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Microsecond Simulations Indicate 3_10 Helix Transition Facilitates Translation of the S4 Segment of Kv1.2 and Suggest a Hydrophobic Gating Barrier

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The stabilization and motion of the arginine-containing S4 segment have been of considerable interest for studies of voltage-regulated potassium channels, with several competing models for the gating. Here, we present results from atomistic simulations on microsecond scale both of integral Kv1.2/2.1 channels as well as voltage sensors. The results support a model where the crystal structure reflects the open-inactivated state, but starts a transition towards a normal open state with increasing 3_10 helix structure upon hyperpolarization (but not when depolarized). This is coupled with significant rotation of the upper part of S4 where R294 and R297 break their hydrogen bonds and move away from the lipids.

We have additionally performed non-equilibrium pulling simulation of S4 segments with either alpha or 3_10 helix structure, and find the latter both energetically favorable for pulling and inducing less distortion to the protein. The 3_10 helix alternative would provide a natural explanation for the additional slow inactivation transition, and leads to a fairly simple translation between resting and activated states since the S4 arginines line up to face hydrogen bond partners (E183,E226) in this secondary structure. We observe the main free energy barrier coming from the F233 fenyl ring that rotates back-and-forth for each arginine passing it; the potential importance of this residue was first suggested by MacKinnon who noticed it is universally conserved in voltage-gated ion potassium channels. This result appears to be confirmed by in silico mutagenesis to smaller hydrophobic residues, which reduces the barrier. Through extrapolation we estimate the barrier for the wildtype voltage sensor could be as low as 20kJ/mol, likely a reasonable range for activation to occur on millisecond scale.

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Structural Transition from Alpha- to 3_10-Helix Reduces the Free Energy Required for S4 Translation in Kv1.2/2.1 Voltage Sensors, and F233 Appears to Responsible for the Gating Barrier

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The S4-segment of voltage-gated ion channels is mostly alpha-helical. However, the Kv1.2/2.1 channel's first part forms an alpha-helix, whereas the voltage-sensor-domain's (VSD) intracellular end adopts a 3_10-helix conformation. This has raised the hypothesis that secondary structure alterations could be critical in gating and is supported by a recent experimental study[1]. Additional molecular simulations have shown structural rearrangements when an external potential is applied on microsecond-scale[2].

Here, we study the S4 movement in an isolated VSD through pulling, by molecular dynamic simulation. First, we induce a 3_10-helix conformation and then by pulling on the voltage-sensing-arginines, systems both with alpha- and 3_10-helices are dragged intracellular, over the hydrophobic core that has been proposed to be the energy barrier of gating. By using a series of gradually longer simulations ranging up to the microsecond-scale we collect close to 4 microseconds of data, enabling energy barrier estimations. Moreover, we identify residues responsible for the barrier by utilizing side-directed-mutagenesis systems of the core region.

Pulling the helices towards the VSD's resting conformation showed that the 3_10-helix is quantitatively more energetically favorable. The rearrangement of the hydrogen bond network rotates the arginine side chains and enables an alpha/3_10-conversion, which could explain the barrier. Additionally, F233's phenyl ring is a structural plug for gating charges, since it has to rotate to let the side chains pass. An estimate for R299(R4) crossing the hydrophobic zone results in approximately 20kJ/mol for 3_10-helix, which is in agreement with experimental results of activation lying in millisecond time scale. Such structural transitions could explain the key differences between the open-inactivated-state present and the activated/resting-states.

[1]Villalba-Galea et al., PNAS, 105(46):17600-17607,2008.
[2]Bjelkmar et al., PLoS CompBiol, 5(2):e1000289,2009.

2691-Pos

Effect of Altered Ion Occupancy on Permeation and Inactivation Gating in K^+ Channel

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The selectivity filter of a K^+ channel is comprised of four equally-spaced K^+-binding sites. In the first three sites the main-chain carbonyl atoms provide the octa-coordination for K^+ ions. In the fourth site both main-chain carbonyl oxygen atoms and threonine side-chain hydroxyl oxygen atoms contribute to K^+ coordination. This threonine is highly conserved among K^+ channels with the only alternative being a serine substitution. Surprisingly, this conservative mutation, which preserves the hydroxyl moiety and the side chain polarity, has been shown to produce profound changes in permeation and gating in *Shaker*. Indeed, mutant T442S shows a dramatic destabilization of inactivation along with an increased conductance for Rb^+ over K^+. Although these studies provided early insights into the role of this position in ion coordination, the structural basis for altered single-channel conductances and gating kinetics remain unclear. KcsA has served as an archetypical K+ pore providing molecular insights into understanding selectivity, ion-permeation, gating pore-blocking and C-type inactivation. Equivalent mutation in KcsA (T75S) shows remarkably similar effects as seen in *Shaker*, including effects on inactivation, single-channel conductance, selectivity and rectification. Combining single-channel and macroscopic current measurements along with X-ray crystallography under different ionic conditions we now provide atomistic details that underlie these divergent channel properties and the role of Thr75 in channel function.

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Experimental Constraints for the Resting Structure of the Shaker Voltage Sensor Domain Derived from Engineered High and Low Affinity Zn^{2+} Binding Sites

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Relatively little is known about the structure of the resting conformation of the voltage sensor domain in K^+ channels. We probed the relative proximity of residues in S2, S3b, and S4 in the resting state of Shaker using engineered ion binding sites. I287 in S2 and F324 in S3b correspond to aspartate residues in eag that form a Mg^{2+} binding site; Mg^{2+} binds to the resting conformation to modulate activation. In Shaker I287D+F324D, Mg^{2+} slows activation and increases the delay prior to opening. Because the delay reflects the initial transitions in Shaker activation, this indicates that Mg^{2+} binds to the resting state. In the double histidine mutant, I287H+F324H, Zn^{2+} slows activation significantly with a half-maximal effective concentration of 300-500 nM. Zn^{2+} also shifts the voltage dependence of the delay in the depolarized direction, indicating that Zn^{2+} stabilizes the resting conformation. Recently, Campos et al (PNAS 104:7904, 2007) reported that I287C in S2 forms a disulfide bond with R362C in S4 in the resting state. However, in dynamic proteins, disulfide bonds can form between residues that are up to 15 Å apart. After reduction, I287C+R362C binds Cd^{2+} with moderate (micromolar) apparent affinity. To further probe the proximity of I287 and R362, we characterized the functional properties of I287H+R362H in the presence and absence of Zn^{2+}. Addition of 10 μM Zn^{2+} induced a slow component of activation not present in either single mutant; 2 μM Zn^{2+} had no effect. Thus, the apparent Zn^{2+} affinity of I287H+R362H is significantly less than that of I287H+F324H. These results suggest that I287 in S2 and F324 in S3b are in atomic proximity in the resting conformation, whereas R362 in S4 is somewhat further away.

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Refinement and Validation of Atomic Models of the Kv1.2 Potassium Channel Through Molecular Dynamics and Gating Charge Calculations

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Voltage-gated potassium (Kv) channels are membrane proteins that respond to changes in transmembrane potential through voltage-sensing domains (VSD). These domains are composed of highly charged transmembrane segments that move in response to changes in electric potential and control opening of the ion conduction gate. Complete atomistic models of Kv1.2, in the open and closed states, have been constructed using the structure prediction program Rosetta and available crystallographic structures. By means of molecular dynamics simulations, the two models are refined in the presence of an external voltage bias leading to stable conformations of the channel in an explicit membrane-solvent environment. Salt-bridge interactions stabilizing the VSD are identified within the VSD and between the charged residues of the VSD and lipid head groups. Conformational changes in the VSD result in the transfer of electric charge across the membrane, that can be measured as a gating

current. The magnitude of the gating charge in Kv1.2 potassium channel is calculated from more than 1microsecond of all-atom molecular dynamics simulation. Free energy calculations are performed to determine the individual contribution of several (nine) charged residues of the VSD to the gating charge. The total gating charge obtained for the refined models of the channel is ~10.5e, indicating that the refined model of the closed resting state most likely represents an intermediate conformation that precedes closing of the channel. Through steered molecular dynamics (SMD) simulations we identify a closed conformation of the channel, corresponding to a gating charge of 12.7e, in accord with experimental values obtained for the Shaker potassium channel.

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Pathway Calculation of the Conformational Transition of the Voltage Sensor Domain in the Kv1.2 Channel

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Voltage-gated potassium channels are homotetrameric proteins that undergo conformational transitions in response to variations of the transmembrane potential, resulting in the opening and closing of the channel. Each protein subunit is made of six transmembrane segments (S1 - S6) arranged in two distinct domains, the voltage-sensor domain (VSD, helices S1 - S4) and the pore domain (S5 - S6), with the VSDs surrounding the pore domain. Voltage sensing occurs on S4 helices of the VSDs, where charged residues, four arginines in particular, respond to changes in the transmembrane electric field, triggering the conformational transition in the domain and eventually in the full channel. Using the available X-ray structure of the Kv1.2 full channel in the open state as a reference, both its open and closed states have recently been modeled and refined via molecular dynamics (MD) simulations, but the sequence of events along the transition path is not known in atomic detail. To investigate this path, we employ the string method with swarms-of-trajectories with all-atom MD simulations. Given an initial guess for the path (the string) in the space of a large set of representative variables, the method finds the most probable path by monitoring the average dynamical evolution of each replica along the path. Once the string has converged, we compute the free energy and the rate for the transition using a recently developed variation of the milestoning method. We study the conformational transition for an isolated VSD as well as for one VSD in the full-length channel starting from the open conformation, in the presence of explicit water-membrane environment. [Supported by NIH grant GM062342 and GM067887].

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3D Geometric Monte Carlo Fitting of LRET Data

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We present a novel method to extract 3-dimensional conformational change within select protein systems using a Monte Carlo-based curve fitting algorithm applied to lanthanide resonance energy transfer (LRET) recordings. The key concept is to fit a constrained 3D geometry directly to a multi-exponential LRET decay. The preparation must be an n-subunit homomeric protein with each subunit containing a genetically encoded lanthanide binding tag (LBT), which holds terbium locked to the backbone of the protein. A fluorophore-labeled acceptor-carrier (toxin or ligand) is bound to the protein. The n terbium atoms and single fluorophore create n donor-acceptor pairs. During energy transfer, the acceptor can diffuse about its labeling site, thus producing a cloud of possible acceptor locations. We model the positions of the acceptor bound to the acceptor-carrier/protein complex by a comprehensive dihedral angle scan including energy calculations at each scan position. We compute n effective distances from the donors to the acceptor cloud. We construct a multi-exponential decay by relating each decay component to its effective distance using Forster theory constrained by the decay amplitude relation to time constant for sensitized emission. The computational task is to find the donor geometry that produces a distance combination that best fits the LRET decay. A Monte Carlo approach is used to sample geometries to find the best fit. The resulting geometry is a 3-dimensional solution, which is unique due to the acceptor cloud asymmetry and position. Our experimental application is the Shaker K+ channel with labeled Agitoxin bound to the pore. Our results from Shaker with the LBT located near the top of S4 in the inactivated state are consistent with the open/inactivated Kv1.2 crystal structure. Most interestingly, results in the closed and open states agree with experimental evidence. Support: NIH GM062342,GM068044,GM030376.

2696-Pos

Stabilization of the Relaxed State of the Voltage Sensing Domain of Shaker

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Segments S4 and S5 in Voltage Gated Channels potassium channels are contiguous and specific residues of these segments get in atomic proximity in a state-dependent way (Lainé *et al*., 2003; Lewis *et al*., 2008). In Shaker, the double mutation R362H+A419H stabilizes the conducting state of the channels when a metal bridge is formed in the presence of Zn^{2+} (Lainé *et al.*, 2003). These results were obtained from ionic conduction experiments but gave no direct information on the dynamics of the Voltage Sensing Domain (VSD) of Shaker. As a proxy for the movement of the VSD, we studied the proton currents through the VSD that results by the double mutation R362H+A419H, on the ultra-fast-inactivating Shaker W434F. When the holding potential (HP) was 0 mV, the current-voltage relation of the proton current (Ip-V) was shifted towards negative potentials as compared to the Ip-V when HP was −90 mV, as expected from the relaxation that the VSD undergoes at maintained depolarization. When HP was 0 mV, the proton current was decreased and the Ip-V was further shifted by increasing the concentration of Ni^{2+} or Zn^{2+} (10 μM-100 μM). In contrast, no changes were observed in the Ip-V voltage dependence with Ni^{2+} or Zn^{2+} when holding at −90 mV. In the presence of Ni^{2+} or Zn^{2+} the proton current showed a second slower kinetic component, whose relative amplitude was increased with an increase in Ni^{2+}or Zn^{2}. Fluorescence recordings with a probe in M356C showed that Zn^{2+} decreased the rate of TMRM dequenching when pulsing to negative potentials from an HP of 0 mV, consistent with the proton current results. These observations indicate that the metal bridge between R362H and A419H stabilizes the relaxed state of the VSD (Support NIHGM030376).

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Biophysical Properties of Three Omega Gaps Along the Voltage Sensor S4 of Shaker Potassium Channel

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Omega current is a cation-selective current conducted through the voltage sensor domain of ion channel when the first arginine R1 is replaced by a short residue. We were able to show Omega current for three different gaps along the voltage sensor S4 in Shaker potassium channel. These omega currents appear when two successive arginines were mutated to short amino acids (serines) creating a gap of short residues in between the long residues. The omega current starts to show at different negative potentials according to the position of the mutated arginines and down to approximately −200 mV. While the classical mutant which has Ala359 and R362S occupying the pore show an onset of omega current at −70 mV, the two other mutants with gaps at R362S/R365S and R365S/R368S showed omega onsets at −50 and −30 mV respectively (Gamal El-Din et al. Biophys. J. 96(3) pp. 381a 2009). The Omega current in the three constructs were conducted down to −200 mV and voltage-dependent closing of the gaps seems to occur at potentials less than −200 mV. Fluorescence of EGFP-bound ion channels was used as a measure of number of expressed ion channels and thus to quantify the omega currents. The biophysical properties of these different omega pores (current-voltage, conductance and gating charge-voltage correlations) are presented in this work. Gating charge for the different constructs is correlated with our proposed model of the gating steps of the voltage sensor S4.

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On-Off Conditions for the Omega Currents Caused by 3 Gaps Along S4 in Shaker K-Channel

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We previously have shown that each replacement of a pair of long amino acid residues by short ones at 3 different sequential positions along the arginine thread of S4 in Shaker led to an omega conductance through a proteinaceous leak pore (Gamal El-Din et al., Biophys. J. 96(3) pp. 381a, 2009). For the already known omega current mutation R362S, we showed that it leaks only if