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Current Opinion in Structural Biology

The influence of lipids on voltage-gated ion channels

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Voltage-gated ion channels are responsible for transmitting electrochemical signals in both excitable and non-excitable cells. Structural studies of voltage-gated potassium and sodium channels by X-ray crystallography have revealed atomic details on their voltage-sensor domains (VSDs) and pore domains, and were put in context of disparate mechanistic views on the voltage-driven conformational changes in these proteins. Functional investigation of voltage-gated channels in membranes, however, showcased a mechanism of lipid-dependent gating for voltage-gated channels, suggesting that the lipids play an indispensible and critical role in the proper gating of many of these channels. Structure determination of membrane-embedded voltage-gated ion channels appears to be the next frontier in fully addressing the mechanism by which the VSDs control channel opening. Currently electron crystallography is the only structural biology method in which a membrane protein of interest is crystallized within a complete lipid-bilayer mimicking the native environment of a biological membrane. At a sufficiently high resolution, an electron crystallographic structure could reveal lipids, the channel and their mutual interactions at the atomic level. Electron crystallography is therefore a promising avenue toward understanding how lipids modulate channel activation through close association with the VSDs.

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Current Opinion in Structural Biology 2012, **22**:529–536

This review comes from a themed issue on **Membranes**

Edited by **Tamir Gonen** and **Gabriel Waksman**

For a complete overview see the Issue and the Editorial

Available online 5th May 2012

0959-440X/$ – see front matter,

http://dx.doi.org/10.1016/j.sbi.2012.03.009

Introduction

The superfamily of voltage-gated ion channels consists of integral membrane proteins that contain four voltage-sensor domains (VSDs) and a central ion-conducting pore domain [1,2]. Members of this superfamily have been identified in all cells, and play critical roles in a variety of cellular physiology, from muscle contraction to neuronal activity to T cell activation in inflammatory (immune) response. Voltage-gated ion channels are divided into two broad groups: the hyperpolarization-activated and the depolarization-activated channels. Biophysical studies have shown that the VSDs in these two groups work in a similar way [3]. In both cases, the VSDs undergo significant conformational changes driven by electrical energy. These conformational changes are coupled to the pore domain, to close or open the ion channel in response to electrical stimuli [3–6]. The hyperpolarization-driven state of the VSD is called the 'DOWN' conformation (also resting or closed), and the depolarization-stabilized state is named the 'UP' conformation (also activated or open) [7••]. Understanding the structural basis for the voltage sensor function in membranes not only is fundamentally important for revealing the exquisite electrical control of protein structure, but also will forge the foundation for developing new therapeutical strategy for human diseases caused by the dysfunction of these channels [8,9].

All known VSDs are made of four helical transmembrane segments (S1–S4) with highly conserved charged residues on the second (S2) and fourth (S4) helices. During voltage-dependent gating, the charged residues on S4 translocate from one side of the transmembrane electric field to the other while the VSDs switch their conformations and couple the charge movement to the opening and closing of the channel pore [6,10,11,12]. Within each VSD there are water-accessible crevices from either side of the membrane [13]. The transmembrane electric field penetrates into these crevices to establish a certain degree of electric focusing [14]. In the UP conformation the gating charges (mainly on S4) are in the extracellular crevice and in the DOWN conformation in the intracellular one. Switching between the UP and DOWN conformations requires a significant energy input from the electric field, ~7.5 kcal/mol per VSD [15–18].

While a number of different structures of voltage-gated ion channels have been determined it remains unclear how the VSDs couple the charge movement to the pore opening and closing [6]. Three different groups of mechanistic models have been proposed and experimentally supported: first, the voltage sensor paddle model; second, the transporter-like model; third, the helical-translocation/helical-screw model. The voltage sensor paddle model argues for a 15–20 Å motion of the paddle (the helix-loop-helix motif composed of the S3b, the S3S4 linker and the extracellular half of S4) along the membrane normal [19•,20]. It does not exclude lateral motion or rotation of the S4, nor does it specify how the other

parts of the VSD adjust to accommodate the major structural changes in membranes. The transporter-like model stemmed from intramolecular distance measurements, and argues that the toggling of the fixed gating charges from the outward-facing to the inward-facing state needs a small-scale (4–6 Å or less) vertical movement of S4, traversing a narrow hydrophobic septum (plug) in the gating pore [21•,22•,23]. The transmembrane electric field is thought to be highly focused across such a short distance [14,18]. The third group of models proposed a vertical displacement of the S4 inside the gating pore with varying distances, and the helical screw model adds a ~180° rotation of S4 in order to reorient the charged residues on S4 [17,24].

Besides the uncertainty on the VSD's mode of action, there is mounting evidence that lipids influence the structural stability and function of the VSDs and therefore the opening and closing of the channel pore. Functional studies of voltage-gated channels in membranes highlight a lipid-dependent gating mechanism. Studies indicate that without any change in transmembrane voltage, manipulating the lipid composition in a membrane switches the VSDs between the DOWN and UP conformations [7••]. This and other studies suggest that the lipids exert strong gating effects on the voltage-gated channels [7••,25••,26••,27••].

In this review we highlight some of the key structural features of voltage-gated ion channels and discuss how lipids were shown to influence channel structure and function. We then highlight electron crystallography as a structural biology technique that could provide information about how the lipids interact with the VSDs to affect channel gating.

An overview of voltage-gated ion channel structures

Structures of four channels that contain VSDs or VSD-like domains have been determined to date: KvAP, Kv1.2 (and its chimera), MlotiK and NavAb [28••,29••,30••,35••]. MlotiK is a ligand-gated channel with VSD-like domains, but has not been found to be functional yet. KvAP, Kv1.2 and NavAb are functional voltage-gated channels. The four structures confirm the common topology that was previously proposed for the superfamily of voltage-gated ion channel. The channels are tetrameric assemblies (Figure 1a,b). The first four helices in each monomer constitute the VSD, and the sequence between helices 5 (S5) and 6 (S6) forms the pore domain. The loop connecting S5 and S6 forms the ion selectivity filter. Four pore domains (S5S6 from each monomer) assemble together around the 4-fold axis to create an ion-conducting pore.

The conformation of the VSD from the full-length KvAP is significantly different from those in the Kv1.2 and its chimera, MlotiK and NavAb (Figure 1a,b). It is fully splayed with helices S1 and S2 wrapped along the side of the pore (Figure 1b, red and green helices). In other three cases the VSDs are folded into a compact 4-helix bundle neatly tucked to the lateral side of the pore (Figure 1a). The structure of the isolated KvAP VSD resembles closely that of the VSDs from Kv1.2 (as well as the Kv1.2/2.1 chimera; Figure 1c, overlay). S1, S2 and the top part of S4 overlay very well, but the position of S3b is different, displaying approx a 90° rotation between the two VSDs. Moreover, the positions of S4 arginines differ between KvAP and Kv1.2 as if they are shifted down by one register in the latter (Figure 1c), and the intracellular half of the S4 in the Kv1.2 chimera structure shows a short 3_{10} helix, which is absent in the same location of the other three VSDs.

The VSD structures of MlotiK and NavAb exhibit good overall fit among all four helices (Figure 1d). MlotiK has only one conserved charge in its S4. Its VSDs appear to be in a permissive 'UP' state, leaving the control of the channel pore to the intracellular nucleotide binding domains. In both channels, the N-terminal halves of their S4 segments harbor a short 3_{10} helix. Charged residues along the 3_{10} helix face the same side, which has implications for sliding the S4 across a newly named charge transfer center without much rotation [29••,30••,31•].

Although the conformational change that ensues in the VSDs in response to voltage is not clear, what is agreed upon is that the movement in the VSD helices is tightly coupled to the pore opening/closing. Exactly how the VSD and pore are coupled is not entirely clear. Two different coupling schemes were proposed for Kv1.2 and NavAb [28••,29••]. The first is based on the observed interaction between the S4–S5 linker and the intracellular half of S6. It was suggested that the sliding motion of S4 pushes the S4S5 linker intracellularly as well as the intracellular end of S6, leading to pore closure at a conserved PVP motif [28••]. This coupling scheme gained support from both structural and functional studies [32]. The second coupling scheme is based entirely on structural comparison between Kv1.2/2.1 chimera and NavAb, whose pore domains are in the open and closed states, respectively. It was suggested that wobbling the VSD could lead to a lateral rotation of the S4S5 linker, which in turn exerts a torque on the S5 and S6 to gate the pore with only a limited vertical movement of the S4. In previous biophysical analysis, the first closing step was found to bear weak voltage-dependence (0.5–1.0 e_0), which seemingly agrees with the small adjustment of the VSD to close the pore even though it is unclear what contributes to the small charge displacement [33,34].

While the available structures helped tremendously in understanding voltage-gated ion channels, there are still