF PHOSPHOLIPID VESICLES BY PHOSPHOLIPASE C. Shastri P. Bhamidipati and James A. Hamilton, Biophysics Institute, Departments of Medicine and Biochemistry, Housman Medical Research Center, Boston Univ. Sch. of Med., Boston, MA 02118

Phospholipase C (PLC) catalyzes the hydrolysis of phospholipids to form diacylglycerol(DG) and monoester phosphoric acid, both of which may act as second messengers. We report a new approach using ^{13}C and ^{31}P NMR spectroscopy for detecting products, determining reaction kinetics, and monitoring structural changes during lipolysis of phospholipid bilayers. PLC-mediated hydrolysis of small unilamellar vesicles[7mol% dipalmitoyl phosphatidylcholine(DPPC) with ^{13}C enrichment at sn-2 chain carbonyl and 93mol% egg-PC] was monitored by ^{13}C and ^{31}P NMR over 6-8 hrs. The substrate ^{13}C NMR spectrum showed signals at 173.90 and 173.60 ppm from outer and inner monolayer phospholipid carbonyl carbons, respectively. Following the addition of PLC (B. Cereus), these two peaks decreased, and a new peak at 172.80 ppm increased, in intensity over time. This peak was assigned to 1,2-DG, and the product identity was confirmed by thin layer chromatography and analytical ^{13}C NMR. The ^{31}P NMR spectrum of substrate vesicles showed two signals (-0.96 and -1.08 ppm) from the outer and inner monolayer PC respectively. Following addition of enzyme, a narrow peak appeared at 2.10 ppm at the expense of total PC signal intensity. This new peak was assigned to phosphorylcholine, the other product of hydrolysis. A ^{31}P NMR spectrum obtained 6 hrs after PLC addition showed ~30% hydrolysis, in agreement with chemical analysis. The vesicles retained a bilayer structure throughout the course of hydrolysis, as indicated by ^{13}C and ^{31}P spectra. These results suggest that PC in both monolayers of the vesicles was hydrolyzed by PLC and that DG may play a significant role in making the inner monolayer accessible to PLC action.

M-AM-F3 DEUTERIUM NMR EVIDENCE OF A UNIQUE SURFACE BINDING SITE FOR ALCOHOLS IN DPPC/GM_1 LIPOSOMES. Cindy Graham-Brittain and George P. Kreishman, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172 and Robert J. Hitzemann, Department of Psychiatry and Behavioral Sciences, S.U.N.Y.-Stony Brook, Stony Brook, New York 11794-8101.

Deuterium NMR spectroscopy has been used to demonstrate that ethanol not only partitions to the interior of DPPC liposomes, it also binds in an apparently cooperative manner to the surface of these membranes. The effect on alcohol-binding of addition of monosialoganglioside (GM_1) to DPPC liposomes has been recently investigated, as GM_1 has been shown to sensitize PC membranes to the disordering effects of alcohols (Molec. Pharm. 25, 410-417, 1984). A third, distinct alcohol-binding site has been detected in the liquid-crystalline phase of DPPC/GM_1 liposomes. Exchange between the free state and this bound state is fast on the NMR time scale. The number of these sites increases with increasing temperature, and with both increasing GM_1 and alcohol concentrations. It is proposed that this binding site is near the membrane surface and is the result of packing defects in the lipid bilayer induced by GM_1. Increases in alcohol concentration and/or temperature causes breakup of GM_1-containing lipid clusters in the bilayer, and thus creates more of these surface sites. The implications of binding of alcohol to these sites will be discussed. In addition, the possible role of GM_1 in determining the sensitivity of mice to alcohol will be presented.

M-AM-F4 ELECTRON TRANSFER REACTIONS IN MONOLAYERS AND MULTILAYERS. Cynthia A. Palmer and Janos H. Fendler, Department of Chemistry and Graduate Biophysics Program, Syracuse University, Syracuse, New York 13244-1200.

Monolayers and multilayers were prepared from redox-active phospholipid analogues. Mixed monolayers containing various ratios of the redox-active and inert surfactants, with a quaternary ammonium or phosphate headgroup, were characterized by surface pressure-surface area isotherms, ellipsometry at the air-water interface, and surface-potential measurements. FTIR-LD transmission techniques were used to assess the two-dimensional molecular orientation of the deposited films.

Semiconductor precursors such as $Cd(EDTA)^{2-}$ or $TiO(Ox)_2^{2-}$ anions have been incorporated into these multilayers containing redox-active surfactants during Langmuir-Blodgett (LB) deposition processes, and have been converted to small semiconductor particles either by subsequent chemical reaction or photolysis. Photoinduced electron transfer processes have been observed in these semiconductor-incorporated LB films. Results suggest the possible organization and alignment of the compounds in the multilayer, as well as the mechanism of formation of controlled-sized semiconductor particles and photoinduced structural transformations in mixed monolayers. (Support of this work by the National Science Foundation and Syracuse University's Center for Membrane Engineering and Science is gratefully acknowledged).

M-AM-F5 PHYSICAL CHARACTERIZATIONS OF A HYDROLYZED THERMOACIDOPHILIC ARCHAEBACTERIAL LIPID IN AQUEOUS SUSPENSION. C. Montague*, Shi-Lung Lo*, and E. L. Chang+. *Geo-Centers, Newton Upper Falls, MA 02164; +Bio/Molecular Engineering Branch, Code 6190, Naval Research Laboratory, Washington, D.C. 20375-5000.

The lipid membranes of high-temperature archaebacterial microorganisms such as, *Sulfolobus acidocaldarius*, contain tetraether, bipolar lipids as the predominant lipid. Hydrolysis of the total lipid extract from the membranes yields two classes of tetraether lipids. The class with asymmetric polar headgroups (GDNT) contains glycerol on one end and nonitol, a multi-hydroxyl polar group on the other. This lipid is extremely difficult to hydrate and does not form lipsomes by the usual methods. We describe our procedure for inducing GDNT to suspend spontaneously in excess water. Light scattering results show that the sizes of these liposomal particles are between 1000-3000 Å. It is found that, similar to diether lipids derived from archaebacterial halophiles, GDNT will only form suspensions in low salt and close to neutral pH. We further describe the results of electron micrographic and permeability studies to determine the integrity of the GDNT membranes and the existence of internal compartments within the GDNT liposomal structures. Partial support by ONR N0001488wx24088 is gratefully acknowledged.

M-AM-F6 THE KINETIC MECHANISM OF CATION CATALYZED PHOSPHATIDYLGLYCEROL TRANSBILAYER MIGRATION IMPLIES CLOSE CONTACT BETWEEN VESICLES AS AN INTERMEDIATE STATE. Barry R. Lentz[1], Nancy A. Whitt[1], Dennis R. Alford[1], Stephen W. Burgess[1], Julie C. Yates[1], and Shlomo Nir[2]. [1] Department of Biochemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. [2] The Hebrew University Faculty of Agriculture, Rehovot 76100, ISRAEL. Intr. by Brad Chazotte.

We have investigated variations in the rate of Mn^{2+}-catalyzed phosphatidylglycerol transbilayer migration (Lentz, Madden, and Alford, *Biochemistry* 21, 6799) with changes in phospholipid and cation concentration, over more than a hundred fold range of both parameters. The slope of a double logarithmic plot of the rate of trans-bilayer lipid migration versus lipid concentration was 1.7, suggesting that lipid redistribution was dependent on vesicle aggregation or collision. A model involving transitory dimerization of vesicles was able to account for the concentration dependence of trans-bilayer redistribution rate. The observed variation in rate with the logarithm of Mn^{2+} concentration was complex: linear above 0.4 μM (corresponding to roughly 2.5 Mn^{2+} per vesicle) but involving a steeper dependence on Mn^{2+} below 0.04 μM (roughly four vesicles per Mn^{2+}). The rate of trans-bilayer redistribution increased substantially between 37 and 56° C, yielding a non-linear Arrhenius plot. There was no evidence of either fusion or lipid exchange between vesicles at the low concentations of Mn^{2+} needed for trans-bilayer redistribution. The data are consistent with a model suggesting local domains of a dehydrated, interbilayer complex as involved in the transition state, and are inconsistent with a model involving an inverted micelle-type structure for the transition state. Supported by GM32707 to BRL.

M-AM-F7 **MEMBRANE PARTITIONING OF N-ALKANOLS AND PHENCYCLIDINE: VARIATION WITH TEMPERATURE, DRUG CONCENTRATION, AND MEMBRANE COMPOSITION.** Martha Sarasua, Karla Faught, M. Daniel Gordin, and William Boggan, Department of Surgery, Cleveland Metropolitan General Hospital, Case Western Reserve University, Cleveland, Ohio

The partitioning of a series of n-alkanols (n = 1,2,3 and 4) and phencyclidine was characterized as a function of temperature and solute concentration in model and biological membranes. Centrifugation was utilized to separate membrane and aqueous phases. ^{14}C-alcohol and 3H-phencyclidine concentrations in the two phases were detected by liquid scintillation counting after correcting the membrane pellets for trapped solvent water. Membranes utilized included multilamellar vesicles composed of phosphatidylcholine with saturated fatty acid chains of varying lengths from 14 to 18 carbon atoms and mouse brain synaptosomes. Partition coefficients for all solutes tested in the model membranes were higher above the gel to liquid crystalline phase transition of the phospholipids indicating preferential partitioning of these solutes into fluid state lipid. Alcohol partition coefficients increased with chain length of the alcohol. Phencyclidine displayed ideal partitioning in all membranes tested. Ethanol partition coefficients varied with ethanol concentration indicating that the partitioning of ethanol was not ideal.

M-AM-F8 TEMPERATURE-INDUCED FUSION OF SHORT-CHAIN LECITHIN / LONG-CHAIN PHOSPHOLIPID UNILAMELLAR VESICLES ("SLUVs"), Ki Min Eum, Gerard Riedy, & Mary F. Roberts, Department of Chemistry, Boston College, Chestnut Hill, MA 02167

Small unilamellar vesicles (R_h ~ 85-90 Å) form spontaneously upon mixing micellar lecithins, such as diheptanoyl-PC, with saturated long-chain PC's, such as dipalmitoyl-PC, below their their gel-to-liquid crystalline transition. When these particles (SLUVs) are heated to the melting temperature of the long-chain species, they become extremely large. QLS studies show particle growth to 2000-5000 Å radius. Above T_m the particles decrease slightly (10-20%) in size. The change is a reversible phenomenon, does not depend on ionic strength, but does depend on the total concentration of phospholipid. Using fluorescence energy transfer studies with the lipid soluble probes N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-PE and N-(lissamine rhodamine B sulfonyl)-PE, we have shown that the large size change is due to vesicle fusion and not aggregation. Fluorescence assays at room temperature also show that the presence of the short-chain component accelerates exchange of the membrane-soluble (but not aqueous encapsulated) dyes between membrane populations. About 75% of the total dye concentration randomizes over a time scale of hours. This corresponds to the amount of dye in the outer monolayer of these small vesicles. We have also examined ^{2}H NMR spectra of d_{26}-diheptanoyl-PC in excess dipalmitoyl-PC (1:20 to 1:50). Below T_m only an isotropic line is detected; above T_m a quadrupolar splitting pattern is observed and order parameters for the short-chain PC can be extracted. The results of these different studies are used to construct a model for SLUV packing which is critical to understanding the activity of water-soluble phospholipases.

M-AM-F9

DIFFERENTIAL SCANNING CALORIMETRIC STUDIES OF THE TRANSITIONS OF PHOSPHATIDYLCHOLINES BETWEEN THE BILAYER AND INTERDIGITATED PHASES

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The saturated like-chain phosphatidylcholines (PC's) exist in a fully interdigitated gel phase in the presence of various ligands including ethanol. We have previously demonstrated, using a fluorescence method, that the transitions of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) from the bilayer (B) to the interdigitated (I) phase are temperature dependent with higher temperature favoring the I phase [Nambi et.al.(1988) Biochemistry: In Press]. In the present investigation, we have used differential scanning calorimetry (DSC) to investigate the B to I transitions of DPPC and DSPC. In order to establish the thermodynamic reversibility of these phase changes we have investigated this transition using both heating and cooling scans. Our results demonstrate that the transitions of DPPC and DSPC between the B and I phases are extremely slow under some conditions. One consequence of this is that these lipids can exist in a metastable state which is not the lowest free energy state for long periods of time. Therefore, under certain conditions, whether the lipid is in the B or I phase is generally a function of not only temperature and ethanol concentration, but also sample history and time. (Supported by the NIAAA and by the Veterans Administration.)

M-AM-F10 INTERMIXING OF DIPALMITOYL PHOSPHATIDYLCHOLINE WITH PHOSPHO- AND SPHINGOLIPIDS BEARING HIGHLY ASYMMETRIC HYDROCARBON CHAINS

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We have used high-sensitivity differential scanning calorimetry to investigate the mixing of dipalmitoyl phosphatidylcholine (DPPC) with N-lignoceroyl galactocerebroside (T_m = 85.2°C), N-lignoceroyl sulfogalactocerebroside (T_m = 52.8°C) and 1-lauroyl-2-lignoceroyl phosphatidylcholine (T_m = 49.5°C). All three lignoceroyl species are completely miscible with DPPC in the liquid-crystalline state but show phase separation in the gel state. Mixtures of DPPC with N-lignoceroyl sulfatide show a limited solid-solid phase separation (from <10 mole% to slightly over 40 mole% sulfatide), while a much more extensive phase separation is observed in mixtures of DPPC with N-lignoceroyl cerebroside (<10 mole% to >90 mole% cerebroside). Interestingly, the phase diagram for the DPPC/1-lauroyl-2-lignoceroyl PC system shows a eutectic point, with the liquidus curve reaching a minimum temperature of 35°C at roughly 37 mole% PC. This behavior can probably be attributed to the ability of the 1-lauroyl-2-lignoceroyl PC to form an interdigitated gel phase. Notwithstanding the unusual behavior of mixtures of DPPC with 1-lauroyl-2-lignoceroyl PC, we find no evidence that the N-lignoceroyl sphingolipids are markedly more prone to segregate laterally in PC-rich bilayers than are previously studied sphingolipid species with shorter N-acyl chains. We conclude that it is unlikely that the incorporation of very long N-acyl chains into animal cell sphingolipids serves to favor their lateral segregation in the plane of the plasma membrane. (Supported by the Medical Research Council of Canada and les Fonds F.C.A.R. du Québec).

M-AM-F11 THE FORMATION AND SOLUBILIZATION OF VESICLES FROM BILE SALT-LECITHIN MIXTURES. Charles H. Spink, Vinny Lieto & Lisa Woytowich. Chemistry Department, State University of New York, Cortland, NY, 13045.

Vesicle structures and related mixed micelles formed by the dilution of concentrated micellar solutions of taurocholate and dipalmitoylphosphatidylcholine (DPPC) can be studied by differential scanning calorimetry (DSC). Thermal transitions are observed for the vesicles which change peak parameters as the composition of the mixed vesicle is varied. In addition, there is thermal activity in the composition regions where large mixed micelles prevail. Because these thermal transitions are so sensitive to the compositions and conditions in solution, a systematic study was initiated to examine the effects of preparation method, equilibration time, solution composition and equilibration temperature on the thermal behavior. The results indicate that (1) there are some minor differences in the observed transitions for solutions prepared by dilution compared with those which are prepared by suspension of the dried film into buffer; (2) solutions are generally equilibrated at room temperature after dilution within 48 hours; (3) in the coexistence region (micelles and vesicles) any external changes produce more drastic changes in the equilibrated forms present; (4) cholesterol has a drastic effect on all of the observed thermal transitions in either vesicle or micelle regions; (5) solubilization of vesicles can be directly followed by changes in the vesicle peak in the DSC. It is concluded that DSC can be used as a monitor of micelle-vesicle transformation processes in systems which are of interest in model membrane reconstitution.

M-AM-F12 LAMELLAR/INVERTED CUBIC (L_α/I_{II}) PHASE TRANSITIONS IN N-MONO-METHYLATED DIOLEOYLPHOSPHATIDYLETHANOLAMINE (DOPE-ME).

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It is shown that an I_{II} phase in DOPE-ME is thermodynamically stable, using high-sensitivity DSC and X-ray diffraction (XRD) techniques. Previous theory [1] and experiment [2] indicated that I_{II} phases in DOPE-ME might be metastable. We observed a reproducible endotherm via DSC, with a peak temperature of ca. 61°C and an enthalpy of ca. 40 cal/mol in DOPE-ME (25-33 wt. % in pH 7.4 buffer, scan rate = 9°C/hr). An endotherm (ca. 200 cal/mol) corresponding to the L_α/H_{II} transition [5] occurs at 66°C (T_H). XRD patterns from samples incubated for 20 hours at 58° and 63°C show that the 61°C endotherm corresponds to formation of a cubic lattice, probably of Pn3m symmetry and lattice constant ≈ 180 Å (63°C). At 58°C, the XRD pattern is that of an L phase. In 50 wt. % DOPE-ME, a different lattice (long spacing ≈ 120 Å) is observed at 63°C. In 33 wt. % DOPE-ME, a shoulder (ca. 65°C) on the L_α/H_{II} endotherm may indicate a transition between two I_{II} phases, as observed in monoglycerides [3]. The XRD and DSC data will be compared with the temperature dependence of fluorescence from samples labeled with 0.1 mol % NBD-PE. The quantum yield of NBD-PE substantially increases at $T \approx T_H$ in many systems [4]. Preliminary evidence [5] indicates that in DOPE-ME this increase occurs closer to the endotherm at 61°C. These data will be discussed in terms of a recent theory of L_α/I_{II} phase transition mechanisms [1]. (1) Chem. & Phys. Lipids 42:279; (2) Biochemistry 27:2332; (3) Biochemistry 26:6349; (4) Biochemistry 26:2105; (5) Ellens et al., Membrane Fusion and Inverted Phases, Biochemistry (in press).

M-AM-G1 CYTOCHROME c OXIDASE: STRUCTURES OF MONOMERS AND DIMERS AND LOCATION OF CYTOCHROME c BINDING, T.G. Frey and J.M. Murray*, Dept. of Biol., San Diego State Univ., San Diego, CA 92182, *Dept. of Anatomy, Univ. of PA, Phila., PA 19104.

We have analyzed electron micrographs of 2-dimensional crystals of frozen-hydrated cytochrome oxidase monomers and dimers. Both crystal forms contain information out to approximately 15 A, and reconstructed images resemble those of similar crystals imaged in negative stain albeit with opposite contrast. Comparison of the monomer and dimer structures in projection has allowed us to identify the orientation of monomers within dimers. Comparison of the structure of frozen-hydrated monomers with the structure of negatively stained monomers determined by Fuller et al. (J. Mol. Biol. 134, 305-327, 1979) suggests that the new arrangement of monomers within dimers differs from previously published models. Incubation of monomer crystals with cytochrome c, a substrate of cytochrome oxidase, results in tight binding of cytochrome c to the crystals and a marked improvement in crystal size and preservation of structural order in the frozen-hydrated state. Monomer crystals containing tightly bound cytochrome c are also characterized by a greater tendency to form multilayered crystals. We have simulated the appearances of multilayered cytochrome oxidase/cytochrome c crystals by cross-correlation analysis vs. single-layered cytochrome oxidase crystals to determine the orientations of individual layers. Difference maps of cytochrome oxidase/cytochrome c images minus the simulated images show well-defined positive peaks which can be interpreted as a single cytochrome c binding site per monomer. The location of the cytochrome c binding site appears to be near the region of the monomer designated "x" by Fuller et al. which corresponds to a position near the center of cytochrome oxidase dimers.

M-AM-G2 HEAT TREATMENT OF CYTOCHROME c OXIDASE PERTURBS THE Cu_A SITE AND AFFECTS PROTON PUMPING BEHAVIOR.

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It has been previously reported that mild heat treatment of cytochrome c oxidase (43^{o} C for 60 min) abolishes the proton pumping activity of the enzyme while leaving the oxidase activity and the cytochromes a and a_3 intact (Sone, N., & Nicholls, P. (1984) Biochemistry, 23, 6550-6554). We find that heat treatment of cytochrome c oxidase causes a local perturbation at the Cu_A site. After heat treatment, the enzyme sample contains three subpopulations, each with a different structure at the Cu_A site. These include (i) native Cu_A, (ii) a type 2 copper species, and (iii) a novel type 1 copper species. In addition to the structural perturbation at the Cu_A site, we find that heat treatment results in accelerated cyanide binding to cytochrome a_3 and removal of subunit III. When heat the heat treated enzyme is reconstituted into phospholipid vesicles, the resultant proteoliposomes exhibit an unusually high permeability to protons. By contrast, if the heat treatment is carried out on the fully reduced enzyme under anaerobic conditions, the Cu_A site remains intact even though subunit III is lost. Reconstitution of this enzyme derivative with phospholipid produces vesicles with respiratory control ratio (RCR), membrane orientation, and activity comparable to those obtained with the native oxidase. These vesicles exhibit proton pumping actvity with an apparent H^+/e^- stoichiometry of 0.4-0.5 and a faster rate of transmembrane pH gradient dissipation as compared to similar vesicles containing the native enzyme.

M-AM-G3 COMPARISON OF PROTON TRANSLOCATING ACTIVITIES OF PHOSPHOLIPID VESICLES CONTAINING SUBUNIT III-DEFICIENT BOVINE HEART MITOCHONDRIA CYTOCHROME c OXIDASE PREPARED BY NATIVE GEL ELECTROPHORESIS AND α-CHYMOTRYPSIN TREATMENT. L.J. Prochaska and J. Rush, Biochemistry, Wright State University, Dayton, OH 45435

Subunit III (M_r 29918) can be removed from bovine heart cytochrome c oxidase by native gel electrophoresis in Triton X-100 (TX-100) (Prochaska & Reynolds (1986) Biochemistry 25, 741) and also by treatment of the enzyme (20 μM heme aa_3) with α-chymotrypsin in Triton X-100 at pH 8.5. The native gel treatment removed 85-95% of subunit III (SIII) from the enzyme, while α-chymotrypsin treatment cleaves > 90% of SIII as determined by SDS-PAGE. All of components b (M_r 9419) and c (M_r 10063) were also removed by both treatments. These preparations have been incorporated into asolectin phospholipid vesicles (40 mg/ml) formed by cholate dialysis and their endogenous proton permeability measured by the respiratory control ratio (RCR). The α-chymotrypsin-treated enzyme in liposomes had RCR values of 10.3 ± 1.6 (n = 6), while the native gel preparation of SIII-depleted enzyme had a RCR value of 5.4 ± 1.8 (n = 26), suggesting a similar low proton permeability in the liposomes of both preparations. Upon the addition of ferrocytochrome c, the native gel preparation of SIII-depleted enzyme in liposomes exhibited a 60-90% decrease in proton translocating activity, while the α-chymotrypsin-treated preparation showed a 60-75% decrease in proton translocating activity under all conditions of assay [2-10 enzyme turnovers ($4e^-$/heme aa_3) when compared to liposomes containing control enzyme. These results suggest that SIII may be a proton conducting pathway in the enzyme or SIII facilitates proton translocation in the enzyme by regulating the aggregation state of the enzyme (Puettner et. al. (1985) J. Biol. Chem. 260, 3719).

M-AM-G4 CHANGES IN REACTIVITY OF BOVINE HEART MITOCHONDRIAL CYTOCHOME c OXIDASE WITH A CHEMICAL CROSSLINKING REAGENT INDUCED BY TRITON X-100 OR REMOVAL OF SUBUNIT III. L.A. Estey and L.J. Prochaska, Biochemistry, Wright State University, Dayton, OH 45435

The chemical crosslinking reagent n-succinimidyl(4-azidophenyl)1,3'-dithiopropionate (N-SP) was used to measure changes in the reactivity of cytochrome c oxidase (COX) when the enzyme was dispersed in variable amounts of Triton X-100 (TX-100) or upon removal of subunit III. N-SP contains a lysine-reactive succinimidyl group and a light-activated azido group that can react with many amino acid residues to crosslink adjacent subunits of the COX complex. COX (5.0 mg/ml) was dispersed in 8 mg TX-100/mg aa_3 or 16 mg TX-100/mg aa_3, chromatographed over Sepharose 2B in a pH 8.0 buffer containing 0.1% TX-100, and 0.75 μM heme aa_3 aliquots reacted with N-SP. Using inhibition of electron transfer activity as a criterion for reactivity, it was observed that increasing amounts of TX-100 caused a decrease in the reactivity of COX with N-SP. 50% inhibition of activity was observed at 20 μM N-SP when COX was dispersed in 8 mg TX-100/mg aa_3, while a shift to 68 μM N-SP occurred in the 16 mg/mg aa_3 dispersed enzyme. The TX-100 treated COX exhibited all 13 subunits on SDS-PAGE. The effects of 16 mg TX-100/mg aa_3 appear to be reversible since this enzyme chromatographed over a cytochrome c affinity column demonstrated reactivity similar to the 8 mg TX-100/mg aa_3 enzyme. COX depleted of subunit III (3.75 mg TX-100/mg aa_3) by α-chymotrypsin treatment showed reactivities similar to 16 mg TX-100/mg aa_3 enzyme. These results suggest that CO-III may resemble the 16 mg TX-100/mg aa_3 enzyme topologically, and dispersion of COX in increasing amounts of TX-100 could drive the enzyme into the monomeric form as shown previously by Robinson and Talbert [Biochemistry (1986) 25, 2328-2335].

M-AM-G5 ULTRASTRUCTURAL COMPARTMENTATION, REGULATION, STRUCTURE AND FUNCTION OF CREATINE KINASE (CK) ISOENZYMES IN TISSUES OF SUDDEN HIGH ENERGY DEMAND

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Immunolocalization of CK isoenzymes in skeletal and cardiac muscle, brain, photoreceptor cells, spermatozoa and electrocytes show that the different CK isoforms are compartmentally localized and in part associated at a subcellular level (a) with sites of high energy demand where ATP is rapidly utilized (e.g. at myofibrils, SR, postsynaptic membrane etc.) and (b) at sites of energy production (mitochondria, glycolysis) where ATP is generated. Mitochondrial CK (Mi-CK) at the producing side of the CP shuttle forms octameric molecules that are cube-like structures with a central cavity or channel. This regularly sized high M_r form of Mi-CK was characterized by UC, STEM mass measurement and electron microscopy and was also crystallized recently. The localization of Mi-CK at the inner mitochondrial membrane and at the contact-sites as well as the structure of the Mi-CK octamer support the function of Mi-CK as an energy channeling enzyme. We propose a dynamic regulatory cycle involving (a) an octamer-dimer equilibrium directed in part by pH, salt and nucleotides and (b) a specific interaction of the octamer, but not the dimer, with the inner mitochondrial membrane. Furthermore, evidence is provided that cytosolic CK is regulated by tissue-specific dimer formation and by post-translational modification, thus enabling the cell to kinetically modify the enzyme according to cellular requirements and possibly to target CK to subcellular sites. Compartmentation and regulation of CK isoenzymes allow the directed transport of high energy phosphate, and the fine-tuning of ATP-regulated processes. (Schnyder et al. and Schlegel et al., J.Biol.Chem.263) (in press)

M-AM-G6 SITE OF INHIBITION OF CYTOCHROME c OXIDASE BY DIBUCAINE. B.K. Stringer and H.James Harmon. Department of Zoology, Oklahoma State University, Stillwater, OK 74078.

The local anesthetic dibucaine inhibits electron transport in cytochrome c oxidase in intact mitochondria with 50% inhibition occurring at 1.1 mM. Dibucaine increases K_m from 23.4 μM to 204 μM and increases V_{max} from 390 nmol O_2/min/mg to 880 nmol O_2/min/mg. Cytochrome oxidase reduced with ascorbate plus tetramethyl-p-phenylenediamine (TMPD) does not bind carbon monoxide in the presence of dibucaine. The absorbance at 445 nm indicates only a 50% reduction of the oxidase by ascorbate plus TMPD when dibucaine is present, yet total reduction is seen upon the addition of dithionite. The absorbance at 604 nm is not affected by dibucaine. The 830 nm copper is not seen upon reduction by ascorbate plus TMPD when dibucaine is present but is visible when reduced with dithionite. The rate of CO recombination is not affected, suggesting that cytochrome a_3 is not affected. These findings indicate that dibucaine blocks the transport of electrons in cytochrome oxidase at a site between cytochromes a and a_3.

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M-AM-G7 THE F_1-SECTOR OF ATP-SYNTHASE: MODEL FROM A 3.8 A DENSITY MAP

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An electron density map to 3.8 A resolution was calculated for the F_1-sector of ATPase from rat liver mitochondria. X-ray diffraction data from native and one single isomorphous mercury derivative crystals were combined with information obtained from packing considerations to produce an initial map that was improved with several cycles of solvent flattening. The resulting map shows very clear molecular boundaries. The overall dimensions of the molecule are 120x120x74 A. With each molecule three regions related by a three-fold axes of symmetry can be clearly separated. These regions are in close proximity at the "top" of the molecule and become separated at the "bottom", leaving an apparently empty central region. The model suggests two possible arrangements between the F_1-sector and the F_0 portion of the ATP synthase. Molecular replacement techniques indicate that within a region there is a pseudo two-fold axis, consistent with the presence of the α and β subunits which are expected to have a similar fold based on their primary sequences. The density map at this resolution shows regions that can be clearly fitted by to α helices. Tracing of the polypeptide chain is currently in progress.

M-AM-G8 EXPRESSION OF RAT LIVER ATP SYNTHASE BETA SUBUNIT AND BETA SUB U-NIT PEPTIDES IN AN E. COLI SECRETION SYSTEM.

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The x-ray crystal structure of the rat liver mitochondrial ATP synthase is now near completion at a resolution of 3.8 A. Further insight into the structure and function of individual ATP synthase subunits may be obtained from site-directed mutagenesis and crystallization of subunit proteins and peptide fragments thereof. To obtain pure beta subunit proteins in sufficient quantities, we have expressed the beta subunit and two fragments thereof in E. coli. One fragment, C4, extends from Glu122 to the C-terminus (358aa) and the other, Xho, extends from Asp141 to Ile275 (135aa). Using the strong E. coli alkaline phosphatase promoter and leader peptide to direct the proteins into the E. coli periplasmic space, high levels of all three proteins were produced. Proteolytic removal of the leader peptide in E. coli enabled the purification of large amounts of the C4 protein, (Garboczi et al., J. Biol. Chem. in press), which is competent in binding nucleotides. Nucleotide binding to mutant C4 proteins bearing site-directed amino acid changes is now being examined to identify critical amino acid residues involved in binding. [Studies supported by NIH grant CA10951 to P.L.P.]

M-AM-G9 FACTORS AFFECTING THE ACTIVITY OF THE MITOCHONDRIAL ATPase AND THE RELEASE OF ATPase INHIBITOR PROTEIN DURING AND FOLLOWING THE RE-ENERGIZATION OF MITOCHONDRIA FROM ISCHEMIC CARDIAC MUSCLE.

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During the re-energization in vitro of intact mitochondria from 20 min ischemic cardiac muscle there is a rapid reactivation of the mitochondrial ATPase and a release of inhibitor protein from the enzyme (Rouslin, W., J. Biol. Chem. 262, 3472-3476, 1987). In the present study we examined the effects of varying [MgATP], pH and pCa upon the extent of ATPase reactivation and inhibitor protein release during and following the re-energization in vitro of intact mitochondria from 20 min ischemic canine cardiac muscle. Consistent with earlier work, increasing the [MgATP] of the sonication medium from zero to 2 mM reduced the extent of the ATPase activity reactivation by approx. one-third. The effect upon inhibitor release was more dramatic. Two mM MgATP reduced inhibitor release by approx. two-thirds. Increasing the pH of the sonication medium above 7.0 increased ATPase activity reactivation and inhibitor protein release to similar extents. However, while decreasing the pH below 7.0 caused a reduction in ATPase activity, it also resulted in the apparent loss of a portion of the ATPase inhibitor. The pCa of the sonication medium was varied between 9.0 and 3.5 using Ca-EGTA buffers. Decreasing the pCa from 9.0 to 3.5 had a paradoxical effect. It resulted in increases in both ATPase activity and in the amount of ATPase inhibitor bound to the particles. Such a paradoxical effect may be explained if one assumes the existence of two kinds of inhibitor-enzyme interaction sites, namely, regulatory and binding sites. Thus, decreasing the pCa may decrease interaction at regulatory sites while enhancing interaction at inhibitor binding sites. (NIH grant HL30926).

M-AM-G10 DOES INTRAMITOCHONDRIAL Ca MOBILIZATION REGULATE OXIDATIVE PHOSPHORYLATION? Reers M., Kelly R.A. and Smith T.W., Harvard Medical School, Boston, MA 02115.(INTR.BY J.D.MARSH)

Although changes in mitochondrial matrix Ca^{2+} activity ($[Ca]_m$) have been implicated in the regulation of substrate flux through serveral key matrix dehydrogenases, there have been no direct means to measure whether $[Ca]_m$ is ever within the range of Ca^{2+} activities known to be around the $K_{0.5}$ of Ca^{2+} for the isolated enzymes. Recently we succeeded in loading the Ca^{2+} sensitive probe fura-2 into isolated rat cardiac mitochondria (mitos), thus avoiding the complication of having fura-2 accumulate in the extramitochondrial space, by attaching dye loaded mitos to a glass coverslip on the stage of an epifluorescence microscope. (Reers M. et al., Biochem. J. In Press). Superfusing immobilized, fura-2 loaded mitos with buffers within the physiological range for cytosolic Ca^{2+} ($[Ca]_i$), the $[Ca]_m$ was always lower than the $[Ca]_i$ in the presence of Mg^{2+}, whether or not Na^+ was present in the buffer. The ratio of $[Ca]_m$ to $[Ca]_i$ followed an ascending sigmoidal curve; the inward Ca^{2+} gradient was maximal at 200-500 nM $[Ca]_i$. The observed $[Ca]_m$ was always below the $[Ca^{2+}]$ known to enhance substrate flux through isolated matrix dehydrogenases, and, in fact, no change in NADH fluorescene was observed even at 1 uM $[Ca]_i$. However, a rapid rise in $[Ca]_m$ could be induced by superfusing mitos with CCCP in the presence of ruthenium red. This was observed even when the $[Ca]_i$ was lowered to 0, presumably due to Ca^{2+} release from a matrix Ca^{2+} buffer. Importantly, the rise in $[Ca]_m$ could be mimicked transiently by ADP in the presence of oligomycin. We propose that changes in cytosolic [ADP] could regulate matrix dehydrogenases by releasing bound Ca^{2+} from a pH and/or $\Delta\Psi$ dependent matrix Ca^{2+} buffer.

M-AM-G11 CA^{++} REGULATION OF PROTON FLUXES IN CHLOROPLASTS. THE 8 KDA CF_0 SUBUNIT IS A CA^{++}-CONTROLLED H^+ FLUX GATING STRUCTURE. G. G. Chiang and R. A. Dilley, Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana, 47907.

Proton electrochemical potential gradients in chloroplasts and mitochondria couple the redox potential energy to ATP formation via the H^+-ATP synthase-ATPase complex (CF_0-CF_1). Recent evidence with chloroplasts shows that ionic conditions used to store thylakoid membranes can reversibly regulate the expression of either a localized or a delocalized proton gradient (1,2). The assay used to detect localized or delocalized energy coupling is, respectively, the absence or presence of permeable buffer effects on the number of single-turnover light flashes required to attain the threshold proton gradient for driving ATP formation. Treatments which perturb Ca^{++} ions from certain membrane-binding sites cause a delocalization of the energy coupling proton gradient (3). With Ca^{++} occupying the site(s), localized energy coupling is favored. The calmodulin-directed Ca^{++} antagonists, chlorpromazine or trifluopirazine, perturb the H^+ gating function in a way consistent with the Ca^{++} gating action involving a membrane protein analogous to a calmodulin-Ca^{++} complex functioning as the closed-gate structure. [^{3}H]Chlorpromazine was used as a photoaffinity probe and three membrane proteins were shown to bind the chlorpromazine in a Ca^{++}-dependent way. One of the proteins was identified as the 8 kDa CF_0 subunit by its DCCD labeling, isolation through a specific butanol extraction-ether precipitation procedure and by its co-labeling with [^{14}C]DCCD. References: (1) Beard, W.A. & Dilley, R.A. (1986) FEBS Lett. 201, 57; (2) Beard, W.A. & Dilley, R.A. (1988) J. Bioenerg. Biomembr. 20, 129; (3) Chiang, G. & Dilley, R.A. (1987) Biochemistry 26, 4911.

M-AM-G12 THE EFFECTS OF ELEVATED INTRACELLULAR CALCIUM AND OF MYOGLOBIN INACTIVATION ON THE ENERGY RESERVES OF CARDIAC MYOCYTES. Jeannette E. Doeller and Beatrice A. Wittenberg, Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461.

The contents of the high energy phosphates ATP and phosphocreatine (PC) in aerated suspensions of resting isolated myocytes are comparable to those of the whole heart. Heart cells maintained nearly constant intracellular calcium concentration (Ca_i), 150 nM, at different extracellular calcium concentrations (0.003-5 mM) without affecting energy reserves. Changes in the rate of oxidative phosphorylation were detected by measuring changes in the content of ATP and PC and changes in the steady state rate of respiratory oxygen uptake. ATP synthesis was depressed by iodoacetate or rotenone to test the effects of changing Ca_i and myoglobin inactivation in energetically challenged heart cells. Iodoacetate, an inhibitor of glycolytic ATP synthesis, did not itself affect ATP or PC. Rotenone, an inhibitor of complex I of the electron transport chain, lowered PC but did not change ATP. When Ca_i was elevated to 300-500 nM by exposing the cells to low extracellular sodium or to veratridine, sarcomere length diminished significantly, and cells were reversibly stimulated to beat. The high energy phosphate contents were not significantly changed by elevated Ca_i even in the presence of iodoacetate or rotenone. Myoglobin inactivation by sodium nitrite significantly reduced respiratory oxygen uptake of aerated suspensions of resting heart cells to 76% of control and reduced PC in resting and beating heart cells with or without the two inhibitors. Analogous to findings in whole heart, these data demonstrate that elevated intracellular calcium does not affect energy reserves, but that myoglobin inactivation significantly diminishes energy reserves of isolated heart cells. Supported in part by NY Heart Assoc. Participating Lab Award and NIH HL19299.