Voltage dependence of rate functions for Na+ channel inactivation within a membrane

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Abstract

The inactivation of a Na+ channel occurs when the activation of the charged S4 segment of domain IV, with rate functions $\alpha_i$ and $\beta_i$, is followed by the binding of an intracellular hydrophobic motif which blocks conduction through the ion pore, with rate functions $\gamma_i$ and $\delta_i$. During a voltage clamp of the Na+ channel, the solution of the master equation for inactivation reduces to the relaxation of a rate equation when the binding of the inactivation motif is rate limiting ($\alpha_i \gg \gamma_i$ and $\beta_i \gg \delta_i$). The voltage dependence of the derived forward rate function for Na+ channel inactivation has an exponential dependence on the membrane potential for small depolarizations and approaches a constant value for larger depolarizations, whereas the voltage dependence of the backward rate function is exponential, and each rate has a similar form to the Hodgkin-Huxley empirical rate functions for Na+ channel inactivation in the squid axon.
INTRODUCTION

The action potential in nerve and muscle membranes is generated by the opening and subsequent inactivation of Na+ channels and the activation of K+ channels [1]. The Na+ channel transient current during a depolarizing voltage clamp is proportional to the expression $m^3h$ where the activation variable $m$ and inactivation variable $h$ satisfy first order rate equations with rate functions dependent on the potential difference across the membrane. Support for the assumption that activation and inactivation are separate processes was provided by the removal of Na+ inactivation from the squid axon membrane by the internal perfusion of pronase without affecting activation kinetics [2]. However, more recent experimental data has shown that there is a delay in the onset of Na+ channel inactivation that is dependent on the time-course of channel activation, and that Na+ channel inactivation partially immobilizes the gating charge associated with activation [3] and therefore, activation and inactivation are interdependent or coupled processes.

Based on voltage clamp fluorometry, it has been shown that, in response to membrane depolarization, the transverse motion of the charged S4 segments of the Na+ channel domains I to III is associated with fast gating current, whereas the slower movement of domain IV (DIV) S4 is correlated with inactivation and the binding of an intracellular hydrophobic motif which blocks conduction through the ion pore [4, 5]. This may occur for small depolarizations when the ion channel is usually closed (closed-state inactivation) or for larger depolarizations when the S4 segments in domains I to III are activated (open-state inactivation). In this paper, it is assumed that Na+ channel inactivation is independent of activation, and that the rate functions for the activation of DIV S4 are large relative to those of the binding of the inactivation motif to the ion pore, and it is shown that the solution of the master equation for inactivation satisfies a rate equation where the derived rate functions have the same voltage dependence as empirical Hodgkin-Huxley (HH) inactivation functions for the squid axon [1].

INDEPENDENT ACTIVATION AND INACTIVATION OF A Na CHANNEL

Inactivation of a Na+ channel is correlated with the transverse motion of the charged S4 segment of the domain IV, with rate functions $\alpha_i$ and $\beta_i$, followed by the binding of an intracellular hydrophobic motif which blocks conduction through the ion pore, with rate functions $\gamma_i$ and $\delta_i$ (see Fig. 1). The occupation probabilities of the permissive states $h_1$, $h_2$ and the inactivated state $h_3$ are determined by

$$\frac{dh_1}{dt} = -\alpha_i h_1(t) + \beta_i h_2(t),$$

$$\frac{dh_2}{dt} = \alpha_i h_1(t) + \delta_i h_3(t) - (\beta_i + \gamma_i) h_2(t),$$

$$\frac{dh_3}{dt} = \gamma_i h_2(t) - \delta_i h_3(t).$$

If the Na+ channel is depolarized to a clamp potential $V$ from a large hyper-
polarized holding potential \((h_1(0) = 1, \text{and } h_2(0) = h_3(0) = 0)\), the solution of Eqs. (1) to (3) is

\[
\begin{align*}
\frac{dh_1(t)}{dt} &= \beta_i \delta_i - \frac{\alpha_i \gamma_i + \delta_i - \omega_1}{\omega_1 \omega_2} \exp(-\omega_1 t) + \frac{\alpha_i \gamma_i + \delta_i - \omega_2}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \\
\frac{dh_2(t)}{dt} &= \frac{\alpha_i \gamma_i}{\omega_1 \omega_2} - \frac{\alpha_i \gamma_i - \delta_i}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) + \frac{\alpha_i \gamma_i - \delta_i}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \\
\frac{dh_3(t)}{dt} &= \frac{\alpha_i \gamma_i}{\omega_1 \omega_2} + \frac{\alpha_i \gamma_i}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_1 t) - \frac{\alpha_i \gamma_i}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t). \tag{4}
\end{align*}
\]

where \(\omega_1 \text{ and } \omega_2 (\gg \omega_1)\) are solutions of the characteristic equation

\[
\omega^2 - \omega(\alpha_i + \beta_i + \gamma_i + \delta_i) + \alpha_i \gamma_i + \delta_i (\alpha_i + \beta_i) = 0. \tag{5}
\]

Assuming that the rate functions for the transition of the DIV S4 sensor are larger than those for binding of the inactivation motif \((\beta_i \gg \delta_i \text{ and } \alpha_i \gg \gamma_i)\), it may be shown from Eq. (5) that \(\omega_1 \approx \gamma_i \alpha_i/ (\alpha_i + \beta_i) + \delta_i\) and \(\omega_2 \approx \alpha_i + \beta_i \gg \omega_1\), and Eq. (4) becomes

\[
h_3(t) \approx \frac{\alpha_i \gamma_i}{\alpha_i \gamma_i + \delta_i (\alpha_i + \beta_i)} [1 - \exp(-\omega_1 t)], \tag{6}
\]

which is a solution of the rate equation (1)

\[
\frac{dh_3}{dt} = \beta_{h,2} - (\alpha_{h,2} + \beta_{h,2}) n, \tag{7}
\]

where

\[
\alpha_{h,2}(V) \approx \delta_i, \tag{8}
\]

\[
\beta_{h,2}(V) \approx \frac{\gamma_i}{1 + \beta_i/\alpha_i}. \tag{9}
\]

Therefore, the probability of the permissive states \(h = 1 - h_1 - h_2\) satisfies

\[
\frac{dh}{dt} = \alpha_{h,2} - (\alpha_{h,2} + \beta_{h,2}) h, \tag{10}
\]

and Eqs. (8) and (10) provide a good fit to the empirical rate functions \(\alpha_h\) and \(\beta_h\) for the squid axon Na channel \([1]\) (see Figs. 2 and 3). Similarly, if we assume that \((\beta_i \gg \delta_i \text{ and } \gamma_i \gg \alpha_i)\), it may be shown that

\[
\alpha_{h,2}(V) \approx \frac{\delta_i (\alpha_i + \beta_i)}{(\gamma_i + \beta_i)}, \tag{11}
\]

\[
\beta_{h,2}(V) \approx \frac{\alpha_i}{1 + \beta_i/\gamma_i}. \tag{12}
\]

and therefore, for a large depolarization, the forward rate \(\beta_{h,2} \approx \alpha_i = \alpha_{i0} \exp(q_{\alpha_i} (V - V_0)/kT)\), where \(kT/e \approx 25\) mV and \(k\) is Boltzmann’s constant, and if
\( q_{a_i} \ll e, \beta_{h,2} \) has a quasilinear voltage dependence, consistent with the determination of \( \beta_h \) for myelinated nerve [6].

Based on the measurement of a rising phase of the gating current in a squid axon membrane and the chemical structure of a Na channel, it has been proposed that at least two transitions are required for the activation of each voltage sensor [7]. Therefore, for each voltage sensor from domains I to III, the occupation probabilities of the closed states \( m_1, m_2 \) and the open state \( m \) (see Fig. 4) are determined by [8, 9, 10].

\[
\frac{dm_1}{dt} = -\alpha_a m_1(t) + \beta_a m_2(t), \tag{13}
\]

\[
\frac{dm_2}{dt} = \alpha_a m_1(t) + \delta_a m(t) - (\beta_a + \gamma_a)m_2(t), \tag{14}
\]

\[
\frac{dm}{dt} = \gamma_a m_2(t) - \delta_a m(t). \tag{15}
\]

where the rate functions \( \alpha_a, \beta_a, \gamma_a \) and \( \delta_a \) are exponential functions of the membrane voltage \( V \). The solution of Eqs. (13) to (15) during activation \((m_1(0) = 1)\), and deactivation \((m(0) = 1)\) is dependent on the frequencies \( \omega_1 \) and \( \omega_2 \) which satisfy

\[
\omega^2 - \omega(\alpha_a + \beta_a + \gamma_a + \delta_a) + \alpha_a \gamma_a + \delta_a (\alpha_a + \beta_a) = 0. \tag{16}
\]

and if \( \omega_2 \gg \omega_1 \), the solution for the open state \( m \) may be approximated by

\[
m_A(t) \approx \frac{\alpha_a \gamma_a}{\alpha_a \gamma_a + \delta_a (\alpha_a + \beta_a)} [1 - \exp(-\omega_1 t)], \tag{17}
\]

\[
m_D(t) \approx \frac{\alpha_a \gamma_a + \delta_a (\alpha_a + \beta_a) \exp(-\omega_1 t)}{\alpha_a \gamma_a + \delta_a (\alpha_a + \beta_a)}. \tag{18}
\]

and satisfy the rate equation [11]

\[
\frac{dm}{dt} = \alpha_{m,2} - (\alpha_{m,2} + \beta_{m,2})m. \tag{19}
\]

If the rate functions of the first step are larger than the second step \((\beta_a \gg \delta_a\) and \(\alpha_a \gg \gamma_a\)), we may write

\[
\alpha_{m,2}(V) \approx \frac{\gamma_a}{1 + \beta_a / \alpha_a}, \tag{20}
\]

\[
\beta_{m,2}(V) \approx \delta_a, \tag{21}
\]

which are in accord with the empirical rate functions \( \alpha_m \) and \( \beta_m \) for the squid axon Na channel [1] (see Figs. 5, 6(a) and (b)), whereas if we assume that \( \beta_a \gg \delta_a \) and \( \gamma_a \gg \alpha_a \)

\[
\alpha_{m,2}(V) \approx \frac{\alpha_a}{1 + \beta_a / \gamma_a}, \tag{22}
\]

\[
\beta_{m,2}(V) \approx \frac{\delta_a (\alpha_a + \beta_a)}{(\gamma_a + \beta_a)}. \tag{23}
\]
CONCLUSION

In this paper, we assume that Na+ channel inactivation occurs when the activation of the DIV S4 sensor provides a binding site for the intracellular hydrophobic motif which blocks conduction of ions through the ion pore. During a voltage clamp of the Na+ channel, assuming that the rate functions for the activation of DIV S4 are large relative to those of the binding of the inactivation motif to the ion pore, the solution of the master equation for the inactivation process may be approximated by the solution of a rate equation where the backward transition rate is an exponential function of the membrane potential $V$ and the forward rate increases exponentially with $V$ for small depolarizations but approaches a constant saturated value for a larger depolarization, reflecting the voltage-independence of the rate function $\gamma_i$ for the binding of the inactivation motif. These functions have a similar form to those determined empirically for Na+ channel inactivation in the squid axon [1], but by comparison, the rate-limiting step for activation of the S4 sensor in K+ and Na+ channels has a small voltage dependence, and therefore, for a large membrane depolarization, the derived forward transition rate for activation is quasilinear.

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Figure 1: Inactivation model of a Na+ ion channel, where the occupation probabilities of the permissive states \( h_1(t) \), \( h_2(t) \), and the inactivated state \( h_3(t) \) satisfy a master equation, and the rate functions \( \alpha_i \), \( \beta_i \) and \( \delta_i \) are voltage dependent whereas the forward rate \( \gamma_i \) for the binding of the inactivation motif is voltage independent.

\[
\begin{array}{c c c c c c}
\alpha_1 & \gamma_1 & \beta_1 & \delta_1 \\
h_1 & \Rightarrow & h_2 & \Rightarrow & h_3 \\
\end{array}
\]

Figure 2: The derived rate functions \( \alpha_{h,2}(V) \) and \( \beta_{h,2}(V) \) (solid line) in Eqs. (8) and (9) provide a good approximation to the HH rate functions (ms\(^{-1}\)) \( \alpha_h = 0.07 \exp(-(V+50)/20) \) and \( \beta_h = 1/(1 + \exp(-(20+V)/10)) \) (dotted line) when the inactivation rate functions are \( \alpha_i(V) = 3 \exp(0.25V/25) \gg \gamma_i(V) = 1 \), and \( \beta_i(V) = 0.406 \exp(-2.25V/25) \gg \delta_i(V) = 0.07 \exp(-(V+50)/20) \).
Figure 3: Survival probability of the permissive states $h(t)$ during a voltage clamp for a two stage model of Na$^+$ channel inactivation (solid line) and for the HH squid axon model (dotted line) (see Fig. 2).

Figure 4: Activation model of a Na$^+$ channel, where the occupation probabilities of the closed states $m_1$, $m_2$, and the open state $m$ satisfy a master equation, and $\alpha_a$, $\beta_a$, $\gamma_a$ and $\delta_a$ are voltage-dependent rate functions between states.
Figure 5: The derived rate functions $\alpha_{m,2}(V)$ and $\beta_{m,2}(V)$ (solid line) in Eqs. (22) and (23) provide a good approximation to the HH rate functions (ms$^{-1}$) $\alpha_m = 0.1(V+25)/(1 + \exp[-0.1(V+25)])$ and $\beta_m = 4 \exp[-(V+50)/18]$ (dotted line) when the activation rate functions are $\alpha_a(V) = 30 \exp(0.27(V+50)/25) \gg \gamma_a(V) = 2.65 \exp(0.27(V+50)/25)$, and $\beta_a(V) = 251 \exp(-0.95(V+50)/25) \gg \delta_a(V) = 4 \exp(-(V+50)/18)$. 
Figure 6: Survival probability of the open state $m(t)$ during a voltage clamp for a two stage model of activation of a Na$^+$ channel (solid line) and the HH model (dotted line) during (a) activation and (b) deactivation, for the rate functions of Fig. 5.