Time Delay in Structural Shifts: Modeling Multiple States

Andrej Savol[1]
Mentor: Carlos Camacho[2]
[1]*Bioengineering & Bioinformatics Summer Institute, Dept. of Computational Biology, University of Pittsburgh, 15260*
Dept. of Computational Biology, University of Pittsburgh, 15260

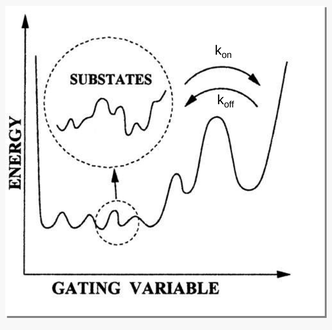
Abstract

The reaction rates of most biological processes are linked to the concentrations of reactants and surrounding conditions. Even in systems with well-defined conformations, state mediation may take place via any number of intermediary states. Although direct observation is potentially difficult experimentally, these intermediate states can more accurately model entire systems where only equilibria were before considered. We specifically consider Shaker K+ channels, and see this approach as providing more variability than models which only predict equilibria.

Introduction

Voltage-dependent ion channels of biological membranes are formed by pore-like proteins that extend through the cell membrane [2]. When dwelling in open conformations (activated), specific ions are allowed to pass through the membrane and participate in important cellular processes (neural excitability, etc.). In closed (resting/deactivated) conformations, ion flow is prevented. Channels can additionally be blocked by inactivating peptides that prevent ion flow in either conformation.

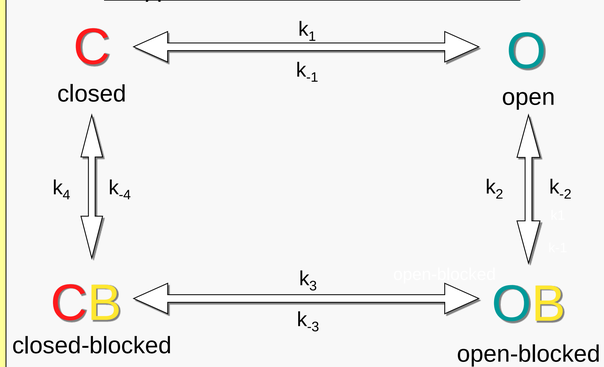
Shaker K+ dynamics are governed by ion concentration and transmembrane potential. Once a (concentration dependent) voltage threshold is surpassed, opening and closing does not take place instantly, though the *in vivo* delay (~ 1 ms) is shortened by hyperpolarization [5]. We hope to introduce new intermediate states that accurately consider opening/closing delay and still provide agreement with past voltage clamp studies.



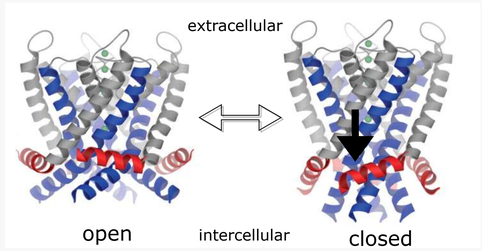
Two global minima correspond to open and closed macroconformations. One assumes a large number of quasidegenerate and voltage-independent closed substates separated from the open conformation by a voltage-dependent potential barrier [1].

Methods

N-type Shaker K+ channel model*



All differential equations were solved numerically using *Mathematica.*



Proposed deactivation mechanism[3]. S4-S5 (red) is mechanically linked to outer voltage sensing helices, and pushes inward, blocking pore when triggered. The time delay for this process is one motivation of the study, since ion current cannot alone predict all physically relevant parameters[1].

*Recovery to C from OB is 92% via CB and 8% via O at 160mM external K+ and -80mV [5].

Results

$$\frac{d[C]}{dt} = k_{-1}[O] - k_1[C]$$

$$\frac{d[O]}{dt} = k_1[C] + k_{-2}[OB] - (k_{-1} + k_2)[O]$$

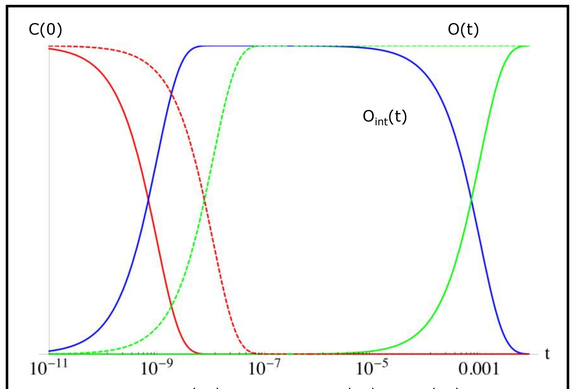
$$\frac{d[OB]}{dt} = k_2[O] - k_{-2}[OB]$$

the same system (primary recovery pathway) with intermediate (int) steps becomes

$$C \underset{\bar{k}_{-1}}{\overset{\bar{k}_1}{\rightleftarrows}} O_{\text{int}} \underset{\bar{k}_{-2}}{\overset{\bar{k}_2}{\rightleftarrows}} O \underset{\bar{k}_{-3}}{\overset{\bar{k}_3}{\rightleftarrows}} OB_{\text{int}} \underset{\bar{k}_{-4}}{\overset{\bar{k}_4}{\rightleftarrows}} OB$$

at equilibrium the systems are equal when

$$k_n = \frac{\bar{k}_{(2n-1)}\bar{k}_{2n}}{\bar{k}_{-(2n-1)} + \bar{k}_{2n}}, \quad k_{-n} = \frac{\bar{k}_{-(2n-1)}\bar{k}_{-2n}}{\bar{k}_{-(2n-1)} + \bar{k}_{2n}}$$



dashed: O ⟺ C, solid: O ⟺ O_{int} ⟺ C

$$k_1 = 10^9,\ k_{-1} = 10^4,\ k_2 = 10^3,\ k_{-2} = 10^{-4}$$

Any intermediate state N must have sufficiently small

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

to "avoid" detection at equilibrium

$\log k_1$	$\log k_{-1}$	$\log k_2$	$\log k_{-2}$	O(t) equil.	80%	95%	99%
9	4	3	-4	5.067×10^8	t = .0016	t = .0030	t = .0046
9	3	2	-4	5.067×10^8	t = .016	t = .030	t = .046
8	4	3	-5	5.067×10^7	t = .0016	t = .0030	t = .0046
7	4	3	-6	5.067×10^6	t = .0016	t = .0030	t = .0046

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

Conclusions and Future Research

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

Intermediate states affect energy landscape but not equilibria or overall ΔG

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

Acknowledgements

The national BBSI program (http://bbsi.eeicom.com) is a joint initiative of the NIH-NIBIB and NSF-EEC, and the BBSI @ Pitt is supported by the National Science Foundation under Grant EEC-0234002.

1) Carlos Camacho and lab members: Marta Bueno, Lidio Forward, Alpay Temiz

2) Department of Computational Biology, University of Pittsburgh: Judy Wieber and Ryan Moslein

References

1. Goychuk, Igor; Hanggi, Peter (2002) *Proc. Natl. Acad. Sci.* USA 99, 3552-3556.
2. Cherry, J.; Adler, F, *J. Theor. Biol*. (2000) 203, 117-133.
3. MacKinnon, R.; Campbell, E.; Long, S. *Science* (2005). 309, 903-908.
4. MacKinnon, R.; Campbell, E.; Long, S. *Science* (2005). 309, 897-903.
5. Kuo, C. *The Journal of Neuroscience* (1997). 17, 3436-3444.
6. Coalson, Rob; Department of Chemistry. *Modeling Ion Transport through Biological Channels*. PowerPoint Presentation: 2007.
7. Liebovitch, L. S.; Czegledy, F. P. *Ann. Biomed. Engr.* 20 (1992), pp. 517-531.
8. Liebovitch, L. S.; Krekora. *Proceedings of the Institute for Mathematics and its Applications*.