Voltage-gated K Channels-Gating II

1626-Pos
Does the Linker in Ci-VSP Function as a PI(4,5)P2 Binding Domain?
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Proteins are often composed of multiple domains which confer specialized functions to the full length protein. The voltage sensing domain (VSD) from voltage-gated ion channels is one such domain. Originally thought to be restricted to ion channels, we now know that it can also confer voltage control to enzymes. The Ciona intestinalis voltage sensor-containing phosphatase (Ci-VSP) is composed of the unique combination of a VSD coupled to a lipid phosphatase. The coupling between these two domains is intriguing since it means that a modular VSD can control two very different effectors, a pore or an enzyme. The original characterization of Ci-VSP suggested that the inter-domain linker played a role in activating the protein while more recent work has shown evidence supporting the hypothesis that the inter-domain linker functions as a phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) binding domain (PBM) (1,2). This function is similar to that of the N-terminus of PTEN, a well-known lipid phosphatase that shares a high degree of homology to Ci-VSP. We chose disease-causing mutations from PTEN, introduced them into Ci-VSP and probed Ci-VSP's voltage-dependent movements and phosphatase activity using voltage clamp fluorometry, two electrode voltage clamp, and biochemical methods. We find that the linker composition is critical for activity where single amino acid mutations either decrease or eliminate activity. We also found that upon PI(4,5)P2 depletion, the voltage dependent motions of the VSD were altered when the linker was intact, but not when the linker was mutated. Our data suggests that the linker both couples to the two domains and also serves as a PBM, regulating via its interaction with PI(4,5)P2.
1. Murata, Y., et al, (2005) Nature 435, 1239-1243
2. Villalba-Galea, C. A., et al, (2009) J Gen Physiol 134, 5-14

1627-Pos
Sensing Charges of Ci-VSP
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The *Ciona intestinalis* Voltage Sensor-containing Phosphatase (Ci-VSP) is a member of the Voltage Sensitive Phosphatase family that exhibits membrane potential-controlled enzymatic activity. Alignments of the amino acid sequence of Ci-VSP against Voltage Gated Channels (VGC) indicate that its Voltage Sensing Domain (VSD) is formed by four transmembrane segments. The putative fourth segment (S4) of Ci-VSP extends between the arginine (R) in position 217 and the glutamine (Q) in position 239, containing five arginines, which might be the voltage sensing charges. Although it has been shown that R229 and R232 are critical for voltage sensing in Ci-VSP (Murata *et al.*, 2005), the role of the remaining charges is still unclear. To address this issue, we have performed a partial Histidine Scanning of the S4 of Ci-VSP, following the paradigm established for the VGC *Shaker* (Starace and Bezanilla, 2004). The voltage dependence of the sensing current of the R217H mutant was modulated by pH. Decreasing the external pH shifted the Q-V curve towards positive potentials, while a pH increase had the converse effect, consistent with the finding that neutralizing R217 (R217Q) produces a negative shift of the voltage dependence of Ci-VSP (Kohout *et al.*, 2008). However, the total net charge of R217H did not change with pH, indicating that R217 does not participate in sensing the membrane potential. When the second arginine is replaced by histidine, the resultant mutant (R223H) exhibits a voltage dependent proton current which closes at positive potentials, resembling the current recorded from *Shaker*-W434F with its first gating charge replaced by histidine (R362H). This result strongly suggests that R223 has access to both the intra- and the extracellular media depending on voltage. Taken together, our results indicate that R223 is the most extracellularly located sensing charge of the Ci-VSP S4 segment. (Support: NIHGM030376)

1628-Pos
Modular Nature of the Main Domains in Voltage Sensitive Phosphatases
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Voltage Sensitive Phosphatases (VSPs) constitute a family of enzymes controlled by membrane potential. Its members have a Voltage Sensing Domain (VSD) in the N-terminus and a Phosphatase Domain (PD) in their C-terminus, both connected by a Phospholipid Binding Motif (PBM). Remarkably, N- and C-terminal domains display high homology to two different types of proteins. The VSD of VSPs resembles the VSD of voltage gated channels; while their PD shares a striking homology to the tumor suppressor phosphoinositide phosphatase PTEN. This feature of VSPs makes them look like natural chimeras. Recently, it has been shown that the catalytic activity of Ci-VSP, a member of the VSP family, depends on the binding of the PBM onto the membrane, which is, in turn, controlled by the membrane potential-driven movement of the VSD. For PTEN, it is known that the binding of PTEN onto the plasma membrane mediated by its N-terminus is *sine qua non* for enzymatic activity.Based on this similarity, we built a chimera by replacing the PD of Ci-VSP by PTEN. This chimera, Ci-VSPTEN, was successfully expressed in *Xenopus* oocytes and displayed sensing currents resembling those observed in Ci-VSP. As for its enzymatic characteristics, Ci-VSPTEN was expressed in CHO cells and its activity tested by measuring membrane association of GFP-tagged phosphoinositide sensors by TIRF microscopy. Depolarization-induced decline in membrane fluorescence indicated that Ci-VSPTEN has voltage dependent $PI(3,4,5)P_3$ 3'-phosphatase activity. Because the binding of the PBM induces an allosteric activation of PTEN, these observations strongly support the idea that the binding of the PBM onto the membrane is a key step in the activation of Ci-VSP. In a broader view, these results show that the VSD and the PD of Ci-VSP, and presumably other VSPs, are naturally modular. (Support: GM030376, DFG OL240/2)

1629-Pos
Two Structurally Distinct Pathways for the Voltage-Sensing S4 Helices
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In voltage-dependent ion channels, the movement of the voltage-sensing S4 helices produces gating currents. The charge displaced as a function of the membrane potential (Q-V) is well described by a sequential two-state Boltzmann relation, indicating that there are at least two steps of gating charge movement from their Resting state to the Active state. In addition, it has been shown that at a maintained positive potential, the S4 helices of voltage-gated Na, Ca and K channels and the voltage sensitive phosphatase Ci-VSP, undergo a slower secondary conformational transition stabilizing the sensor in a Relaxed (inactivated) state. From the Relaxed state, the Q-V relation exhibits a strong shift towards negative potentials when compared to the Q-V relation measured from the resting state. We engineered gating perturbations in the Shaker potassium channel, by substituting specific aromatic residues in positions spatially close to the S4. One of these mutants, in position I241 of S1, part of the hydrophobic plug of the voltage sensor, when mutated to tryptophan (I241W), produces a strong split in the Q-V when measured from the resting state. By labeling M356C with TMRM we also find the same split in the fluorescence-voltage curve. We propose that the presence of the tryptophan in the 241 position favors an interaction with one of the positively-charged arginines along the S4, thus stabilizing a fleeting intermediate state in the gating pathway. However, in the I241W mutant, the split in the Q-V almost disappears when the gating currents are measured from the relaxed state and the same result is seen with the fluorescence-voltage curve. This result and the effect of other tryptophan perturbations near the S4 segment strongly support the existence of two structurally distinct gating pathways for the movement of the S4 helices. Supported: NIHGM030376.

1630-Pos
Zinc Inhibition of Monomeric and Dimeric Proton Channels Suggests Cooperative Gating
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Voltage gated proton channels are strongly inhibited by Zn^{2+}, which binds to His residues. However, in a molecular model based on similarity between proton channels and the voltage sensing domain of K^+ channels, the two externally accessible His are too far apart to coordinate Zn^{2+}. In view of the proton channel existing as a dimer, we hypothesize that a high affinity Zn^{2+} binding site is created at the dimer interface by His residues from both monomers. Consistent with this idea, Zn^{2+} effects are weaker on monomeric channels. In addition, monomeric channels opened exponentially, and dimeric channels opened sigmoidally, suggesting a Hodgkin-Huxley type process in which multiple