The proton channel can also help set the membrane potential. The Hv1 channel is proposed to play a role in the capacitation process of the sperm, in addition to and in the innate immune system during the respiratory burst, in pH regulation, in spermatozoa, in apoptosis, and in cancer metastasis. This channel is highly cooperative. Here, by measuring Foster Resonance Energy Transfer (FRET) mechanisms, allowing distance measurements between the membrane and the C-terminus, we have been able to determine the relative separation between the C-termini of the Hv1 proton channel. The ability to block them selectively is an important target for drug development and for biophysical studies of channel function. Recently, 2-guanidobenzimidazole (2-GBI), a selective and state-dependent blocker of Hv1 channels was identified. However, the exact location of the binding site for a blocker as well as its apparent state-dependence has yet to be established. Recently, we have investigated the structure of the closed and open states of the voltage-gated proton channel through a combination of modeling and experimental analysis. To understand the mode of binding and blocking, and to hopefully identify the binding pocket, an initial docking study was performed for all of the ligands reported by Hong et al (25) as having significant binding affinity with the open channel monomer. We tested whether our open- and closed-state models could explain the state-dependence of the binding of the guanidine analogue 2-guanidobenzimidazole (2-GBI) from the cytoplasmic face of the channel. A combination of free-energy simulations and molecular docking established the binding affinity of this ligand to the open state of the proton channel.
of functional Hv1 as shown by established fluorescence based proton flux. To understand Hv1 gating, we have developed a simple biochemical preparation of the S4-containing voltage sensing domains (VSD). As a way to functional conduction of proton. Hv1 carries out this function on the structural blue-side of the membrane only when the channel is open. Here, we examine the apparent binding affinities of a series of 2GBI derivatives on Hv1 channels mutated at positions located in the core of the VSD and apply mutant cycle analysis to determine the most likely interactions between channel and inhibitor. We identify four residues involved in 2GBI binding and determine the orientation of the blocker inside the VSD. Our data provide a simple explanation for the very large difference in binding affinity between 2GBI and the related compound 2-guanidinobenzoxazole (2GBOZ), and suggest ways to design more effective Hv1 inhibitors. We also investigate the molecular characteristics that allow guanidine derivatives to reach the binding site when added from the extracellular side of the membrane so that they can be used to block endogenous proton channels under physiological conditions. This work is supported by NIH -National Institute of General Medical Sciences, grant R01GM098973.

**3766-Pos Board B494**

Electromechanical Coupling in Gating of the Hv1 Voltage Sensor is Revealed by Resting-State Currents in an S4 Arg to His Mutation (R205H) I. Scott Ramsey, Aaron L. Randolph.

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In the tetrameric Shaker voltage-gated K⁺ channel, the gating of a resting-state proton currents in the S4 mutant R362H reports a conformational change associated with voltage sensor (VS) activation (Starace et al., 2004). Here we show that the analogous R205H mutation in the Hv1 proton channel mediates a novel H⁺ ‘shuttle’ conductance (GSH) that is gated off by membrane depolarization. The similarity of GSH in Hv1 R205H to the channel-like Shaker R362H suggests that GSH gating can be used to measure VS activation in Hv1. The intrinsic or ‘aquous’ H⁺ conductance (Gaq) in Hv1 R205H occurs mainly at more positive voltages than GSH, producing a distinctive U-shaped G-V relation. The separation of GSH from Gaq gating along the voltage axis implies that initial voltage sensor activation precedes the opening of Gaq in Hv1. Changes in the transmembrane pH gradient that are known to shift the apparent voltage dependence of Gaq also affect GSH, indicating that pH-dependent gating occurs early in the Hv1 activation pathway. Second-site mutations of an S3 acidic residue (D185) that is selectively conserved in Hv1 orthologues shift the voltage dependence of Gaq gating, but do not alter GSH gating. D185 mutations are thus interpreted to perturb coupling between VS activation and the opening of Gaq. Another second-site mutation that results in voltage-dependent block of Gaq (R205H-N214R) does not affect the voltage dependence of either GSH or Gaq gating. The existence of VS-pore coupling in Hv1, which lacks a canonical pore domain, demonstrates that electromechanical coupling is not exclusive to tetrameric voltage-gated cation channels.

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Forming Conformational Rearrangements in a Novel Voltage-Sensing Protein


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Through bioinformatic searches, we have identified a protein coded by the C15orf27 gene that we named NVS (Novel Voltage Sensor). NVS contains 531 residues and consists of 3 parts: an S1-S4 domain, a 90 residue N-terminus and a 307 residue C-terminus, both of which are predicted to be intracellular. The most critical residues found in S1-S4 domains of other voltage sensors are conserved in NVS, including 3 Arg and a Lys in the S4 helix, 4 conserved acidic residues in S1-S3 and the charge-transfer Phe in S2. In addition, the C-terminus contains a conserved acidic residue. A working hypothesis is that NVS functions as a voltage sensor that couples to intracellular signaling pathways (as yet undefined) or interacts with Hv1 to form hetero-oligomers through the C-terminal coiled-coil domain. In the present study we used site-specific voltage-clamp fluorometry to look for evidence that NVS may undergo conformational rearrangements in response to changes in membrane potential. We identified several positions on the S3-S4 loop where introducing an unlabeled Cys residues produced changes in fluorescence as a function of membrane potential. Several positions give complex fluorescence responses, starting with a rapid increase in fluorescence followed by slower decrease in fluorescence. Additionally we applied fluorescence quenchers extracellularly and examined the voltage dependence of quenching. Our results show that depolarization increases fluorescence quenching, suggesting the quencher has increased access to the fluorophore at positive membrane potentials. Taken together, our results support the hypothesis that NVS undergoes a conformational change in responses to membrane depolarization, and we are currently investigating the oligomeric state of NVS testing whether it can form heteromeric complexes with Hv1.

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Long Alpha-Helices Projecting from the Membrane as the Dimer Interface in the Voltage-Gated H⁺ Channel

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The voltage-gated H⁺ channel (Hv) is a H⁺ permeable voltage-sensor domain (VSD) protein that consists of four transmembrane segments (S1-S4). Hv assembles as a dimeric channel and two transmembrane channel domains function cooperatively; which is mediated by the coiled-coil assembly domain in the cytoplasmic C-terminus. However, the structural basis of the inter-domain interactions remains unknown. Here, we provide a picture of the dimer configuration based on the analyses of interactions among two VSDs and a coiled-coil domain. Systematic mutations of the linker region between S4 of VSD and the coiled-coil showed that the channel gating was altered in the helical periodicity with the linker length, demonstrating that two domains are linked by helices. Cross-linking analyses revealed that the two S4 helices were situated closely in the dimeric channel. Thus, continuous helices stretching from the transmembrane to the cytoplasmic region in the dimeric interface regulate the channel activation in the Hv dimer.

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Ph Sensitivity of Voltage Sensing Domain Relaxation

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Voltage sensing domain (VSD) relaxation is a process involving a voltage-independent transition from the activated state into the relaxed state. This process has been shown to shift the voltage dependence for sensing charge movement (sensing current) in the voltage sensitive phosphatase (VSP) known as Ci-VSP and to slow down the deactivation of potassium currents in Shaker and Kv1.2. Given the effect of VSD relaxation on the dynamics of these voltage sensitive proteins, it is thought that the relaxed and the active states are comprised of different set of conformations. Thus, it is possible that these states are sensitive to different physiological parameters. Here, it is shown that VSD activation and relaxation in Ci-VSP display different sensitivity to external pH.