Editorial: Graphic Representation of the Results of Kinetic Analyses

The mission of *The Journal of General Physiology* is to publish articles that elucidate basic biological, chemical, and physical principles of broad physiological significance. Physiological significance usually means mechanistic insights, which often are obtained only after extensive analysis of the experimental results. The significance of the mechanistic insights therefore can be no better than the validity of the theoretical framework used for the analysis—and it is usually better to be vaguely right than precisely wrong.

The uncertainties associated with data analysis are well illustrated in the *Perspectives on Ion Permeation* through membrane-spanning channels (*J. Gen. Physiol.* 113:761–794) and the related *Letters-to-the-Editor* in this issue. This exchange moreover identified a particular problem that can be resolved by a change in editorial policy.

The problem is the graphic representation of the results of kinetic analyses of ion permeation based on discrete-state rate models—and similar kinetic analyses of other physiological processes. It seems to have become de rigueur to summarize such results in a so-called energy profile (see Fig. 1), where the rate constants (k) deduced from the kinetic analysis are converted into free energies ($\Delta G^{\ddagger}$)—almost invariably using Eyring's transition state theory (TST):

$$\Delta G^{\ddagger} = -k_B T \cdot \ln[k \cdot (h/k_B T)], \qquad (1)$$

where k_B is Boltzmann's constant, T the temperature in kelvin, and h Planck's constant. The problems arise because Eq. 1 will be valid only for elementary transitions; e.g., transitions over distances less than the mean free path in aqueous solutions, ~0.1 Å. Whether or not one can use a discrete-state rate model to analyze a permeation process, for example, the (in)validity of Eq. 1 depends primarily on the distances ions have to traverse in the transitions between the different kinetic states.

The limitations inherent in the use of Eq. 1 are well known, but energy profiles have taken on a life of their own because they provide a convenient graphic representation of the results, as opposed to the more tedious (albeit more correct) tabulation of the rate constants. Assuming the experimental results justify the use of a discrete-state model, which would entail a demonstration that the model and the deduced rate constants satisfactorily describe the results, the problem becomes, how can one represent the results graphically in a manner that avoids the errors associated with the use of Eq. 1?

One such representation of linear kinetic schemes can be implemented by noting that free energy profiles based on the Eyring TST (i.e., on the use of Eq. 1) formally can be expressed as:

$$\Delta G(p) = -k_B T \cdot \ln\left\{\frac{\prod_{i=1,3,\ldots}^{p} [k_i/(k_B T/h)]}{\prod_{i=2,4,\ldots}^{p} [k_i/(k_B T/h)]}\right\}, \qquad (2)$$

where p (= 1, 2,…,n, where n is the total number of rate constants in the scheme) denotes the sequential position of the energy peaks and wells in the kinetic scheme (beginning with the first peak and ending outside the pore on the other side), and k_i is the ith rate constant in the scheme (forward rate constants are odd numbered and reverse rate constants are even numbered). That is, $\Delta G(p)$ for p = 1, 3,…, n − 1 denotes the peak energies, whereas $\Delta G(p)$ for p = 2, 4,…, n denotes the well energies. The interrupted line in Fig. 1 (right-hand ordinate) shows such an energy profile. The generalization of Eq. 2 is immediate, as the rate constant "profile" along the kinetic scheme can be represented by the function:

$$RCR_{ff}(p) = -\log\left\{\frac{\prod_{i=1,3,\ldots}^{p} (k_i/ff)}{\prod_{i=2,4,\ldots}^{p} (k_i/ff)}\right\}, \qquad (3)$$

where ff is an arbitrary "frequency factor." The three lines in Fig. 1 (left-hand ordinate) show rate constant representations (RCR) for ff = 1, 10^9, and $6 \cdot 10^{12}$ s^{-1} (= $k_B T/h$). (ff = 1 s^{-1} denotes the simplest version of Eq. 3, ff = 10^9 s^{-1} was chosen to approximate the frequency of diffusional transitions over a distance of 1 nm, and ff = $k_B T/h$ was chosen for comparison to Eq. 2.)

It is instructive to consider briefly some features of Eq. 3 and Fig. 1. First, the heights of the "peaks" vary with the choice of ff. The peaks shift in parallel up or down as ff is increased or decreased, which serves to emphasize how arbitrary a "barrier height" is—and to underscore the difficulties inherent in deducing an energy profile from a set of rate constants (compare Fig. 1 and the two different energy profiles deduced for ff = $6 \cdot 10^{12}$ and 10^9 s^{-1}). Second, the differences in height among the peaks are invariant, suggesting that they have mechanistic significance. It is unlikely that the frequency factors associated with each barrier crossing will be identical,

J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/99/10/589/2 $5.00
Volume 114 October 1999 589–590
Available online 27 September 1999
http://www.jgp.org

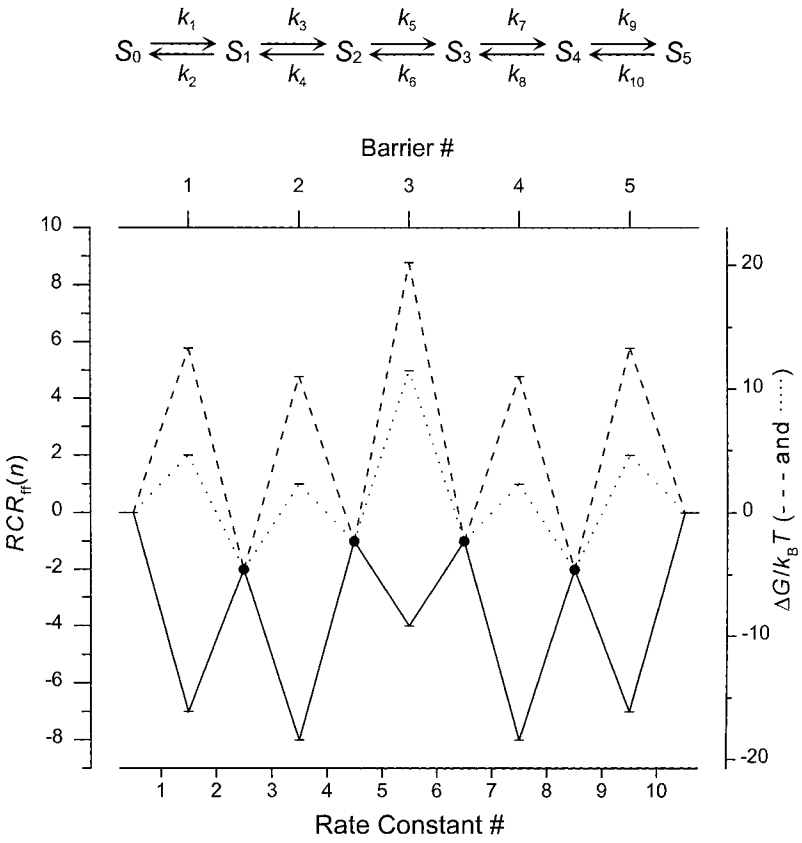

Figure 1. Graphic representation of the rate constants in a linear kinetic scheme. (Top) The kinetic scheme denoting the kinetic states (S_0 – S_5) and the rate constants (k_1 – k_{10}). (Bottom left-hand axis) The three lines denote rate constant representations using Eq. 3 and $ff = 1\ s^{-1}$ (——), $10^9\ s^{-1}$ (·····), and $6 \cdot 10^{12}\ s^{-1}$ (- - - -), respectively (for $k_1 = k_{10} = 10^7\ s^{-1}$, $k_2 = k_9 = 10^5\ s^{-1}$, $k_3 = k_8 = 10^6\ s^{-1}$, $k_4 = k_7 = 10^7\ s^{-1}$, and $k_5 = k_6 = 10^3\ s^{-1}$). (Note that for $ff = 1\ s^{-1}$, the peaks are below the wells.) (Bottom, right-hand axis) The free energy profile deduced using Eq. 2 (- - - -) and a similar profile deduced using $ff = 10^9\ s^{-1}$ (·····).

however, and one cannot relate differences in peak height to differences in free energy without knowing the variation in *ff*. Third, the "well" depths relative to the electrolyte solution outside the pore are invariant, again suggesting that they have mechanistic significance. The different behaviors of the peaks and "wells" arise because of the qualitative difference between $RCR_{ff}(p)$ for odd and even *p*: only for odd *p* does the value of $RCR_{ff}(p)$ depend on *ff*. Visually, the peaks probably should be above the wells; compare the profile for $ff = 1\ s^{-1}$ vs. those for $ff = 10^9$ and $6 \cdot 10^{12}\ s^{-1}$, which justifies the use of physically plausible, albeit arbitrary, frequency factors.

Eq. 3 applies generally, meaning that it is possible to provide graphic representations of the results of kinetic analyses without invoking the Eyring TST to describe situations where that theory is inapplicable—whether it be ion permeation, channel gating, protein conformational transitions, or other physiological processes. *The Journal of General Physiology* therefore will publish rate constant representations based on Eq. 3, or some equivalent, but will no longer publish energy profiles deduced from kinetic analyses unless the authors explicitly justify their choice of the underlying model using "generally accepted" physico-chemical reasoning.

Olaf Sparre Andersen
Editor
The Journal of General Physiology