Time Delay in Structural Shifts: Modeling Multiple States

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N-type Shaker K+ channels are composed of four subunits.
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deactivation site

side view
top view
outside of cell

open

inside of cell

closed
N-type Shaker K+ channel functional states

- open (activated) ↔ closed (deactivated)
- unblocked ↔ blocked (inactivated)
“recovery”: two routes to C

- C (closed)
- C (open)
- CB (closed-blocked)
- OB (open-blocked)

Substantial ionic ("tail") currents

No currents
“recovery”: two routes to C

*160 mM ext. K+ at -80mV

Demo and Yellen, 1991

substantial ionic (“tail”) currents

no currents
Experimental Detection of States

Early (lasting) assumptions:
- thermal (energy) fluctuations and ligation drive state shifts
- shift probability, but not exact time, knowable
- channel unchanged by ion current

Why consider intermediate steps?

(1) \[ [A] + [C] \rightleftharpoons [AC] + [B] \rightleftharpoons [ABC] \]
\[ \begin{align*}
&\quad k_1 \quad k_1^- \\
&\quad k_2 \quad k_2^- \\
&\quad k_3 \quad k_3^- \\
&\quad k_4 \quad k_4^- \\
\end{align*} \]
(2) \[ [A] + [C] \rightleftharpoons [AC_{int}] \rightleftharpoons [AC] + [B] \rightleftharpoons [ABC_{int}] \rightleftharpoons [ABC] \]
\[ \begin{align*}
&\quad k_1 \quad k_1^- \\
&\quad k_2 \quad k_2^- \\
&\quad k_3 \quad k_3^- \\
&\quad k_4 \quad k_4^- \\
\end{align*} \]

at equilibrium:

(1) \[ 0 = k_1 [A][C] + k_2 [ABC] - k_1^- [AC] - k_2^- [AC][B] \]

(2) \[ 0 = \frac{k_2 k_1}{k_1^- + k_2} [A][C] + \frac{k_3 k_4}{k_3^- + k_4} [ABC] - \frac{k_1 k_2}{k_1^- + k_2} [AC] - \frac{k_3 k_4}{k_3^- + k_4} [AC][B] \]
at equilibrium:

(1) \[ 0 = k_1[A][C] + \frac{k_2}{k_1+k_2}[ABC] - \frac{k_1}{k_1+k_2}[AC] - \frac{k_2}{k_3+k_4}[AC][B] \]

(2) \[ 0 = \frac{k_2k_1}{k_1+k_2}[A][C] + \frac{k_3k_4}{k_3+k_4}[ABC] - \frac{k_1k_2}{k_1+k_2}[AC] - \frac{k_3k_4}{k_3+k_4}[AC][B] \]
Adjustable Delay, $\Delta G$ Preserved

Equilibrium and delay values for varying rate coefficients.

$$\frac{k_{-1}k_{-2}}{k_1k_2}$$ was maintained to preserve $\Delta G \sim 6$ kcal/mol.
Adjustable Delay, $\Delta G$ Preserved

Any intermediate state $N$ must have sufficiently small $k_{N-1}[N-1] + k_N[N+1]$ to "avoid" detection at equilibrium:

$$
\frac{k_{N-1}[N-1] + k_N[N+1]}{k_{(N-1)} + k_N}
$$

Equilibrium and delay values for varying rate coefficients:

<table>
<thead>
<tr>
<th>$k_1$</th>
<th>$k_{-1}$</th>
<th>$k_2$</th>
<th>$k_{-2}$</th>
<th>$O(t)$ equil.</th>
<th>$80%$</th>
<th>$95%$</th>
<th>$99%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>$5.067 \times 10^{-8}$</td>
<td>$t = .0016$</td>
<td>$t = .0030$</td>
<td>$t = .0046$</td>
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Equilibrium and delay values for varying rate coefficients.

$\frac{k_{-1}k_2}{k_1k_2}$ was maintained to preserve $\Delta G \sim 6$ kcal/mol.
Hyperpolarization shortens delay

Initial delay is likely coupled to movement of voltages sensors (gating charges) on outer helices

Future Work

Short lifetimes and low $k_{off}$ values explain experimental difficulties of intermediate detection

Intermediate states affect energy landscape but not equilibria or overall $\Delta G$

50% of current drugs target ion channels[6].

Our understanding of channel dynamics and treatment discovery would be improved by:

1. Determining Shaker $K^+$ channel crystal structure in closed conformation[3]

2. Models that describe mechanical coupling of gating charges to pore inactivation

3. Describing selectivity mechanisms in Na+ and K+ channels
References


