Voltage clamp fluorimetry studies of mammalian voltage-gated $K^+$ channel gating

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Abstract
VCF (voltage clamp fluorimetry) provides a powerful technique to observe real-time conformational changes that are associated with ion channel gating. The present review highlights the insights such experiments have provided in understanding Kv (voltage-gated potassium) channel gating, with particular emphasis on the study of mammalian Kv1 channels. Further applications of VCF that would contribute to our understanding of the modulation of Kv channels in health and disease are also discussed.

Introduction
Structural rearrangements associated with transitions between channel states (i.e. closed, open and inactivated states) can be studied using VCF (voltage clamp fluorimetry) by observing changes in the fluorescence emission of a fluorophore [e.g. TMRM (tetramethylrhodamine-5-maleimide)], which is attached at a specific site within the channel. Protein reorganization alters the environment of the fluorophore and this is reported directly as a change in the fluorescence emission. Using VCF, observation of channel rearrangements can be obtained during voltage clamp experiments at the same time as the recording of ionic current flow through the channel. Thus VCF studies have pioneered a new way to directly observe the structural dynamics associated with Kv (voltage-gated potassium) channel gating.

Direct observation of Kv channel conformational changes by using VCF
The first VCF studies were performed using the archetypal Kv channel, Shaker [1,2]. These pioneering studies demonstrated that changes in fluorescence emission from TMRM attached in the S3–S4 linker accurately reported movement of the voltage sensor during channel activation. Upon depolarization, the fluorescence report from TMRM attached at A359C in this linker is rapid and mono-exponential (Figure 1A), and its voltage dependence is left-shifted from that of channel opening. These results showed that fluorescence changes recorded using VCF provide a faithful report of voltage sensor movement upon channel activation. Since these initial experiments, further VCF studies and other fluorescence-based approaches, such as FRET (fluorescence resonance energy transfer) and LRET (lanthanide-based resonance energy transfer), have led to quantitative definitions of the distance and direction of voltage sensor movement [3–5], although there remains considerable debate regarding the exact nature of this movement [6]. Thus fluorescence experiments have made a significant contribution to our understanding of the dynamic structural events that determine Kv channel activation gating.

Using VCF, the conformational changes of the outer pore that are associated with P/C-type inactivation (Figure 1B) have also been observed [5,7,8]. The slow collapse of the outer pore is reported by TMRM when attached in the pore or in the S3–S4 linker [5,7–10]. These fluorescence observations have led to the proposal of a mechanism by which voltage sensor movement upon activation stabilizes inactivated conformations of the ion-conducting pore [5,7,8].

Thus, during a single voltage clamp recording, VCF enables direct observation of fast gating events (e.g. voltage sensor movement), as well as slow gating events (e.g. inactivation). This provides direct visualization of conformational changes that are associated with channel gating transitions. In addition, because there is no requirement for ion conduction to record fluorescence measurements, VCF provides a powerful assay to readily observe conformational changes that occur during electrically silent transitions, such as between closed or inactivated channel states [7,9,10].

Application of VCF to the study of mammalian Kv channel gating
Recently, fluorescence measurements of gating-associated conformational rearrangements have been reported in three human Kv channels: ether-a-go-go-related gene (hERG) channels [11], large-conductance $Ca^{2+}$-activated (BKCa) channels [12] and CNG (cyclic nucleotide-gated) channels [13], in addition to the insect Drosophila ether-a-go-go (eag) channel [14]. Fluorescence measurements from TMRM attached within the S3–S4 linker of mammalian channels [11,12] show similar voltage sensor conformational changes that are associated with channel opening to those in Shaker channels. However, recent VCF measurements of the...
Figure 1 | VCF measurements of Kv channel conformational changes

(A, B) Representative ionic currents (above) and fluorescence signals (below) recorded from Shaker Δ6–46 A359C channels in response to 100 ms (A) or 7 s (B) voltage clamp pulses to the indicated potential (holding potential = −80 mV). The N-terminal deletion of Shaker (Δ6–46) was used to prevent rapid N-type inactivation. TMRM was attached at A359C in the S3–S4 linker.

structural dynamics of voltage sensor gating in a mammalian Kv1 subfamily channel report quite different fluorescence signals (T.W. Claydon, M. Vaid, S. Rezazadeh and D. Fedida, unpublished work). Figure 2 shows the fluorescence report of voltage sensor movement from TMRM attached in the S3–S4 linker of Kv1.2, Kv1.4 and Kv1.5 channels. These fluorescence signals show that, although the report of voltage sensor movement in Kv1.2 and Kv1.4 channels is Shaker-like, the report from Kv1.5 is unique and complex (T.W. Claydon, M. Vaid, S. Rezazadeh and D. Fedida, unpublished work). Following a transient downward deflection, there is a rapidly decaying component of fluorescence. Using manipulations that inhibit inactivation, these authors suggest that the decaying component reported on a rapid inactivation process that occurred upon channel opening. In order to identify the ionic correlate of this process, the authors investigated the effects of raising the extracellular K⁺ concentration, which abolished the decaying component of fluorescence (T.W. Claydon, M. Vaid, S. Rezazadeh and D. Fedida, unpublished work). These studies demonstrated that rescue of channels from the rapid inactivation by raising external K⁺ increased macroscopic Kv1.5 channel conductance. This suggests that the ionic correlate of the putative decaying component is the reduction of peak macroscopic conductance and that channel availability, and therefore current amplitude, is critically regulated by changes in extracellular K⁺ levels, which may occur, for example, as a consequence of myocardial ischaemia. Further studies are required to determine whether the unique fluorescence report is a novel rapid inactivation process or a rapid component of P/C-type inactivation that has not been observed previously. In addition, it is not yet clear whether such an inactivation process might occur in other channels, and simply is not reported on because of subtle structural differences, or whether the process is unique to Kv1.5 channels.

Modulation of Kv channel gating revealed by VCF

VCF promises to provide unique insights into ion channel function in health and disease as it is applied to report on the modulation of channels by drugs and by physiological and pathophysiological agents. For example, VCF was recently used to observe the structural consequences of 4-AP (4-aminopyridine) drug binding to Kv channels [9,15]. Because the fluorophore reports conformational changes even in the absence of ion conduction, the authors could directly observe the states to which 4-AP bound as well as the effect of 4-AP binding on transitions between channel states. These studies demonstrated, for example, that 4-AP binding prevents inactivation, but that inactivation does not prevent 4-AP binding [9]. In addition, VCF was recently applied to directly observe the effect of low pH, which is associated with ischaemia, on Kv channel gating [10]. Fluorescence measurements from closed and inactivated channels demonstrated that ionic current is reduced at low pH because protons stabilize channels in closed inactivated states. There is, therefore, a bright future for fluorescence-based approaches in the study of ion channels. In particular, the application of such techniques to understand the mechanisms
Figure 2 | Fluorescence measurements of voltage sensor movement in Kv1 subfamily channels

Representative fluorescence signals recorded from Kv1.2 A291C, Kv1.4 Δ2-147 A441C and Kv1.5 A397C in response to 100 ms voltage clamp pulses to +60 mV (the holding potential was −80 mV). The N-terminal deletion of Kv1.4 (Δ2-147) was used to prevent rapid N-type inactivation. A291C, A441C and A397C occupy the equivalent position in the S3–S4 linker to A359C in the Shaker channel.

Figure 2 content:

- **Kv1.2 A291C**
  - Trace: Fluorescence signal recorded from Kv1.2 A291C.
  - Voltage Change: +60 mV.
  - Time Span: 25 ms.
  - Scale: 1% ΔF/F.

- **Kv1.4 A441C**
  - Trace: Fluorescence signal recorded from Kv1.4 A441C.
  - Voltage Change: +60 mV.
  - Time Span: 25 ms.
  - Scale: 0.75% ΔF/F.

- **Kv1.5 A397C**
  - Trace: Fluorescence signal recorded from Kv1.5 A397C.
  - Voltage Change: +60 mV.
  - Time Span: 25 ms.
  - Scale: 0.75% ΔF/F.

- **τ_decay** = 3.7 ± 0.4 ms

References


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