

Topical Review

Diffusion Theory and Discrete Rate Constants in Ion Permeation

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Introduction

Discrete models of membrane transport have provided a great deal of insight into the process of ion movement through channels (Hille & Schwartz, 1978; Urban & Hladky, 1979; Finkelstein & Andersen, 1981; Latorre & Miller, 1983; Eisenman & Horn, 1983; Läuger, 1987). Such models consist of a set of occupancy states and a set of rate constants for transitions between the states. The states represent conformations of the channel with ions bound at specific sites, i.e., potential energy wells. The rate constants describe the movement of an ion from one well to the next over an intervening energy barrier. This set of elements—states and rate constants—comprises a kinetic model of the channel, which can be solved using standard techniques, to yield an equation that specifies the current through the channel as a function of the bath concentrations and the rate constants. Even without further specification of the rate constants, many interesting conclusions about channel behavior can be drawn, such as: current saturation with increasing permeant concentration, interaction of unidirectional fluxes, competition between different permeants and between permeants and blockers, and the complicated behavior of a channel with conformational fluctuations. Such conclusions are independent of the model for the rate constant and are simply a consequence of assuming a set of discrete occupancy states.

The voltage dependence of the current is a consequence of the voltage dependence of the rate constants and its study therefore requires a model of a rate constant. The model currently in use derives

from Eyring transition state theory (Läuger, 1973). It gives the rate constant over a barrier as an exponential of the free energy difference between the barrier and its associated well. This exponential is multiplied by a constant which we will refer to as the pre-exponential factor. Many of the current users of Eyring theory do not use the standard form of the pre-exponential factor (i.e., kT/h), instead they use it as an adjustable parameter assumed identical for all barriers. This represents an implicit assumption that even if Eyring theory is not the proper theory for barrier crossing in a channel, the correct theory is of the same form as Eyring theory. We know of no theoretical justification for this view. If this view is not accepted, then using an Eyring form with an arbitrary pre-exponential factor can be thought of as a convenient way of parameterizing data. This may be a useful thing to do, but a consequence of doing so is that no inferences about channel structure can be made from the model parameters, because they have no physical basis. What is needed is either a justification for the Eyring form of the rate constants or some other more appropriate theory.

In this article we examine the underlying physical assumptions on which Eyring theory rests in order to better understand the origin of its form and its applicability to channel permeation. Based on this analysis we hope to convince the reader that a more reasonable model for these rate constants should come from diffusion theory. As an example of this approach we present a diffusion model using the mean first passage time theory (Schulten, Schulten & Szabo, 1981; Weiss, 1986; Cooper, Gates & Eisenberg, 1988). It is well known that, in the limit of large barriers (the so-called Kramer's limit), this theory yields a form similar to Eyring theory. We investigate this limit to see if it might justify the use of an Eyring-like form for rate constants in channels. Other diffusion theory based ap-

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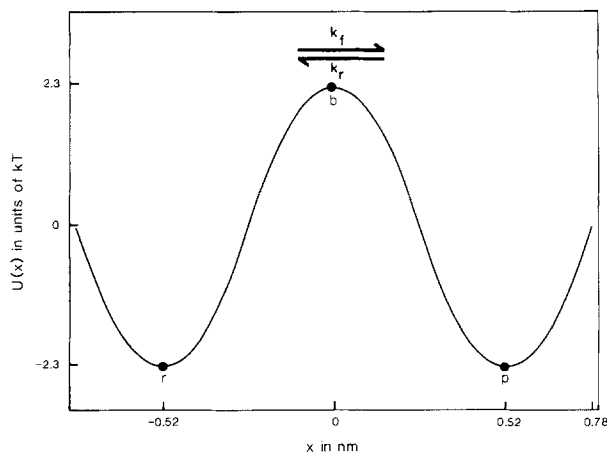


Fig. 1. Potential energy $U(x)$ as a function of position for use in rate constant computations. r is the minimum in the reactant well, p is the minimum in the product well, and b is the maximum of the intervening barrier

proaches are certainly possible (Levitt, 1986, 1987; Jakobsson & Chiu, 1987; Gates, Cooper & Eisenberg, 1988). We choose this approach because it allows a natural comparison with Eyring theory.

Theory

In this section we: discuss the physical assumptions involved in Eyring rate theory and how they relate to an ion moving through a channel; present the basic equations of the mean first-passage time theory; and, finally, derive the large barrier limit of that theory. In order to make computations with the theories, we use the barrier structure shown in Fig. 1. It represents the potential energy, $U(x)$, seen by an ion as it traverses one barrier in a channel. We add to it a component due to an externally applied voltage, assuming that this component does not alter $U(x)$ but only adds to it and further that it can be described as a constant field. Thus, the total potential, $W(x)$, can be written

$$W(x) = U(x) + zq\Delta Vx/\delta \quad (1)$$

where z is the ion valence, q is the fundamental unit of charge, ΔV is the voltage difference across the system, and δ is the width of the system.

EYRING RATE THEORY

Standard derivations of the rate constant for barrier crossing using Eyring theory yield the following well-known equation (Hynes, 1985a)

$$k_f(TST) = \frac{kT}{h} \exp(-\Delta G'/kT) \quad (2)$$

where k is Boltzmann's constant, T is the absolute temperature, and $\Delta G'$ is the free energy difference between the well and the barrier ($G'(b) - G'(r)$). Note that we have identified the potential energy $W(x)$ with the free energy $G'(x)$, hence $\Delta G' = \Delta W$. Also it should be clear from the above that the voltage dependence of the rate constant comes from the difference in the voltage at points b and r .

The derivation of Eq. (2) requires two essential assumptions (*see* Appendix A). The first assumption is that the current through the system is so small that the spatial distribution of ions remains close to the equilibrium distribution. We call this the quasi-equilibrium assumption. This assumption should be valid in a limiting sense as ΔW gets large and thus currents get small. The second assumption involves the specific model used for the process by which an ion crosses the peak of the barrier. The usual assumption is that the particle moves across the peak like a gas molecule moving between collisions, i.e., it moves at a constant velocity (given by the mean thermal velocity) in the direction of crossing and *undergoes no collisions during the crossing*. This assumption is often referred to as the ballistic crossing assumption.

Neither of these assumptions is likely to be valid in a channel. The quasi-equilibrium assumption requires that ΔW be on the order of 6–10 kT or larger for a simple barrier shape and no applied voltage (*see* Results and Cooper et al., 1985). Various lines of argument indicate that this is not generally the case for channels (Finkelstein & Andersen, 1981; Lecar, 1981; Jordan, 1984; Yellen, 1987). If an applied voltage is present, then ΔW is changed simply due to superposition, being decreased in size in one direction and increased in size in the other direction (Fig. 2). Thus even if ΔW satisfies the quasi-equilibrium assumption at zero applied voltage, it could easily fail to do as ΔV is made large.

The ballistic crossing assumption is the weaker of the two assumptions. It requires that the ion not interact with solvent as it crosses the barrier. An ion inside a channel is in a liquid-like environment, implying that the ion does not cross the top of the barrier ballistically; it diffuses across. This is a much slower process and involves many recrossings of the peak (Frauenfelder & Wolynes, 1985). Thus, the Eyring theory pre-exponential factor overestimates the crossing rate. Since barrier heights and well depths are not known independently, data can be fit by assuming a larger ΔW , offsetting the overestimate of the pre-exponential factor.

Transition state models such as Eyring theory are best suited to describe chemical reactions in the gas phase (Hynes, 1985*a*). Their application to liquid state problems has been criticized for diffusion through membranes (van Dijk & de Levie, 1985), for diffusion through channels (Levitt, 1982, 1986; Cooper, Jacobsson & Wolynes, 1985; Cooper et al., 1988), for chemical reactions in liquids (Kramers, 1940; Skinner & Wolynes, 1980; Hynes, 1985*b*), and for enzymatic reactions (Debrunner & Frauenfelder, 1982; Hanggi, 1983; Frauenfelder & Wolynes, 1985). These diverse references agree that the preferred models are based on diffusion theory. Diffusion theory was abandoned in the study of channels because it was not thought to be a molecular theory and because it was not clear that it could describe the interactions between ions as they move through a channel (nonindependence) (Hille, 1979). Diffusion theory can be founded on molecular level considerations (reviewed in Cooper et al., 1988) and recent work has shown that a diffusion theory can describe deviations from independence in both a one-ion and a two-ion channel (Levitt 1986, 1987; Gates et al., 1987, 1988). Therefore if discrete models are used, the Eyring form for the rate constants should be replaced with a diffusion model.

MEAN FIRST-PASSAGE TIME THEORY

The mean first-passage time (MFPT) theory for rate constants mentioned in the introduction is a diffusion theory. The fundamental result of that theory is the following equation (Schulten et al., 1981):

$$k_f(\text{MFPT}) = \left[\tau_f + \tau_r \frac{Z(p)}{Z(r)} \right]^{-1} \quad (3)$$

where τ_f is the average time an ion takes to diffuse from its initial position in the reactant well to the peak of the barrier; τ_r is the analogous time from the product well to the peak of the barrier; and $Z(p)$ and $Z(r)$ are integrals over the potential $W(x)$. The rate constant $k_f(\text{MFPT})$ depends on the initial position (i) of the diffusing ion. This dependence is absent from Eyring theory because it assumes an equilibrium distribution in the system. In a channel, ions enter the well from its end points, so in τ_f , $i = r$ and in τ_r , $i = p$. τ_f and τ_r can be computed from the standard equations of time-dependent diffusion in a potential (Goel & Richter-Dyn, 1974; Schulten et al., 1981; Gardiner, 1983; Cooper et al., 1988). The resulting equations are given below. They specify the passage times in terms of the particle diffusion coefficient D (here assumed to be position independent) and $W(x)$.

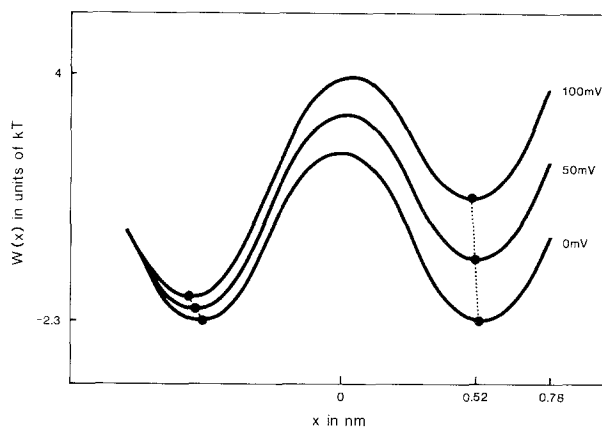


Fig. 2. The potential profile from Fig. 1 with various applied voltages. Note that the positions of the minima and maxima shift with the applied voltage and that the well-to-peak energy difference changes

$$\tau_f, \tau_r = \frac{1}{D} \int_i^b \int_x^b \exp((W(y) - W(x))/kT) dy dx \quad (4)$$

$$Z(r) = \int_r^b \exp(-W(x)/kT) dx \quad (5)$$

$$Z(p) = \int_p^b \exp(-W(x)/kT) dx. \quad (6)$$

Note that if the barrier is symmetric $Z(r) = Z(p)$, and $\tau_f = \tau_r = \tau$ yielding the simpler result $k_f(\text{MFPT}) = 1/(2\tau)$. However, even if $U(x)$ is symmetric, $W(x)$ is not when a potential is applied, so this simple result is not very useful in describing a channel. Note also that the MFPT rate constant depends on the entire function $W(x)$, and not just on the parameters $W(r)$, and $W(b)$ as in Eyring theory. Finally, since the MFPT is a nonequilibrium theory, the pre-exponential factor depends on a transport parameter, D , not found in Eyring theory.

One use of the above equations is to assess the validity of the quasi-equilibrium assumption. Under this assumption, the ions have initial positions determined by a Boltzmann distribution. Thus the MFPT, in this case, will be a weighted average of the passage times for all these initial positions, with the weighting being the fraction of ions starting from that point as given by the Boltzmann distribution. This equilibrium passage time can be obtained from the above by integrating the product of the Boltzmann distribution and τ . The resulting equation can be simplified to the following form (Schulten et al., 1981):

$$\tau_f(\text{eq}) = \frac{1}{D} \int_r^b e^{W(x)/kT} \left[\int_r^x e^{-W(y)/kT} dy \right]^2 dx / Z(r). \quad (7)$$

An analogous equation for τ_r (eq) also exists.

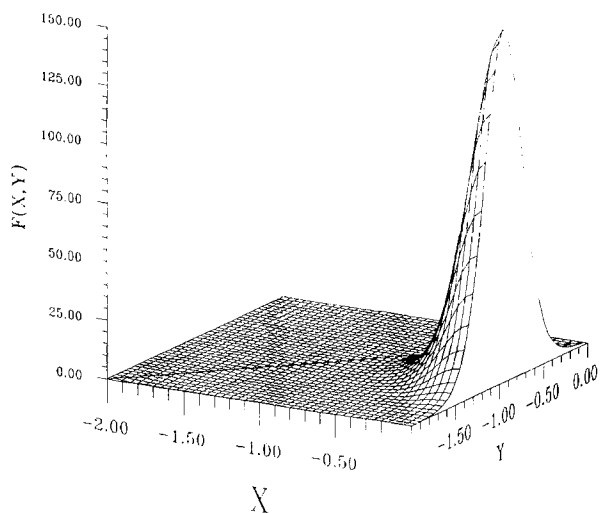


Fig. 3. The integrand in Eq. (4) for $\Delta U = 5$ kT

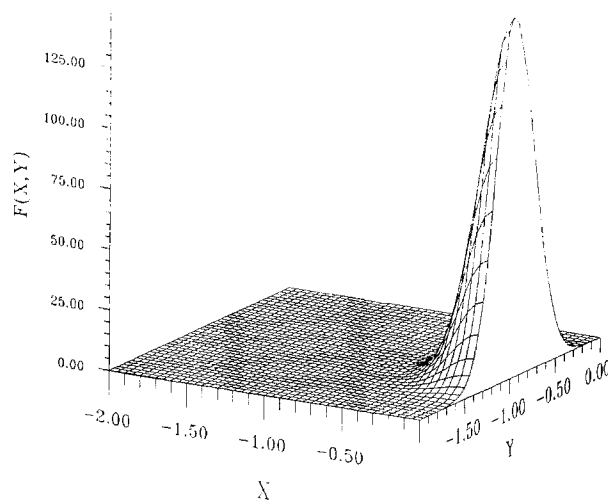


Fig. 4. The Gaussian approximation to the surface of Fig. 3

KRAMERS' LIMIT

There exists a simple limit of the MFPT theory that has a form similar to that of Eyring theory (Schulten et al., 1981). This limit describes the behavior of the MFPT as ΔW gets large. This well-known limit was first derived, in a different manner than we will use, by Kramers (1940). One can obtain the limit by applying the method of steepest descent to Eq. (3) (for details *see* Appendix B).

This method consists of approximating the argument of the exponential in Eq. (4) by a three-term Taylor series in the neighborhood of the point where the integrand is largest. The exponential of the Taylor series expansion in the neighborhood of that peak yields a Gaussian function that approximates the integrand fairly well in the limit of large barriers, as can be seen by comparing Figs. 3 and 4. With this Gaussian for the integrand, the multiple integral can be evaluated analytically and yields the following equation, known as Kramers' equation.

$$k_f(K) = \frac{Dm\omega(r)\omega(b)}{2\pi kT} \exp(-\Delta W/kT) \quad (8)$$

where m is the ion's mass, and the ω 's are given by

$$\omega^2(s) = \left| \frac{1}{m} \frac{\partial^2 W}{\partial x^2} \Big|_s \right| \quad (9)$$

ω has the units of a frequency and can be thought of in simple terms as an attempt frequency for a particle trying to reach the barrier (if $s = r$) or for a particle leaving the barrier and going to the well (if $s = b$). This interpretation is similar to that usually given for the pre-exponential term in Eyring theory.

Note that this equation has the same dependence on well depth and barrier height as does Eyring theory, but the pre-exponential factor depends on the diffusion coefficient and on the shape of $W(x)$ in the neighborhood of the well minimum and the barrier maximum. This result is significant because it implies that even though the exponential dependence in Eyring theory is asymptotically correct, the use of a single pre-exponential factor for all barriers is not in general correct, because this factor should also depend on the shape of the potential function. It is perhaps worth reiterating that this result is only valid in the limit of large ΔW and, as we shall see later, how large ΔW has to be depends on $W(x)$. If $W(x)$ is composed of parabolic segments, then ΔW need not be enormous; but if there are ledges in the profile or if $W(x)$ is very flat and broad at the minimum or maximum, then the Kramers' approximation might require a substantial ΔW .

Results

MFPT THEORY VERSUS EYRING THEORY IN THE ABSENCE OF AN APPLIED POTENTIAL

We begin comparing the two approaches by seeing what they predict when fit to an experimentally derived rate constant. The best characterized rate constant in the channel literature is that for Na^+ crossing the central barrier of the gamicidin channel (Levitt, 1978; Urry et al., 1980; Finkelstein & Andersen, 1981; Eisenman & Sandblom, 1983; Hladky & Haydon, 1984). The literature values, at zero transmembrane voltage, vary from $3.2 \times 10^6 \text{ sec}^{-1}$

Table 1. Rate constant as a function of ΔU for the potential shown in Fig. 1

| ΔU (kT) | Equilibrium rate (sec ⁻¹) | Nonequilibrium rate (sec ⁻¹) | Kramers' rate (sec ⁻¹) |
|-----------------|---------------------------------------|--|------------------------------------|
| 2.0 | 1.027×10^8 | 9.287×10^7 | 8.922×10^7 |
| 4.0 | 2.453×10^7 | 2.407×10^7 | 2.415×10^7 |
| 6.0 | 4.913×10^6 | 4.899×10^6 | 4.864×10^6 |
| 8.0 | 8.851×10^5 | 8.847×10^5 | 8.846×10^5 |
| 10.0 | 1.497×10^5 | 1.497×10^5 | 1.496×10^5 |

to $2.5 \times 10^7 \text{ sec}^{-1}$. We use the value $1.5 \times 10^7 \text{ sec}^{-1}$. The standard form of Eyring theory, Eq. (2), requires a well-to-barrier free energy difference of 12.9 kT to give this rate.

To perform this calculation with the MFPT theory we use the Kramers' limit, Eq. (8). This requires more information about $W(x)$. For simplicity, assume a symmetric barrier with a simple quadratic shape (Fig. 1), thus ω in the well will be the same as ω at the peak of the barrier. To calculate ω we need to know the well-to-peak distance. Since this distance is not known we investigate several values. As a first approximation assume that the gramicidin channel has three barriers, one in the center and one at each mouth, and also two binding sites inside the entry barriers. If we assume all five structures are the same size, then a well-to-peak distance of 0.52 nm would be obtained from the 2.6 nm overall length of the channel. The one remaining unknown is the diffusion coefficient for Na^+ . We use the experimentally derived value $1.4 \times 10^{-10} \text{ m}^2/\text{sec}$ (Dani & Levitt, 1981). These values yield a peak-to-well energy difference of 4.6 kT. This seems more in line with the observation that an ion sees a fairly small barrier as it traverses the gramicidin channel (Finkelstein & Andersen, 1981; Jordan, 1984). A broader central barrier seems more likely; this would require an even smaller peak-to-well height but would be so small that the Kramers' limit would not be accurate and the full integral expression would be needed. If the barrier is only 0.1 nm wide then the well-to-peak height is 8.5 kT. To get the Kramers' result to agree with the Eyring result ($\Delta G' = 12.9 \text{ kT}$) requires that the barrier be $1.4 \times 10^{-2} \text{ nm}$ wide. It takes 185 such barriers to fill a gramicidin channel.

These results show that the MFPT theory and the Eyring theory can give orders of magnitude different estimates for the rate constant *over a given* $W(x)$. This difference comes about because of the vast overestimate of the barrier crossing rate implied by the Eyring model. The Kramers' limit of the MFPT contains a more reasonable model for barrier crossing but maintains the same exponential dependence on ΔW ; thus it might be a reasonable

replacement for the Eyring form. It is necessary to check this assertion by examining how good an approximation the Kramers' limit is to the full MFPT equation. This requires two types of tests; first, investigate the limit for various barrier shapes of interest; and second, compare the voltage dependence of the Kramers' expression and the full MFPT theory. This is done in the next two sections.

RATE CONSTANT AS A FUNCTION OF BARRIER SHAPE

We begin investigating the Kramers' limit by comparing it to the full MFPT result for barriers of differing height but constant well and barrier positions. We use the same barrier structure used in the last section but vary ΔU . To do this we must specify a starting point for the ions. The obvious choice is the left boundary, but it is also of some interest to start the ions from an equilibrium distribution. This allows an estimate of the error made in using the quasi-equilibrium assumption when ΔW is not large enough to ensure an equilibrium distribution.

The first column of Table 1 shows the results of a set of such computations. The $k_f(\text{MFPT})$ is shown for various values of ΔU with an equilibrium initial distribution of particles in the well. As expected, the rate constant falls as ΔU increases. In the second column the particles are started initially at the left boundary; note that this gives a smaller rate constant since, with an equilibrium initial distribution, many of the particles start closer to the peak and thus have a shorter distance to diffuse before leaving the system. The table shows that, for this barrier structure, the difference between the equilibrium and nonequilibrium initial distributions is never more than about 10%. The third column shows the rate constant as calculated from the Kramers' equation. As ΔU gets large, columns 1 and 2 approach the Kramers' equation as they should, and the Kramers' equation does not differ by more than 15% for any of the cases.

One of the problems with the Kramers' limit is that it only depends on $W(x)$ in the vicinity of r and b . To see the problems this can cause, consider three other possible barrier structures for the gramicidin channel. If the barriers at the mouth are outside the membrane field, then the two wells and the central barrier fill the entire 2.6 nm. This could occur in several ways. The most obvious way would be to have the wells and barriers wider. This would clearly require a smaller barrier and the Kramers' limit would be a worse approximation. Somewhat more interesting is the possibility that the barrier and wells are the same shape as before but that a ledge fills the rest of the channel. Such a ledge could

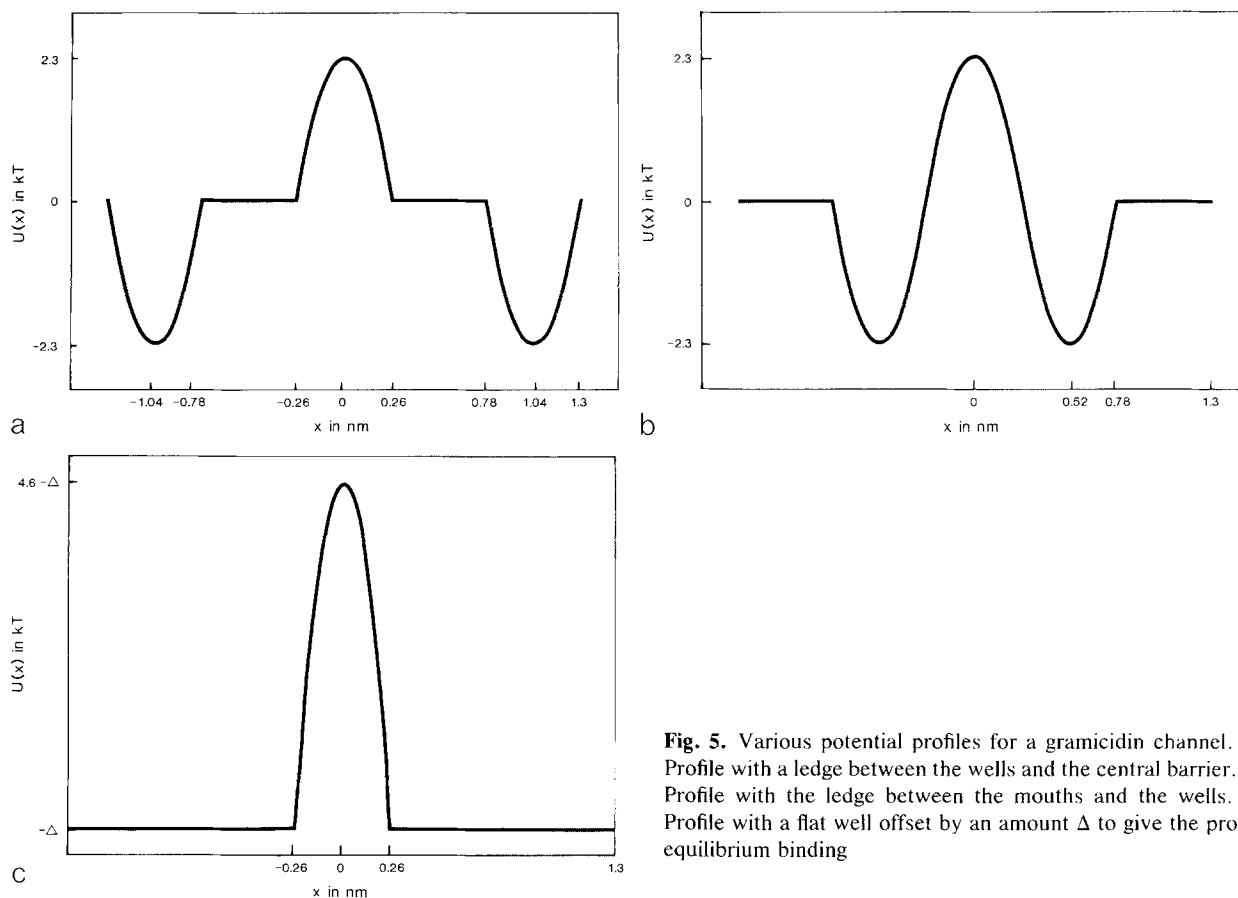


Fig. 5. Various potential profiles for a gramicidin channel. (a) Profile with a ledge between the wells and the central barrier. (b) Profile with the ledge between the mouths and the wells. (c) Profile with a flat well offset by an amount Δ to give the proper equilibrium binding

Table 2. Rate constant as a function of ΔU for the potentials shown in Fig. 5

| ΔU (kT) | k_a (sec ⁻¹) | k_b (sec ⁻¹) | k_c (sec ⁻¹) |
|-----------------|----------------------------|----------------------------|----------------------------|
| 2.0 | 4.088×10^7 | 5.348×10^7 | 3.863×10^7 |
| 4.0 | 1.447×10^7 | 1.878×10^7 | 9.611×10^6 |
| 6.0 | 3.777×10^6 | 4.416×10^6 | 1.712×10^6 |
| 8.0 | 7.863×10^5 | 8.480×10^5 | 2.720×10^5 |
| 10.0 | 1.424×10^5 | 1.471×10^5 | 4.138×10^4 |

The a , b , c subscripts on the rate constants refer to the potential profiles in parts a , b , c of Fig. 5.

be located between the channel mouth and the well or between the well and the central barrier (Fig. 5a,b). The ledge would be 0.52 nm wide. Since it does not affect any of the terms in Kramers' equation, *the Kramers' equation has no way of taking the ledge into account*. Table 2 shows the effect of such a ledge on the rate constant. The first column is the rate constant for the case where the ledge is between the well and barrier and the second column is for the case where the ledge is located before the well. There is little difference in rate constant be-

tween these two cases but a comparison with the third column of Table 1 shows that Kramers' equation may now be off by more than a factor of two. The error in using the Kramers' equation again decreases as ΔU increases.

As a final example of the problems with the Kramers' equation, consider a model for the channel (Fig. 5c) where the wells are relatively structureless regions depressed some amount below the potential in the bath (to give the proper equilibrium binding). Such wells have zero curvature, and the Kramers' equation gives a zero rate for crossing the barrier. The third column of Table 2 gives k_f (MFPT). Comparison with the rate constants in the first two columns shows that this profile can give rate constants between 0.6 and 0.3 times as large as the previous profiles.

MFPT THEORY VERSUS EYRING THEORY WITH AN APPLIED VOLTAGE

In the traditional application of Eyring rate theory to channels, an applied voltage is simply added to the free energy at the well minimum and barrier

maximum, which are assumed not to change position with applied voltage (Lauger, 1973). We use the same technique for $k_f(K)$ and then compare the results with $k_f(\text{MFPT})$. We use the same profile as in the calculations in Table 1. The results are shown in Table 3. The first column shows $k_f(\text{MFPT})$ with $\Delta U = 4.6$ kT and voltages in the range of ± 100 mV. The second column is the expected value of the rate constant using Kramers' equation. This rate constant is always larger than the actual rate constant, and the discrepancy becomes larger with increasing applied voltage. In the range shown in the table the error can be as large as 45%.

One obvious source of this discrepancy is the fact that *the positions of the minima and maxima shift as a function of the applied voltage* (see Fig. 2). Thus using the voltage at two fixed locations will obviously cause some error. Simple equations can be derived for the position of the maxima and minima as a function of applied voltage for the polynomial potential used here. From these equations one can derive a correction to $k_f(K)$ (see Appendix C for details). The equation for the corrected forward rate constant is

$$k_f(KC) = k_f(K) \exp\{-\Delta V^2/36\Delta U\} \quad (10)$$

where $K_f(KC)$ is the corrected Kramers' rate constant, $k_f(K)$ is the standard Kramers' rate constant, ΔV is the applied voltage difference and ΔU is the well-to-peak energy difference at zero voltage. Clearly the smaller ΔU is, the larger the correction needed. The third column in Table 3 shows that the corrected rate constant is a distinct improvement. Over the voltage range used the error is less than 6%. This improves the agreement but does not make the two results identical; thus, there is some inherent error in obtaining the voltage dependence of the rate constant by this simple addition method. The agreement would be less dramatic if ΔU were smaller, since then the Kramers' equation itself would be a worse approximation.

Discussion

The main conclusion of this paper is that the use of Eyring theory for rate constants in channels is not appropriate. Its underlying assumptions do not reflect the physical processes occurring in a channel, resulting in an overestimate of the rate. This occurs because the Eyring model predicts a much more efficient and rapid barrier crossing than would a more realistic diffusion description. This can be seen dramatically by comparing the rates calculated using Eq. (2) with any of the results in Tables 1 and

Table 3. Rate constant as a function of applied voltage

| ΔV (mV) | k (sec ⁻¹) | k_k (sec ⁻¹) | k_{k_c} (sec ⁻¹) |
|-----------------|--------------------------|----------------------------|--------------------------------|
| -100 | 1.530×10^8 | 2.212×10^8 | 1.503×10^8 |
| -75 | 9.009×10^7 | 1.136×10^8 | 9.140×10^7 |
| -50 | 5.262×10^7 | 5.831×10^7 | 5.294×10^7 |
| -25 | 2.898×10^7 | 2.994×10^7 | 2.922×10^7 |
| 0 | 1.537×10^7 | 1.537×10^7 | 1.537×10^7 |
| 25 | 7.792×10^6 | 7.891×10^6 | 7.703×10^6 |
| 50 | 3.755×10^6 | 4.051×10^6 | 3.678×10^6 |
| 75 | 1.761×10^6 | 2.080×10^6 | 1.674×10^6 |
| 100 | 7.670×10^5 | 1.068×10^6 | 7.257×10^5 |

k is the full MFPT rate constant, k_k is the Kramers' rate constant, and k_{k_c} is the Kramers' rate corrected for the shift in well and barrier position via Eq. (10).

2 (this is made easier by using Fig. A1). The difference can be orders of magnitude.

In addition to this problem, there appears to be an inherent error in obtaining the voltage dependence of any exponential rate constant by adding the applied voltage at the barrier peak and the well minimum to the intrinsic potential at those points. Some of the discrepancy is due to the fact that the minima and maxima in the potential are not fixed, but rather shift in response to the applied voltage. The remainder of the discrepancy comes about because the rate is not dominated by processes at the peak when the barrier heights are moderate. These problems justify the use of the more accurate rate constants obtained from the MFPT theory.

While it may be tempting to use Kramers' limit, it is *only* valid for large barriers and suffers from the same problems in specifying voltage dependence as Eyring theory. With the increasing availability of powerful lab computers and good quality numerical analysis programs it seems reasonable to calculate rate constants using the full MFPT theory. However, to do so requires the specification of a diffusion coefficient as well as the shape of the entire potential function $W(x)$. This is more information than required by Eyring theory.

The specification of $D(x)$ is problematic. If $U(x)$ is not known, but is to be determined by fitting a model to data, then it seems reasonable to use a constant D and absorb the spatial variation into $U(x)$. If $U(x)$ is known, then the question of how to determine $D(x)$ is more critical. It could, in principle, be computed from the underlying theory of the diffusion equation (see Cooper et al., 1988). In that case $D(x)$ is related to the variance of the distribution function of particle position. A molecular dynamics simulation could be carried out, starting an ensemble of particles at a point x in the potential in question. The variance in position after some time

would yield a measure of $D(x)$. $D(x)$ could also be computed from molecular dynamics simulations via the velocity autocorrelation function (*see* Rodger, Sceats & Gilbert, (1988) for a discussion of these methods). However, it appears that such computations do not always give very good agreement with experimental data (Berkowitz & Wan, 1987). A less computationally demanding technique would certainly be valuable.

One particularly nice feature of this theory is that it predicts a shift in minima and maxima with voltage. Such a shift implies that a semi-log plot of an individual rate constant versus voltage is nonlinear (unlike Eyring theory; *see* Fig. A1). This phenomenon might explain the nonlinearity sometimes seen in semi-log plots of open channel block (Tomlins & Williams, 1986; Tang et al., 1988¹).

We have presented a detailed description of the assumptions of Eyring theory and would be remiss if we did not mention the assumptions underlying the diffusion approach to rate constants (for a more complete discussion *see* Cooper et al., 1988). There are three assumptions necessary for the validity of the diffusion theory used in this paper. First, it must be assumed that the diffusing ion is always at velocity equilibrium with its environment; in a liquid this is a reasonable assumption. Second, the potential function $U(x)$ cannot vary too rapidly with position, but given the small amplitude of channel barriers this does not seem to be much of a problem either. Finally, the potential function must be static; this requires that the channel protein not have conformational fluctuations (relevant to permeation) on the time scale of ion movement through the channel. This is a questionable assumption. Diffusion models of rate constants not using this assumption are under development (reviewed in Hynes, 1985*a,b*).

An important issue remains to be resolved. If one believes that diffusion models are indeed the correct physical approach to channel modeling, then one has at least two choices when modeling a specific channel: one can either use the Nernst-Planck approach (Levitt, 1986; Gates et al., 1987, 1988) or one can use a discrete approach with a diffusion model for the rate constants. It is not obvious to us that these approaches will always give the same results. Further work on this question needs to be done.

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References

- Berkowitz, M., Wan, W. 1987. The limiting ionic conductivity of Na^+ and Cl^- ions in aqueous solutions: Molecular dynamics simulation. *J. Chem. Phys.* **86**:376–382
- Cooper, K.E., Gates, P.Y., Eisenberg, R.S. 1988. Surmounting barriers in ionic channels. *Q. Rev. Biophys.* **21**:331–364
- Cooper, K.E., Jakobsson, E., Wolynes, P.G. 1985. The theory of ion transport through membrane channels. *Prog. Biophys. Molec. Biol.* **46**:51–96
- Dani, J., Levitt, D. 1981. Water transport and ion-water interaction in the gamicidin channel. *Biophys. J.* **35**:501–508
- Debrunner, P.G., Frauenfelder, H. 1982. Dynamics of proteins. *Annu. Rev. Phys. Chem.* **33**:283–299
- Dijk, C. van, Levie, R. de 1985. An experimental comparison between the continuum and single jump descriptions of non-actin-mediated potassium transport through black lipid membranes. *Biophys. J.* **48**:125–136
- Eisenman, G., Horn, R. 1983. Ionic selectivity revisited: The role of kinetic and equilibrium processes in ion permeation through channels. *J. Membrane Biol.* **76**:197–225
- Eisenman, G., Sandblom, J.P. 1983. Energy barriers in ionic channels: Data for gramicidin A interpreted using a single-file (3B4S^o) model having 3 barriers separating 4 sites. *In: Physical Chemistry of Transmembrane Ion Motions*. T.G. Spach, editor. Elsevier Science, Amsterdam.
- Finkelstein, A., Andersen, O.S. 1981. The gramicidin A channel: A review of its permeability characteristics with special reference to the single-file aspect of transport. *J. Membrane Biol.* **59**:155–171
- Frauenfelder, H., Wolynes, P.G. 1985. Rate theories and puzzles of heme protein kinetics. *Science* **229**:337–345
- Gardiner, C.W. 1983. *Handbook of Stochastic Methods*. Springer-Verlag, New York
- Gates, P.Y., Cooper, K.E., Eisenberg, R.S. 1987. Diffusive flux through ionic channels. *Biophys. J.* **51**:48a
- Gates, P.Y., Cooper, K.E., Eisenberg, R.S. 1988. Analytical diffusion models for membrane channels. *In: Ion Channels*. Vol. 2. T. Narahashi, editor. Plenum, New York (*in press*)
- Goel, N.S., Richter-Dyn, N. 1974. *Stochastic Models in Biology*. Academic, New York
- Hanggi, P. 1983. Physics of ligand migration in biomolecules. *J. Stat. Phys.* **30**:401–412
- Hille, B. 1979. Rate theory models for ion flow in ionic channels of nerve and muscle. *In: Membrane Transport Processes*. Vol. 3, pp. 5–16. C.F. Stevens and R.W. Tsien, editors. Raven, New York
- Hille, B., Schwarz, W. 1978. Potassium channels as multi-ion single-file pores. *J. Gen. Physiol.* **72**:409–442
- Hladky, S.B., Haydon, D.A. 1984. Ion movements in gramicidin channels. *Curr. Topics Membr. Transp.* **21**:327–368
- Hynes, J.T. 1985*a*. The theory of reactions in solution. *In: Theory of Chemical Reaction Dynamics*. Vol. IV, pp. 171–234. M. Baer, editor. CRC, Boca Raton

¹ Tang, J.M., Wang, J., Eisenberg, R.S. 1988. K^+ -selective channel form sarcoplasmic reticulum of split lobster fibers. *J. Gen. Physiol.* (*submitted*)

- Hynes, J.T. 1985*b*. Chemical reaction dynamics in solution. *Annu. Rev. Phys. Chem.* **36**:573–597
- Jakobsson, E., Chiu, S.-W. 1987. Stochastic theory of ion movement in channels with single-ion occupancy. Application to sodium permeation of gramicidin channels. *Biophys. J.* **52**:33–52
- Jordan, P.C. 1984. The total electrostatic potential in a gramicidin channel. *J. Membrane Biol.* **78**:91–102
- Kramers, H.A. 1940. Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica* **7**:284–304
- Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71**:11–31
- Läuger, P. 1973. Ion transport through pores: A rate theory analysis. *Biochim. Biophys. Acta* **311**:423–441
- Läuger, P. 1987. Dynamics of ion transport systems in membranes. *Physiol. Rev.* **67**:1296–1331
- Lecar, H. 1981. Single-channel conductance and models of transport. In: *The Biophysical Approach to Excitable Systems*. W. Adelman and D. Goldman, editors. Plenum, New York
- Levitt, D.G. 1978. Electrostatic calculations for an ion channel: II. Kinetic behavior of the gramicidin A channel. *Biophys. J.* **22**:221–248
- Levitt, D.G. 1982. Comparison of Nernst-Planck and reaction-rate models for multiply occupied channels. *Biophys. J.* **37**:575–587
- Levitt, D.G. 1986. Interpretation of biological ion channel flux data: Reaction-rate versus continuum theory. *Annu. Rev. Biophys. Biophys. Chem.* **15**:29–57
- Levitt, D.G. 1987. Exact continuum solution for a channel that can be occupied by two ions. *Biophys. J.* **52**:455–466
- Rodger, P.M., Sceats, M.G., Gilbert, R.G. 1988. Stochastic models for solution dynamics: The friction and diffusion coefficients. *J. Chem. Phys.* **88**:6448–6458
- Schulten, K., Schulten, Z., Szabo A. 1981. Dynamics of reactions involving diffusive barrier crossing. *J. Chem. Phys.* **74**:4426–4432
- Skinner, J.L., Wolynes, P.G. 1980. General kinetic models of activated processes in condensed phases. *J. Chem. Phys.* **72**:4913–4927
- Tomlins, B., Williams, A.J. 1986. Solubilisation and reconstitution of the rabbit skeletal muscle sarcoplasmic reticulum K⁺ channel into liposomes suitable for patch clamp studies. *Pfluegers. Arch.* **407**:341–347
- Urban, B.W., Hladky, S.B. 1979. Ion transport in the simplest single file pore. *Biochim. Biophys. Acta* **554**:410–429
- Urry, D.W., Ventkatachalam, A., Spisni, R.J., Bradley, T.L., Prasad, K.U. 1980. The malonyl gramicidin channel: NMR-derived rate constants and comparison of calculated and experimental single-channel currents. *J. Membrane Biol.* **55**:29–51
- Weiss, G. 1986. Overview of theoretical models for reaction rates. *J. Stat. Phys.* **42**:3–36
- Yellen, G. 1987. Permeation in potassium channels: Implications for channel structure. *Annu. Rev. Biophys. Biophys. Chem.* **16**:227–246

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Appendix A

Derivation of Eyring Theory

Consider a particle in well r of Fig. 1, moving across the barrier to well p . During this process it must cross the point b , a point known as the transition state. Standard chemical kinetics gives the rate of disappearance of particles in well r as

$$\frac{d[r]}{dt} = -k_f(TST) [r]. \quad (A1)$$

However, this rate can also be obtained as the product of the concentration of particles at b times the rate at which such particles decay into well p (call this rate R_f). Therefore

$$\frac{d[r]}{dt} = -k_f(TST) [r] = -R_f [b] \rightarrow k_f(TST) = \frac{R_f [b]}{[r]}. \quad (A2)$$

Quasi-Equilibrium Assumption

Assume that the rate over the barrier is so small that it does not significantly perturb the equilibrium of particles in the well at r , then $[r]$ and $[b]$ can be related by an equilibrium constant K

$$K = \frac{[b]}{[r]} \rightarrow k_f(TST) = R_f K. \quad (A3)$$

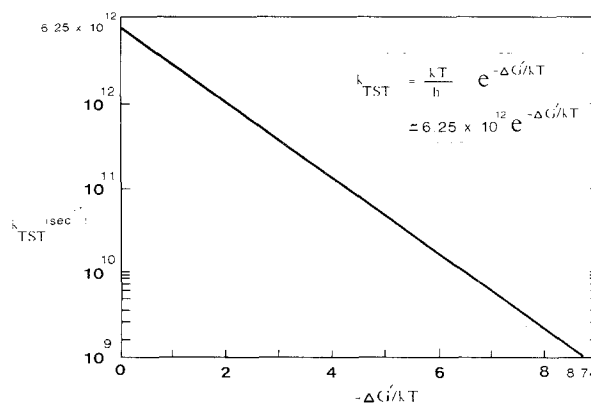


Fig. A1. Barrier crossing rate given by Eq. (A11) as a function of ΔG^\ddagger

A standard thermodynamic result relates an equilibrium constant to the Gibbs' free energy difference between products and reactants. If this result is applied to the $r - b$ equilibrium we obtain

$$\Delta G = -kT \ln K \quad \Delta G = \Delta H - T\Delta S \rightarrow K = e^{-\Delta H/kT} e^{\Delta S/kT}. \quad (A4)$$

Equilibrium statistical mechanics relates the above entropy difference to the partition functions of the states r and b as follows:

$$\Delta S = k \ln \frac{Q(b)}{Q(r)}. \quad (\text{A5})$$

Factor out of the partition function of b its translational term in the direction of crossing, q_t , then

$$Q(b) = q_t Q'(b) \quad (\text{A6})$$

where

$$q_t = \sqrt{2\pi mkT} \lambda / h \quad (\text{A7})$$

m is the particle mass, h is Planck's constant, and λ is the length of the transition state region. Thus

$$K = \frac{q_t Q'(b)}{Q(r)} e^{-\Delta H / kT} = q_t e^{-\Delta G' / kT} \quad (\text{A8})$$

where

$$\Delta G' = \Delta H - kT \ln \frac{Q'(b)}{Q(r)}. \quad (\text{A9})$$

Ballistic Crossing Assumption

Having an equation for K we now need an equation for R_f . Model R_f by assuming that any particle entering a region of length λ centered at the top of the barrier goes over to well p unhindered by collisions. This can be expressed as follows:

$$R_f = \frac{V}{2\lambda} \quad \text{where} \quad V = \sqrt{\frac{2kT}{\pi m}} \quad \text{is the 1-D thermal velocity.} \quad (\text{A10})$$

Thus we can write for $k_f(TST)$

$$k_f(TST) = K R_f = \frac{V q_t}{2\lambda} e^{-\Delta G' / kT} = \frac{kT}{h} e^{-\Delta G' / kT}. \quad (\text{A11})$$

Note that $kT/h = 6.25 \times 10^{12} \text{ sec}^{-1}$. Equation (A11) is shown graphically in a semi-log plot in Fig. A1

Appendix B

Derivation of the Kramers' Limit

In this appendix we derive the Kramers' limit to Eq. (4). As the energy difference between barrier and well (ΔU) gets large, the integrand in Eq. (4) becomes dominated by a Gaussian-like peak (see Fig. 3). Let $f(x, y)$ denote the integrand. A Taylor series expansion of $f(x, y)$ about its peak yields

$$f(x, y) = \exp[\Delta U / kT] \exp[-a(x-r)^2 + cy^2] \quad (\text{B1})$$

where

$$a = \frac{1}{2} \frac{\partial^2 U}{\partial x^2} \Big|_r, \quad c = \frac{1}{2} \frac{\partial^2 U}{\partial x^2} \Big|_b. \quad (\text{B2})$$

The region of integration can be extended to $\pm\infty$ with little error when ΔU is large (see Fig. 4). Thus

$$\begin{aligned} \tau &\equiv \frac{1}{2D} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) dy dx \\ &= \frac{e^{\Delta U / kT}}{2D} \int_{-\infty}^{\infty} e^{-a(x-r)^2} dx \int_{-\infty}^{\infty} e^{-cy^2} dy. \end{aligned} \quad (\text{B3})$$

These integrals are of the following standard form

$$\int_{-\infty}^{\infty} e^{-ax-r^2} dx = \sqrt{\pi/a}. \quad (\text{B4})$$

Combining the above yields Kramers' equation

$$k_f(K) = \frac{1}{2\tau} = \frac{Dm\omega(r)\omega(b)}{2\pi} e^{-\Delta U / kT} \quad (\text{B5})$$

where

$$\omega(z)^2 = \frac{1}{m} \frac{\partial^2 U}{\partial x^2} \Big|_z. \quad (\text{B6})$$

Appendix C

Derivation of the Correction to Kramers' Equation

In this appendix we derive an expression for ΔW as a function of ΔU and ΔV for a simple piecewise quadratic potential like that of Fig. 1. If we normalize the length of the system such that the left and right endpoints are at $x = \pm 3$, then the following expression are obtained

$$\text{left well: } U(x) = \frac{\Delta U}{2} \{x^2 + 4x + 3\} \quad (\text{C1})$$

$$\text{barrier: } U(x) = \frac{\Delta U}{2} \{1 - x^2\} \quad (\text{C2})$$

$$\text{everywhere: } V(x) = \frac{\Delta V}{6} \{x + 3\} \quad (\text{C3})$$

For each region, compute $W(x) = U(x) + V(x)$ and find the new extremum for that region.

$$\text{left well: } x_w = -2 - \frac{\Delta V}{6\Delta U} \quad (\text{C4})$$

$$\text{barrier: } x_b = \frac{\Delta V}{6\Delta U}. \quad (\text{C5})$$

Substitute these equations into the $W(x)$ expressions and compute the difference $\Delta W = W(x_b) - W(x_w)$.

$$\Delta W = \Delta U + \frac{\Delta V}{3} + \frac{\Delta V^2}{36\Delta U}. \quad (\text{C6})$$

The first two terms are the result obtained if there is no shift in extrema; the last term is the correction for the shift.