

ELECTRIC FIELDS AND THE COOPERATIVITY OF BIOLOGICAL MEMBRANES*

By TERRELL L. HILL

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OREGON, EUGENE

Communicated May 15, 1967

In an earlier paper,¹ we discussed the possible effects of an electric field on the separation of the two strands of DNA and on the elasticity of a protein or polypeptide chain consisting of a number of units, each of which can exist in two states (short or long). In connection with the latter case, it was pointed out that a small change in electric field could cause a large change in length of an elastic system (at constant force), if a cooperative or phase transition were involved. It was suggested that this kind of effect could conceivably be responsible for muscle contraction, as an alternative to a prior proposal² that contraction might involve a phase transition triggered by a small change in the concentration of a bound ion or molecule.

It was also stated in the electric-field paper¹ that "perhaps a more likely application of the principles outlined here would be to changes in state and properties of biological membranes resulting from changes in membrane potential."

In a recent paper, Changeux, Thiéry, Tung, and Kittel³ discuss cooperative effects in a membrane triggered by a change in the concentration of a bound ligand. Mathematically, this problem is the same as the *elasticity-bound ion* system referred to above,² in the special case f (force) = constant = 0.

The object of the present note is to combine, in as simple a way as possible, our treatment of electric fields¹ with the work of Changeux *et al.*³ We should like to suggest the rather obvious possibility that if indeed there are cooperative effects in membranes, the trigger might be, in some cases at least, a change in membrane potential or electric field (with ligand concentration constant), or a simultaneous change in electric field and in ligand concentration.

Formally, the treatment here corresponds to generalizing the *elasticity-bound ion* problem from $f = 0$ to an arbitrary force.² That is, the electric field plays the role of the force.

The general thermodynamic principle involved here and earlier¹ is that, in an equilibrium between two forms or states of a system or subsystem, imposition of an electric field will cause a greater decrease in free energy in the more polarizable of the two states, and hence will shift the equilibrium in a direction favoring that state. When the equilibrium is a cooperative one, a "landslide" or phase transition may occur.

The Model.—We use the Bragg-Williams (or "molecular field"³) approximation, and the methods and notation we have adopted elsewhere.⁴ The membrane consists of a two-dimensional lattice of M units, each with c nearest-neighbor units, and each of which can exist in state A or state B . The partition function of a unit in state A is $j_A(T)$, and in state B is $j_B(T)$. Each unit has one site for the binding of a ligand. The absolute activity of the ligand is $\lambda = e^{\mu/kT}$; λ is proportional to the concentration of ligand, in a dilute solution. The partition function of a bound ligand on an A site is $q_A(T)$, and on a B site is $q_B(T)$. The polarizability of a unit

in state A is α_A ; it is α_B in state B . These polarizabilities may include contributions from the individual atoms, molecular configuration changes, rotation, proton migration,^{1, 5} etc. In general, α_A and α_B will be functions of T and of the proton absolute activity, λ_{H^+} . For simplicity here, we assume that the ligand is not a proton, and that λ_{H^+} is constant throughout. Several examples of the opposite, more complicated case are treated explicitly elsewhere.¹

Also, for simplicity we neglect induced dipole-dipole interactions in the main argument, but provide a correction for this effect later.

We have not yet introduced interactions (van der Waals, hydrogen bonds, etc.) between nearest-neighbor units (which lead to cooperative effects), but it may be instructive at this point to write down the basic statistical mechanical and thermodynamic equations for the system as outlined so far. The grand partition function for a single unit⁴ is

$$\xi(T, E, \lambda) = j_A e^{\alpha_A E^2/2kT} (1 + q_A \lambda) + j_B e^{\alpha_B E^2/2kT} (1 + q_B \lambda), \quad (1)$$

where E = electric field strength. The first term on the right is proportional to the probability of observing a unit in state A at equilibrium; the second term has a similar significance for state B . The effects of λ and E on these "weights" are included explicitly. In an analogous way, $q_A \lambda$ is the weight of an occupied A site while 1 is the weight of an empty A site, and so on.

The grand partition function for the whole system of M units is

$$\Xi(T, E, \lambda, M) = \xi^M. \quad (2)$$

The pertinent thermodynamic equations are

$$dU = TdS - PdE + \mu'dM + \mu dN, \quad (3)$$

$$U = TS + \mu'M + \mu N, \quad (4)$$

$$-d(\mu'M) = d(kT \ln \Xi) = SdT + \bar{P}dE - \mu'dM + \bar{N}d\mu, \quad (5)$$

where U = energy, P = total polarization, μ' = chemical potential of a unit, and N = number of bound ligand molecules. Given ξ , as in equation (1), equation (5) tells us how to calculate, for example, the mean values \bar{P} and \bar{N} .

Let us now introduce nearest-neighbor interactions between units. The nearest-neighbor interaction free energies are $w_{AA}(T)$, $w_{BB}(T)$, and $w_{AB}(T)$. In the Bragg-Williams approximation, a random distribution of neighbors is assumed in calculating the average interaction free energy between a unit and its nearest neighbors. For an A unit, this free energy is

$$u_A = \delta c w_{AA} + (1 - \delta) c w_{AB}, \quad (6)$$

and for a B unit,

$$u_B = \delta c w_{AB} + (1 - \delta) c w_{BB}, \quad (7)$$

where $\delta = \bar{M}_A/M$ = equilibrium fraction of A units. Equation (1) becomes then

$$\xi = j_A e^{-u_A/kT} e^{\alpha_A E^2/2kT} (1 + q_A \lambda) + j_B e^{-u_B/kT} e^{\alpha_B E^2/2kT} (1 + q_B \lambda). \quad (8)$$

Note that in this simple treatment we are multiplying four factors together in each

term: the four contributions are assumed to be noninteracting. From the two terms on the right of equation (8), the respective weights of *A* and *B*, we obtain

$$\frac{\delta}{1 - \delta} = \frac{\bar{M}_A}{\bar{M}_B} = \frac{j_A e^{-u_A/kT} e^{\alpha_A E^2/2kT} (1 + q_A \lambda)}{j_B e^{-u_B/kT} e^{\alpha_B E^2/2kT} (1 + q_B \lambda)} \tag{9}$$

Equations (2) to (5) still apply.⁶ We find, for example, as we should expect,

$$\bar{P}/M = [\delta \alpha_A + (1 - \delta) \alpha_B] E \tag{10}$$

$$\theta = \bar{N}/M = \delta \theta_A + (1 - \delta) \theta_B, \tag{11}$$

where

$$\theta_A = \text{fraction of occupied } A \text{ sites} = q_A \lambda / (1 + q_A \lambda) \tag{12}$$

$$\theta_B = q_B \lambda / (1 + q_B \lambda). \tag{13}$$

For computational purposes, equation (9) can be rewritten as follows:⁷

$$\frac{\delta}{1 - \delta} e^{c(2\delta - 1)w/2kT} = y, \tag{14}$$

where

$$y = \left(\frac{j_A e^{w_{AA}/2kT}}{j_B e^{-w_{BB}/2kT}} \right) \left(\frac{1 + q_A \lambda}{1 + q_B \lambda} \right) e^{E^2(\alpha_A - \alpha_B)/2kT} \tag{15}$$

and

$$w = w_{AA} + w_{BB} - 2w_{AB}. \tag{16}$$

We see that if $q_A > q_B$, an increase in λ increases y and hence also increases δ (fraction of *A* units). Furthermore, if $\alpha_A > \alpha_B$, an increase in E increases y and δ . Both of these results are as anticipated. If $\lambda = 0$ and $E = 0$, the second and third factors in y , equation (15), become unity, and we obtain the "resting" value of δ from equation (14). Since λ and E are both included in y , either or both can serve to trigger the phase transition, which occurs at $y = 1$ (if $T < T_c$).

Figure 1 shows δ as a function of $\ln y$, as calculated from equation (14). If w is negative (i.e., if *AA*, *BB* pairs are more stable than *AB* pairs), there is a phase transition at a low enough temperature. One phase is rich in *A*, the other in *B*. The critical temperature (Fig. 1) is given by^{3, 7} $cw/kT_c = -4$.

When $T < T_c$, there is a discontinuity in δ at $y = 1$ (the vertical line rather than the dashed loop in Fig. 1 is the stable equilibrium path). Therefore, there is also, at $y = 1$, a discontinuity in \bar{P} and in θ , from equations (10) and (11).

An order of magnitude calculation based on reasonable polarizabilities^{1, 5} and mem-

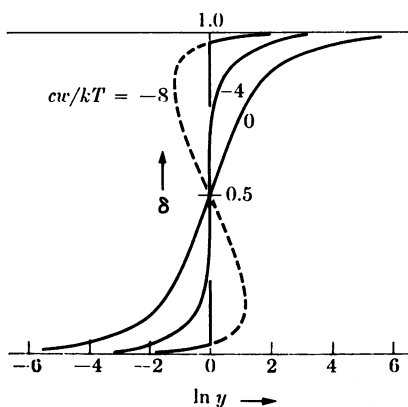


FIG. 1.—Fraction of *A* units as a function of $\ln y$.

brane potentials shows that $\alpha E^2/kT$ for a unit can easily be of order unity. Hence the λ and E factors in equation (15) are typically similar in magnitude. But this is not an essential point since only a very small change in γ suffices for "trigger" action.

In the problem of the elasticity of a single A, B chain with binding, we put $c = 2$ in equation (14) and $e^{f(l_A - l_B)/kT}$ in place of $e^{E^2(\alpha_A - \alpha_B)/2kT}$ in equation (15), where $f =$ force, $l_A =$ length of an A unit, etc. Actually, an exact treatment is easy and possible for a one-dimensional system; this is given by equations (14-21) of reference 4. The two-dimensional elasticity problem (a sheet of A, B chains) is made complicated by the effect of the differing lengths of the A and B units on nearest-neighbor interchain interactions.²

If we replace the random lattice of M dipoles (all perpendicular to the lattice plane) by a continuous distribution outside of a circle of area equal to the area per dipole, we can easily obtain a correction to equation (14): E is replaced by

$$\frac{E}{1 + 2(\pi M/\alpha)^{3/2} [\delta\alpha_A + (1 - \delta)\alpha_B]}$$

where E is the external field and α/M is the area per molecule (or dipole) in the lattice.

Summary.—It is suggested that if membranes exhibit cooperative transitions, the trigger may be a change in electric field (membrane potential), or in a ligand concentration, or both.

* Supported in part by research grants from the Heart Institute of the U.S. Public Health Service and the National Science Foundation.

¹ Hill, T. L., *J. Am. Chem. Soc.*, **80**, 2142 (1958).

² Hill, T. L., *J. Chem. Phys.*, **20**, 1259 (1952); Hill, T. L., *Discussions Faraday Soc.*, **13**, 132 (1953).

³ Changeux, J.-P., J. Thiéry, Y. Tung, and C. Kittel, these PROCEEDINGS, **57**, 335 (1967).

⁴ Hill, T. L., *Statistical Thermodynamics* (Reading, Mass.: Addison-Wesley, 1960), pp. 140-143, 201-205, 241-242, 246-249, and 252.

⁵ Kirkwood, J. G., and J. B. Shumaker, these PROCEEDINGS, **38**, 855, 863 (1952).

⁶ In carrying out the differentiations $\bar{P} = kT \partial \ln \Xi / \partial E$, etc., δ in eq. (8) is held constant. This is a necessary feature of this approximation when an *open* system partition function is used. See p. 252, ref. 4. In effect, we introduce the value of δ in eq. (8) in order to calculate δ in eq. (9).

⁷ Equation (14) is formally the same as eqs. (14-47), ref. 4 (for a "lattice gas").