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Initial Response of the Potassium Channel Voltage Sensor to a Transmembrane Potential

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Abstract

Early transition events of the voltage sensor (VS) of Kv1.2 potassium channel embedded in a lipid membrane are triggered using full atomistic molecular dynamics (MD) simulations. When subject to an applied hyperpolarized transmembrane (TM) voltage, the VS undergoes conformational changes and reaches a stable kinetic intermediate state, β' , within 20 ns. The gating charge ($\sim 2e$) associated with this fast transition results mainly from salt-bridge rearrangements involving negative charges in S2 and S3 and all but the two top residues R²⁹⁴ and R²⁹⁷ of S4. Interactions of the latter with phospho-moieties of the lipid head groups appear to stabilize the kinetic state β'

In voltage-gated ion channels, the voltage-sensor (VS) domain senses the variations of the transmembrane (TM) voltage and triggers the conformational changes leading to the opening and closing of the pore.¹ During the voltage-sensing process, the displacement of the charges tethered to the VS gives rise to transient currents: the so-called gating currents. The time integral of this current is Q , the “gating charge” translocated across the membrane capacitance. Since the seminal work of Hodgkin and Huxley,² gating currents and gating charges have been measured for a variety of channels, as discussed extensively in recent reviews.^{3,4} For channels selective to potassium (Kv), the total gating charge Q is $\sim 13e$ (~ 3.25 per subunit).⁵ Kinetic models devised to describe the time course of gating currents are very diverse but all indicate that the gating process is complex, encompassing many *transitions* occurring at various characteristic time scales;^{3,4,6–8} the fastest component resolved so far, the “loose” charge occurs within 10 μ s.⁹ Identification of the charges participating in gating currents has long served to signal potential conformational changes of the VS. Several basic residues in the S4 segment and negative charges in S2 and S3 have been implicated in voltage sensing.^{10–14} Furthermore, salt bridges involving these charges appear to stabilize various gating *states* during activation.¹⁵

Accordingly, channel molecular models are testable against gating experiments to examine the robustness of the sensor geometry and gating motions.^{16–18} Models of the closed states of Kv channels^{19,20} have been hence proposed given the known open state of the Kv1.2 channel and considering the total gating charge Q .²¹ However, determination of the closed state topology does not allow full understanding of the gating mechanism. Key questions remain. What are the intermediate states? What is the pathway for charge gating? What elements of the structure can affect it? To begin to answer such questions, the dynamical, time-resolved gating processes need to be uncovered.

In this study, early *transition* events of the voltage sensor of the Kv1.2 potassium channel embedded in a lipid membrane are triggered using full atomistic molecular dynamics (MD)

simulations spanning 50 ns. We follow the VS conformational changes when the system is subject to an applied hyperpolarized TM voltage and measure the corresponding gating charges. The results show that the channel reached a stable kinetic intermediate state, β' , within 20 ns. The gating charge ($\sim 2e$) associated with this fast *transition* resulted mainly from a *zipper-like* salt-bridges rearrangement involving negative charges in S2 and S3 and all but the two top residues R²⁹⁴ and R²⁹⁷ of S4. Interactions of the latter with phospho-moieties of the lipid head groups appear to stabilize the intermediate kinetic state β' . The gating charge measured here ($\sim 0.5e$ per subunit) is about the same magnitude as the elementary charge movements estimated from measurements of gating current fluctuations^{7,22} and as the “loose” charges ($\sim 1e$ per subunit) indicating that the β' intermediate is of functional significance.

The Kv1.2 open (α) conformation has been thoroughly examined in previous simulations performed at depolarized potentials ($\Delta V = 0$ mV).²³ The VS domain (residues Pro¹⁵⁶ to Ile³¹⁶) was shown to remain stable within a 10 ns time scale, in agreement with other simulations.²⁴ In contrast to an earlier investigation, in which an electric field was applied to generate ΔV ,²⁵ here we used a method recently introduced²⁶. In this setup, one considers a bilayer surrounded by electrolyte baths (150 mM KCl) each terminated by an air/water interface (Fig. 1). As the bilayer behaves as a condenser, a finite transmembrane voltage ΔV may be imposed at $t = 0$ by creating a net charge imbalance q_0 between the intra- and extra-cellular electrolytes, i.e. displacing a given number of cations (depending on the system capacitance and total area) from the lower to the upper solution (c.f. SM for details). As shown below, such a protocol, allows for direct estimates of the gating charge Q .

Experimentally, the closed state is obtained within the ms time scale when ΔV is maintained at -100 mV.¹ Here, we applied ΔV about six times larger in order to promote a faster response of the system (Fig. 2). The channel was initially subject to a TM potential $\Delta V_{in} = -675$ mV. Given the size of the system, this necessitated displacing 4 K⁺ ions ($q_0 = 8$) from the lower to the upper electrolytes (cf. SM). Within 20 ns, the TM potential relaxed to $\Delta V_{fin} \approx -325$ mV due to protein charges rearrangements (Fig. 3).

In the α conformation, the top residues R²⁹⁴ and R²⁹⁷ (S4) interact with lipid-PO₄⁻ groups at the membrane-water interface and R³⁰⁰ is close to E¹⁸³ (S1). Deeper within the VS, R³⁰³, K³⁰⁶ and R³⁰⁹ are involved, respectively in salt bridges with E²²⁶ (S2) D²⁵⁹ (S3) and E²³⁶ (S2). During the α - β' transition, R³⁰⁰, R³⁰³, K³⁰⁶ and R³⁰⁹ undergo a concerted downward motion relative to acidic residues. They moved from external to internal-binding sites along the VS forming a different electrostatic network involving the pairs R³⁰⁰-E¹⁸³/E²²⁶, R³⁰³-D²⁵⁹, K³⁰⁶-E²³⁶ and R³⁰⁹-E¹⁵⁷.

The total rmsd between initial (α) and final (β') conformations of the VSD is 6.2 ± 0.7 Å. About 50% of this deviation is accounted-for by displacements along the membrane normal in which several S4 charged residues experienced side-chain rather than main-chain movements (Table 1). Note that the pore domain remained close to its initial state, *e.g.* open, and that most of the structural changes in the VS occurred during the first 20 ns (see SM).

As no free (K⁺, Cl⁻) ions translocated through the membrane during the MD run, the concomitant drop in ΔV (~ 350 mV) is mainly associated with rearrangement of tethered channel charges with respect to the membrane capacitor. In a closed circuit, *e.g.*, a patch clamp experiment, maintaining ΔV constant requires balancing the capacitive current and leads therefore to a measure of gating currents. In this simulation protocol, as the capacitance of the system remains constant (see SM), the voltage drop provides a *direct* measure of a gating charge Q , that is simply the measured change of ΔV . Evaluated as $Q = (q_0/2) (1 - (\Delta V_{fin}/\Delta V_{in}))$, it amounts here to $\sim 2e$.

For a structure undergoing a general α - β transition under ΔV , the gating charge Q may be linked^{17,24} to the variation of the free energy of the channel through $Q \cdot \Delta V = \Delta G(\beta, \Delta V) - \Delta G(\alpha, \Delta V)$. In each conformational state λ , $\Delta G(\lambda, \Delta V) = G(\lambda, \Delta V) - G(\lambda, 0) = \Delta V \cdot \sum q_i \cdot \delta_i^\lambda$ is the excess free energy due to the applied ΔV . Here, δ_i^λ is the so-called “electrical distance” that accounts for the degree of coupling between the local electrostatic potential $\phi_i^\lambda(r_i)$ and ΔV ^{16,22,27,28}: $\delta_i^\lambda \equiv \frac{\partial}{\partial V} (\phi_i^\lambda(r_i))$.

This formulation allows the identification of the specific molecular components that contribute to the gating charges (Fig 4). It shows that gating currents do not measure the displacement of charges across the membrane (i.e. across physical distances), but rather the product of the charges times the fraction of the membrane potential each traverses. Hence as previously noted, while some S4 residues change their position from an intra to extra-cellular accessible space,^{29,30} charge gating between transition states may involve limited displacements of the TM segments. The total gating charge per VS for the α - β' transition estimated from the MD trajectory (for details see SM) is $Q_{\alpha-\beta'} \sim -0.45e$ and is very consistent with the direct measure (Fig. 2). The electrical displacement during the transition $\delta^{\beta'} - \delta^\alpha$ is substantial for most of the VS charged residues and marginal in the loop regions. Importantly, several S2-S3 negatively charged residues - along with the S4 basic residues - contribute substantially to $Q_{\alpha-\beta'}$. These results are overall consistent with experiments¹⁰⁻¹⁵ showing that mutations of the S2-S3 and S4 residues influence the gating currents and with recent simulations of the VS domain.³¹

In conclusion, the present MD study reveals the motions in the VS triggered by a TM voltage (ΔV) indicative of the existence of an early, fast transition state in which, S4 charged residues move relative to rather static acidic amino acids tethered to other VS segments. The Kv channel is formed by a tetramer in which the voltage-sensors are de-correlated in the main structure (except through the tetramerization T1 intracellular domain). As such, the simulation results should in fact be viewed as four independent dynamical events in one single MD trajectory. Here, the alpha-beta transition was observed in three of the four VS subunits (movies depicting the conformational change in each of the VS subunits are provided in the SM). This suggests that the transition described in the manuscript is not a rare event. The excess free-energy difference involved in the α - β' transition was directly estimated from the energetic formulation described above (equation [2] in the SM). For a ΔV value in the range of physiological TM potentials, i.e., 100 mV (3.86 kT/e), the stabilization of the β' state relative to the α state ($\Delta G(\beta', \Delta V) - \Delta G(\alpha, \Delta V)$) amounts to ~ 1.5 kT. Note that this energy is related only to the transfer of protein charges across the TM electrical field and it does not reflect the overall-conformational free energy due to the α - β' transition.

Further analysis of the structure of the β' state indicates that the top S4 arginines R²⁹⁴ and R²⁹⁷ are in close contact with lipid-phosphate groups (cf. SM). The salt bridges interactions involved are likely to play a key role in stabilizing the VS domain in its open state in agreement with recent experiments³². This may explain why the expected following transition, involving probably screw motions on the top of S4³³ in order to bring R²⁹⁴ and R²⁹⁷ from the membrane-water interface near the next binding site E¹⁸³ (S1), requires longer time scales. Given suitable computational resources a time resolved atomistic description of such transitions, using the same protocols as in the present study, is within reach and should allow one to decipher further key steps and mechanisms underlying the exquisite sensitivity of Kv channels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Left: initial MD configuration of Kv1.2 embedded in a POPC bilayer (grey) and ~150 mM KCl solution (VS: green, S4: yellow, pore: red, T1 intracellular tetramerization domain: blue, K⁺: orange, Cl⁻: cyan). For clarity, 2 of 4 channel subunits are depicted and water is not shown. The MD simulation box is drawn in light blue. Note that the electrolytes extend up to the air/water interfaces. A net charge imbalance between the upper and lower electrolytes created by displacing cations from the lower to the upper bath induces a finite TM potential. Right: One of the four voltage-sensor domains highlighting the charged residues (blue) of the S4 helix: from top to bottom: R²⁹⁴ R²⁹⁷ R³⁰⁰ R³⁰³ K³⁰⁶ and R³⁰⁹.

Figure 2.

Top: Electrostatic potential profile $\Phi(z)$ along the bilayer normal (z) of the initial configuration. $\Phi(z)$ is derived directly from the MD simulation as a double integral of the charge distribution of all atoms averaged over the membrane plans, $\rho(z)$, as $\Phi(z) - \Phi(0) = -\epsilon_0^{-1} \int \int \rho(z'') dz'' dz'$. Here, as a reference Φ is set to zero in the upper electrolyte. The arrows indicate the approximate position of the lipid-water interfaces. Bottom: Relaxation of the TM potential during MD run. Every point corresponds to the TM voltage estimated from the average over a simulation-time window of ~ 1.2 ns - the error associated with each estimate is ± 50 mV. The ΔV reduction provides a direct estimates of the gating charge (see main text).

Figure 3. Initial (left) and final (right) conformations of a VS domain that are representative of respectively the α and β' states. The arrow highlights the collective motion of the lower charged residues of S4. Note the specific salt bridges between basic residues (blue) of S4 (yellow) and acidic residues (red) of segments S1, S2 and S3 (green).

Figure 4.

Top: Electrical distances δ_i^A for each TM residue in the α (black) and β' (orange) conformations and net charge per residue (green) along the Kv channel sequence (excluding the T1 domain). The position of the TM segments S1 to S6 and the P loop are indicated by arrows. δ_i^A were normalized assuming $\delta_i^A = 1$ and 0 for residues positioned respectively above 25 Å and below -25 Å from the bilayer centre. Bottom: Cumulative (yellow line) and per-residue (bars) gating charges. The S4-basic residues (blue) and the VS-negative residues (red: E157, E183, E226, E236 and D259) are highlighted. Contributions of the mobile loop residues (gray) were not included in the estimates of the cumulative charge.

Table 1

Root mean square deviations (rmsd) ([Å])

atoms	direction	VSD	S4	Pore
heavy	xyz	6.2 ± 0.7	3.4 ± 0.6	2.4 ± 0.2
	z	3.2 ± 0.5	1.5 ± 0.3	1.0 ± 0.2
backbone	xyz	2.9 ± 0.3	1.4 ± 0.2	1.0 ± 0.1
	z	1.5 ± 0.3	0.6 ± 0.1	0.4 ± 0.1

The rmsd values are estimates of the overall displacement of selected protein segments between the initial and final conformations of the channel and averaged over the four monomers.