Minisymposium 3: Inactivation and Desensitization Mechanisms in Ion Channels

1787-Minisymp A Structural And Functional View Links Toxin-induced Conformational Changes In A Potassium Channel To C-type Activation

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A recent solid-state NMR study [1] revealed that high-affinity scorpion toxin binding induces conformational changes in the selectivity filter of potassium channels. The exact nature of these conformational changes, however, remained elusive. We combined all-atom molecular dynamics simulations with solid-state NMR and electrophysiological measurements to investigate these changes. Our simulations covered the complete pathway of spontaneous toxin approach and binding to the membrane-bound channel. The obtained structural model of the complex revealed conformational changes in the selectivity filter that account for the observations made in solid-state NMR. They also explain tight channel blockade and enhanced toxin affinity. We show that these changes are not only structurally, but also functionally closely related to C-type activation [2]. Our study points to heterogeneity in the binding modes that might serve to stabilize the complexes entropically.

References

[1]. Lange, A., et al. Nature *440*, 959–962 (2006).
[2]. Cordero-Morales, J.F., et al., Nat. Struct. Mol. Biol. *13*, 311–319 (2006).

1788-Minisymp Structural basis of K^+ channel C-type inactivation: Crystal Structure of KcsA in the Open/C-type inactivated Conformation

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It is well established that in KcsA, proton dependent activation leads to a widening of the inner helical bundle (IHB). In addition, channel activation triggers C-type inactivation at the selectivity filter (SF), a feature common among all potassium selective channels. By establishing the molecular elements responsible for proton sensitivity in KcsA (Cuello et al., this meeting), we have engineered a constitutively open-channel in which the IHB is fully open at basic pH. Additionally, we have demonstrated that deletion of the KcsA C-terminal domain enhances C-type inactivation. These two findings have given us a unique opportunity to structurally evaluate the conformational changes underlying KcsA proton-dependent activation, C- type inactivation at the SF and the conformational coupling between these two processes. By using Fab-assisted crystallization methods we have solved 17 KcsA structures "trapped" in different stages along the transition pathway, from closed to the fully open state (open-inactivated state), including at least four classes of gating intermediates. Analysis of these structures have revealed:

1. The KcsA IHB can be stabilized with several degrees of gate opening, ranging from about 11.5 Å (Cα-Cα distance at residue 112) to 32 Å;
2. We find no ion occupancy at the channel aqueous cavity in the fully open conformation;
3. Major conformational changes at the SF include flipping of carbonyl groups at V76 and a progressive loss of ions at potassium binding sites S2 and S3. Ion occupancy at these sites is strongly correlated with the degree of opening at the IHB;
4. a small but significant conformational change of the P-loop is seen when the IHB is fully open.

These structures reveal, at unprecedented detail, the molecular basis of K^+ channel C-type inactivation and its coupling to activation gating.

1789-Minisymp Allosteric Ion Binding Sites In Kainate Receptors

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Almost all fast excitatory signaling in the brain is due to the neurotransmitter glutamate. Hence, the ion channel receptors for glutamate (iGluRs) are cornerstones of excitatory transmission throughout the brain. This family of receptors, with subtypes named AMPA, kainate and NMDA, underlies synaptic currents that have decay times ranging from milliseconds to seconds. Understanding the regulation of their finely tuned kinetic regimes by endogenous modulators is critically important for unraveling their complex roles at central synapses. The recent discovery of an anion binding site in the intersubunit dimer interface of kainate receptors (Plested and Mayer, *Neuron* 2007) provided a simple explanation of how chloride ions control receptor availability and kinetics. Monovalent cations also modulate kainate (but not AMPA) receptors. However, no bound cation was apparent in either the Cl^-- or Br^--bound structures, despite the similarities between modulation of iGluRs by anions and cations (Bowie, *J. Physiol* 2002). We present data that allow us to estimate the coupling between the cation and anion binding sites. These data suggest that kainate receptors are not fully bound by sodium and chloride at physiological salt. We show that this renders a significant fraction of kainate receptors unavailable for activation by glutamate. Using deletion mutagenesis and non-desensitizing mutant receptors, we locate the allosteric cation binding site and demonstrate clearly that external ions are not coactivators of kainate receptors (Wong *et al. J. Neurosci* 2006). We present structural data that illustrate the cation binding site for the first time, and suggest that a simple allosteric mechanism accounts for the apparent concomitant modulation of kainate receptors by anions and cations.

1790-Minisymp Activation and Inactivation Of TRPA1 By Permeating Calcium Ions

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The transient receptor potential A1 (TRPA1) channel is expressed by sensory neurons in the pain pathway and is the molecular target for a number of pungent chemicals and environmental irritants. TRPA1 channels can be activated by pungent chemicals in the absence of external Ca^{2+}, but the currents are greatly potentiated in its presence. Moreover external Ca^{2+} is required for inactivation of TRPA1 currents. External Ca^{2+} could regulate TRPA1 activity through binding to extracellular regions of the channel or by permeating the channel and acting on the cytoplasmic side of the membrane. To distinguish between these possibilities, we studied the effects of manipulating intracellular Ca^{2+} on heterologously expressed rTRPA1 channels. In the absence of external Ca^{2+}, transient elevation of intracellular Ca^{2+} by flash photolysis elicited a long-lasting activation of TRPA1 currents, consistent with a putative internal Ca^{2+} activation site. Surprisingly, currents evoked by transient elevation of intracellular Ca^{2+} did not inactivate unless external Ca^{2+} was added. Thus, activation and inactivation are separable Ca^{2+}-dependent processes. To determine whether inactivation was regulated by binding of Ca^{2+} to the outside of the channel, we mutated acidic residues predicted to be on extracellular domains. One mutation dramatically reduced both Ca^{2+}-induced activation and inactivation of TRPA1. This mutation mapped to the putative pore of the channel and significantly reduced Ca^{2+} permeability. Activation and inactivation of mutant TRPA1 channels by external Ca^{2+} could be restored by conditions where internal Ca^{2+} was allowed to rise, indicating that both processes are mediated by internal sites of action. Together our data predict a model whereby permeant Ca^{2+} ions act at the inner surface of the channel to enhance ionic currents and at a different, lower affinity site to promote inactivation of the channel.

1791-Minisymp The Guanylate Kinase Domain of the Beta-Subunit of Voltage-Gated Calcium Channels Suffices to Inhibit Voltage-Dependent Inactivation

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The β-subunit of voltage-gated calcium channels ($Ca_V\beta$) contains two conserved interacting modules, a Src homology 3 (SH3) and a guanylate kinase (GK) domain, flanked by variable regions. GK binds to a site highly conserved in all $Ca_V\alpha_1$-pore-forming subunits, the so-called AID motif, through a hydrophobic pocket located opposite to the surface that interact with SH3. While SH3 appears to control membrane protein internalization by direct interaction with dynamin, the functional competence and the extent to which GK alone is able to sustain $Ca_V\beta$ functions it is not clear. Here we report the effect on $Ca_V2.3$ mediated currents of GK domain of two $Ca_V\beta$ isoforms, $Ca_V\beta_{1b}$ and $Ca_V\beta_{2a}$, that exhibit opposite effects on channel voltage-dependent inactivation. Both, $Ca_V\beta_{1b}$-GK and $Ca_V\beta_{2a}$-GK were expressed in bacteria and refolded from inclusion bodies by size exclusion chromatography and batch dilution, respectively. The prevalent view is that palmitoylation of two N-terminal cysteine residues in $Ca_V\beta_{2a}$ is responsible for the unique ability of this isoform to inhibit inactivation of $Ca_V2.3$ channels. Our data show instead that injection of refolded $Ca_V\beta_{2a}$-GK module alone, devoid from the N-terminal region, is nearly as efficient as the full-length protein to slow down inactivation of $Ca_V2.3$ channels expressed in *Xenopus* oocytes. Moreover, $Ca_V\beta_{1b}$-GK is undistinguishable from $Ca_V\beta_{2a}$-GK in inhibiting inactivation of $Ca_V2.3$ channels. These findings demonstrate that inhibition of inactivation is fully encoded by the GK domain, and indicate that the $Ca_V\beta$ modules correspond to biological functional units. In full-length $Ca_V\beta_{1b}$ and other $Ca_V\beta$ isoforms, variable regions would antagonize GK inactivation-inhibiting capability and accelerate the rate of channel-inactivation.

1792-Minisymp The Role of Hydrophobic Interhelical Association in the Adaptive Gating Mechanism of the Mechanosensitive Channel MscS

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The E. coli MscS channel, acting as a tension-activated osmolyte release valve, shows adaptive behavior involving transitions between closed, open, desensitized and inactivated states. The MscS crystal structure, solved in the absence of lipids, revealed a heptameric three-transmembrane-domain architecture with a substantial (30o) splay of the peripheral transmembrane helices (TM1-TM2) relative to the central pore formed by TM3s. The resulting deep crevices interrupt the connection between the stress-receiving peripheral helices and the gate. Here we address the question of whether the separated state of TM helices observed in the crystals may bear functional significance for the inactivated state, in which the channel is irresponsive to tension. The resting and open states of MscS reconstructed through modeling and simulations predicted a more parallel packing of TM helices creating a tight neighborhood of hydrophobic residues on TM2 (V65, F68, L69) forming a dehydrated buried contact with L111 and L115 on the gate-bearing TM3s. Steered molecular dynamic simulations demonstrated that tension applied only to the TM1-TM2 helices effectively transmits through this interface and opens the gate. Hydrophilic or small sidechain substitutions in these locations profoundly changed the channel behavior in patch-clamp experiments. The L69S mutation increased the rate of inactivation from the open state, whereas V65S, F68S, L111S, L111A and L115T also opened an unnatural route for 'silent' inactivation under moderate tensions directly from the closed state. The L115S mutant displayed retarded closing and 'silent' inactivation. Based on these data and statistical analysis of over 80 homologous sequences, we conclude that the close hydrophobic contact between the residues lining the interhelical crevice is responsible for the tension transmission to the gate and that the

separation of helices associated with hydration of the crevice promotes inactivation.

Platform AM: Exocytosis & Endocytosis

1793-Plat Vesicle Diffusion Close to Supported Lipid Bilayers: A Model For Endo- and Exocytosis

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Understanding underlying mechanisms of vesicle diffusion near membranes can provide a comprehensive interpretation of endocytosis, exocytosis and synaptic fusion. We experimentally and theoretically characterize the dynamics of freely diffusing small vesicles near supported planar bilayers using total internal reflection-fluorescence correlation spectroscopy (TIR-FCS). The population distributions of vesicles diffusing near planar bilayers are affected by changing ionic strength, buffer pH, and the composition of the planar bilayers. As a result, hydrodynamic interactions of vesicles with the planar bilayers are changed, altering vesicle movements near the bilayers. These experimentally determined dynamics of vesicles at physiological conditions agree with theoretical expectations. Effective surface charge on neutral bilayers are also analyzed by comparing experimental and theoretical data, and we demonstrate the possibility that vesicle dynamics can be modified by surface charge redistribution of the planar bilayer. Based on these results, we hypothesize that small vesicles, when they are not in contact with cellular membrane, do not randomly diffuse around membranes, but may diffuse in a controlled manner depending upon the local and dynamic biological conditions. These studies allow us to further investigate the molecular dynamics involved in membrane fusion and fission.

1794-Plat Nanometer-scale Rearrangements Of Fission Pore During Normal And Aborted Endocytosis

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We report measurements, with a time resolution of milliseconds, of fission intermediates during formation and pinching-off of single endocytic vesicles. Fission pore conductance changes are detected by patch-clamp admittance measurements and associated with nanometer-scale rearrangements of the surrounding membrane structure (membrane neck). Principle modes of membrane neck deformation/transformation are considered and compared to resolved fission pore dynamics and electron microscopy of endocytic intermediates. Two kinetically distinct stages of the conductance time-course were designated to correspond to:

1. the uniform elongation of a highly constricted membrane neck (calculated fission pore diameter < 3 nm), followed by
2. the abrupt decrease of pore diameter finalizing the pinching-off event.

Disruption of the endocytic machinery by the dynamin-interacting drug dynasore precluded elongation of membrane neck and resulted in oscillations of fission pore diameter insufficient to complete fission. Energetic aspects of membrane neck elongation and constriction will be discussed.

1795-Plat Membrane Lipid Segregation in Endocytosis

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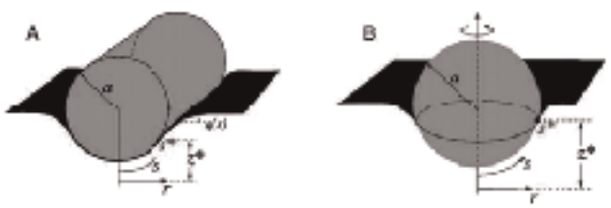
We explore the equilibrium mechanics of a binary lipid membrane that wraps a spherical or a cylindrical particle. One of the lipid membrane components induces a positive intrinsic spontaneous curvature, while the other induces a negative local curvature. Using a Hamiltonian approach, we derive the equations governing the membrane surface shape and lipid concentrations near the wrapped object.

Asymptotic expressions and numerical solutions for membrane shapes are presented. We determine the regimes of bending rigidity, surface tension, intrinsic lipid curvature, and effective receptor binding energies that lead to efficient wrapping and endocytosis. Our model is applicable to the invagination of Clathrin coated pits and to receptor-induced wrapping of colloids such as spherical virus particles.



1796-Plat The Relationship Of The Membrane Of Docked Synaptic Vesicles To The Presynaptic Membrane At Neuromuscular Junctions At Nanometer Spatial Resolution

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We examined by electron tomography the relationship of the membrane of docked vesicles to the presynaptic membrane in tissue sections from resting frog and mouse neuromuscular junctions fixed in aldehyde and stained with heavy metals with the aim of determining its constancy. We first measured the distance from the external surface of the presynaptic membrane to the luminal surface