The voltage-sensor structure in a voltage-gated channel

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A recent electron paramagnetic resonance study of KvAP, a prokaryotic voltage-gated channel, in its lipid native environment has revealed the location of the transmembrane segments, the connecting loops and the relative position of the voltage-sensing charges. The results confirm that the previously reported crystal structure does not represent a native conformation and give us structural constraints that will help in determining the molecular structure of the voltage sensor.

The nerve impulse and voltage-dependent channels

In the generation of a nerve impulse, the membrane potential of the axon undergoes a rapid change from its negative resting potential to a positive potential followed by a repolarization to its resting level. These changes are produced by voltage-dependent conductances described in a classical series of papers by Hodgkin and Huxley [1]. Today, we know that these conductances are the result of the contribution of billions of voltage-dependent ion channels, which are specialized membrane proteins with differing ion selectivity and kinetics depending on their type. Of particular interest in the generation of the action potential are sodium channels, which are responsible for the upstroke of the action potential, and potassium channels, which repolarize the membrane towards its resting level. Voltage-gated channels have four subunits or domains each containing six transmembrane (TM) segments, the first four of which are considered to function as the voltage sensor and the last two of which form the segments, the first four of which are considered to function or domains each containing six transmembrane (TM) segments, the first four of which are considered to function as the voltage sensor and the last two of which form the conducting pore of the channel, giving origin to the voltage dependence of the conductance. The movement of these charged residues generates the transient gating current [5], and the opening of one channel is associated with a total charge of 13 elementary charges (e0) across the electric field [6].

The crystal structures and the EPR study

Until recently, the location and movement of the gating charges was not known with any certainty because there was no crystal structure available for voltage-gated channels. The pioneering work of the MacKinnon group presented the first crystal structure of KvAP, a voltage-gated channel from the archea Aeropirum pernix [7]. The crystal was obtained in detergent and in the presence of Fab fragments, and now there is wide consensus that it is distorted and does not represent a conformation found in the native bilayer. Even with the crystal structure of the first four TM segments (i.e. without the pore region), which was presented by the same group [7], the crystals available today do not address the structural issues of how voltage-gated channels are assembled and, to a lesser extent, how they operate. A recent paper by Cuello et al. [8] of the Perozo group has approached the study of the structure of KvAP in its open-inactivated conformation in a bilayer environment using electron paramagnetic resonance (EPR). In this work, ~140 mutant channels were created, each of which with a cysteine residue substituted in the voltage-sensor region, which was then reacted with a cysteine-reactive EPR probe. The spectrum of each mutant revealed three basic parameters: (i) the mobility of the probe attached to each residue, (ii) the exposure to lipid using oxygen and (iii) the exposure to water environment using nickel-ethylenediamine-N,N’-diacetic acid (NiEdda). These measurements enabled the authors to infer the relative positions of the residues and whether a particular residue was in contact with the lipid bilayer, the internal or external solutions, or whether it was immobilized within the protein core. The first clear result was that the linkers between the TM segments were highly mobile and exposed to water – in particular, this applied to the S1–S2 loop (Figure 1). This result confirms the classical view of the arrangement of the TM segments, but it is incompatible with the crystal structure of the full KvAP channel. Not surprisingly, it is also incompatible with the proposed structure of the voltage sensor of the ‘original’ paddle model that was built by using the lipid-buried S2 and S1–S2 loops [9] (Figure 1a). It should be noted that the paddle model has changed from its original inception [9]; it now describes the S1 and S2 as TM segments [10].

The location of the S4 segment and its gating charges

The S4 segment carries most of the gating charge of the voltage sensor [3,4], therefore, its location is crucial to our understanding of how the voltage sensor might operate. In

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The paddle model as presented by Jiang et al. [9] down and extracellular is up. Segments S1–S4 are color-coded as follows: S1, the S4 segment, only the first and fourth charges are shown. the transition region of the lipid bilayer. Because most of the charges are covered by the bilayer and into the protein core, with the most extracellular charge exposed to water.

Figure 1. Schematic representations of the ‘original’ paddle model [9] and the topological model of Cuello et al. [8] in the open-inactivated state. Intracellular is down and extracellular is up. Segments S1–S4 are color-coded as follows: S1, white; S2, pink; S3, red; S4, light blue; S5–S6 pore region, green. Only one of the four voltage sensors is represented, the rest of the channel is shown in orange. (a) The paddle model as presented by Jiang et al. [9]. In this model the S1 and S2 segments are embedded in the bilayer and the S4 charges (dark blue circles) are pointing into the bilayer. (b) Topology as proposed by Cuello et al. [8]. S1 and S2 segments are transmembrane and S1 is surrounded by the rest of the protein. The S4 segment is located in the periphery but its charges are pointing away from the bilayer and into the protein core, with the most extracellular charge exposed to the transition region of the lipid bilayer. Because most of the charges are covered by the S4 segment, only the first and fourth charges are shown.

The location of the S1 TM segment
An unexpected result from the EPR study of Cuello et al. [8] was the accessibility and mobility of S1 in KvAP. It is clear from their results that the S1 segment is surrounded by protein (Figure 1b). This is in contrast to the inference obtained by perturbation analysis done in eukaryotic channels, which locates S1 in the periphery of the channel that is making contact with the lipid bilayer [14,15]. Note that the residues that are tolerant of being replaced by tryptophan are also poorly conserved in genomic analysis. There are several possible explanations for this discrepancy. Perturbation analysis studies the effect of residue replacement on the functional properties of the channel. Thus, a region that has no impact on the function after replacement is considered non-specific and, in consequence, is normally attributed to exposure to the lipid bilayer. However, this might not be case if the region in question is in contact with a face of the protein that does not have an important influence in channel function. Perturbation analysis studies the effect of residue replacement on the functional properties of the channel. Thus, a region that has no impact on the function after replacement is considered non-specific and, in consequence, is normally attributed to exposure to the lipid bilayer. However, this might not be case if the region in question is in contact with a face of the protein that does not have an important influence in channel function. Perturbation analysis studies the effect of residue replacement on the functional properties of the channel. Thus, a region that has no impact on the function after replacement is considered non-specific and, in consequence, is normally attributed to exposure to the lipid bilayer. However, this might not be case if the region in question is in contact with a face of the protein that does not have an important influence in channel function.
a conformational change that is not associated with charge displacement in the electric field. Furthermore, electrophysiological experiments on KvAP show that prolonged exposure to zero membrane potential causes the channel to enter a deep inactivated state and that it takes several minutes at hyperpolarized potential to recover activity (which is immensely long on a molecular timescale). In this regard, it should be noted that this conformational change induced by prolonged depolarization might affect other TM domains including the position of the S4 segment. Finally, another possibility is that KvAP differs from eukaryotic K⁺ channels. For example, in eukaryotic K⁺ channels, there is an intracellular domain – the T1 domain – [19] that is not present in KvAP. This domain precedes the S1 segment by a few residues, thus, it could force S1 to be located in the periphery. These possibilities can be tested experimentally by performing perturbation analysis in KvAP or by adding the T1 domain to KvAP. If indeed the location of S1 differs between channel types, it is possible that the arrangement of TM segments inferred by the EPR analysis might not apply directly to eukaryotic channels.

Concluding remarks and outlook
The arrangement of the TM segments of voltage-gated channels, which was not determined by the crystal structures of KvAP, have now been better defined by EPR studies on the same channel, at least for the open-inactivated state of the channel. Important conclusions are (i) that S1, S2, S3 and S4 are helical TM segments, (ii) that the loops are solvent-exposed, and (iii) that the charges carried by S4 are not exposed to the lipids (Figure 1b). Contrary to what was recently stated [10], the center of the controversy between the paddle model and the traditional models did not hinge as much on the location of S4, but on whether or not its charges were exposed to the bilayer. The EPR results provide incontrovertible evidence that, although S4 is in the periphery of the KvAP channel protein, the gating charges are protected from the lipid hydrocarbon and partially exposed to the external solution. This result is at odds with the concept of a hydrophobic cation, which is central to the paddle model. The other controversial point concerns the magnitude of the translation of S4 across the membrane upon channel activation: it is ~15–20 Å for the paddle model, whereas in some traditional models it does not exceed 5 Å. Because the EPR results are from just one conformation of KvAP, competing models of sensor operation cannot be excluded. However, the results are consistent with the available evidence from functional and spectroscopic studies that strongly suggest a focused electric field near the gating-charge location, thus requiring a small movement to carry 13 e₀ per channel [13,20–21].

The experiments of Cuello et al. [8] are a important step in understanding the topology of voltage-gated channels. They form the basis for future EPR studies performed at different membrane potentials, which are expected to establish the conformational rearrangements of the voltage sensor in response to changes in membrane potential.

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