The KCNQ1 channel is a voltage-dependent potassium channel and is ubiquitously expressed throughout the human body including the heart, lung, kidney, pancreas, intestine and inner ear. Gating properties of the KCNQ1 channel are modulated by KCNE auxiliary subunits. For example, the KCNQ1-KCNE1 channel produces a slowly-activating potassium current, while KCNE3 makes KCNQ1 a voltage-independent, constitutively open channel. Thus, physiological functions of KCNQ1 channels are greatly dependent on the type of KCNE protein that is co-expressed in that organ. It has long been debated how the similar single transmembrane KCNE proteins produce quite different gating behaviors. Recent applications of voltage-clamp fluorometry (VCF) for the KCNQ1 channel have shed light on this question. The VCF is a quite sensitive method to detect structural changes of membrane proteins and is especially suitable for tracking the voltage sensor domains of voltage-gated ion channels. In this short review, I will introduce how the VCF technique can be applied to detect structural changes and what have been revealed by the recent VCF applications to the gating modulation of KCNQ1 channels by KCNE proteins.

Key words: ion channel, potassium channel, auxiliary subunit, voltage sensor, electrophysiology

The heart is an organ for pumping blood through the entire body. The atria and ventricles alternately contract in each cardiac cycle and that is how the heart works as an efficient and rhythmic pump. These rhythmic activities are produced by electrical activity (i.e., action potentials), and each action potential is shaped by voltage-gated ion channels including Na+ channels, Ca2+ channels and K+ channels. Ventricular myocytes are depolarized by Na+ channels and Ca2+ channels, leading to contraction. Depolarization is typically maintained for 200–300 ms in the human heart. K+ channels subsequently repolarize the myocytes, leading to relaxation. Therefore, any defect of these ion channels or their associated proteins can cause prolongation (or shortening) of ventricular depolarization. As a result, the QT interval of an electrocardiogram is prolonged (or shortened) and is diagnosed as long (short) QT syndrome (LQTS, SQTS). LQTS (or SQTS) increases the risk of atrial and ventricular arrhythmias and is potentially lethal. Therefore, it is important that these ion channels work at proper timings in cardiac cycles.

Structure and function of KCNQ1 channel and KCNE proteins

The KCNQ1 channel is a member of the voltage-gated K+ channel family. As KCNQ1 is known as a causative gene for long QT syndrome (LQTS type 1 or LQT1), it is obviously important for shaping cardiac action potentials. KCNQ1 α subunit is a membrane protein having six transmembrane
The segments (S1–S6) and four α subunits form a single KCNQ1 channel. The first four transmembrane segments (S1–S4) form a voltage-sensing domain (VSD) and the remaining two segments (S5–S6) form a pore domain (PD) (Fig. 1a). The S4 segments (red helices) voltage-dependently move up and down as indicated by a cyan arrow in the right figure. (b) Representative currents and G-V curves of the KCNQ1 channel in Xenopus oocytes with or without KCNE1 co-expression. G/G_{max} indicates apparent open probability of the channel. Co-expression of KCNE1 slows the activation of the channel and shifts the G-V curve in the positive direction (The channel requires higher membrane potential to be activated.). Inset shows the voltage clamp protocol. The currents at 0 mV are colored in red for comparison. Figures and the data presented are adapted from [26,40].

Figure 1 Structure and ionic currents of the KCNQ1 channel with KCNE1. (a) Top view (left) and side view (right) of the tetrameric KCNQ1 structures [37]. S1–S3, S4, S4–S5 linker, and S5–S6 are colored in green, red, orange and yellow, respectively. Dotted circles indicate putative KCNE1 sites. S4 segments (red helices) voltage-dependently move up and down as indicated by a cyan arrow in the right figure. (b) Representative currents and G-V curves of the KCNQ1 channel in Xenopus oocytes with or without KCNE1 co-expression. G/G_{max} indicates apparent open probability of the channel. Co-expression of KCNE1 slows the activation of the channel and shifts the G-V curve in the positive direction (The channel requires higher membrane potential to be activated.). Inset shows the voltage clamp protocol. The currents at 0 mV are colored in red for comparison. Figures and the data presented are adapted from [26,40].

Modulation of KCNQ1 voltage sensor movement by KCNE1

It has also long been debated how KCNE1 changes the KCNQ1 gating. Initial reports suggested that KCNE1 directly interacts with the PD of KCNQ1 and prevents it from opening [11–14]. We and other groups showed that VSD movement (or state transition) was affected by co-expression of KCNE1 by utilizing the substituted cysteine accessibility method (SCAM) [15–17]. Those studies led to works focusing on the VSD movement of KCNQ1 channels, and works in which voltage-clamp fluorometry (VCF) was used to track the KCNQ1 VSD revealed the detailed mechanisms by which KCNE1 regulates KCNQ1 gating via the VSD [18–20]. In VCF experiments, a cysteine residue was introduced at the extracellular side of the S4 segment (S3-S4 linker) by site-directed mutagenesis. Fluorescent dye such as tetramethylrhodamine maleimide or Alexa488 maleimide was then attached to the site of the introduced cysteine residue to track voltage-dependent conformational rearrangement by detecting change of fluorescence intensity under a voltage-clamp (Fig. 2a) [21–23]. VCF experiments clearly showed that KCNQ1 VSD movement was greatly changed by KCNE1, though the change was far more complicated than expected [18,20]. In KCNQ1, the fluorescence-voltage (F-V) relationship (representing the VSD movement) and the G-V relationship (representing the pore opening) are mostly overlapped (Fig. 2b upper panel) [19]. This is because each individual VSD movement contributes sequentially to channel conductance in KCNQ1 channel [24,25], not like other shaker-type potassium channels in which all four VSD must be in the up state before opening. On the other hand, KCNE1 shifts the G-V relationship in the positive direction, while the F-V relationship is split into two components, a steep component F1 and a shallow component F2 (Fig. 2b bottom panel). F1 reflects a non-conducting state(s) from −160 to −50 mV and F2 nearly overlaps the G-V relationship. It is assumed that there are three states in KCNQ1 gating: closed state (S4 segments in the down state and the PD closed), intermediate state (S4 segments in the up state and the PD closed), and open state (S4 segments in the “full” up state and the PD open). The results of VCF studies indicated that KCNE1 may stabilize the intermediate state and that is why the F-V relationship is split into two (Fig. 2c). Since the intermediate state is non-conducting, channel opening requires higher and longer depolarization. Conversely, the channel requires much larger hyperpolarization (~200 mV) to enter the “fully...
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bulkiness dependence in the G-V curves. Phe232 affects the G-V curve only in the presence of KCNE1 [26]. According to the closed state and open state of KCNQ1-KCNE1 structural models by Sanders’s group [27], Phe232 on the S4 segment of the VSD faces the S5 segment of the PD of an adjacent subunit, and Phe232 seems to have to hurdle another bulky Phe279 on the S5 segment during the transition from the closed state to the open state. Therefore, we hypothesized that Phe232 collides with Phe279 during upward movement of the S4 segment (Fig. 3b). It turned out that was probably the case because the size of the Phe279 side chain affected the G-V relationship as the size of the Phe232 side chain did. The larger the introduced side chain at Phe279 was, the greater was the shift in the G-V relationship in the positive direction and, again, the G-V relationship was shifted only in the presence of KCNE1 (Fig. 3c). We concluded that the presence of KCNE1 created a steric hindrance between two phenylalanine residues of the S4 and S5 segments, and this could be the reason why activation of the KCNQ1-KCNE1 channel becomes more difficult than that of the KCNQ1 channel.

We next applied VCF to reveal which gating step was mostly affected by steric hindrance of the two phenylalanine residues. By attaching Alexa 488 maleimide to G219C mutation on the S3-S4 linker, ionic currents and fluorescence intensity were simultaneously recorded from wild-type and down state [18,20]. Since membrane potential of cardiomyocytes rarely reaches −200 mV, most of the KCNQ1 channels with KCNE1 subunits should be in the intermediate state.

Steric hindrance between the VSD and PD of KCNQ1 induced by KCNE1

As discussed in the previous section, co-expression of KCNE1 dramatically changes the voltage-dependence of the KCNQ1 VSD and that is at least one of the mechanisms by which KCNE1 slows KCNQ1 gating kinetics. However, it still remains largely unknown how KCNE1 changes the voltage-dependence of KCNQ1. We previously made a series of cysteine mutants of the KCNQ1 S3-S4 linker and the first half of the S4 segment (G219C-Q234C) to identify the best site for SCAM study [15], and we noticed that the F232C mutant did not show the characteristic positive G-V shift induced by KCNE1. Phe232 (F232) is located next to the second positive charge (R231) of the S4 segment. We therefore made several other Phe232 mutants (mostly hydrophobic amino acid residues) to explore the role of Phe232 in the positive G-V shift induced by KCNE1. Remarkably, the G-V curve shifts induced by KCNE1 were dependent on the bulkiness of the amino acid side chain (Fig. 3a). Interestingly, KCNQ1 mutants without KCNE1 did not show bulkiness-dependence in the G-V curves. Phe232 affects the G-V curve only in the presence of KCNE1 [26]. According to the closed state and open state of KCNQ1-KCNE1 structural models by Sanders’s group [27], Phe232 on the S4 segment of the VSD faces the S5 segment of the PD of an adjacent subunit, and Phe232 seems to have to hurdle another bulky Phe279 on the S5 segment during the transition from the closed state to the open state. Therefore, we hypothesized that Phe232 collides with Phe279 during upward movement of the S4 segment (Fig. 3b). It turned out that was probably the case because the size of the Phe279 side chain affected the G-V relationship as the size of the Phe232 side chain did. The larger the introduced side chain at Phe279 was, the greater was the shift in the G-V relationship in the positive direction and, again, the G-V relationship was shifted only in the presence of KCNE1 (Fig. 3c). We concluded that the presence of KCNE1 created a steric hindrance between two phenylalanine residues of the S4 and S5 segments, and this could be the reason why activation of the KCNQ1-KCNE1 channel becomes more difficult than that of the KCNQ1 channel.

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trophophysiology and simulation study [29]. F279I was also examined in our study. Isoleucine is slightly smaller than phenylalanine, and the G-V curve of the F279I mutant is therefore shifted in the negative direction (easier to be activated) by 10 mV (Fig. 3c, blue curve). This 10-mV shift may be large enough to cause short QT syndrome, which is a result of upregulation of the potassium current and shortened cardiac action potential.

Comparison to KCNE3 and other recent advances revealed by VCF

F232A and F279A mutants of KCNQ1 co-expressed with KCNE1 (Fig. 4a and b). In the wild-type KCNQ1-KCNE1 channel, an apparent delay of ionic currents (PD opening) after the increase of fluorescence (upward movement of the VSD) was observed. On the other hand, the delay was substantially diminished or almost disappeared in F232A or F279A mutants (Fig. 4a). In addition, the ratio of a shallow (presumably F2) component was increased in the F-V relationships of the mutants, indicating the transition from the intermediate state to the open state requires less depolarization in the mutants (Fig. 4b). Therefore, it was concluded that the steric hindrance mostly affects the gating transition from the intermediate state to the open state and prevents the channel from opening.

Why does steric hindrance occur between Phe232 and Phe279 in the KCNQ1 channel only when KCNE1 is co-expressed? In movies of MD simulation of the voltage-gated K+ channel gating provided by Jensen et al. in 2012, four VSDs are moving around slightly and have a loose coupling with the PD [1]. We hence propose that the distance between the S4 and S5 may become smaller due to the interaction of KCNE1 with both the VSD and PD [28]. Only when KCNE1 interacts with the KCNQ1 channel, Phe232 on S4 and Phe279 on S5 become close enough to collide with each other during gating, and that would prevent the channel from entering the open state (Fig. 4c).

F279I, a novel mutation of KCNQ1, was recently reported and shown to be responsible for short QT syndrome by electrical physiology and simulation study [29]. F279I was also examined in our study. Isoleucine is slightly smaller than phenylalanine, and the G-V curve of the F279I mutant is therefore shifted in the negative direction (easier to be activated) by 10 mV (Fig. 3c, blue curve). This 10-mV shift may be large enough to cause short QT syndrome, which is a result of upregulation of the potassium current and shortened cardiac action potential.
in the negative direction by 100 mV, and the VSD therefore requires large hyperpolarization (lower than −100 mV) to start entering the down state. This means that KCNQ1-KCNE3 channels virtually stay in the open state in the physiological range of membrane potentials.

The KCNQ1 channel is known to require phosphatidylinositol-4,5-bisphosphate (PIP$_2$) for activation [34]. Recent VCF experiments have revealed that the VSD still shows voltage-dependent movement without PIP$_2$. However, the channel loses functional coupling between the VSD and PD in the absence of PIP$_2$, and that is why KCNQ1 is unable to open the pore [35]. PIP$_2$ may directly interact with the S4-S5 linker to control coupling of the VSD and PD of the KCNQ1 channel. Therefore, it is possible to functionally separate the VSD from the PD by abolishing PIP$_2$. VCF experiments were performed in the absence of PIP$_2$ to reveal which part(s) of the KCNQ1 channels was/were affected by KCNE proteins [36]. The results suggested that KCNE3 functionally interacted only with the VSD and does not need an interaction with the PD to make the channel constitutively open. On the other hand, KCNE1 functionally interacted with both the VSD and PD. While the VSD is stabilized at the intermediate state, KCNE1 also directly affects the PD [36].

**Future direction of KCNQ1 study**

It still remains unknown how KCNE1 (and KCNE3) interacts with KCNQ1 and modulates the gating. Recently, Sun and MacKinnon reported the first tetrameric KCNQ1 structure determined by cryo-EM (Fig. 1a) [37]. Because the structure lacks PIP$_2$, there is no coupling between the PD and VSD, and the VSD is therefore in the up state (at 0 mV) and the PD is closed. Because it is a homo tetrameric structure, it does not offer any information on how and where KCNE proteins interact. Structures of the KCNQ1-KCNE complex are, therefore, eagerly awaited. On the other hand, as I discussed in this short review, the KCNQ1 channel goes back and forth among many states to produce a voltage-dependent (or independent) potassium current with KCNE proteins. Electrophysiological experiments including current recording and VCF should remain powerful tools for studying state transitions of ion channels.

**Applications of VCF to other membrane proteins**

Although VCF is most suitable for tracking voltage-dependent conformational change of voltage-gated ion channels, applications of VCF to other membrane proteins such as pumps and transporters have also been reported [38,39]. For example, conformational changes of the Na+/K+ -ATPase induced by extracellular potassium are detectable by VCF [38]. Thanks to the recent advancement of cryo-EM, structural information of membrane proteins has been explosively increasing. This simple yet powerful tool for tracking real-time structural changes will be applicable to more diverse membrane proteins by utilizing these new structural information.

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**Conflict of Interest**

The author declares no conflict of interests.

**Author contributions**

K. N. wrote the manuscript.

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