Nano-environmental changes by KCNE proteins modify KCNQ channel function

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Abbreviations: FRET, fluorescence resonance energy transfer; VSD, voltage-sensor domain; SCAM, substituted cysteine accessibility method; TIRF, total internal reflection fluorescence

The KCNQ1 channel is a voltage-dependent potassium channel, which is widely expressed in various tissues of the human body including heart, inner ear, intestine, kidney and pancreas. The ion channel properties of KCNQ1 change remarkably when auxiliary subunit KCNE proteins co-exist. The mechanisms of KCNQ1 channel regulation by KCNE proteins are of longstanding interest but are still far from being fully understood. The pore region (S5–S6 segments) of KCNQ1 is thought to be the main interaction site for KCNE proteins. However, some recent reports showed that the voltage-sensing domain (S1–S4 segments) is critically involved in the regulation of KCNQ1 by KCNE proteins. In addition, we recently re-examined the stoichiometry of the KCNQ1-KCNE1 complex and found that the stoichiometry is not fixed but rather flexible and the KCNQ1 channel can have up to four associated KCNE1 proteins. We will review these recent findings concerning the mechanisms of KCNQ1 regulation by KCNE proteins.

Functions and Structures of KCNQ1 and KCNE1

KCNQ1 is one of the five members of KCNQ family (KCNQ1-5 or K,7.1–7.5) and also a member of the conventional voltage-gated potassium (K) channel family.1 KCNQ1 (K,LQT1) was first identified as a causative gene of cardiac arrhythmia.2 cDNA of KCNE1 (also known as Isk or minK) was first isolated as that of a putative single transmembrane potassium channel.3 Soon after the cDNA cloning of KCNQ1, KCNE1 was found not to be a pore-forming main subunit but to serve as an auxiliary subunit of the KCNQ1 channel.4-5 The complex of KCNQ1 and KCNE1 underlies the slowly activating delayed-rectifier K+ current I\textsubscript{Ks}, which regulates action potentials in cardiac myocytes. Various mutations of either gene are known to cause long QT syndrome in human. The complex is also known to be expressed in the apical membrane of strial marginal cells in cochlea, where KCNQ1-KCNE1 channels contribute K+ transport to maintain the endochochlear potential, which is essential for auditory function.6-7 Hearing loss along with cardiac arrhythmia is seen in Jervell and Lange-Nielsen syndrome, a type of long QT syndrome caused by a defect of either KCNQ1 or KCNE1 genes.8,9

Functional homomeric KCNQ1 channels can be expressed on the plasma membrane without KCNE1 co-expression in Xenopus oocytes or mammalian cells. However, by co-expressing KCNE1, the current amplitude of KCNQ1 becomes 5- to 20-times larger than that of KCNQ1 alone (Fig. 1A). There are more prominent changes induced by co-expression of KCNE1; such as 100-times slower kinetics (both activation and deactivation), positive shift of voltage-dependence and absence of inactivation.1-5 To understand the mechanisms by which these changes are induced by KCNE1, it has been an attractive subject in the ion channel biophysics field, and also important for cardiac physiology and drug development.

Like other types of K channels, the KCNQ channel has six transmembrane segments (S1–S6); the former four segments (S1–S4) serve as a voltage sensor and the latter two segments (S5–S6) form the pore region (Fig. 1B). The N-terminal intracellular region is relatively short and lacks the T1 domain which is important for tetramerization and gating in Shaker-type K channels.10-12 Instead, the KCNQ channel has a relatively long C-terminal intracellular region possessing coiled-coil domains, which is required for tetramerization and trafficking to the plasma membrane.13-17 Although the crystal structure of the transmembrane region of KCNQ1 has not been solved yet, structural models for the KCNQ1 channel based on the crystal structure of K,1.2 are available.18-20 KCNE1 is a membrane protein with a single transmembrane domain (Fig. 1B). The structure of solitary KCNE1 protein reconstituted in micelles has been solved by NMR analysis.21 However, the structure of the molecular complex of KCNQ1 and KCNE1 remains speculative.

KCNE1 Subdomains Required for the Interaction with KCNQ1 Channel

The transmembrane domain of KCNQ1 has been thought to be required for the interaction with the pore domain of the KCNQ1 channel. Introduction of cysteine residues in the transmembrane region of KCNE1 makes the KCNQ1-KCNE1 channel Cd\textsuperscript{2+}-sensitive, suggesting that the transmembrane region of KCNE1
lines the conduction pore of the channel. Later, it turned out that KCNE1 does not form the ion conduction pathway, but just interacts with the pore domain of the KCNQ1 channel. By co-immunoprecipitation experiments, Melman et al. clearly showed that the pore region of KCNQ1 is actually the site of direct physical interaction for KCNE1 subunits. Assuming that the structure of KCNQ1 is similar to the structure of K_\text{v}1.2, KCNE1 is thought to be located between a gap of two adjacent voltage-sensing domains, where KCNE1 can physically access the pore domain of KCNQ1 (see Fig. 2B, yellow arrow).

The transmembrane domain of KCNE1 includes some functionally important amino acid residues. An early mutagenesis study has already revealed that the transmembrane domain of KCNE1 plays an important role in channel activities and gating. A serial perturbation study also indicated that the transmembrane domain of KCNE1 plays an important role in channel activities and gating. By a chimeric approach using KCNE1 and KCNE3, a different type of KCNE subunit which makes KCNQ1 a constitutively-open K^+ channel, Melman et al. identified that Thr-58 of KCNE1 (Val-72 of KCNE3) may be the molecular determinant for the different outcomes of KCNQ1 modulation by KCNE proteins. Because these amino acid residues are located in the middle of the transmembrane domain of KCNE proteins, they may be the site where direct interaction between KCNQ1 and KCNE proteins occurs. Tryptophan scanning of the KCNQ1 S6 segment suggests that Phe-340 of KCNQ1 may be the partner for the functional interaction with Thr-58 of KCNE1.

Compared with the pore domain, the C-terminal cytoplasmic region right after the transmembrane domain of KCNE proteins (amino acid residues 68–82 in KCNE1) is relatively well conserved. Mutations of the C-terminal domain of KCNE1 have revealed that this region is required for a proper modulation of gating even though it is not essential for binding. Actually, two initial KCNE1 mutations identified in patients (S74L and D76N) are located in this region. This region forms alpha-helical secondary structure which interacts with the C-terminal cytoplasmic domain right after the S6 segment of the KCNQ1 channel, and thus may directly control gating. By using FRET analysis, Haitin et al. showed that the C-terminal cytoplasmic region is tightly coupled with the cytoplasmic region of KCNE1 and observed voltage-dependent FRET changes between them, indicating that the C-terminal cytoplasmic region of KCNQ1-KCNE1 complex is dynamic upon gating.

**Role of the Voltage-Sensor Domain (VSD) in KCNQ1 Modulation by KCNE Proteins**

Just like other K_\text{v} alpha subunits, KCNQ1 subunit has a voltage-sensor domain (S1–S4 segments; VSD). In terms of voltage dependence, co-expression of KCNE1 shifts the conductance-voltage (G-V) relationship rightward (i.e., higher voltage is required to open KCNQ1-KCNE1 channels than that required to open homo KCNQ1 channels). Activation kinetics are obviously slowed in KCNQ1/KCNE1 channels. Therefore, the kinetics and the voltage-dependence of KCNQ1 channel during the activation process may be modulated by association with KCNE1 subunits. In addition, KCNE3 (MiRP2) makes KCNQ1 constitutively active and voltage-independent channel, suggesting that KCNE3 might also modulate the voltage sensing mechanism of KCNQ1.

Panaghie et al. examined whether the positive charges in the S4 segment have a role in the modulation by KCNE proteins. Substitutions of arginine with alanine (R231A or R237A) make KCNQ1 constitutively active even with KCNE1. This study implies that movement of the S4 segment (or VSD) is influenced by the presence of KCNE proteins. Two groups including us then examined if the VSD movement is actually affected by the presence of KCNE proteins. By using FRET analysis, Haitin et al. showed that the C-terminal cytoplasmic region is tightly coupled with the cytoplasmic region of KCNE1 and observed voltage-dependent FRET changes between them, indicating that the C-terminal cytoplasmic region of KCNQ1-KCNE1 complex is dynamic upon gating.
into Ala-226 (A226C) at the top of the S4 segment, membrane-impermeable cysteine-attacking MTS reagents can only attack those introduced cysteine residues when they are exposed to the extracellular milieu. By applying this method, we and others confirmed that the reaction speed of MTS reagent was slowed down in the presence of KCNE1. More importantly, the accessibility of MTS reagent was voltage-independent in the presence of KCNE3, indicating that the VSD is immobilized in the presence of KCNE3 (Fig. 2A). The evidence of involvement of the VSD in KCNQ1 modulation by KCNE proteins has been growing. Recently, Osteen et al. directly observed the KCNQ1 VSD movement with voltage clamp fluorometry with alexa488 bound to the S3–S4 linker (G214C). According to their results, KCNE1 actually alters the VSD movements; however, in the presence of KCNE1, the VSD unexpectedly starts to move from -160 mV which is 140 mV below the threshold of KCNQ1-KCNE1 current. The fluorescence-voltage (F-V) profile of KCNQ1-KCNE1 is gradual and continues to rise until +80 mV, while the F-V curve of KCNQ1 without KCNE1 is as steep as its G-V curve. This is surprising because VSD was thought to be stabilized in the “down state” in the presence of KCNE1. The regulation of VSD movement by KCNE1 seems to be more complex than previously thought.

If KCNE1 affects the VSD movements, KCNE1, either directly or indirectly, should interact with the VSD. We observed that a cysteine residue introduced into the extracellular domain of KCNE1 (E44C) can make a disulfide bond with A226C of KCNQ1 only when the cell is depolarized (Fig. 2B and green arrow). This indicates that the location of KCNE1 is close enough to make a disulfide bond with the S4 segment of KCNQ1 at least when the S4 segment is in the “up state”. KCNE1 also interacts with the S1 segment of KCNQ1 (Fig. 2B and blue arrow), and the contacts may be state-dependent similar to the contact between KCNE1 and the S4 segment. Located in the “binding pocket” between two adjacent VSDs, KCNE1 may be able to interact with S1, S4 and S6 segments in a state-dependent manner (Fig. 2B).

**Stoichiometry of KCNQ1 and KCNE Proteins**

Stoichiometry of KCNQ1 and KCNE1 or number of KCNE1 subunits in one ion channel has been a matter of debate since KCNE1 (minK) cDNA was isolated, even before KCNE1 was found out to be an auxiliary subunit for KCNQ1 channels. Some experiments based on the macroscopic currents suggested that four KCNQ1 subunits (one ion channel) associate with two KCNE1 subunits (4:2 stoichiometry), although the KCNQ1 channel has an obvious 4-fold symmetrical structure. On the other hand, the experiment of tandem fusion constructs of KCNE1 and KCNQ1 revealed that any stoichiometry of KCNQ1 and KCNE1 (4:1, 4:2, 4:4) is capable of producing I_Ks-like slowly activating current. Therefore, we reexamined the stoichiometry of KCNQ1 and KCNE1 by direct subunit counting using single molecule imaging under total internal reflection fluorescence (TIRF) microscopy. By counting photo-bleaching events of fluorescent GFP, tagged to a target protein (e.g., KCNE1), one can determine how many GFP-tagged target proteins (subunits) exist in a single fluorescent spot (one protein complex) (Fig. 3A and B). We found that (1) the KCNQ1 tetramer can have up to four KCNE1 subunits, (2) the stoichiometry is not fixed but rather flexible (multiple stoichiometries are allowed), and (3) the stoichiometry is dependent on relative expression densities of KCNQ1 and KCNE1. In other words, when more KCNE1 is expressed, each KCNQ1 channel has more KCNE1 subunits (up to four) (Fig. 3C). Their relationship may be analogous to the relationship between a receptor and a ligand although KCNQ1 and KCNE1 reside together in a two-dimensional membrane. We also confirmed the functional differences among different stoichiometries. Gradual increase in injected mRNA of KCNE1 gradually shifted the G-V curve of KCNQ1-KCNE1 current, and excess amount of KCNE1 lowered maximum current amplitude. This result implies that more KCNE1 makes the channel harder to be opened or even inhibits the channel.
These previous studies are mostly done in Xenopus oocytes as a heterologous expression system. Stoichiometry of KCNQ1-KCNE1 channels in vivo such as cardiac myocytes still remains to be seen. In cardiac myocytes, multiple KNCE genes (KCNE1-5) are all expressed. Each KCNE protein has a strikingly different effect on KCNQ1 modulation. Recent studies indicate that two different KCNE proteins can bind to one KCNQ1 ion channel simultaneously. If the stoichiometry of KCNQ1/KCNE1 is actually flexible and can be changed by relative expression densities of both subunits as we reported, the number of possible combinations of KCNQ1/KCNE complex would be enormous. We observed a lot of free KCNE1 (not bound to KCNQ1) subunits which seemed to be looking for a docking partner on the plasma membrane of Xenopus oocytes. Association and dissociation of KCNE proteins may dynamically occur on the plasma membrane. If that is the case, controlling the relative expression densities between KCNQ1 and KCNE proteins should be critical for heart beat regulation.

Conclusions

Although the mechanisms of how KCNE1 regulates KCNQ1 are not yet fully understood, roles of each domain of KCNQ1 and KCNE1 are slowly being elucidated. The modulation of VSD movement is especially intriguing because it not only can give an insight into KCNE1 modulation but also has the potential to give an insight into how the gating occurs in voltage-gated ion channels. In terms of stoichiometry and diversity, KCNQ1 may have up to four KCNE proteins and these four KCNE proteins can be different. The mechanism of how the binding of those different KCNE proteins is regulated in vivo would be an interesting future question.

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