prefers to open to the intermediate and large conductance states. In the presence of the willardiine partial agonists, the channel opens more frequently to the smallest and intermediate conductance states. Kinetic modeling using maximum interval likelihood rate optimization revealed two time constants in each open state and at least three in the closed state for the partial and the full agonists. These data suggest the mode of channel activation is similar for both glutamate and willardiine compounds with varying rates of activation. Supported by NIH NS049223.

2533-Pos Board B503

Energetics of the Cleft Closing Transition and the Role of Electrostatic Interactions in Conformational Rearrangements of the Glutamate Receptor Ligand Binding Domain

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The ionotropic glutamate receptors are localized in the pre- and postsynaptic membrane of neurons in the vertebrate central nervous system. Activation by the principal excitatory neurotransmitter glutamate allows the ligand binding domain to change conformation, communicating opening of the transmembrane channel for ion conduction. The free energy of the GluR2 S1S2 ligand binding domain (S1S2) closure transition was computed using a combination of thermodynamic integration and umbrella sampling modeling methods. A path that involves lowering the charge on E705 was chosen to clarify the role of this residue. A continuum electrostatic approach in S1S2 is used to show E705, located in the ligand binding cleft, stabilizes the closed conformation of S1S2. Molecular dynamics simulations reveal: (1) in the closed conformation, in the absence of a ligand, S1S2 is somewhat more closed than reported from X-ray structures; (2) a semi-open conformation characterized by disruption of a single cross-cleft interaction differing only slightly in energy from the fully closed S1S2; (3) the fully open S1S2 conformation exhibits a wide energy well, sharing structural similarity to the apo S1S2 crystal structure. Hybrid continuum electrostatics/MD calculations along the chosen closure transition pathway reveal solvation energies, as well as electrostatic interaction energies between two lobes of the protein increase the relative energetic difference between the open and the closed conformational states. By analyzing the role of several cross-cleft contacts and binding site residues we demonstrate how S1S2 interactions facilitate formation of the closed conformation of the ligand binding domain. A molecular model of the full GluR2 receptor is currently being constructed to reflect a consistent physical and biochemical picture based on an evolutionary comparison and all available biophysical data.

2534-Pos Board B504

Hinge and Twist Rigid Body Domain Motions in Ionotropic Glutamate Receptor GluR6 and the Hydrogen Bond Interactions that Switch Them On and Off

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Ionotropic glutamate receptors are tetrameric ligand-gated ion channels found in pre and postsynaptic cell membranes of the central nervous system. There are three pharmacological classes of ionotropic glutamate receptor, namely N-methyl-D-aspartate (NMDA); alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA); and kainate receptors. Ionotropic glutamate receptors play an important role in neuronal development and synapse plasticity as well as in higher order processes such as memory and learning. Ionotropic glutamate receptors are also implicated in several neurological and neurodegenerative disorders such as epilepsy, Parkinson's and Alzheimer's diseases. Only the structure of the isolated ligand binding domain is known: it comprises two lobes that enclose a ligand binding cleft. Using this isolated domain it has been possible to extract a great wealth of information concerning the relationship between functional and structural states of the receptor. Here, we characterize the intrinsic conformational dynamics properties of the ligand-binding domain of GluR6, a kainate receptor, in the absence of glutamate. Notably, we identify three inter-lobe hydrogen bonds interactions that govern and regulate the opening of the binding cleft via two distinct mechanisms: an hinge-like and a twist-like rigid-body domain motion. The computational studies reveal how the interplay between these interactions promotes either one or the other form of rigid-body motion. Moreover, the pattern of evolutionary conservation of these inter-lobe interactions suggests a putative role in the differential functional properties of the distinct ionotropic receptors classes.

2535-Pos Board B505

Purification and crystallization of iGluR Amino Terminal Domains

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The glutamate receptor ion channels which mediate excitatory synaptic transmission in the mammalian brain have a unique architecture distinct from that for other ligand gated ion channels. Ten years ago the 1st crystal structure was solved for an AMPA receptor ligand binding domain[1], with members of other iGluR gene families following over the next few years[2-4]. The ligand binding domain is preceded by a large amino terminal domain which controls assembly, but which does not bind neurotransmitter. Despite its key biological role structures of the ATD have not been solved. A major impediment to this is the poor expression of iGluR ATDs in *Escherichia coli*. To address this we screened ATD expression in HEK cells using constructs designed for secretion of soluble proteins and focused on the GluR6 subtype for which we can obtain 4 mg/l of glycosylated protein. The results of crystallization screens and data collection with synchrotron radiation indicate that it will be possible to solve a structure of the GluR6 ATD and explore its role in subtype specific assembly.

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2536-Pos Board B506

Structure And Stability Of Ligand Binding Core Dimer Assembly Controls Desensitization In A Kainate Receptor

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Ionotropic glutamate receptors couple free energy of agonist binding to opening and desensitization of a transmembrane ion channel. Central to their function is a structural unit formed by a dimer assembly of the ligand binding domains. The rates of transitions between resting, conducting, and desensitized states is controlled by conformational changes in the dimer. The serendipitous discovery that the GluR2 L483Y mutant blocks desensitization by stabilizing dimer assembly has profoundly influenced understanding of AMPA receptor gating. Paradoxically, GluR5-GluR7 subtype kainate receptors have an aromatic amino acid at the equivalent position, but desensitize rapidly and completely. Using a library of GluR6 dimer interface mutants, we used analytical ultracentrifugation to show that for kainate receptors there is a direct correlation between the rate of onset of desensitization and the stability of dimers formed by ligand binding cores, establishing that the gating mechanisms of AMPA and kainate receptors are conserved. Crystal structures for a series of 5 mutants were solved to reveal the underlying molecular mechanisms. Visualized in the crystal structures is a rich complexity of interactions across the dimer interface, illuminating how small sequence differences within the ligand binding domain function to diversify receptor properties. Our results indicate that even following extensive engineering, the stability of kainate receptor dimers is at most half of that of their AMPA counterparts, and that even if it were possible to generate dimers as stable as those for GluR2 L483Y, these would be insufficient to block kainate receptor desensitization. We show this is because the desensitized state in kainate receptors acts as a deep energy well offsetting the stabilizing effects of dimer interface mutants. Our results reveal how receptors with similar structures and gating mechanisms can exhibit strikingly different functional properties.

Muscle: Fiber & Molecular Mechanics & Structure I

2537-Pos Board B507

Computational Energetic Analysis of Intrafacial Binding Energies in Interpolated Myosin States

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In order to understand how myosin interacts with ATP and actin to produce motion during structural rearrangements corresponding to progression from one biochemical/structural state to the next in the ATPase crossbridge cycle, we examined the energetic differences in interfaces within structural models of myosin. So that these stages of the crossbridge cycle are logically and energetically realistically connected we generated, by interpolating in an energetically realistic way, structures between existing crystal structure states of individual myosin heads representative of different biochemical/structural states of the crossbridge cycle. We computationally alanine scanned[1] these structures to objectively examine myosin energetics and test hypotheses of force production and regulation. Combining these computed energies with displacement measurements as examined by distance displacement contact maps, we gained important insights into the complex interplay of energy, strain, elastic energy storage and release, and force generation within myosin and how structural elements interact to create the complex regulated mechanochemical behavior of the myosin motor. In particular, we focused on the recovery stroke connecting the Dictyostelium myosin structures 1MMD and 1VOM using intermediates generated by the conjugate peak refinement method[2] to find areas of strain creation, strain maintenance (elastic energy storage) and strain release, as well as elastic recovery. This contributes to a quantitative view of the regulatory mechanisms; interface formations, deformations and dissolutions; force producing motions, and the energy landscape on which these changes take place.

2538-Pos Board B508

Molecular Dynamics Simulations Of The Hydrolysis Transition State Intermediate In Myosin

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We have used molecular dynamics simulations to analyze the nucleotide site of myosin and its interaction with ATP and a catalytic water. Simulations used the Dictyostelium myosin ADP●VO4 x-ray crystal structure. This structure is widely hypothesized to be an analog of the hydrolysis transition state intermediate for an in-line water attack on the γ-phosphate position. The trigonal bi-pyramidal VO4 moiety was replaced by PO3 covalently bound to ADP and a water molecule oxygen. Surprisingly, the MD simulation indicated that the x-ray structure was not capable of controlling the position of the modeled attacking water as required for hydrolysis. Instead the water molecule rattled around a catalytic pocket formed by the γ-phosphate of ATP, elements of switch 1, switch 2, and the salt-bridge between R238 and E459. The salt-bridge has been postulated to serve to help stabilize the closed conformation of switch 2. The simulated double alanine R238A/E459A mutation eliminated this salt-bridge. There was little resulting change in the conformation of switch 2 (0.55Å r.m.s. deviation, Cα-Cα, a.a. D454-L495) and the crucial hydrogen bond distance between the backbone amide of G457 and the γ-phosphate oxygen of ATP increased from 1.9Å to only 2.0Å in the mutated structure. However, the modeled catalytic water rapidly escaped from the catalytic pocket in the mutated myosin. Thus the simulations suggest that the closed-switch 2 structure is stabilized by a number of interactions in addition to the salt-bridge. The function of the salt-bridge is to serve as a lid to sequester water in the catalytic pocket.

2539-Pos Board B509

Analysis Of The Interaction Of The Nucleotide Base With The Myosin Catalytic Pocket And The Effect On Substrate Efficacy

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Myosin has been shown it to be a promiscuous enzyme that can utilize a number nucleotide and non-nucleoside triphosphate substrates. However, the chemomechanical efficacy of the alternative substrates varies widely. In particular, the ability of ''nanalog'' substrates (an amino-ethyl or -propyl linker and a substituted phenyl ring in lieu of the ribose and the nucleotide base) to completely decouple substrate hydrolysis from motility clearly indicates that the correct interaction of myosin with the ribose and base is essential for myosin function. As a first step to understand better the myosin-substrate interaction and how interactions other than with the triphosphates are essential for motility, we have used molecular dynamics simulations of the Dictyostelium ADP●BeFx x-ray structure to investigate the interactions of myosin with ATP, CTP (effective substrate) and GTP (poor substrate) docked at the active site. The simulations with ATP suggest a ''troika'' of conserved amino acids lining the nucleotide site that form a cyclical chain of nucleotide-protein hydrogen bonding interactions: ATP (N6) → Y135 → Y116 → N188 → ATP (N7). In the simulations, substitution of CTP at the active site maintains this coordination. Substitution of GTP at the active site perturbs the interaction. Thus the simulations suggest a set of crucial protein-substrate interactions that must be maintained for an effective substrate. The modeling has predictive power. ''In silico'' mutation experiments suggest that the mutation Y135K preserves troika-mediated pattern with GTP at the active site, but destroys it with ATP at the active site. The prediction would be that the Y135K mutation would make myosin a more effective GTPase and a less effective ATPase.

2540-Pos Board B510

Myosin II Trapped In A Weak Actin-binding State Through A Chemical Crosslink Across The Actin-Binding Cleft

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We have trapped *Dictyostelium* myosin II in a weak actin-binding conformation by chemically crosslinking two engineered Cys across the actin-binding cleft using a bifunctional spin label (BSL). With sites in the lower and upper 50 kDa domains, the crosslink restricts the conformation of the actin-binding cleft. The crosslinking reaction was monitored by electron paramagnetic resonance (EPR) based on the spin label immobilization that occurs upon reaction of both Cys. The EPR spectrum of crosslinked myosin is sensitive to structural changes induced by both nucleotide- and actin-binding. Functional assays demonstrate that crosslinking partially impairs actin binding and actin-activation but has negligible effects on basal ATPase activity. We propose that crosslinked myosin is trapped in a weak actin-binding structure in which phosphate release is inhibited by the presence of actin. This conformation presumably binds actin weakly but cannot transition to the ''closed'' cleft structure that is populated with strong actin-binding (Klein et al, 2008, *PNAS* 105:12867-72). The weak actin-binding structure has proven difficult to characterize because of its transient nature; BSL-crosslinked myosin provides a stable model system for analysis of structural dynamics. We are using EPR to analyze the orientation of BSL-crosslinked myosin attached to actin in skinned muscle fibers, and we are using nucleotide probes to investigate the coupling between the actin-binding cleft and the nucleotide-binding pocket. This work is complementary to a study in which BSL was used to crosslink SH1 (C707) and SH2 (C697) in the force-generating domain of myosin, producing a stable complex that bound weakly to actin with slow orientational disorder (Thompson et al., 2008, *Biophys. J.*, in press). This work was supported by grants from NIH (AR32961, AR07612) and the Minnesota Supercomputing Institute.

2541-Pos Board B511

Analysis of Conformation of Skeletal Muscle Myosin Cross-linked by pPDM Using FRET

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Previously biochemical studies have demonstrated that the highly reactive cysteine residues SH1 and SH2 can be crosslinked by variety of bifunctional reagents with different spans (3-14 Å) in the presence of nucleotides, suggesting that the region is highly flexible. The SH1-SH2 region is believed to play a key role in the conformational changes that occur in the myosin head during the force generation coupled to ATP hydrolysis. We have previously shown that the HMM, which SH1-SH2 was crosslinked by p-Phenylene-dimaleimide (pPDM) in the presence of ADP, have a novel conformation using quick freeze deep etch electron microscopy (QFDE-EM). We have also demonstrated that conformational change of the myosin motor domain during ATP hydrolysis can be monitored by measuring the FRET using fluorescent ATP analogue NBD-ATP. In the present study, we analyzed the conformation of the myosin crosslinked by pPDM using the FRET between the ATP binding site and the A1 essential light chain (ELC) and compared with the 3D structure models of ATPase intermediates derived from electron microscopic analysis. We prepared the skeletal muscle myosin subfragment-1 (S1), which ELC was labeled by 6-bromoacetyl-2-dimethylaminonaphthalene (BD) at the Cys 177. And fluorescent ADP analogue NBD-ADP was trapped in the ATPase site of S1 labeled by BD. The FRET efficiency was estimated by measuring the change of fluorescence intensity of BD comparing with control BD-S1. FRET efficiency of pPDM-S1-NBD-ADP was apparently different from other nucleotides and nucleotide analogues bound states.