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.

2694-Pos

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].

2695-Pos

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).

2697-Pos

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.

2698-Pos

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