Environment of the Gating Charges in the Kv1.2 *Shaker* Potassium Channel

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ABSTRACT Recently, the structure of the *Shaker* channel Kv1.2 has been determined at a 2.9-Å resolution. This opens new possibilities in deciphering the mechanism underlying the function of voltage-gated potassium (Kv) channels. Molecular dynamics simulations of the channel, embedded in a membrane environment show that the channel is in its open state and that the gating charges carried by S4 are exposed to the solvent. The hydrated environment of S4 favors a local collapse of the electrostatic potential, which generates high electric-field gradients around the arginine gating charges. Comparison to experiments suggests furthermore that activation of the channel requires mainly a lateral displacement of S4. Overall, the results agree with the transporter model devised for Kv channels from electrophysiology experiments, and provide a possible pathway for the mechanistic response to membrane depolarization.

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Three molecular models have been proposed so far for the activation of Kv channels (1). These models disagree, in particular, by the fashion in which the voltage-sensor and the pore domains are coupled. In the conventional model, S4 helices are buried in the protein and slide in a large piston-like motion (2–4). In the transporter model, a specific hydration of S4 shapes the electric field in the transmembrane domain region and small upward motion of S4 leads to the channel opening (5–8). The paddle model is based on the x-ray structure of the archeabacterial KvAP channel (9), in which the so-called voltage-sensor paddle undergoes a large upward movement. This model disagrees, however, with several experiments on eukaryotic channels (10–17). Furthermore, the very recent x-ray structure of the Kv1.2 *Shaker* channel (18) reveals that the paddle model does not describe the activation mechanism of this eukaryotic channel. In the Kv1.2 structure, S4 is perpendicular to the membrane in agreement with the classical view. With this structure at hand, it is still unclear how Kv channels function, and what possible conformational changes take place during activation.

Here we study, using molecular dynamics (MD) simulations, the molecular properties of the Kv1.2 *Shaker* channel embedded in a membrane environment considering as a framework the x-ray structure (cf. Fig. 1 and Supplementary Material). The MD simulation was performed at constant pressure (1 atm) and constant temperature (300 K) for 9 ns. Analysis of the pore volume highlights the conductive (open) state of the channel. The largest accessible volume of the conduction pathway occurs in the intermediate region between the T1 and the TM domains. The volume becomes then narrower in the region of the activation gate, where Val^{410} constitutes the major constraining element along the pathway. This residue has been suggested to constitute a hydrophobic gate obstructing the ion-conduction pathway in the closed state of the channel (19). For the present conformation, this gate delineates a pore of radius ~4.5 Å, e.g., large enough to allow ion translocation.

One major controversial issue in the literature concerns the environment of the gating charges (arginines in S4), especially their exposition to the solvent and to the lipid acyl-chains (20–22). Recent electron paramagnetic resonance measurements on KvAP show that Arg^{294}, Arg^{297} are, respectively, fully and partially exposed to the lipid whereas Arg^{300} and Arg^{303} are not (23). This is consistent with the accessibility to the lipid derived from the simulation (Fig. 2). Using NiEdda to probe exposure to water, Cuello et al. showed that, at the inverse of the top charges Arg^{294} and Arg^{297}, Arg^{300} and Arg^{303} are not accessible to NiEdda and are therefore buried in the protein (23). Simulations indicate, however, that while buried in the protein, e.g., protected from the lipid, Arg^{300} and Arg^{303} are in contact with extracellular water crevices. Despite inaccessibility of Arg^{300} and Arg^{303} to Niedda reagent, solvent accessibility of these gating charges cannot be excluded as previously mentioned by Cuello et al. (23). Indeed, the existence of water crevices in contact with Arg^{300} and Arg^{303} is expected given the ability of *Shaker* channels to behave as proton transporters and proton pores in depolarized potentials (5,8,20,24). Here, Arg^{303} bridges between intracellular and extracellular crevices (Fig. 2 *c*) in agreement with its involvement in proton conduction (24).

Note that MD results depend on how one initially packs the lipid/water around S4. One could have attempted to place a distorted lipid in the central cavity of the sensor domain (*arrow* in Fig. 2 *c*). We have, however, discarded such configuration as it disagrees with the electron paramagnetic resonance measurements showing no accessibility of Arg^{300} and Arg^{303} to lipids.

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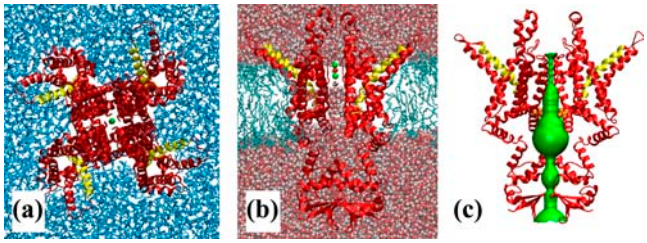

FIGURE 1 (*a*) Configuration of the macromolecular system containing the Kv1.2 channel (*red*, S4 in *yellow*) embedded in a POPC bilayer (*cyan*). (*b*) Lateral view. (*c*) Contour of the pore volume (*green*) along the ion conduction pathway (31). Val^{410} forms the constriction region of the channel's gate (*orange*).

The local environment (specific hydration) of the gating charges changes drastically the morphology of the electrostatic potential (EP). As shown in Fig. 3, the EP collapses around S4 helices. The hydrated environment of S4 favors a focused electric field around the arginines. This has been suggested to explain the exquisite electric sensitivity of Kv channels (20,25).

In summary the Kv1.2 structure corroborates several experiments. The channel is in an open state, but it is not clear how far its present conformation is from the physiologically membrane-bound state. It is not clear either how this structure differs from the closed state. In the *Shaker* B active state, cysteine pair mutations involving Ala^{291}-Phe^{348}, Arg^{294}-Phe^{348}, and Arg^{294}-Ala^{351} produce disulphide bridges (11,12,14) and those involving Val^{408}-His^{418} produce a metal bridge (19). Arg^{294}-Phe^{348} and Val^{408}-His^{418} distances in Kv1.2 (<9 Å) are consistent with the probed bridges (Fig. 4). In contrast, the C_β-atoms of Arg^{294}-Ala^{351} and Ala^{291}-Phe^{348} (~14 Å) are too far away to allow spontaneous formation of a disulphide bridge. These interacting pairs join the top region of segments S4 and S5 of adjacent subunits. This region is quite rigid as revealed by a root mean square deviation analysis, raising the possibility that S4 may be positioned too far from S5.

We are left with a key question: what conformational changes of S4 take place during activation? Several experiments indicate that in *Shaker* B, S4 undergoes a small (2–5 Å) vertical displacement (16,25,26). Very recently, it was shown that S4 does not translocate across the lipid bilayer (27,28). In contrast, using avidin binding to a biotinylated

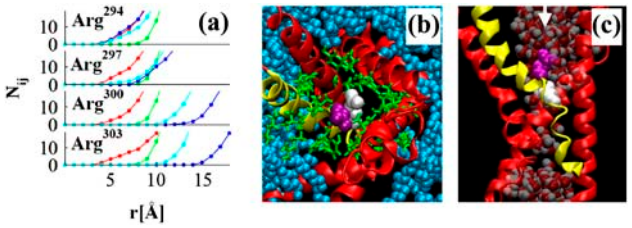

FIGURE 2 Environment of the gating charges. (*a*) Coordination number around arginines as a function of distance from the residue center for: water (*red*), protein but S4 (*green*), lipid acyl chains (*cyan*), and headgroups (*blue*). (*b*) Packing of lipids (*cyan*) and protein side chains (*green*) around Arg^{303} (*white*) and Arg^{300} (*purple*). (*c*) Water crevice around Arg^{300} and Arg^{303}.

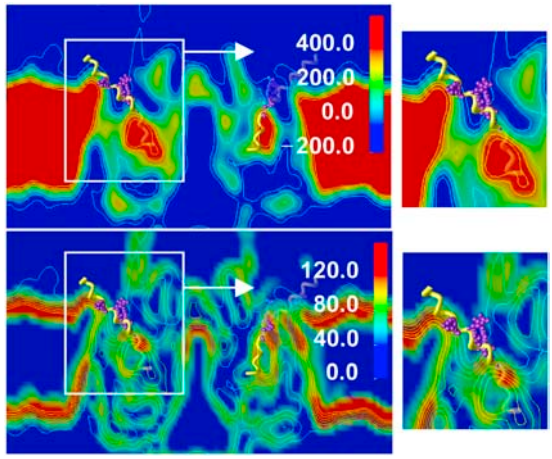

FIGURE 3 (*Top*) Two-dimensional electrostatic potential maps (mV) of the system. The channel is located in the center of the panel and for clarity only S4 helices (*yellow*) are drawn. Note the aqueous (*blue*) environment of the gating charges (ball-sticks in *purple*) carried by S4. Bottom: corresponding two-dimensional maps of the electrostatic field (mV A^{-1}).

channel, it was shown that S4 of the KvAP channel undergoes displacements of at least 15 Å under activation (9). Indeed, a displacement of S4 larger than the length of the biotin tether, e.g., ~10 Å, is required to expose or to protect biontinylated sites. Given the original KvAP structure in which S3-S4 forms a paddle, it was assumed that such displacement of S4 is vertical.

One possible interpretation to reconcile these experimental findings is an activation mechanism in which S4 tilt and/or displace laterally. To make our point we consider specific interactions between S4 and S5 identified in the resting (closed) state. An intersubunit disulphide bridge involving Ser^{289}-Glu^{350} was measured in *Shaker* B (13). Short distances were also identified for Val^{295}-Phe^{342} and Phe^{305}-Phe^{336} in the homologous KAT1 channel (29). For the present ''open'' Kv1.2 structure, these distances average, respectively, to 16, 20, and 14 Å.

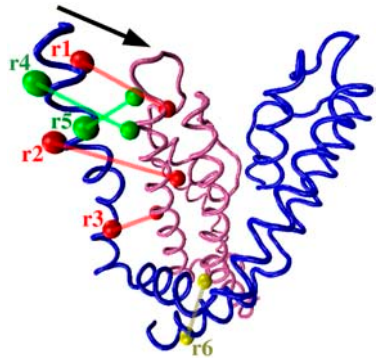

FIGURE 4 Representation of intersubunit distances between residues of S4 and S5 forming disulfide or metal bridges (c.f text) for the closed state (*red*), the open state (*green*), and both conformations (*yellow*) in Kv1.2. r1, Ser^{289}-Glu^{350}; r2, Val^{295}-Phe^{342}; r3, Phe^{305}-Phe^{336}; r4, Ala^{291}-Phe^{348}; r5, Arg^{294}-Ala^{351}; and r6, Val^{408}-His^{418}. For clarity, Arg^{294}-Phe^{348} is not shown.

Fig. 4 shows clearly that a lateral displacement of S4 toward S5 would shorten those distances to comply with the above experiments. We argue, therefore, based on this, that a possible route from the closed to the open state is a lateral displacement of S4 and not necessarily a large vertical displacement.

How such mechanism, involving a limited vertical displacement of S4, may explain the well-known gating current in Kv channels? In the transporter model, it is proposed that gating current results from changes in the dielectric environment during activation (27,28). Chanda et al. (27) used a molecular model of a *Shaker* channel embedded in a low dielectric membrane continuum that mimics a lipid bilayer. Gating charges of ~14e were measured considering a small (2 Å) vertical displacement of S4, when the local dielectric was distorted by protrusion of solvent crevices. Using an atomistic model of the *Shaker* B (30), we find indeed that the protrusion of water around S4 changes drastically the morphology of the local electrostatic potential during activation (cf. Supplementary Material).

In conclusion, the simulation studies of the Kv1.2 in a realistic membrane environment reveal many interesting features that appear to comply with the transporter model.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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