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On the Theory of Ion Transport Across the Nerve Membrane, VI. Free Energy and Activation Free Energies of Conformational Change*

(protein complex/electric field/net charge/polarizability/time constant maximum)

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ABSTRACT **Empirical functions, such as $n_\infty(V)$ and $\tau_n(V)$ (of the Hodgkin-Huxley type), can be recast in terms of more fundamental functions $F(V)$ (related to a conformational free energy change) and $\theta(V)$ (related to the corresponding free energies of activation). Examples of $F(V)$ and $\theta(V)$ are given, for squid and frog node. $F(V)$ is essentially a quadratic function of V. The possible molecular origin, for protein-like subunits, of the linear (e.g., net charge) and quadratic (e.g., polarizability) terms in $F(V)$ is discussed. The $F(V)$, $\theta(V)$ kind of analysis leads rather automatically to a simple explanation of the well-known approximate coincidence in location (V value) of the maximum in $\tau_n(V)$ (time constant) and the steeply rising part of $n_\infty(V)$ (also m, $1 - h$).**

In a previous paper (1) we introduced the working hypothesis that the separate potassium and sodium channels or gates in the membrane of the squid axon are protein complexes. Further, we assumed that the subunits of which the complexes are constructed undergo V-dependent (V = membrane potential) conformational changes which, in turn, determine whether a channel is open or closed to K^+ or Na^+ transport.

The composition of the channel material has still not been demonstrated experimentally, let alone its molecular structure. However, protein, lipoprotein, or polypeptide seem the most likely candidates at the present time. In any case, whatever the nature of the molecules, voltage clamp kinetics, as first analyzed by Hodgkin and Huxley (2), suggest a multisubunit organization, though the number of subunits is also not certain. The fact that the conformational change, in Hodgkin–Huxley (HH) kinetics, occurs on a time scale of milliseconds probably requires that the subunits be fairly large molecules.

The possible origin of the rather sensitive V-dependence of the conformational equilibrium constant was considered briefly in ref. 1. The analysis given there made it clear, however, that it is necessary to first establish the degree of cooperativity, if any, between the subunits of a complex before the "intrinsic" equilibrium constant, as a function of V, can be deduced from the experimental data.

We considered this preliminary question in previous publications (3, 4). We found that K^+ channels (or gates) are not likely to have any significant amount of cooperativity. We have not attempted a similar study of Na^+ channels.

With cooperativity (in K^+ channels) excluded, we can return, then, to the subject introduced in ref. 1 and push our analysis a little further. Actually, what we shall do here amounts merely to a recasting of illustrative experimental data into a form suitable for possible future theoretical analysis. We shall discuss the appropriate theory very briefly, but a serious attempt to "explain" the empirical functions $F(V)$ and $\theta(V)$ seems to us to be premature. For this, one really needs much more information about channel composition and structure than is presently available.

In the remainder of the paper, we shall have K^+ channels in mind, primarily, but the same approach would apply to Na^+ channels if the intrinsic (i.e., with interaction effects separated out) properties of the (presumably) two kinds of subunit were known. Actually, in the absence of information to the contrary, we shall assume that the "m" subunits (1) in the Na^+ channel can be treated in the same way (i.e., with interactions omitted) as the "n" subunits in the K^+ channel. However, there now seems to be ample evidence (5–9), especially in the paper by Goldman (9), that the "h" subunit interacts with the "m" subunits. Hence, we deemphasize h below, to some extent.

I. GENERAL CONSIDERATIONS

The quantity

$$K(V) = \frac{n_\infty(V)}{1 - n_\infty(V)} = e^{-\Delta G(V)/RT} = \frac{\alpha(V)}{\beta(V)} \qquad [1]$$

is the equilibrium constant between two conformations ($i \rightleftarrows ii$) of a (presumably protein) subunit, expressed in Hodgkin–Huxley notation. The dependence of K on V arises from the V-dependence of a free energy change, $\Delta G(V)$ (this is the negative of ΔG in Eq. **20** of ref. 1). Also, $K(V)$ is related to the rate constants of the process, as shown. The V-dependence of $\alpha(V)$ and $\beta(V)$ have their origin in the V-dependences of the two corresponding free energies of activation.

We define the functions $F(V)$, $f(V)$, and $\theta(V)$, in that order, by

$$F(V) = \Delta G(V)/RT \qquad [2]$$

$$F(V) = F(0) + f(V) \qquad [3]$$

$$\alpha(V) = \alpha(0)e^{-\theta(V)f(V)},\ \beta(V) = \beta(0)e^{[1-\theta(V)]f(V)}. \qquad [4]$$

Eqs. **4** are consistent with Eq. **1**, and only one of the pair is independent. It follows from the definitions that $RTF(0) = \Delta G(0)$ is the "unperturbed" (i.e., $V = 0$) free energy change (for $i \rightarrow ii$) while $RTf(V)$ is the additional contribution to

* The fifth paper in this series is to be published in the *Biophys. J.*

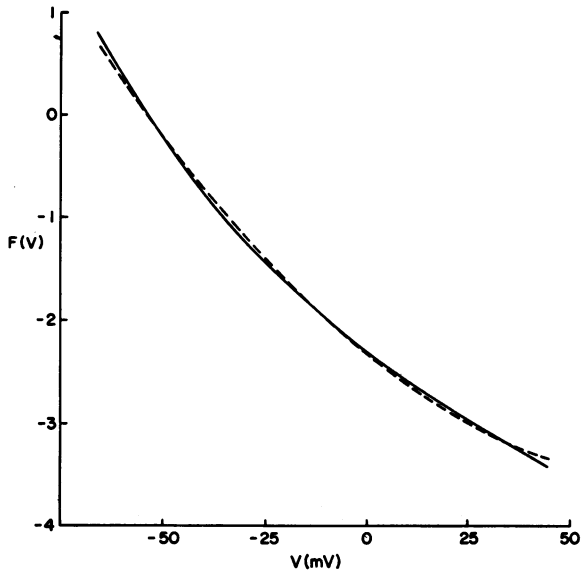

FIG. 1. The function $F(V)$ from n_∞ (Hodgkin–Huxley) (*solid curve*). The *dashed curve* is the least squares best fit of a quadratic function to $F(V)$.

$\Delta G(V)$ that arises on imposition of a nonzero membrane potential V (the "perturbation"). The factor $\theta(V)$ "splits" $RTf(V)$ between the two (forward and backward) activation free energies; $\alpha(0)$ and $\beta(0)$ are the unperturbed ($V = 0$) rate constants for the conformational change. The most obvious simple guess (10–12) about θ would be θ = constant = $^1/_2$ (i.e., the effect of V on the free energy of the activated complex is the average of its effect on the two states, *i* and *ii*). As we shall see, this is a considerable oversimplification here.

Given the empirical functions $n_\infty(V)$ and $\tau(V) = (\alpha + \beta)^{-1}$, from experiment, one can easily calculate $F(V)$ and $\theta(V)$ from Eqs. 1–4. These latter functions contain no new information, of course, but they express the V-dependence of the conformational change in a more fundamental way than the original functions (n_∞ and τ). The theoretical problem is then to try to understand $F(V)$ and $\theta(V)$ [as well as $\alpha(0)$ and $\beta(0)$]. The $F(V)$ problem (see below) does not seem fundamentally difficult, but the explanation of $\theta(V)$ [and $\alpha(0)$, $\beta(0)$] will presumably require detailed structural information about the subunit conformational change, including the intermediate activated complex.

To illustrate, we use the n and m empirical functions from Hodgkin–Huxley (2) (squid) and from Dodge (13) as modified by Hille (14) (frog node). The *solid curves* in Figs. 1–4 show the functions $F(V)$. The four $\theta(V)$ functions are given in Fig. 5. The somewhat atypical behavior of m(DH) (DH = Dodge–Hille) in Figs. 4 and 5 originates in the form of the experimental $\alpha_m(V)$ (13, 14). Otherwise, $F(V)$ appears to be a simple quadratic function of V (see below), and $\theta(V)$ is rather insensitive to V (but θ = constant = $^1/_2$ is clearly inapplicable).

The particular curves shown in these figures obviously depend (via n_∞, τ_n, etc.) on the number of subunits assumed (four for n, three for m), but not on the assumption that the subunit material is protein. This kind of analysis should be useful whatever the number of subunits and whatever the molecular nature of the subunit.

We have suggested previously (1, 11, 12, 15) that $F(V)$ might be a quadratic function of V. The possible physical basis for this is the subject of the next section. Thus, we approximate $F(V)$ by

$$F(V) = q + \delta V + \gamma V^2 \qquad [5]$$

where q, δ, and γ are constants. The *dashed curves* in Figs. 1–4 show the best possible (least squares) fit of Eq. 5 to the *solid curve* in each case. We would, of course, have preferred to fit the original data points. [Incidentally, in addition, excellent fits were obtained to the empirical functions from $1 - h$(HH) and $1 - h$(DH).] Table 1 gives the values of q, δ, and γ found. The preliminary values for n(HH) found in ref. 1 (Table 1, $y^2 = 1.0$, convert x to V and Q to q) were -2.37, -3.44, and $+2.12$, respectively.

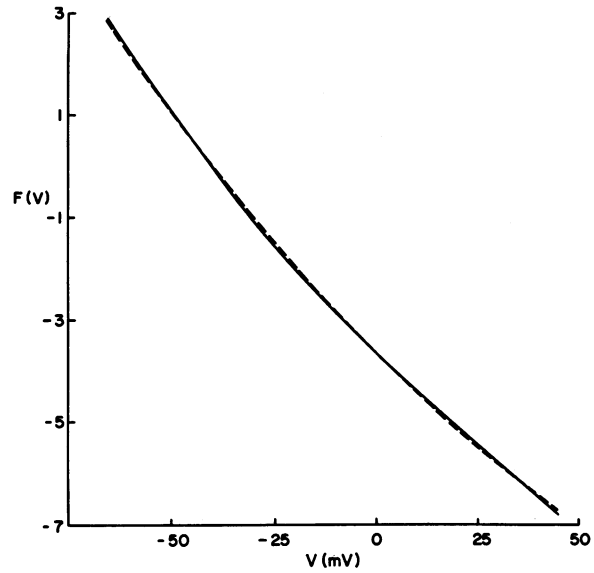

FIG. 2. The function $F(V)$ from m_∞ (Hodgkin–Huxley) (*solid curve*). The *dashed curve* is the least squares best fit of a quadratic function to $F(V)$.

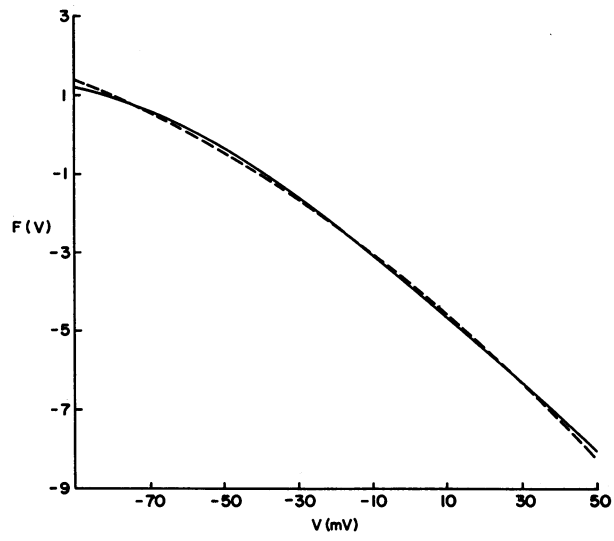

FIG. 3. The function $F(V)$ from n_∞ (Dodge–Hille) (*solid curve*). The *dashed curve* is the least squares best fit of a quadratic function to $F(V)$.

Of course $q = \Delta G(0)/RT$, and $K(0) = e^{-q}$ is the equilibrium constant ($i \rightleftarrows ii$) at $V = 0$ (see q values in Table 1). The often remarked steep rise of the functions $n_\infty(V)$, $m_\infty(V)$, and $1 - h_\infty(V)$ is reflected here in a negative δ and a positive γ, since dn_∞/dV at $n_\infty = {}^1/_2$ is equal to $-(\delta + 2\gamma V_{1/2})/4$. Incidentally, $\gamma > 0$ is necessary to obtain an iso-osmotic K^+ steady-state negative conductance at *positive* V (1, 11, 12, 16). The function $n_\infty(V)$ reaches a maximum, in this case ($\delta < 0$, $\gamma > 0$), at $V_{max} = -\delta/2\gamma$. Table 1 gives, for n(HH), $V_{max} = +78$ mV which happens to agree with the location of the maximum in $g_K(V)$ found by Gilbert and Ehrenstein (16) (*curve IV*, Fig. 2). Table 1 suggests that this phenomenon would be absent in frog node since $\gamma < 0$ for n(DH).

II. δV AND γV^2 CONTRIBUTIONS TO $\Delta G(V)/RT$

We have discussed this subject, or parts of it, on previous occasions (1, 11, 15, 17), but we recapitulate and add a few further comments here. The essential point is that elementary numerical examples, which we omit to save space, show that it is possible to obtain the observed orders of magnitude of δ and γ (Table 1) from the ionic properties of protein-like molecules. We give below a list (1, 15) of some rather obvious possible contributions to the δV and γV^2 terms in $F(V)$. It is important to remember that δ and γ represent differences (e.g., $\delta = \delta_{ii} - \delta_i$), and hence both can have either sign (in principle).

(*A*) *Net Charge.* If the two conformations differ in net charge by only several charges (e.g., from a difference in proton or ion binding), a linear term δV of adequate magnitude can arise (see Eq. 7). Also possible, but numerically less plausible, is a translation (as a consequence of the conformational change) of a fixed set of charges in the subunit to a location with a significantly different local electrostatic potential.

A quadratic term γV^2 (a kind of polarizability) would also occur here if the electric field pulls the charged part (or parts) of the subunit out of its equilibrium ($V = 0$) location or configuration, against a restoring force (this being the force responsible for the equilibrium configuration in the first place). As a simple example, consider (in a subunit conformation) a charge $z\epsilon$ (ϵ = charge on proton) located at a distance y from the outside surface of the membrane, in a linear electrostatic potential $\psi = yV/d$ (d = width of membrane), under a restoring force $-a(y - y_0)$ (y_0 = equilibrium location of $z\epsilon$ when $V = 0$). Then the total potential energy with $z\epsilon$ at y is

$$U(y) = (z\epsilon yV/d) + ({}^1/_2)a(y - y_0)^2. \quad [6]$$

The equilibrium position y' at V follows from $(dU/dy)_{y'} = 0$. Then U at y' is

$$U(y') = (z\epsilon y_0 V/d) - (1/2a)(z\epsilon V/d)^2, \quad [7]$$

which has terms in V (see above) and V^2. The V^2 term will contribute to γV^2 if either z or a is different in the two conformations. Numerical examples indicate that the latter case is a very good possibility, but the former is less likely. That is, a reasonable $z_{ii} - z_i$ will give an adequate value for δ (see above) but an additional source, for example $a_{ii} - a_i$, is probably needed for γ (see also below).

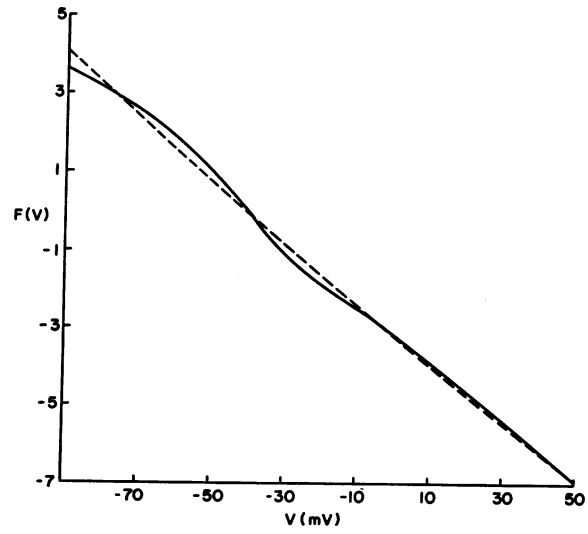

FIG. 4. The function $F(V)$ from m_∞ (Dodge–Hille) (*solid curve*). The *dashed curve* is the least squares best fit of a quadratic function to $F(V)$.

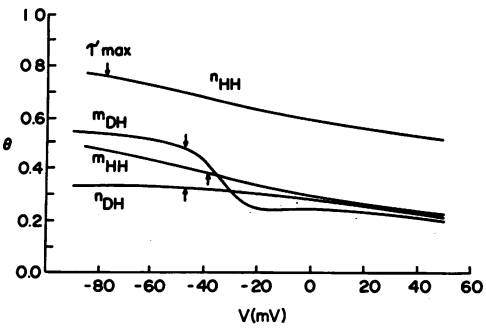

FIG. 5. The functions $\theta(V)$ for n_∞(HH; Hodgkin–Huxley); m_∞(HH), n_∞(DH; Dodge–Hille), and m_∞(DH). The *arrows* show the location (V value) of τ_{max}.

(*B*) *Polarizability.* This source of a contribution to γV^2 has already been discussed in some detail (1, 11, 17, 18). Proton migration in the electric field (18) is an especially good possibility for proteins. The two conformations might differ in polarizability, electric field strength at the location of the subunit, or orientation of subunit axes (if the polarizability is different along different axes).

(*C*) *Second Wien Effect.* This is the effect of an electric field on the degree of dissociation of a weak electrolyte (19, 20). The contribution to $F(V)$ is proportional to $|V|$. Because of

TABLE 1. *Free energy parameters*

	q	$\delta \times 10^2$ (mV^{-1})	$\gamma \times 10^4$ (mV^{-2})
n (HH)	−2.33	−3.24	+2.08
m (HH)	−3.66	−8.14	+2.76
n (DH)	−3.80	−7.85	−2.34
m (DH)	−3.22	−7.69	+0.526
$1 - h$ (HH)	(−6.10)	(−6.16)	(+5.61)
$1 - h$ (DH)	(−11.06)	(−14.34)	(+1.54)

( ) = see last sentence of introduction.

symmetry about $V = 0$, this would appear (approximately) as a contribution to γV^2.

(*D*) *Rotation of Permanent Moment.* Suppose the subunit has a permanent dipole moment and that the axis of the dipole has a different orientation to the field in the two conformations. This is the rotational analogue of the "translation of charges" [see II (*A*)]. Further, if the axis orientation of each conformation at $V = 0$ can be altered somewhat by the field ($V \neq 0$), but resisted by a restoring force, then we would also have the rotational analogue of the γV^2 term ($a_{ii} - a_i$) [see II (*A*)].

Another possibility, used in effect by a number of authors, is that the two orientations are the same but the two moments are different.

III. LOCATION OF THE TIME CONSTANT MAXIMUM

Appreciable interest has been expressed in the approximate coincidence in the location (V value) of the maximum in the $\tau_j(V)$ curve and the steeply rising part of the $j_\infty(V)$ curve, where $j = n, m, 1 - h$ and $\tau_j = (\alpha_j + \beta_j)^{-1}$ = time constant. The kind of analysis in Section I provides a simple explanation. The case $\theta = 1/2$, below, as applied to black lipid membranes with EIM (excitability inducing material), has been considered independently and in a different way by H. Lecar and G. Ehrenstein (personal communication).

To begin with, let us make the approximation that $\theta \cong$ constant around the maximum in $\tau(V)$ (dropping the subscript j). The *arrows* in Fig. 5 locate τ_{max}. These examples indicate that the approximation ought to be fairly good (see also below). We substitute Eqs. **4** into $\tau = (\alpha + \beta)^{-1}$, set $d\tau/dV = 0$, and find

$$\frac{1 - \theta}{\theta} = \frac{\alpha(0)e^{-f(V')}}{\beta(0)} \qquad [8]$$

where V' is the value of V at τ_{max}. Comparison with the result of substituting Eqs. **4** into Eq. **1** shows that $j_\infty(V') = 1 - \theta$ is the value of j_∞ at τ_{max}. Thus, if θ is, say, in the range $0.2 < \theta < 0.8$ (see Fig. 5, for example), then V' would fall within the steeply rising portion of $j_\infty(V)$ (roughly $0.2 < j_\infty < 0.8$). Also, it is easy to show that, at $V = V'$,

$$\left(\frac{d^2\tau}{dV^2}\right)_{V'} = \frac{-\tau_{max}}{\theta(1 - \theta)}\left(\frac{dj_\infty}{dV}\right)^2_{V'} < 0. \qquad [9]$$

Hence, the maximum in τ should be (as a rough rule) sharper the steeper $j_\infty(V)$ at V'. This correlation can be noticed in the squid (2) and modified frog node (13, 14) curves.

The exact relation (i.e., with θ a function of V) between θ and j_∞ at V' is found to be

$$\theta(V') = 1 - j_\infty(V') - f(V')(d\theta/dV)_{V'}(df/dV)_{V'}^{-1}. \qquad [10]$$

The approximation used above follows on setting $(d\theta/dV)_{V'} = 0$.

A fair test of the approximation (as it applies to the present question) is to compare V' with V'', where V'' is defined as the value of V at which $1 - j_\infty$ is equal to $\theta(V')$. From the squid (2) and modified frog node (13, 14) empirical functions, we find six values of $V'' - V'$ which range between +3.3 and +7.8 mV, a rather small difference. The six $\theta(V')$ values vary from 0.31 to 0.76 (see *arrows* in Fig. 5 for four of these values). The six $j_\infty(V')$ values vary from 0.63 to 0.16.

We are indebted to Dr. Kenneth Cole for first bringing the problem in Section III to our attention, and to Dr. F. A. Dodge for providing us with information from Hille's thesis (14). One of us (Y. C.) was supported in part by a grant from the National Science Foundation to the University of California (Santa Cruz).

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