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Working Model for the Structural Basis for KCNE1 Modulation of the KCNQ1 Potassium Channel

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Abstract

The voltage-gated potassium channel KCNQ1 (Kv7.1) is modulated by KCNE1 (minK) to generate the I_{Ks} current critical to heartbeat. Defects in either protein result in serious cardiac arrhythmias. Recently-developed structural models of the open and closed state KCNQ1/KCNE1 complexes offer a compelling explanation for how KCNE1 slows channel opening and provides a platform from which to refine and test hypotheses for other aspects of KCNE1 modulation. These working models were developed using an integrative approach based on results from nuclear magnetic resonance spectroscopy, electrophysiology, biochemistry, and computational methods an —approach that can be applied iteratively for model testing and revision. We present a critical review of these structural models, illustrating the strengths and challenges of the integrative approach.

Introduction

KCNQ1 (also known as Kv7.1 or KvLQT1) is a widely-expressed tetrameric voltage-gated potassium (Kv) channel that is involved in heartbeat and is associated with hundreds of mutations linked to deafness and cardiac arrhythmias[1,2]. Defects in KCNQ1 function are most commonly associated with long QT-syndrome (LQTS), a disease associated increased duration of the activation and recovery cycle of the ventricular myocardium[3]. KCNQ1 is a member of the Kv channel superfamily in which each KCNQ1 α -subunit has the archetypical six transmembrane architecture, with helices S1–S4 forming the voltage sensor domain and S5 and S6 encompassing the pore domain (Figure 1). KCNQ1 is modulated by KCNE1 to form the slow delayed rectifier current (I_{Ks}) that is critical for cardiac repolarization[4]. KCNE1 modulates KCNQ1 activity by delaying channel opening, shifting

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Conflicts of interest

The authors declare that they have no conflict of interest.

Supplementary information available

Coordinate and video files of KCNQ1 and KCNE1 complex structural models from the Smith et al. [48] and Kang et al. [33] studies are available online.

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the voltage-dependence of channel activation ($V_{1/2}$) to more positive potentials, increasing single-channel conductance, and suppressing channel inactivation. (Figure 2A)[5–7].

KCNE1 (previously minK or I_{sK}) is the founding member of the KCNE family of membrane proteins, having been identified in 1988 and originally thought to be an ion channel[8]. The actual role of KCNE1 as a channel *modulator* was recognized almost a decade later when experiments revealed that KCNE1 assembles with the KCNQ1 channel to generate the I_{Ks} current associated with the cardiac action potential[5,6,9,10]. Other KCNE family members were identified via bioinformatics studies and reported in 1999[11]. The KCNE protein family consists of five homologous members (KCNE1–5), each believed to have a single α -helical transmembrane domain (TMD) with extracellular N-terminal and cytoplasmic C-terminal domains[12–14]. Genetic studies have revealed that all KCNE family members are linked to human disorders[5,6,11,13,15–26]. Each KCNE protein seems to be able to modulate several Kv channels by altering properties such as membrane expression levels, channel activation, conductance, selectivity, and pharmacology. KCNQ1 is unique in that it can be modulated by all 5 KCNE proteins (Figure 2). For example, KCNE3 constitutively opens KCNQ1, abolishing voltage-dependent gating and dramatically increasing whole-cell current[27]. In contrast, KCNE4 is an inhibitor of KCNQ1 activity[28]. Elegant chemical subunit counting experiments indicate that association of a KCNQ1 channel tetramer with two KCNE1 proteins leads to full modulation of channel function [29,30], although this has recently been challenged in favor of a 4:4 stoichiometry[31,32]. Our KCNQ1 KCNE1 structural models offer a rationale for a 4:2 ratio based on steric clashes involving KCNE1 extramembrane domains[33], but alternative stoichiometries are not ruled out. KCNE1 remains by far the most commonly studied family member. Unlike Kv channel modulators such as the Kv β subunits and the KChIPs, which alter channel function via interactions occurring in the cytoplasm, KCNE proteins act at least partly via transmembrane interactions[34–38]. For an excellent general review of proteins that modulate potassium channels see[34].

Elucidating the structural mechanisms by which members of the KCNE family modulate Kv channels such as KCNQ1 and HERG is an important step in revealing their roles in human physiology and related diseases. Structural information provides a platform for interpreting existing functional and biochemical data while guiding future studies. Unfortunately there are several technical limitations that currently hinder high-resolution structural characterization of the I_{Ks} channel. As a result we have employed an integrative approach involving the use of solution NMR, comparative structural biology, electrophysiological and biochemical data, and computational methods to generate models for the KCNQ1-KCNE1 complex that are consistent with available data. In this critical review we will focus on these working models and the hypotheses they generate regarding how KCNE1 associates with KCNQ1 and modulates channel function. We will also highlight some of the difficulties associated with synthesizing seemingly disparate data into viable structural models.

Challenges in Kv channel structural biology

While spectacular advances have been made over the past 12 years in ion channel structural biology, only a limited number of eukaryotic channels have been expressed and purified at levels required for structural biological studies. Rat Kv1.2 crystals grown in the absence of an electrical potential have led to structures that appear to reflect open channel structures[39]. However, it has not yet been possible to crystallize a Kv channel in the closed-state that would predominate when the channel is exposed to the hyperpolarized transmembrane electrical potentials present at the physiological resting state. For this reason, the structures associated with the closed state have traditionally been pursued by building models based on data obtained from a combination of biophysical methods, structure-

function studies, closed state structures of pore domains from bacterial channels, and computational biology[40–44].

Although there has been significant progress in the structural biology of the KCNQ family cytosolic domains [45–47] there are no reports of high-level expression of any full length KCNQ channel or of the transmembrane domains, much less high resolution structural studies of this channel in complex with any KCNE family member. This is despite the fact that almost 1,000 articles have been published on KCNQ1.

Bridging structure and function with theory and experiment

The integrative approach taken to develop “high resolution” working structural models for the KCNE1-KCNQ1 channel complexes employs (1) new KCNQ1 channel models, (2) an experimentally determined structure for KCNE1, (3) structure-function and biochemically-derived structural restraints, and (4) computational methods capable of integrating these disparate forms of data to generate globally consistent structural models for the complexes.

As a starting point, structural models of the open and closed state KCNQ1 channels were generated[48] (and are available on-line as supplementary information to this review). For the open state, the Kv1.2 open state crystal structure was used as a template for homology modeling. Rosetta software [49] was then used to “fill in” connecting loop structures that could not be modeled based on the Kv1.2 template due to poor resolution in the crystal structure or gaps in the aligned sequences[39,48]. A virtue of using Rosetta in this manner is that it leads to generation of an *ensemble* of roughly equally “acceptable” structures rather than just a single favored structure. Perusal of these ensembles allows regions of the channel to be readily identified that are flexible, structurally uncertain, and/or for which multiple conformations are predicted.

A similar approach was used to develop the model for the closed state KCNQ1 structure except that the template was selected from among several competing models for the closed state of Kv1.2 [44,48]. These models tend to share the same pore domain structure, which is usually based on KcsA, but have widely varying structures of the voltage sensor domain.

Solution NMR methods were used to determine the structure of KCNE1 in micelles composed of a mild and functionally-validated detergent[33,50]. These studies demonstrated that the TMD of KCNE1 is helical, but curved and that the extramembrane N- and C-terminal domains consist of flexibly-linked alpha helices. We have hypothesized that the curved TMD of KCNE1 is retained within the KCNE1-KCNQ1 channel complex, but that both the N- and C-terminal extramembrane domains of KCNE1 are likely subject to dramatic structural rearrangements upon binding to the channel. In light of this hypothesis and also because the TMD of KCNE1 appears to be largely responsible for delaying KCNQ1 channel activation [51] and likely contributes both to increased single-channel conductance and shifting of $V_{1/2}$, we have focused our initial studies on the interactions of the TMD of KCNE1 with the KCNQ1 channel.

There have been numerous structure-function studies of KCNQ1 and KCNE1, which typically involve mutagenesis followed by electrophysiological measurements, ideally with the support of cell biological/biochemical control experiments to verify correct trafficking of channel complexes. Other biochemical studies have also identified a limited number of KCNE1-KCNQ1 inter-residue proximities. From these studies it was possible to glean a number of restraints for use in modeling the structures of the open and closed state KCNE1-KCNQ1 complexes.

With the KCNE1 TMD structure, KCNQ1 open and closed models, and restraints in hand we then conducted calculations where the KCNE1-TMD was computationally docked into the open and closed state KCNQ1 structures. Rather than employing one closed and one open state model, the top 20 models from Rosetta ensembles (see above) for each state were used. This approach allows the complications of segmental flexibility to be partly factored into docking. Using Rosetta-Dock [52], 80,000 candidate complex structures were generated for both the open and closed states. These candidate structures were then filtered using structure-function and biochemically-derived restraints to eliminate all structures of the complex that were inconsistent with the restraints. Remarkably, although only a limited number of restraints were available, most of the candidate structures were ruled out at this stage, leaving roughly 20 open and 20 closed state KCNQ1/KCNE1-TMD structures that satisfied all restraints. Within each of these ensembles it was observed that all structures were very similar in regard to the location of KCNE1 within the channel, indicating that the available data led to precise convergence.

Working model for KCNE1-TMD modulation of KCNQ1

Most of what is known about the structural basis for the activation of KCNQ1 has been inferred from observations obtained from studies with other Kv channels[42,43,53]. It is generally accepted that in the closed state the S4–S5 linker interacts with the C-terminal end of S6 from another subunit (which serves as the activation gate) to lock it in the closed configuration [39,53–55]. Upon changes in the conformation of the voltage sensor in response to depolarization, the S4–S5 linker is pulled up and away from the S6 segment and the activation gate opens. Within the context of this mode of channel gating our Q1/E1-TMD models offer an explanation for how the presence of KCNE1 interferes with channel activation to slow down opening of the gate. Specifically, KCNE1 appears to directly impede the movement of the S4–S5 linker after membrane depolarization.

The closed state Q1/E1-TMD model predicts that the curvature in the KCNE1-TMD allows its C-terminal end to sit on the end of the S4–S5 linker while simultaneously permitting its N-terminal end to make extensive (and presumably adhesive) contacts in the cleft between the voltage sensor and pore domains of the channel (Figure 4). This binding mode is likely to be energetically avid, leading to a stabilization of the closed channel state relative to the open state, resulting in at least in part of the shift in the $V_{1/2}$ for channel activation towards more positive potentials. Binding to this cleft is also proposed to be a major factor that contributes to the delay in channel activation induced by KCNE1. When the KCNQ1 channel tries to open in response to membrane depolarization, activation is initially impeded by the binding of KCNE1 and must wait until KCNE1 micro-dissociates away from its binding site (Figure 4A). When KCNQ1 opens, KCNE1 is unlikely to completely dissociate from the channel; rather, the model predicts that the KCNE1-TMD locally re-associates to a binding site in the open state channel (Figure 4). Videos showing the interconversion of the closed and open state Q1/E1-TMD models have been generated [33] and are available online as supplementary information to this review.

KCNE1 also enhances the conductance of the channel open state, with KCNE1's TMD appearing to contribute to this effect. The open state model for the complex suggests the N-terminal segment of KCNE1 binds to an interface between the voltage sensor and pore domains that we have dubbed the “gain-of-function” (GOF) cleft. When the model for the KCNQ1 open state was developed it was immediately observed that a majority of the disease-associated mutations known to result in channel GOF are located at the voltage sensor/pore domain interface near the extracellular face of the channel[48]. Mutations at this GOF cleft are found in both the pore and voltage sensor domains. Interestingly, these mutations result in inappropriate channel opening (slower deactivation and more negative

voltage-dependence of activation when compared to wild-type I_{Ks}) only when KCNE1 is present [56,57]. Restier et al. [58] proposed that salt bridge formation between residue E160 in the S2 helix and residue R237 in the S4 helix of KCNQ1 favors the open state and that KCNQ1 GOF mutations stabilize this salt bridge when KCNE1 is present. Both the open state stabilizing salt bridge and the docked position of KCNE1 in the open state to the GOF cleft are recapitulated in our structural models. The fact that the open state structural model places the N-terminus of the KCNE1-TMD in the gain-of-function cleft suggest that the presence of KCNE1 stabilizes this interface in the mutant channel open state (relative to wild type KCNQ1/KCNE1), which is consistent with the slower deactivation rates observed for the GOF mutant I_{Ks} channels[58]. It is also possible that binding of KCNE1 to the open state GOF cleft may contribute to KCNE1's enhancement of single channel conductance by perturbing the "turret" of the channel, which includes both the pore helix and the selectivity filter, which are immediately proximal to the GOF cleft. However, the exact nature of such perturbations cannot be inferred with confidence from the current models.

A critical evaluation of the integrative approach used to construct the current working model

The KCNQ1/KCNE1-TMD closed and open state models described above are appealing from several standpoints. First, they are high resolution in the sense that they are associated with actual 3-D molecular coordinates, not cartoons. This provides a level of detail that allows for very specific testing of these models. Moreover, to the degree that these models or components thereof are correct, the models provide site-specific insight into the structural basis for channel function and dysfunction. For example, details of the model-predicted residue-residue interactions involving >80 KCNQ1 disease mutation sites in both open and closed states have been tabulated (see on-line supporting information to [48]). In some cases, such as the disease-associated gain-of-function mutations alluded to earlier, the models provide the basis for formulating hypotheses regarding the molecular basis for disease phenotypes. Such hypotheses must, of course, be experimentally tested. The same is true for generating hypotheses regarding how KCNE1 modulates channel function, as currently being pursued in our lab and others. However, the availability of working models can dramatically facilitate experimental design.

Any model building exercise, even one that is as experimentally-based as possible, is associated with assumptions that lead to uncertainties in the output model. We acknowledge that this is the case for our first generation Q1/E1-TMD models, with key assumptions being:

1. *The templates used for homology modeling of KCNQ1 were accurate.* We believe that this is generally the case for the open state, although the Kv1.2 template on which the current model is based failed to recognize that the upper part of the voltage sensor is a 3_{10} helix, as was later revealed[59]. For the closed state template we employed a closed state model of Kv1.2 that was developed by Yarov-Yarovoy and Baker[44]. This model is one of several competing models for the canonical closed state of Kv channels. The conformation and positioning of the voltage sensor domains in the competing models varies dramatically. There does not yet seem to be an emerging consensus regarding which closed state model best reflects reality, such that inferences drawn from analysis of our closed state KCNQ1 structures should be considered with reservations.
2. *The functionally-critical components of KCNQ1 and KCNE1-TMD structures do not undergo significant changes in backbone conformation upon complex formation. As a corollary it is also assumed that the KCNE1-TMD has the same structure in both open and closed state complexes.* The validity of these

assumptions is uncertain. There has been debate about whether KCNE1 might actually be directly involved in the channel conduction pathway (c.f. [60] and [61]). A direct role for the KCNE1 TMD in the ion conduction pathway would require a major rearrangement of the channel pore domain. Whether this is feasible is not obvious from available potassium channel structures and is certainly at variance with our working model. However, Gouaux and co-workers have recently provided an impetus to reconsider this possibility based on their structural observations for the AMPA receptor [62]. A second example of uncertainty is found in the work of Rocheleau et al. [63] who provided data that suggest that the voltage sensor of KCNQ1 can undergo transitions between its resting state and membrane-depolarized configurations even in the presence of KCNE1 in a manner that is rapid compared to channel opening—in other words just because the sensor is in its depolarized configuration does not necessarily mean that the channel gate is open. Among the possible explanations for how this observation can be structurally rationalized is that the linker between the voltage sensor and channel domains may be capable of adopting multiple conformations, with extended conformations allowing flexible tethering between sensor and pore domains. Other recent work describes KCNE1 as “remodeling” the KCNQ1 voltage sensor [64] although it is not yet clear whether this necessarily means that the conformation of the voltage sensor is altered by KCNE1. Clearly, experiments are needed that will provide insight into potential structural changes that take place in KCNE1 or in KCNQ1 upon complex formation. Of course, modeling approaches exist that can factor in the potential for structural changes, and these can be implemented in future iterations of the working model if necessary.

3. *Experimental protein-protein interresidue distance restraints can be applied equally well to judge the veracity of both open and closed state complexes.* Some available experimental distance restraints were specific to either the open or to the closed state complexes and were employed accordingly—for example, restraints based on the formation of disulfide bonds that lock the channel in either an open or a closed state. However, for some restraints it was not clear whether the measured interresidue proximity applied only to a single state or to both. In light of the very limited number of available data, the decision was made to apply such restraints to both states, albeit with very loose tolerances. In future interactions of model revision/refinement it will be necessary to replace these general restraints in favor of improved state-specific measurements.

In addition to the assumptions described above an additional challenge is that it is often not clear exactly how structure-function data can best be converted into state-specific structural restraints. Part of the reason for this is that the number of coupled equilibria involved in this system is formidable. The channel complex populates two states (not to mention the reported sub-conductance or inactivated states [65]), with the equilibrium between these two states being dependent both on the binding of KCNE1 and on the transmembrane voltage. Moreover, the functional open channel state output (potassium current) is dependent on both membrane expression level (often not quantified) and whether KCNE1 is present or not. It is therefore often difficult to correlate functional data to the energy and/or structure of a single state.

The iterative future

Following the presentation of the initial models for the Q1/E1-TMD complexes, new data from structure-function electrophysiology and biochemical studies have become available [37,38,54,66–68]. These recent results will provide the basis for further iterations of model building/refinement. One set of studies generated restraints regarding interactions of the

cytosolic juxtamembrane domain of KCNQ1 with the C-terminal domain of KCNE1[66–69]. These results will help restrain the C-terminal end of KCNE1-TMD, which is currently imprecisely located within the complex structures, particularly in the open state. The KCNQ1/KCNE1-TMD complex models will then be expanded to include these domains of the channel complex, enhancing the power of these models to generate hypotheses for how KCNE1 modulates KCNQ1 activity. Although the TMD of KCNE1 appears to be mostly responsible for delaying channel activation, the C-terminal domain contributes to enhancing ion conductance, suppressing channel inactivation, and contributing binding energy to complex formation [51,60,70].

Other newly available data provide restraints to validate or revise the structure of the voltage sensor domain, which will be particularly welcome for the closed state channel model. It is encouraging that the current KCNQ1/KCNE1-TMD models for these domains have already been helpful in explaining recent structure-function data [38,69,71] and are also consistent with results from a recent study of the nature of conformational changes that occur in the KCNQ1 voltage sensors upon membrane depolarization[72].

Finally, we note that alternative models for the KCNE1-KCNQ1 complexes have been proposed [37,38,69,71,73,74] that are primarily based on our models of KCNQ1, but that have employed distinct (and typically new) sets of restraints to predict the locations of KCNE1 with the closed and open channel states. In considering these competing models, we note that while we regard our first generation Q1/E1-TMD models as reflecting the output of a state-of-the-art approach that is as experimentally-based as possible based on the data available in 2008, we are proposed to abandon or dramatically revise these models as warranted by new data and/or by a more definitive interpretation of existing data. Indeed, our first major iteration to produce second generation models is planned for the near future. However, we humbly offer our opinion that the integrative approach summarized in the review is a relatively bias-free way of iteratively compiling all available restraints to generate models that are both structurally-fully-detailed and that reflect the consensus of all available data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Voltage-gated potassium ion channel and KCNE protein topology and structural model of KCNQ1

A) KCNQ1 is a 6TM voltage-gated channel that has two membrane integral domains, the voltage-sensor domain (blue, S1–S4) and the pore domain (green, S5–S6). A schematic representation of KCNE1 is shown as a red cylinder that represents the single transmembrane helix common to all KCNE family members. B) Homology models illustrate the tetrameric nature of KCNQ1. Protein Databank-format coordinates for the open and closed states models of KCNQ1 are originally from Smith et al. [48] and can be found in the on-line supplementary information.

Figure 2. Electrophysiology recordings detailing KCNE modulation of KCNQ1

Whole-cell currents elicited from CHO cells transiently expressing KCNQ1 alone or with KCNE proteins (Vanoye et al, unpublished data). A) Current profile of KCNQ1 in the absence of any KCNE protein. B) Effects of KCNE1 modulation of KCNQ1 activity: slower channel activation and increased current density. C) KCNE3 alters the KCNQ1 properties to make channel activation nearly instantaneous and voltage-independent, as well as to increase current density. D) KCNE4 is a strict inhibitor of KCNQ1. E) and F) show the current and time scales for the recorded data and the voltage protocol used, respectively.

Figure 3. Integrative approach to developing structural models

Various types of data obtained by electrophysiology, biochemistry, and biophysical approaches are used in conjunction with experimental and computational structural data to generate hybrid structural models. An important feature of this is that the model accuracy can be cross-validated by new functional studies and iteratively updated as new data and/or improved restrained modeling methods become available.

Figure 4. Structural models of the KCNQ1/KCNE1-TMD open and closed state complexes
A) and **B)** illustrate representative structures from the data-allowable ensembles of structures for the closed and open states model complex where KCNE1 strategically binds to proximal clefts in KCNQ1[33]. According to the structural models, KCNE1 undergoes a modest micro-dissociation/re-association from one cleft to another that is accompanied by partial rotation. However, KCNQ1 undergoes a relatively major conformation change between states. Protein Databank-format coordinates and videos illustrating the conversion between states were published by Kang et al. [33] and are available in the supplementary information of this review. **C)** through **F)** feature shaded residues in KCNQ1 for which Cα are within 10 Å of a Cα from at least one residue in the KCNE1-TMD.