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Effects of β -subunit on gating of a potassium ion channel: Molecular simulations of cardiac IKs activation

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Abstract

Dynamic conformational changes of ion channel proteins during activation gating determine their function as carriers of current. The relationship between these molecular movements and channel function over the physiological timescale of the action potential (AP) has not been fully established due to limitations of existing techniques. We constructed a library of possible cardiac IKs protein conformations and applied a combination of protein segmentation and energy linearization to study this relationship computationally. Simulations reproduced the effects of the beta-subunit (KCNE1) on the alpha-subunit (KCNQ1) dynamics and function, observed in experiments. Mechanistically, KCNE1 increased the probability of “visiting” conducting pore conformations on activation trajectories, thereby increasing IKs current. KCNE1 slowed IKs activation by impeding the voltage sensor (VS) movement and reducing its coupling to pore opening. Conformational changes along activation trajectories determined that the S4-S5 linker (S4S5L) plays an important role in these modulatory effects by KCNE1. Integration of these molecular structure-based IKs dynamics into a model of human cardiac ventricular myocyte, revealed that KCNQ1-KCNE1 interaction is essential for normal AP repolarization.

Keywords

Protein dynamics; Ion channels; Molecular simulation; Protein structure-function; Cardiac action potential

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A. Supplementary data
Supplementary material
Supplementary figures

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Introduction

Voltage-gated ion channels play an important role in generating the transmembrane current during the action potential (AP) in excitable tissues. Their dynamic properties at an atomic scale determine their function during the AP and, in turn, the AP properties and cell excitation. Understanding the relationship between the channel protein structure, its movement during gating and the emergent electrophysiological function is imperative to mechanism-based understanding of channelopathies and development of effective drug and molecular therapies. Crystallography, nuclear magnetic resonance spectroscopy (NMR) and other imaging techniques provided detailed information on ion channels molecular structure. However, the information is limited to protein conformations ‘frozen’ at particular membrane conditions and therefore difficult to relate to kinetic functional data from fluorescence, voltage clamp and other experimental recordings. Molecular dynamics (MD) simulations have provided insights on possible conformational changes of proteins at pico-seconds resolution. However, it required extreme non-physiological membrane voltages and weeks of computing time for a customized super-computer to simulate a single trajectory of conformational changes during 250 micro-seconds gating of a Kv1.2-Kv2.1 chimera [1]. Clearly, simulation of ion-channel gating at the atomistic scale, over the physiological time course of the AP (milliseconds for nerve and muscle; hundreds of milliseconds for the cardiac AP) is impossible with direct computing and commonly used MD methods.

In this article, we present a framework for computing conformational changes of an ion channel protein over the time scale of activation gating and determine mechanistic relationships between conformational changes and electrophysiological function. While it builds on our previous work [2][3][4][5], it advances it in the following major ways that are important for elucidating mechanisms: (1) Simulations are conducted on an atomistic scale, at a resolution of $<1.0\text{\AA}$ and with all molecular details included. (2) Tetrameric symmetry of the protein is not assumed. (3) Concerted movement between the four voltage sensors (VS) and cooperativity in activation gating are also not assumed. (4) A much higher number of degrees of freedom for molecular movement are considered in the computations. (5) KCNE1, the β subunit of IKs, is docked into the KCNQ1 structure and included explicitly in the simulations. This is accomplished by generating a large library of possible protein structures, which covers the conformational space that the ion channel can occupy during gating, and by dissecting the protein into structural segments and linearizing calculations of protein energy. This approach was applied to cardiac IKs for the following reasons: (1) IKs provides an important repolarizing current during the AP [6][7]; it plays a major role in AP adaptation to rate changes and is subject to β -adrenergic modulation [8]. (2) Mutations in IKs can lead to the arrhythmogenic long QT syndrome [9]. (3) Its pore-forming subunit (KCNQ1) is homologous to Kv1.2, for which a crystal structure has been resolved [10]. (4) The structure of the accessory subunit (KCNE1) has been determined using NMR [11]. (3) and (4) provided a starting template for constructing the IKs structures. The recently published cryo-EM structure of the KCNQ1/CaM complex [12] falls within the conformational space of possible structures in the library. (5) Experiments have shown that KCNE1 slows the activation of IKs and increases its macroscopic current [13] but there is no consensus about the structure-based mechanisms of these effects.

We used the computational approach to study mechanisms of KCNQ1-KCNE1 interactions and their effects on IKs activation and the human cardiac ventricular AP. We determined the role of specific segments and residues of KCNQ1 and KCNE1 during channel gating and identified key residues that influence the activation kinetics, with emphasis on the S4-S5 linker that couples the voltage sensor domain of the channel to its pore. The computational approach allowed us to integrate across scales, from the molecular structure of IKs to the whole-cell AP.

Materials and Methods

Achieving the objectives of this study required simulation of KCNQ1 and IKs activation based on their detailed molecular structures at atomistic scale. Direct computing of the gating movement over the relevant time scale (tens of milliseconds) is not possible. To overcome this difficulty, the strategy depicted in Figure 1A was employed. KCNQ1 and IKs structural libraries were constructed and constrained based on biophysical considerations and experimental data. The libraries contained more than 10^{30} conformations and covered the conformational space of possible structures during activation gating. The electrostatic energy of each possible conformation was computed using the Generalized Born Equation. For the energy computation, the protein was divided virtually into structural elements, based on functional role during activation. These included VSL - the VS domain coupled to the S4-S5 linker (S4S5L), the pore domain and KCNE1 (Figures 1 and Supplement Figures S1, S2). Energy was computed for each element and through superposition for the entire protein. This approach was motivated by the need to circumvent the (impossible) intense computing requirement for direct computation of the entire protein energy for all possible conformations. It also allowed us to determine the energy contributions from different elements of the protein and their interactions. To simulate the dynamics of KCNQ1 and IKs during activation, trajectories were generated in conformation space based on the energy landscape. General methodology procedures are described below; details are provided in the Supplement.

Generation of an initial structure for construction of the KCNQ1 and IKs structural libraries.

An initial structure of the KCNQ1 transmembrane domain was constructed as a homology model to the crystal structure of the Kv1.2-Kv2.1 chimera (PDB: 2R9R) [10] except for the S6 segment, which was based on the crystal structure of KcsA (PDB: 3EFF). The S6 of Kv1.2 contains a PVP motif, which is absent in both KcsA and KCNQ1. S6 was extended with Helix A, which together with Helix B were clamped by calmodulin (PDB: 4V0C) [14]. The distal Cterminus beyond Helix B and the N-terminus of KCNQ1 were not included, as there is no evidence for direct interactions between these segments and the VSL or the gating region of the pore domain. The transmembrane segment of KCNE1, with the residues extending its two termini, was constructed from the NMR-determined structure (PDB: 2K21) [11]. The initial structures for KCNQ1 and KCNE1 were inserted into a lipid membrane surrounded by water and then refined by molecular dynamics (MD) simulations [15]. The relevant software packages are listed in Supplement Table S1.

Constructing the structural libraries of KCNQ1 and IKs.

The KCNQ1 initial structure was aligned with a coordinate system referenced to the membrane (Figure 1B). Structural elements were determined by the secondary structure of the protein. Skeletons (backbone atoms) of secondary-structure segments (Figures 1B and S1A) were assumed to be rigid. Dihedral angles of KCNQ1 residues 258–259 (red in Figure S1A and red for all residues mentioned below) were varied to yield structures with different conformations of S4S5L relative to the N-terminus of S5 (Supplement Figure S3). S4 structures with different translations and rotations were generated for each S4S5L structure by varying dihedral angles of residues 244–246 (Supplement Figures S3 and S4). S3 was connected to S4 with a short 4-residue linker and had a very limited movement relative to S4 ($<1.0\text{\AA}$). The pore conformation library was constructed by first computing S6 structures with different dihedral angles of KCNQ1 residues 317–322 and 341–345 and then aligning the proximal C-terminal segments to each S6 (Supplement Figure S5). The final KCNQ1 library was determined after removal of structures with steric clashes (details in Supplement). To generate an IKs structural library, two transmembrane segments of KCNE1 were docked into the KCNQ1 protein in spaces suggested by the experiments listed in Table S2; their position was then refined by side-chain packing for each KCNQ1 conformation (Supplement Figures S6 and S7). Note that the complete protein library was constructed as a combination of VSL and pore libraries. The four VSL and S6 segments of the pore were not assumed symmetric. One structure from the library is shown as an example; Figure 1 shows the transmembrane domain only and Supplement Figure S2 includes the proximal cytosolic domain.

Energy computation.

The KCNQ1 or IKs conformations were inserted into a $140\text{\AA} \times 140\text{\AA} \times 140\text{\AA}$ lipid-water box. The center of the membrane (POPE) was aligned to the x-y plane at $z = 0$. A PIP2 molecule was docked at the lipid-water interface near S4S5L. An energy component analysis of a coarse-grid sample KCNQ1 library (40,490 conformations) was conducted to validate the assumption [16] that electrostatic interactions play a dominant role during channel gating, even at zero membrane voltage (Supplement Figure S8). The electrostatic energy of each conformation in the library was computed using the Generalized Born equation [17]. The effect of membrane voltage was included by calculating the energy of charged atoms in the associated electric field. All-atom van der Waals interactions were also incorporated.

Simulating of trajectories of conformational changes during activation.

Trajectories of KCNQ1, with and without KCNE1, were generated in conformation space during activation. Activation was initiated with the following protocol: holding potential of -80mV for 18 seconds, followed by voltage steps to different membrane potentials from -140mV to $+100\text{mV}$. The starting conformation of each trajectory was selected randomly from the structural library. At each step, the next conformation was randomly chosen from neighboring conformations within $5.0\text{\AA}$ root-mean-square deviation (RMSD) from the present conformation. The probability of selecting the next conformation was computed using Smoluchowski equation [3][4][5] based on atom displacements relative to the present conformation and its energy, computed as the sum of its electrostatic and van der Waals

energies. The probability of a conformation being in a conducting state (P_C) was determined by its pore diameter (D_{pore} ; 0 for $D_{\text{pore}} < 5.0\text{\AA}$, 1 for $D_{\text{pore}} > 8.0\text{\AA}$ and increasing sigmoidally with D_{pore} between these pore diameter values; Supplement S2.1, Equation 1). For simplified analysis, the pore conformations were classified into two groups; conformations with $D_{\text{pore}} < 6.6\text{\AA}$ closed and $D_{\text{pore}} \geq 6.6\text{\AA}$ open, as $6.6\text{\AA}$ is the largest dimension of a hydrated potassium ion. For each protocol, the macroscopic behavior of KCNQ1 and IKs, based on 2000 trajectories, was computed and described in terms of the average translation of voltage sensors, average D_{pore} , total P_C and ionic current. The voltage and time dependence of these properties were compared to experimental data for validation and study of mechanisms.

Computing Macroscopic Currents during the AP.

The macroscopic KCNQ1 and IKs currents were computed from the structure-based simulations and integrated into a human ventricular myocyte model [18] to simulate the effects of KCNQ1-KCNE1 interaction on the AP. The time course and voltage dependence of the channel open probability were used to formulate the activation gate of IKs current, with and without KCNE1, in the model. The relationship between the maximum open probability, simulated from the structure, and the maximum conductance of macroscopic IKs current was computed using an AP-clamp protocol. Details of the approach, including the relevant equations, are provided in Supplement S6.

Results

Structural Properties of the Conformation Space

To study the dynamic conformational changes of the transmembrane domain of KCNQ1 during channel activation, we constructed an all-atom structural library of this domain with the proximal C-terminal segments attached. Following elimination of structures through application of spatial constraints and minimization of van der Waals energies, the library retains 11,547,630 VSL conformations and 615,946 pore conformations (Figure 1), which amounts to more than 10^{30} possible KCNQ1 structures. In the library, the spatial range of the S1-S2 panel is limited to $2\text{\AA}$. The S3-S4 paddle can translocate $22.01\text{\AA}$ in the z direction across the membrane, rotate about the z-axis (ω) within a range of 79.08° and tilt (γ) relative to the z-axis from 0 to 32.24° (Figures 1 and 2 and Supplement Figure S4). The C-terminal half of S6, together with the proximal cytosolic domain, can swing around an axis through the center of the pore, allowing the pore diameter to change over a range from 0 to $15.85\text{\AA}$ (Supplement Figures S5). The library includes conformations of Helix A-Helix B-Calmodulin that occupy a volume consistent with (less than 0.3% difference) from that in a recently published cryo-EM structure of KCNQ1 [12]. When VSL locates at high z positions (H-VSL, top panel in Figure 2A), considered an activated state of the voltage sensor, it is possible to have structures with open or closed pore conformations. It is not necessary that all four VSLs be activated for the pore to be open; a subset of permissible open pore conformations have VSLs at low z positions (L-VSL, bottom panel in Figure 2A). Thus, the KCNQ1 channel can have a conducting (open) pore with some or even all voltage sensors at low positions. Importantly, some combinations of four H-VSLs can assemble with up to 68% of the open pore conformations without steric clashes, compared to only 18% for four

L-VSLs. This results in a greater probability for the channel to be in a conducting state when more VSLs are at high positions.

Effect of Membrane Potential

Based on computed energy landscapes of the structural library, the VSLs favor different clusters of conformations at different membrane potentials, consistent with experimental observations [19]. At resting potentials (-80mV in Figure 2B), VSLs reside preferentially at low z positions, where R228 and R231 on S4 can form salt bridges with E160 on S2, and R237 on S4 is close to E170 on S2 (L-VSL). Following depolarization ($+60\text{mV}$ in Figure 2B), there is an increased probability for VSLs to translocate in the positive z direction, to conformations where R237 is closer to E160 (H-VSL). Hence, at depolarized potentials there is greater probability for VSLs to be in the activated state and for the pore to be open (conducting). Note that the energetically favored z positions of VSL at resting and depolarized potentials are less than $12\text{\AA}$ apart. This distance is much smaller than the permissible range of $22\text{\AA}$ (space unoccupied by other protein segments); it is consistent with the range predicted experimentally for the voltage sensor movement of Kv1.2 [20]. In addition to the z translocation, the energy landscape indicates that VSLs favor conformations with different ω -rotation at different voltages (Figure 2B).

To simulate KCNQ1 activation gating, we computed trajectories of conformational changes in response to membrane depolarization to different voltages. When the membrane is depolarized to $+60\text{mV}$, 1.7% to 2.1% of the KCNQ1 channels became activated with high VSL conformations and large pore diameter ($+60\text{mV}$ trace in Figures 3A and Supplement Figure S9). The trajectories maintained tight coupling between VSLs and pore, a property that underlies KCNQ1 fast activation without substantial delay. The average z position of VSLs and open probability of the channel (P_C) share a similar voltage dependence (Figure 4A, top) and the fast and slow time constants of P_C follow the time constants of VSL translocation in the z direction (Figure 4B). This behavior, indicative of a tight VSL-pore coupling in KCNQ1 channels, is consistent with voltage-sensor fluorescence experiments [21][22].

Modulation of KCNQ1 Activation Gating by Co-assembly with KCNE1

A stoichiometry of 4KCNQ1:2KCNE1 is considered the physiological stoichiometry of IKs [23]. The structural library of IKs was generated by inserting into each tetrameric KCNQ1 conformation two KCNE1 subunits in opposite positions (Figure 1C). Docking of KCNE1 does not eliminate any possible conformations in the KCNQ1 library, as the single-spanning transmembrane helix of KCNE1 occupies less space than the empty pocket between the voltage sensors from two adjacent KCNQ1 subunits (Supplement Figures S6 and S7). However, charges carried by KCNE1 residues change the electrostatic energy landscape of KCNQ1 (Figures 2 and Supplement Figure S10) and consequently the steady-state z -position, ω -rotation and γ -tilt of VSL at resting and depolarized potentials (Figures 3 and Supplement Figures S9, S10). KCNE1 makes H-VSLs energetically more favorable at depolarized voltages, so that IKs achieves a higher average z of VSL and a larger open probability compared to KCNQ1. The computed P_C traces (Figure 3; note different scales of panels) for IKs are 5 times larger than for KCNQ1, as observed in experiments [13][21][22]

[24]. However, conformational changes of IKs are slower to reach steady state (Figures 3). The co-expression of KCNE1 with KCNQ1 separates the voltage and time dependence of VSL z translation from that of pore opening (Figure 4), reducing the coupling between these two motions. The simulations (Figure 4) reproduce experimental measurements [21][22], except at low membrane voltages ($V_m = -60\text{mV}$ and -40mV for $\tau_{z,VS}$ of KCNQ1 and $V_m = 20\text{mV}$ and 40mV for τ_{pc} of IKs) for which the experimentally recorded signals are too small to separate multiple temporal components. Comparison between the simulated KCNQ1 and IKs trajectories shows that at resting membrane potentials, VSLs of IKs reside at lower z positions at steady state, but translocate to higher z upon depolarization. This prolongs the time to steady-state following depolarization of IKs compared to KCNQ1. However, more IKs channels accumulate in conformations with larger pore diameters at a depolarized steady state (Figure 5), accounting for the larger IKs macroscopic current compared to KCNQ1, recorded in experiments.

To study the effect of KCNE1 on IKs activation during a cardiac ventricular AP, we extracted the fast and slow activation time constants τ_{pc} (Figure 4B) from the structure-based simulations. We incorporated these parameters in a human myocyte model [18], which was paced at a cycle length of 300ms (Figure 3B). In the absence of KCNE1 (Figure 3B, left), the KCNQ1 current activates at an early phase of the AP and then declines slowly during the plateau. In contrast, when KCNE1 is present (Figure 3B, right), IKs increases monotonically during the plateau to a higher peak amplitude during late plateau and phase-3 repolarization. With this time course, IKs provides a large repolarizing current when it is most effective for AP repolarization. *The model reproduces the rate adaptation of the AP [18]; the AP duration (APD) with KCNQ1 is 232ms at cycle length CL = 1000ms and 196ms at CL = 300ms (APD = 36ms; 15% shortening), with IKs in the absence of β -adrenergic effect APD is 228ms at CL = 1000ms and 182ms at CL = 300ms (APD = 46ms; 20% shortening) and with β -adrenergic effect APD is 211ms at CL = 1000ms and 165ms at CL = 300ms (APD = 46ms; 22% shortening). As shown below, IKs is more effective in APD shortening at fast rate than KCNQ1 due to its slower gating properties.*

Contribution of Specific Residues and Structural Elements during Activation Gating

The contribution of each protein residue during activation was evaluated by computing the energy of its electrostatic interactions with the rest of the protein and its self-energy (Figure 6). The significant differences of energy contributions by E160 on S2 and by R228 and R237 on S4 in different structures of the VSL library confirm their dominant role in determining conformational changes during activation. KCNE1 does not interact directly with these residues but changes their electrostatic environment (Figure S11). This modification results in different rates of transition between VSL conformations in IKs compared to KCNQ1.

Co-assembly of two KCNE1 subunits with KCNQ1 divides the four subunits of KCNQ1 into two different types, based on the proximity of their VSLs to KCNE1 (Figure 1C): the VSLs with S3-S4 facing KCNE1 were labeled VS_A and those with S1-S2 facing KCNE1 were labeled VS_B . KCNE1 interacts with charges on VS_A and VS_B with different distances and therefore changes the electrostatic energy landscapes of VS_A and VS_B differently, which results in different average behaviors during membrane depolarization (Figure S10). The

electrostatic energy contribution of R249 on S4S5L of VS_A is affected greatly by presence of KCNE1 (see the next section). KCNE1 also influences the electrostatic energy contribution of E146 on S1 of VS_B, and K41 on KCNE1 contributes differently in different VS_B conformations (Figure 6B and 6C). These results indicate an interaction between the N-terminal end of the KCNE1 transmembrane segment and the extracellular portion of S1 in VS_B, as suggested by experiments [25][26].

Importance of S4S5L in Activation Gating and Coupling between VS and Pore

The calculation of energy contributions from specific residues (Figure 6) determines that S4S5L plays an important role in IKs activation (box in Figure 6B). When the membrane is depolarized, S4S5L moves with S4 (Supplement Figure S9); the time constant of its movement coincides with the slow time constant of S4 for both KCNQ1 and IKs (Figure 7A). The S4S5L conformational space is limited by its connection to the relatively static S5. A relative movement between S4 and S4S5L is made possible by the dihedral angles of Gly245-Gly246 (Figure 7C), which can undergo significant changes due to their small side chains. This enables an initial fast response of S4 to membrane depolarization. It then constrains the additional translation of S4 by coupling it to the swinging movement of S4S5L about the N-terminal end of S5. KCNE1 slows the S4S5L movement and the slow component of S4 movement (z_{S4S5L} and $z_{VS,slow}$ respectively, in Figure 7A). The electrostatic interaction between KCNE1 and R249 of S4S5L on VS_A (Figure 6B, VS_A-KCNE1) creates an energy barrier at medium S4S5L z displacement and stabilizes it at extreme (low and high) z positions (Figure 7B). This impedes the z translation of VS_A and slows IKs activation compared to KCNQ1. *During fast pacing of a cardiac myocyte at short CL, the slow S4S5L movement with KCNE1 present could lead to accumulation of channels in conformations with high S4S5L displacements and increased probability for an open pore, creating an “available reserve” of conducting channels. This provides the basis for a larger repolarizing current and shorter APD at fast rate.*

S4S5L plays another important role in coupling VS movement and pore opening. The C-terminus of S6 swings beneath S4S5L between its close and open conformations (Supplement Figure S5). S4S5L contributes to the electrostatic environment of S6; interactions between S4S5L and S6, both positively charged, favor channel conformations with greater distances between these charges on structural elements. When S4S5L is at medium z, repulsion between these elements impedes pore transitions from close to open conformations (Figure 7D, middle). As S4S5L moves up, away from the C-terminal portion of S6, energy preference increases for conformations with a larger pore (Figure 7D, top). The C-terminal end of the KCNE1 transmembrane segment is docked close to S4S5L. It adds positive charge to the space of interaction between S4S5L and S6, decreasing further the probability of transitions from close pore to open pore conformations at medium z positions of S4S5L (Figure 7D). Therefore, the presence of KCNE1 requires a higher z position of S4S5L for the channel to be in a conducting state, thereby slowing activation of IKs relative to KCNQ1.

Discussion

The objectives of this study required an implementation of a computational approach that overcame limitations of existing methods and was capable of simulating ion channel gating over a physiological time scale of its function. This was achieved by dissecting the ion-channel protein into structural segments and linearizing energy computations. We applied this approach to the cardiac IKs channel and studied modulation by the β -subunit KCNE1 of its activation gating. *A recent modeling paper introduced a different approach for simulating IKs gating at the atomic scale [28]. In that approach, artificial intelligence (machine learning) methods were used to overcome the computational enormity of the problem. Reassuringly, the two independent methods generated structural libraries that covered the same conformational space and contained experimentally determined and other simulated structures [3][19][27][29]. The two approaches were applied to study different aspects of IKs activation.*

The simulated trajectories of conformational changes during channel activation reproduced experimental results (Figures 3 and 4). Further exploration of the conformational library provided new mechanistic insights into the workings of the IKs channel and some of its properties that have been the subject of a long-standing debate. An important finding confirms that cooperativity between voltage sensors is not required for pore opening in either KCNQ1 or IKs. Not all voltage sensors need to be in activated (up) positions for the channel to be in a conducting state, as the conformational space contains permissible open-pore conformations with one or more voltage sensors in low z positions. However, there is greater preference energetically for open-pore conformations with greater number of activated voltage sensors, increasing their open-pore probability. This property is consistent with the sequential gating concept, proposed in Meisel et al. 2012 [30]. The KCNE1 transmembrane helix introduces negative charge on the extracellular aspect of the lipid-water phase and positive charge on the intracellular aspect. This modification of the electrostatic environment favors conformations with higher z positions of the positively charged VSLs of IKs, resulting in a larger population of conformations with large pore diameter and larger P_C when KCNE1 is present, resulting in larger macroscopic IKs currents compared to KCNQ1 [13]. To study this property experimentally, it will be useful to devise fluorescent signal recording experiments that can identify channel conformations with different numbers of H-VSLs and record their occurrence together with macroscopic KCNQ1 and IKs currents.

Like other voltage-gated potassium channels [31], the positively charged S4 segment of KCNQ1 moves up across the membrane in response to depolarization. Experimental studies suggested an important role for S4S5L in IKs gating [12][32][33][34]. This functional property of S4S5L is supported by the residue-specific energy analysis of electrostatic interactions (Figure 6). The correspondence of the slow component of VSL movement with the upward movement of S4S5L (Figure 7) implies restriction of late-phase S4 movement by S4S5L due to the short linker between them. The slowing of S4S5L movement and thereby of the slow component of S4 by KCNE1, provides an explanation for the detection of 2-stage VS movement in fluorescence experiments for IKs but not for KCNQ1 [24]. *The fast stage of the VS movement that is not slowed by the S4S5L movement, covers a larger displacement compared to the slow stage. This result is consistent with the observation by*

Ramasubramanian and Rudy [28]. Direct experimental evaluation of the role of S4S5L movement during activation gating is difficult to obtain because of its cytosolic position on the channel protein.

Previous experiments concluded that KCNE1 slows KCNQ1 activation either by slowing the S4 movement [35] or by suppressing the coupling between VS movement and pore opening [21][24]. The simulations demonstrated coexistence of both mechanisms and determined their molecular basis (Figure 4 and 7). Slowing of IKs S4S5L movement, by the interaction between its R249 residue and the C-terminus of the KCNE1 transmembrane segment, has two consequences – it slows S4 movement and delays pore opening. Compared to KCNQ1, IKs requires a larger S4S5L movement to a higher position to achieve P_C larger than 0.5. Coupling between VS movement and pore opening is reduced, delaying IKs activation. A previous experiment suggested the presence of an intermediate-open state in KCNQ1 but not in IKs [19]. The simulated trajectories in the present study support this observation, identifying many more conducting conformations with VSLs at middle-z positions in KCNQ1 than in IKs. While open conformations with VS in middle z are possible, they occur with small probability because of the barrier to transition from close to open pore (Figure 7D, middle).

The ratio of KCNQ1:KCNE1 was assumed 4:2, considered the dominant stoichiometry of IKs in cardiac cells [23]. With two KCNE1 subunits located in opposite pockets of the KCNQ1 tetramer, the four VSLs that are symmetric in KCNQ1, can be divided into two types, VS_A and VS_B . The simulations show that KCNE1 modifies the electrostatic energy landscapes of these two VSLs in two different ways (Supplement Figure S10). Electrostatic interaction with the C-terminus of KCNE1 transmembrane helix favors extreme z positions for S4S5L in VS_A , but does not affect the S4S5L in VS_B significantly. *Consequently, the two types of VSLs in IKs behave differently during channel activation, a behavior that was also observed in recent simulations using a different, independent approach [28].* This property could contribute to the multiple stages of IKs voltage dependent gating observed experimentally [24]. The possibility of variable KCNQ1:KCNE1 has also been suggested [36][37]. On-going simulations, using the computational approach presented here, could examine the hypothesis that variable stoichiometry is a mechanism for modulation of IKs function.

This is the first study that integrates the dynamics of an ion channel protein structure into a model of the whole-cell AP. A previous modeling study used a Markov model of IKs kinetics to examine its role in AP repolarization [7]. Here, starting from the molecular protein structure and its conformational changes during activation, we compare the time course of IKs and KCNQ1 during a human ventricular AP (Figure 3B). The results demonstrate that modulation by KCNE1 is essential for an effective role of IKs in AP repolarization and AP shortening at fast rate.

The computational framework presented in this paper is general and could be applied to study the relationships between physiological function and molecular structure of a variety of proteins. Examples specific to IKs could include: (1) The structural libraries of KCNQ1 and IKs generated here could be used to study other properties of the wild-type ion channel

(e.g., dependence on potassium ion concentration). (2) Following the same scheme, structural libraries could be generated for channels containing mutations to study the structural basis of pathological phenotypes such as the long QT syndrome. (3) Similarly, libraries of structures modified by drug binding could be used to study drug action and drug toxicity. (4) Effects of other regulatory subunits (e.g. KCNE2) could be explored. (5) The mechanism of β -adrenergic stimulation and current amplification could be studied, once methods are developed to extend the approach to the unstructured N-terminus of KCNQ1.

The two key procedures of the presented approach are construction of a structural library and generation of trajectories for conformational changes under various voltage protocols. The time to execute the first step is highly dependent on the number of degrees of freedom of the target protein, the desired resolution of the library and knowledge of the conformational space. The second step is less time consuming and depends on the number of trajectories required for convergence. Taking the IKs conformation library in this study as an example, it requires about 8 weeks for a 64-core super-computer to construct the raw library. Applying restrictions to the library (eliminating steric clashes, etc.) takes additional time. It requires another 2–3 weeks of computing time to generate the trajectories. Building a Hodgkin-Huxley model of the current and incorporation into the cell model requires minimal computational resources and could be accomplished using a desktop computer within one week. Note that it is possible to optimize these procedures by specialized data structures and computational algorithms.

Limitations.

The emphasis in this study was on the transmembrane domain of the KCNQ1/KCNE1 complex. Only spatial occupation by the cytosolic domain was considered in the study, including Helix-A, Helix-B, Helix-C, Calmodulin, KCNE1 C-terminus and a docked PIP2. The electrostatic energy from the cytosolic domain of KCNQ1 was neglected, considering the screening effect of the water phase. In order to linearize the electrostatic energy calculation for the transmembrane domain, parameters were estimated from a coarse-grid sample set of the conformation library (see Supplement). We estimated the error introduced by the linearization to be smaller than 1.5%. We evaluated the channel conductance indirectly, based on the pore diameter as a surrogate. Single-channel recordings detected multiple subconductances of IKs [39]; subconductances will be introduced in the simulations from structural considerations when more detailed information on the pore structure becomes available. *The simulations were conducted with a fixed 4:2 KCNQ1:KCNE1 stoichiometry, considered the dominant ratio under physiological conditions. Studying other stoichiometries (we hypothesize that stoichiometry could be variable and serves to regulate channel function [36][37]) requires building structural libraries for each stoichiometry from 4:1 to 4:4. A study of variable stoichiometry, considering all possible configurations of KCNE1 docking in KCNQ1, is being conducted in our laboratory.*

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Integration from molecular IKs structure to its repolarizing function in the AP
- Two-component movement of VS; the slow component tightly coupled to S4S5L
- Important role for S4S5L in coupling VS to pore opening during activation
- KCNE1 modulates IKs gating through its interaction with S4S5L
- KCNE1 slows IKs activation and delays current for effective AP repolarization

Figure 1,

(A) Flowchart for simulating channel activation. (B) Diagrams defining structural elements of IKs and coordinate system. Linkers with dihedral angles that were varied during construction of the structural library are colored red. (C) Top view of a representative conformation from the IKs library. Cytosolic domain of the channel is omitted here; a complete representative KCNQ1 structure is shown in Supplement Figure S2. Two KCNE1 subunits (brown) are inserted in opposite pockets of the KCNQ1 protein. Two types of voltage sensors (VSA and VSB) are distinguished, based on their proximity and interactions with KCNE1; S3 and S4 (blue) of VSA face KCNE1, S1 and S2 (gray) of VSB face KCNE1. (D) Side view of the same conformation. The alpha carbons of V212, R228 and R243 are shown as red spheres. VS, voltage sensor; S4S5L, S4-S5 linker; VSL, VS and S4S5L combined; TM, transmembrane segment; Nt, N-terminus; Ct, C-terminus. Color code (for Figures 1 and 2) is defined in the diagram of panel B.

Figure 2,

(A) Two KCNQ1 conformations selected from the library with VSL at high z (top, H-VSL) and low z (bottom, L-VSL). Dashed lines mark the membrane boundaries. (B) Energy landscapes for VSL in KCNQ1 across the structural library at -80mV (bottom) and $+60\text{mV}$ (top). Arrows indicate the corresponding z positions for each representative conformation in panel A on the energy landscape. There is lower energy (hence preferred residency) in low z and small ω at -80mV , and high z and large ω at $+60\text{mV}$ (dark blue). (C) Difference energy landscape (IKs - KCNQ1) at $+60\text{mV}$ reveals preferential residency in high z and large ω conformations of IKs compared to KCNQ1. (D) VSL trajectories in z coordinate for KCNQ1 (blue) and IKs (brown), during step depolarization (shown on top).

Figure 3,

(A) Conformational changes of KCNQ1 (left) and IKs (right) in response to a depolarizing step (protocol on top). Traces show VSL z position (top) and estimated probability for the channel to be in a conducting state (P_c , bottom). The stochasticity of conformational changes is reflected in the fluctuations of P_c . Experimental recordings of these currents, for a similar protocol, are shown in the framed panel (right; Nakajo and Kubo 2014 [22]). Note the 5-times greater magnitude of computed IKs P_c and of recorded IKs compared to KCNQ1 (shown with different scales). The simulated delayed activation of IKs (arrow in the IKs P_c panel) reproduces the delay in the recorded IKs current (arrow in the framed panel).

(B) Macroscopic currents during a human ventricular AP [18] paced at two rates: top panels CL = 300ms, bottom panels CL = 1000ms. Left panels: KCNQ1. Middle panels: IKs without ISO. Right panels: IKs with ISO. The currents are computed using the P_C of panel A, which, in turn, is computed from the protein structural changes during activation. Note current accumulation with ISO at fast rate (arrow in the top right panel). Traces in the framed inset are reproduced from Nakajo and Kubo 2014 [22] with permission. ISO, isoproterenol.

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Figure 4,
Quantitative comparison between simulations (line traces) and experimental recordings (symbols) for the protocol in Figure 3. (A) Normalized voltage dependence of VS z translation (dashed line) and conducting-state probability of the channel (Pc, solid line) of KCNQ1 (top, blue) and IKs (bottom, brown). Note separation of z and Pc voltage dependence in IKs but not KCNQ1. (B) Time constants of z (left) and Pc (right) for the same protocol; light or dark color represents fast or slow time constant, respectively. Note the different τ_{Pc} scale in the bottom right panel, reflecting a large increase of the fast time constant (slowing) for pore opening in IKs, compared to KCNQ1. Experimental recordings are from Nakajo and Kubo 2014 [22]; empty circles are fluorescent signals of voltage sensor movement; filled circles are recorded macroscopic current.

Figure 5,
Steady-state residency maps of KCNQ1 (top, blue) and IKs (middle, brown) structures projected on the z - D_{pore} plane of configuration space at -80mV (left) and $+60\text{mV}$ (right). Maps in the bottom row show the difference between IKs and KCNQ1 residency in the two upper rows. The arrows in the bottom right panel indicate the major directions of conformation changes when the channels are depolarized from -80mV to $+60\text{mV}$. D_{pore} , pore diameter.

Figure 6,

Energy contributions from residues on VSL and KCNE1. Energy is computed for interaction between one residue on one subunit and rest of the protein. Median energy is indicated by circle. The bar shows the range of energy over the entire structures in the library. (A) Interactions between residues on VSL and KCNQ1 (top) or IKs (bottom). (B) Interactions between residues on VSL and KCNE1 of IKs (two configurations, VSA – top and VSB – bottom). (C) Interactions between residues on KCNE1 and the two types of VS, VSA – left and VSB – right. E1TM: KCNE1 transmembrane helix.

Figure 7,

(A) Time constants for z translation of VS (z_{VS} , squares) and S4S5L (z_{S4S5L} , x symbols) in KCNQ1 (top) and IKs (bottom) for the protocol in Figure 3. There are two time constants for VS movement. The single time constant of S4S5L movement in KCNQ1 and IKs coincides with the slow time constant of VS in both cases. (B) Electrostatic contribution of R249 on S4S5L, with and without KCNE1, at different z levels of S4S5L (z_{S4S5L}). The presence of KCNE1 close to S4S5L of VSA changes significantly electrostatic energy contribution from R249 and divides the local energy minimum into two minima at extreme positions on the z axis. KCNE1 does not change the electrostatic profile of R249 on S4S5L of VSB significantly, due to the large distance between these two structural elements. (C) An example VSA conformation at a medium level of z shows the small distance between R249 and positively charged residues on KCNE1 C-terminus. (D) Average probability of a pore conformation with a particular diameter (D_{pore}) for KCNQ1 (blue) or IKs (brown), with different heights of VSL, z_{S4S5L} . Note that IKs has a smaller probability than KCNQ1 for having medium-size pore conformations (arrow) at medium z_{S4S5L} . This indicates a greater barrier for pore opening as VSL moves up.