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Hypothesis

On the activation–inactivation coupling in *Shaker* potassium channels

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The 'ball-and-chain' model suggests the existence of a negative site which may attract the positively charged inactivation ball to occlude the pore when the channel is in the open state. For *Shaker* K^+ channels, we propose that the state-dependent negative site be tryptophan-435, which becomes negatively charged after receiving an electron from tyrosine-445. The kinetic scheme for the channel's activation–inactivation coupling as derived from the YW-gated model resembles a successful 'scheme 8' proposed by Zagotta and Aldrich. Our model suggests that the final rapid voltage-independent transition to the open state is due to the deprotonation of tyrosine-445.

Inactivation; Gating current; Potassium channel; Electron transfer

In response to a constant depolarizing voltage, *Shaker* K^+ channels open and then inactivate rapidly. According to the 'ball-and-chain' model originally proposed for the fast inactivation of Na^+ channels, inactivation is due to the pore occlusion by a positively charged 'inactivation ball' [1]. For *Shaker* K^+ channels, the inactivation ball has been identified to be the channel protein's first 20 amino acids, where a few positively charged residues are essential for channel's inactivation [2–4]. Experiments have also indicated that the inactivation occurs mainly from the open state [1,5]. Therefore, the 'ball-and-chain' model implies the existence of a negative site which may attract the positively charged inactivation ball to occlude the pore when the channel is open, or about to open [1,2]. The detailed physical mechanism remains unclear.

In a previous paper [6], we proposed that the *Shaker* K^+ channels could be activated by electron transfer from tyrosine-445 (Y445) to either tryptophan-434 or -435 (W435), or both. There was not enough information to identify the actual electron acceptor between W434 and W435. Recent experiments have demonstrated that the gating current remains even after W434 is mutated to phenylalanine [7]. This result indicates that W434 is not essential in the electron transfer process. Thus, W435 should be the sole electron acceptor from Y445.

In [6], we pointed out that the aperture formed by the hydrogen bonds between four Y445s is quite small. Due to the large size of a tryptophan sidechain, the W435 region is also very narrow [8]. In the resting state, ions are unlikely to pass through either the Y445 or W435 region. After the electron jumps from Y445 to W435, the tyrosine becomes a radical and the tryptophan is negatively charged [6]. The formation of tyrosine radicals could open the Y445 region [6]. In the mean time, the negatively charged W435s in the four subunits can also repel each other to make this region wider, allowing ions to pass through. Since the negatively charged W435s are associated with channel's opening, they could be the 'negative site' postulated in the ball-and-chain model to attract the positively charged inactivation ball. According to this mechanism, the positively charged channel blocker, tetraethylammonium (TEA), may also be attracted to occlude the pore if it is present on the cytoplasmic side. This mechanism agrees with two well-established features. First, internal TEA blocks only open channels, not closed channels [9]. Second, internal TEA may compete with the inactivation ball for the same site or two slightly different sites [10]. As the inactivation ball or TEA approaches W435s from the cytoplasmic side, its positive field may hinder the return of the transferring electron upon repolarization, resulting in 'charge immobilization' [11]. The cationic local anesthetics may also block the Na^+ channels through a similar mechanism [12].

It is very interesting to note that the kinetic scheme (Fig. 1) based on the YW-gated model resembles a successful 'scheme 8' proposed by Zagotta and Aldrich [5]. As discussed in [6], a fully open K^+ channel required four Y445s to become radicals. The formation of each tyrosine radical involves an electron transfer process and a deprotonation process [13,14].

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$$\mathrm{TyrOH} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \mathrm{TyrOH^+} + e^- \quad (1)$$

$$\mathrm{TyrOH} \underset{\beta_H}{\overset{\alpha_H}{\rightleftharpoons}} \mathrm{TyrOH^\bullet} + H^+ \quad (2)$$

where TyrOH represents the tyrosine sidechain. For our system, the electron in Eqn. 1 goes to W435 and the proton in Eqn. 2 is free to move. In the backward reaction of Eqn. 2, the tyrosine radical takes any proton available in its environment.

The deprotonation of $TyrOH^+$ is much faster than the electron transfer between tyrosine and tryptophan [13,14]. Therefore, the rate limiting step in the formation of a tyrosine radical is the electron transfer process (Eqn. 1). The YW-gated model thus leads to a kinetic scheme as shown in Fig. 1, where the fast deprotonation of $TyrOH^+$ has been neglected except in the final transition from the C_4 state to the open state. Experimentally, the existence of the C_4 state is supported by the channels's 'burst' in single channel recordings [5,15]. During a burst, a group of openings are separated by very short closings, which may reflect the rapid back and forth proton transfer between any of four tyrosine radicals and their environment. Kinetic studies have indicated that the final transition to the open state is very fast and voltage-independent [5,15], consistent with our assumption that the transition is due to the deprotonation of $TyrOH^+$.

According to our model, the rate constants for inactivation depend on the electrostatic and hydrophobic interactions between the inactivation ball and the channel pore. Mutations of the residues lining the inner mouth of the pore may change these rate constants [16]. However, during the dynamic gating, they are mainly controlled by W435s. The more W435s become negatively charged, the stronger they may interact with the inactivation ball. Thus, the inactivation rate α_{I4} from the open state (with four negatively charged W435s) should be larger than the inactivation rate α_{I3} from the C_3 state (with three negatively charged W435s). The inactivation rate α_{I3} should be larger than α_{I2}, and so forth. This explains why the inactivation from C_0, C_1 or C_2 state can be neglected [5].

The above mechanism for inactivation suggests that the inactivation rate constants should not depend on membrane voltages, in agreement with experiments [5]. However, the electron transfer rates, α_i and β_i (i=1–4) may be quite voltage-sensitive. From the Marcus' theory [17,18],

$$\alpha_i \propto \exp(-E^*/k_B T) \quad (3a)$$

$$E^* = [\lambda - (E_{Yi} E_{Wi})]^2/4\lambda \quad (3b)$$

where k_B is the Boltzmann constant, T is the absolute temperature, λ is called the 'reorganizational energy', E_{Yi} and E_{Wi} denote the potential energies of the whole

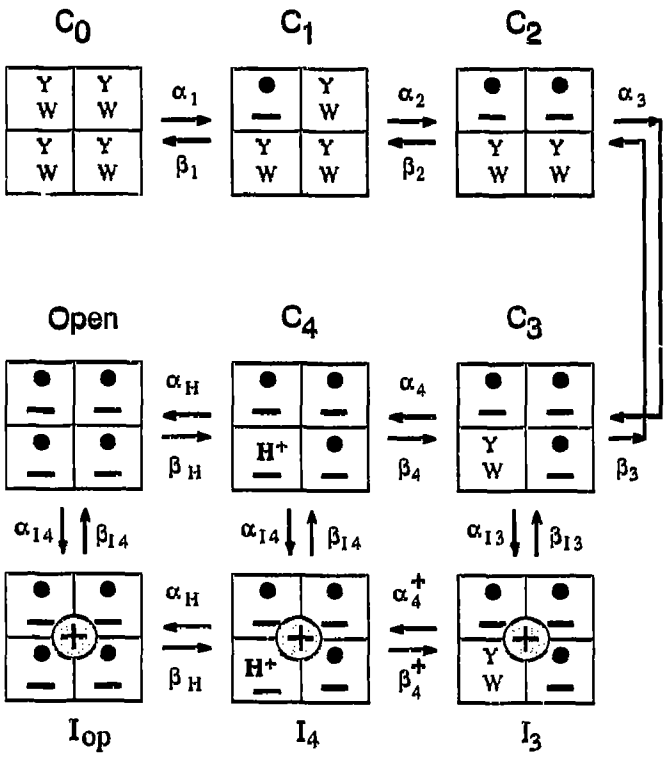

Fig. 1. A kinetic scheme based on the YW-gated model. The *Shaker* K^+ channel consists of four identical subunits. Each electron transfer between Y445 and W435 leads to another closed state, C_i (i=0–4). The deprotonation of $TyrOH^+$ is not explicitly shown except in the final transition to the open state. The deprotonation rate α_H is much faster than the electron transfer rates α_i and β_i. The inactivation rate constants (α_{I3}, α_{I4}, β_{I3} and β_{I4}) depend on the number of negatively charged W435s interacting with the inactivation ball (represented by a circle enclosing a positive sign). The transition rates, α_4^+ and β_4^+, between inactivated states are the electron transfer rates modified by the positive field from the inactivation ball. Representations of other symbols: Y, Y445; W, W435, H^+, $TyrOH^+$; '•', tyrosine radical, and '–', negatively charged W435.

system when the transferring electron is localized at Y445 and W435, respectively. The electric field may alter the energy gap, $E_{Yi} - E_{Wi}$, thereby changing the transfer rate [17,18]. As calculated in [6], the energy gap is not very sensitive to the depolarizing field itself, but may be altered significantly through the change of the electric field from nearby charged residues (the voltage sensors) in response to the depolarizing field. Although the S4 segment has been postulated to be the voltage sensor [19,20], its residues may be tightly bound in the protein interior. Within the framework of the YW-gated model, the major voltage sensor could be aspartate-447 (D447), which is located just above Y445 [6]. According to the molecular structure obtained by Busath's group [8], the sidechain of D447 is pointing toward the pore. To stabilize the structure, the four negatively charged D447s are likely to be coordinated with a Ca^{2+} ion [8]. In this configuration, the D447 sidechains (and the coordinated Ca^{2+} ion) may be flexible to respond to the membrane potential change.

The transitions between inactivated states are similar to those between closed states, except that the electron transfer rates have been modified by the presence of the inactivation ball near W435s. Fig. 1 shows only a couple of these transitions. Due to the positive field from the inactivation ball, the backward electron transfer rate, β_i^+ (i=1–4), should be smaller than β_i at all membrane potentials. This modification will result in apparent charge immobilization.

It was commonly assumed that the voltage dependence of channel activation is determined by the equivalent valence of gating charges [19,20]. Based on this assumption, the total charge displacement per channel has been estimated from the voltage sensitivity. This approach is reasonable if the gating charges moving between closed and open states are also the voltage sensors. Recently, however, the voltage-sensitivity method has been found to be inconsistent with direct measurement [21]. Moreover, mutation of a residue in the *Shaker* K^+ channel changed the voltage sensitivity significantly without affecting the total charge displacement obtained by direct measurement [21]. These results 'call into question an assumption in experiments that support the S4 hypothesis' [21].

According to the YW-gated model, the gating charge moving between Y445 and W435 is not the voltage sensor. From Eqns. 3a and 3b, the voltage sensitivity of activation depend on the energy gap between E_{Yi} and E_{Wi}, which is controlled by the voltage sensors in response to the depolarizing field. Mutations could modify the molecular structure around these voltage sensors so that their effect on the energy gap is altered. For example, the distance between D447 and Y445 may become longer, or the binding between D447 sidechains and the coordinated Ca^{2+} ion may become tighter so that stronger depolarization is needed to push the D447 sidechain toward Y445. In either case, mutations will reduce the voltage sensitivity of the electron transfer rate. Due to the possible allosteric effects, many point mutations could modify the molecular structure around voltage sensors, thereby changing the voltage sensitivity. However, most of them will not change the total charge displacement. The result of Schoppa et al. [21] may not be a special case.

Although the YW-gated model is consistent with many aspects of channel's gating, one may point out that the charge displacement between Y445 and W435 is much less than the directly measured charge displacement per channel, equivalent to 12.3 electronic charges moving across the membrane. As noted by Schoppa et al. [21], their measurement may include charge movement not directly involved in activation. In our model, the negatively charged W435s may induce displacement of charged and dipolar residues, especially those on the cytoplasmic side. The positive charges will be attracted toward W435s and negative charges repelled toward the cytoplasmic side, resulting in a net outward current. Since a channel protein contains hundreds of charged and dipolar residues, the induced charge displacement could be much larger than the actual gating charge displacement between Y445 and W435.

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