A mesoscopic approach to understanding the mechanisms underlying the ion permeation on the discrete-state diagram

Shigetoshi Oiki, Masayuki Iwamoto, and Takashi Sumikama

Department of Molecular Physiology and Biophysics, University of Fukui Faculty of Medical Sciences, Fukui 910-1193, Japan

In the Perspectives series in the June 2010 issue (Bahar, 2010; Bucher and Rothlisberger, 2010; Dror et al., 2010; Roux, 2010; Silva and Rudy, 2010), a broad range of permeation events from femtoseconds to minutes in time scale and sub-angstrom to millimeter in space were discussed. A main message in the Perspectives was to explore each discipline to expand its limitation, such that different levels of knowledge in a hierarchy of ion permeation events are integrated. Here, we propose another approach from a mesoscopic point of view.

Electrophysiologists are familiar with single-channel currents, a macroscopic event in terms of ion permeation (Fig. 1 A). They have postulated the underlying mechanisms of ion permeation processes from a top-down point of view. On the other hand, recent progress of the computer simulation enabled visualization of the ion trajectories through channel molecules, which provided microscopic views of permeation processes (Bernèche and Roux, 2001). One is eager to understand a whole range of events by integrating microscopic trajectories into macroscopic observables, but it has not been successful because the "reality" and these microscopic trajectories have remained far apart.

To bridge the gap, electrophysiologists have developed a tool to relate single-channel currents to the discrete-state Markov model (DSMM) for permeation. Ion distributions are segregated into a few representative "states," and connecting these states create a discrete-state diagram (Fig. 1, A→B). A random walk on the diagram leads to the net flux of the reaction (or permeation; Fig. 1 B) (Andersen, 1989; Miller, 1999). Single-channel current recordings under various experimental conditions were collected, and the set of rate constants for the diagram was optimized for the experimental data. These values have been used to draw the free energy profile of ion permeation (Fig. 1, B→C). In this procedure, the ion trajectory in real space is converted to the free energy profile as a function of the electrical distance. Thus, in this line of thinking, the permeation is considered as a simplified motion on linear coordinates of either the real space (Fig. 1 A) or the electrical distance (Fig. 1 C).

We present here an alternative way of conceptualizing the permeation processes (Ando et al., 2005; Iwamoto, M., H. Shimizu, S. Oiki. The water-ion coupling ratio for ion permeation through the KcsA potassium channel: dependencies on concentration and species of permeating ions. 2009. *Biophys. J.* Abstr. 927; unpublished data). Our proposal is to trace the processes as cyclic paths on the DSMM (Fig. 1, B→D) rather than linear coordinates. This is a slight modification in concept, but it allows intuitive images of ion permeation processes. There are many cycles on the diagram (in a simple case depicted in Fig. 1 B there are three cycles), and a random walk on the diagram leads to a completion of a cycle. The rate of cycle completion is called the cycle flux (Hill, 2004), which is an important thermodynamic concept, where the driving force is conjugated on it. Furthermore, energy couplings, such as water–ion coupling ratio, can be readily treated on the cyclic paths (Ando et al., 2005). The cycle kinetics not only provides a conceptual framework, but it also gives a quantitative evaluation of flux that has not been attained before. It may not have been recognized that, if the diagram becomes complicated such as that for the KcsA channel, the net ionic current, rather than net flux, cannot be calculated from the standard method using the steady-state probability of ion-occupancy states (unpublished data). In the previous method, stoichiometric numbers cannot be implemented, and Monte Carlo simulation was needed (Morais-Cabral et al., 2001). The cycle flux calculated by the diagrammatic method developed by Hill (1988) enabled the calculation of macroscopic observables (Ando et al., 2005; Iwamoto, M., H. Shimizu, S. Oiki. 2009. *Biophys. J.* 179a. Abstr. 927).

The work of T.L. Hill marked a new era of nonequilibrium thermodynamics, and he was enthusiastic to draw diagrams. These efforts are delineated in a seminal book, *Free Energy Transduction and Biochemical Cycle Kinetics* (Hill, 2004). He established a method of evaluating cycle flux using a diagrammatic method rather than Monte Carlo approximation. In the diagrammatic method, a large number of possible paths were decomposed into elementary

Correspondence to Shigetoshi Oiki: oiki-fki@umin.ac.jp

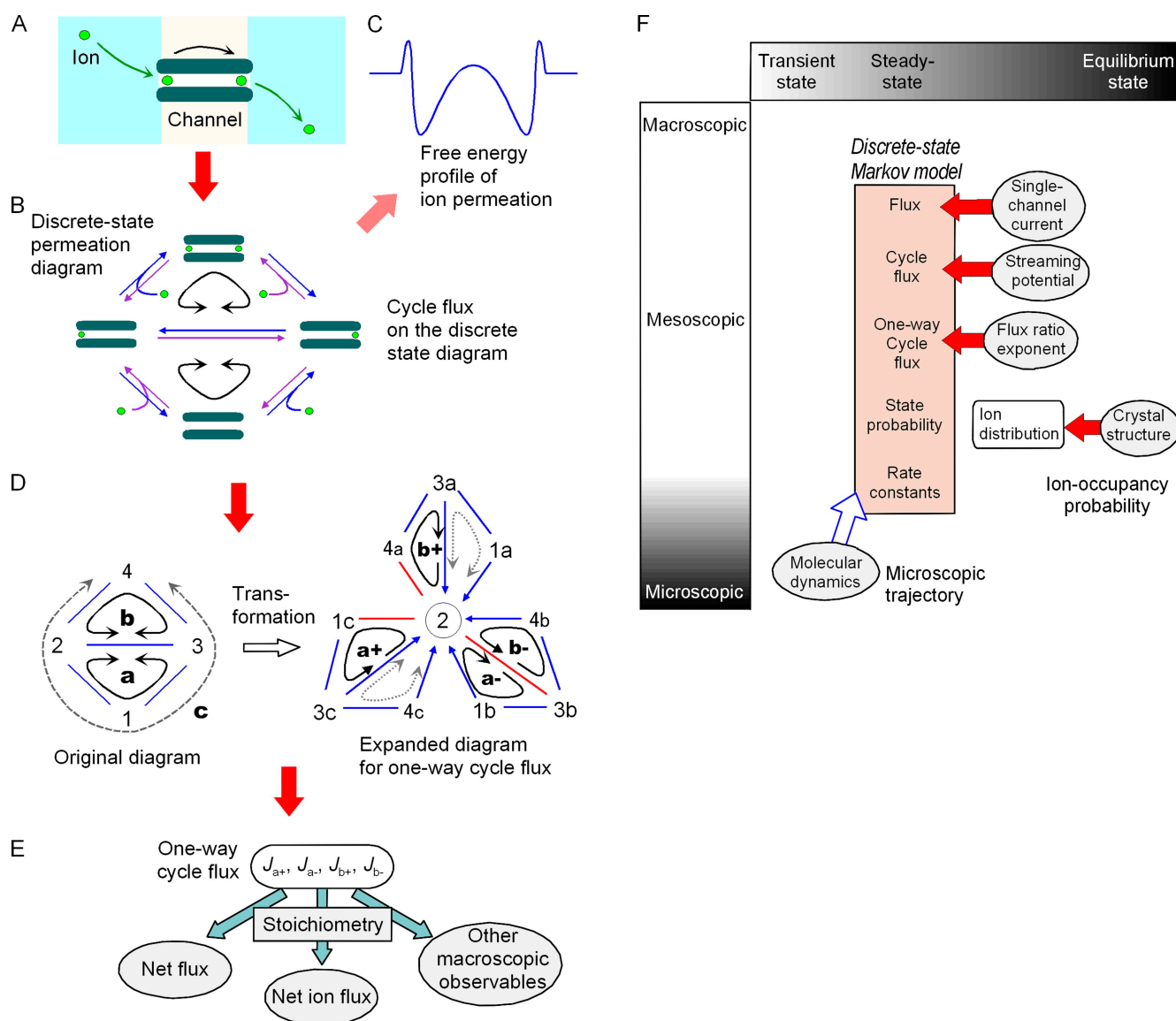Abbreviation used in this paper: DSMM, discrete-state Markov model.

Figure 1. From ion permeation through channel structure to cycle kinetics on DSMM. The ion permeation processes (A) are projected on a discrete-state diagram (B) on which a random walk completing cyclic paths generates net ion flux. Macroscopic observables, such as the single-channel current, can be deduced with a set of rate constants for the diagram. These rate constants have been transformed to the free energy profile of ion permeation (C), and they can be used for calculating the mesoscopic parameter of the cycle flux. There are several cyclic paths on the diagram, some of which generate nonzero net flux (a and b) and others (c) do not (D). From the original diagram, all the cyclic paths were decomposed into one-way cyclic paths (D; right). All the one-way cycle fluxes can be calculated. Given the cycle fluxes, trends of random kinetic processes, such as relative contributions of the cycles, are visualized, and macroscopic observables were calculated using stoichiometric numbers (E). Permeation processes in different levels and experimental observables were related in the hierarchy of ion permeation events (F).

and informative cyclic paths with one-way directionality (Fig. 1 D).

In the steady state of flux, cycle completions of each type, *a*+, *a*−, *b*+, and *b*−, are regarded as independent random events. Thus, random transitions among states (microscopic events) were integrated into random transitions among independent one-way cycles (mesoscopic events). The macroscopic observables can be attained as a linear combination of the independent cycles having stoichiometric numbers as a weight factor (Fig. 1 E). Con-

versely, the relative contribution of each cycle under certain conditions is quantitatively evaluated. In the case of the diagram (Fig. 1 B), predominant operation in cycle *a* at low concentration of permeating ions is replaced by cycle *b* at higher concentration, and those concentration-dependent shifts of cycle contribution are given quantitatively.

We designated the cycle flux as the mesoscopic parameter. The term "mesoscopic" is intended as a relative, rather than strict, concept (Fig. 1 F). In fact, each rate constant in the DSMM might be a mesoscopic parameter when it

is viewed from the trajectories of molecular dynamics. Recently, attempts have been made to build up an ensemble of microscopic trajectories obtained from molecular simulations into more a collective description so that DSMM can be applied (Swope et al., 2004; Bowman et al., 2009; Silva and Rudy, 2010). In any case, Fig. 1 (A–C) can be interpreted such that A represents experimental observables, B is kinetic representation, and C can be related to the results of the molecular dynamics. Fig. 1 B is now a crossover point deducing down from the experimental observables and integrating up from the conformational trajectories. The cycle kinetics mediates messages among these three different languages. This use of the cycle kinetics is a helpful tool for understanding permeation processes, one worthy of the attention of researchers of ion permeation.

Our attempt to connect different levels of events is an example of an approach that is becoming increasingly important in the study of ion permeation. For example, we must address whether (a) quantum mechanics leads to the empirical parameters for molecular dynamics, and (b) elongating molecular dynamics simulations may coalesce into the results of a coarse-grained simulation. Answers to these critically important questions remain elusive. Attempts to achieve a seamless view throughout the hierarchy of ion permeation processes are ongoing, and progress along these lines is much anticipated.

Benoit Roux served as guest editor.

REFERENCES

Andersen, O.S. 1989. Kinetics of ion movement mediated by carriers and channels. *Methods Enzymol.* 171:62–112. doi:10.1016/S0076-6879(89)71007-7

Ando, H., M. Kuno, H. Shimizu, I. Muramatsu, and S. Oiki. 2005. Coupled K^+–water flux through the HERG potassium channel measured by an osmotic pulse method. *J. Gen. Physiol.* 126:529–538. doi:10.1085/jgp.200509377

Bahar, I. 2010. On the functional significance of soft modes predicted by coarse-grained models for membrane proteins. *J. Gen. Physiol.* 135:563–573. doi:10.1085/jgp.200910368

Bernèche, S., and B. Roux. 2001. Energetics of ion conduction through the K+ channel. *Nature.* 414:73–77. doi:10.1038/35102067

Bowman, G.R., K.A. Beauchamp, G. Boxer, and V.S. Pande. 2009. Progress and challenges in the automated construction of Markov state models for full protein systems. *J. Chem. Phys.* 131:124101. doi:10.1063/1.3216567

Bucher, D., and U. Rothlisberger. 2010. Molecular simulations of ion channels: a quantum chemist's perspective. *J. Gen. Physiol.* 135:549–554. doi:10.1085/jgp.201010404

Dror, R.O., M.O. Jensen, D.W. Borhani, and D.E. Shaw. 2010. Exploring atomic resolution physiology on a femtosecond to millisecond timescale using molecular dynamics simulations. *J. Gen. Physiol.* 135:555–562. doi:10.1085/jgp.200910373

Hill, T.L. 1988. Discrete-time random walks on diagrams (graphs) with cycles. *Proc. Natl. Acad. Sci. USA.* 85:5345–5349. doi:10.1073/pnas.85.15.5345

Hill, T.L. 2004. Free Energy Transduction and Biochemical Cycle Kinetics. Dover Publications, Inc., New York. 119 pp.

Miller, C. 1999. Ionic hopping defended. *J. Gen. Physiol.* 113:783–787. doi:10.1085/jgp.113.6.783

Morais-Cabral, J.H., Y. Zhou, and R. MacKinnon. 2001. Energetic optimization of ion conduction rate by the K^+ selectivity filter. *Nature.* 414:37–42. doi:10.1038/35102000

Roux, B. 2010. Perspectives on: molecular dynamics and computational methods. *J. Gen. Physiol.* 135:547–548. doi:10.1085/jgp.201010456

Silva, J.R., and Y. Rudy. 2010. Multi-scale electrophysiology modeling: from atom to organ. *J. Gen. Physiol.* 135:575–581. doi:10.1085/jgp.200910358

Swope, W.C., J.W. Pitera, and F. Suits. 2004. Describing protein folding kinetics by molecular dynamics simulations. 1. Theory. *J. Phys. Chem. B.* 108:6571–6581. doi:10.1021/jp037421y