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Some Possible Biological Effects of an Electric Field Acting on Nucleic Acids or Proteins[1]

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The relative stability of two different states or configurations of a macromolecular system may be altered or reversed by the action of an electric field. The field will shift the equilibrium between the two states in such a way as to favor the state with greater polarizability. Specific examples considered here are (1) possible separation of the two molecular chains in DNA as a result of the application of an electric field (the two states in this case: paired chains and separated chains) and (2) the effect of an electric field on the elasticity of a protein chain (two states: long chain and short chain). These examples may possibly be of interest in mitosis and muscle action, respectively. Perhaps a more likely application would be to changes in state and properties of biological membranes resulting from changes in membrane potential.

I. Introduction

Electric fields are known or presumed to be present in many biological situations. We have been considering, theoretically, some of the possible biological effects of an electric field acting on nucleic acids and proteins. A report on this work may be appropriate at this time, particularly for purposes of comparison with the recent contribution along these lines of Klotz and Horowitz.[2]

We shall use the most elementary possible models here, simply to point out the effects, and confine ourselves to two examples: possible separation of the two molecular chains in DNA (deoxyribonucleic acid) as a result of the application of an electric field (Section II) and the effect of an electric field on the elasticity of a protein chain (Section III). The first example would be of biological significance if the application of an electric field is the trigger for the division of the genetic material in a cell nucleus prior to self-duplication. The second example may be of interest in muscle action. 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. However, we do not pursue this possibility in the present paper.

The general thesis developed here is that an electric field will alter the average distribution of bound ions (*e.g.*, protons) among possible sites on a polyelectrolyte molecule and this, in turn, may affect the relative stability of two different configurations of the molecule.

Incidentally, it is necessary to use an unconventional criterion of thermodynamic stability in this paper because the processes of interest presumably take place in the presence of a reservoir of protons or other bound ions at a fixed chemical potential. This situation is undoubtedly a very common one in biological systems.

Although we shall not use their formalism, the work of Kirkwood and Shumaker[3] provides the general foundation for the present discussion.

II. Stability of Paired Molecular Chains of DNA Relative to Separated Chains

We assume, as seems reasonable, that the two nucleotide chains in DNA *in vivo* are rather delicately balanced, thermodynamically, between pairing and unpairing. Contributions to this "free energy"[4] balance arise from hydrogen bonds between bases, van der Waals interactions, electrostatic repulsion between fixed charges,[5] configurational entropy effects, ion binding, etc. In the absence of an electric field, paired chains are assumed slightly more stable than unpaired chains. We investigate below the possibility of an electric field reversing the relative stability by a shift in location of bound ions. The essential requirement is that the unpaired chains be more polarizable by the field (and hence undergo a greater decrease in "free energy" when the field is applied) than the paired chains. This is the case in the model described below because (a) the polarizability is attributed to the movement of bound ions and (b) inter-chain attractive interactions involving bound ions reduce the tendency of these ions to move with the field in the case of paired chains.

The model for paired chains is shown schematically in Fig. 1. It is essentially the same as that used in an earlier paper,[6] except for the possibility here of the existence of an electric field along the axis of the chains. The system of interest consists of M independent pairs of binding sites. All inter-chain forces (hydrogen bonds, etc.) other than electrostatic interactions involving these sites and the ions bound on them are lumped together in a free energy W per pair of sites, which is zero when the chains are separated. Let j_1 be the partition function (including the binding energy) of an ion bound at site 1 of a pair, and similarly let j_2 refer to an ion bound at site 2. Let W_{AA}, W_{AB}, W_{BA}, and W_{BB} be the free energies of interaction between the sites when both sites are occupied (AA), when site 1 is occupied and site 2 unoccupied (AB), etc., respectively. That is, the W's are the potentials of average force for the pair of sites occupied in each of the four ways possible; the potential zero is chosen at infinite separation in each case.

The external electric field E is assumed to be constant along the axis of the chains and directed toward the $n = 1$ end (Fig. 1). Contributions of bound ions to the field are ignored. If the length of the chains is l, the potential difference between the two ends is

$$\psi = El = \psi(M) - \psi(1)$$

(1) This investigation was supported by a research grant from the Heart Institute, Public Health Service.

(2) I. M. Klotz and M. G. Horowitz, *Science*, **126**, 26 (1957).

(3) J. G. Kirkwood and J. B. Shumaker, *Proc. Nat. Acad. Sci.*, **38**, 855, 863 (1952). For a more general treatment of protein-protein forces, see also T. L. Hill, *J. Chem. Phys.*, **23**, 623 (1955).

(4) We are using the term loosely here. Actually it is not a free energy that is involved. See Appendix I.

(5) T. L. Hill, *Arch. Biochem. Biophys.*, **57**, 229 (1955).

(6) T. L. Hill, THIS JOURNAL, **78**, 3330 (1956).

Presumably the case of most interest is $\psi(M/2)$ (the potential at the middle of the chains) = 0 whether the field is on or off. The more general situation is discussed briefly in Appendix III. The canonical ensemble partition functions for pair n, with zero, one and two ions bound, respectively, are then

$$Q_0(n) = e^{-W/kT}\, e^{-W_{BB}/kT}$$
$$Q_1(n) = e^{-W/kT}(j_1 e^{-W_{AB}/kT} + j_2 e^{-W_{BA}/kT}) e^{\beta(n-\frac{1}{2}M)} \quad (1)$$
$$Q_2(n) = e^{-W/kT}\, j_1 j_2 e^{-W_{AA}/kT}\, e^{2\beta(n-\frac{1}{2}M)}$$

where

$$\beta = -lqE/MkT = -q\psi/MkT$$

and q is the charge on the ion being bound. For separated chains (superscript zero throughout)

$$Q_0^0(n) = 1$$
$$Q_1^0(n) = (j_1 + j_2) e^{\beta(n-\frac{1}{2}M)} \quad (2)$$
$$Q_2^0(n) = j_1 j_2 e^{2\beta(n-\frac{1}{2}M)}$$

For simplicity we have omitted in each of eqs. 1 and 2 a factor

$$e^{\beta'(n-\frac{1}{2}M)}$$
$$\beta' = -l(q_1 + q_2)E/MkT$$

where q_1 and q_2 are the charges on unoccupied sites 1 and 2, respectively. Because of symmetry, the contribution of this factor drops out in eq. 6 (see Appendix III).

In view of the independence of pairs in this model, the two grand partition functions are simply

$$\Xi = \prod_{n=1}^{M} [Q_0(n) + Q_1(n)\lambda + Q_2(n)\lambda^2]$$
$$\Xi^0 = \prod_{n=1}^{M} [Q_0^0(n) + Q_1^0(n)\lambda + Q_2^0(n)\lambda^2] \quad (3)$$

where $\lambda = e^{\mu/kT}$.

The thermodynamic significance of Ξ here is seen from (Appendix I)

$$\mathrm{d}(N\mu - A) = N\mathrm{d}\mu + S\mathrm{d}T + \varphi\mathrm{d}M + P\mathrm{d}E$$
$$= \mathrm{d}(\varphi M) = \mathrm{d}(kT \ln \Xi) \quad (4)$$

According to Appendix I, paired chains are more stable than unpaired chains, at the same T, μ, M and E, if

$$w = (N\mu - A) - (N\mu - A)^0 = kT \ln (\Xi/\Xi^0) > 0 \quad (5)$$

As shown in Appendix I, this quantity is the reversible work which must be done on the system, at constant T, μ, M and E, in order to separate the chains.

Since M is a large number, we can replace the sum by an integral in the expression for $\ln \Xi$

$$\ln \Xi = -\frac{MW}{kT} + \int_{-M/2}^{+M/2} \ln [x_1 + (j_1 x_2 + j_2 x_3) e^{\beta m}\lambda + j_1 j_2 x_4 e^{2\beta m}\lambda^2] \mathrm{d}m \quad (6)$$

where

$$x_1 = e^{-W_{BB}/kT},\ x_2 = e^{-W_{AB}/kT},\ x_3 = e^{-W_{BA}/kT},\ x_4 = e^{-W_{AA}/kT}$$

The integral in eq. 6 cannot be expressed in closed form, so for order of magnitude purposes below we

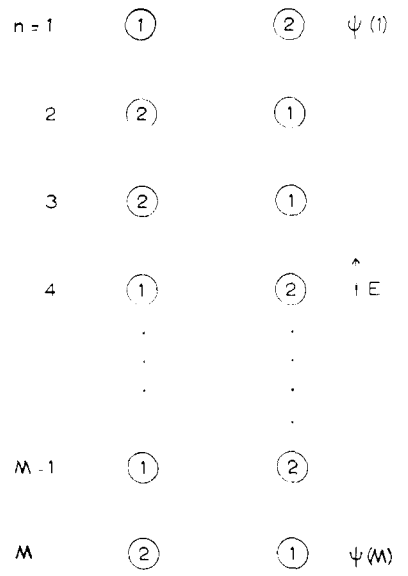

Fig. 1.—Two paired chains of DNA (schematic) with one pair of binding sites for each pair of nucleotides. $\psi(n)$ = electric potential at nth pair.

write it as a power series in E and drop terms above E^2. That is, for

$$\left|\frac{\beta M}{2}\right| = \left|-\frac{lqE}{2kT}\right| << 1 \quad (7)$$

we retain only up to quadratic terms in the expansion of the exponentials in eq. 6. We find

$$\ln \Xi = -\frac{MW}{kT} + M \ln \xi + \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 [\lambda] \quad (8)$$

and

$$\frac{P}{kT} = \frac{\partial \ln \Xi}{\partial E} = \frac{M}{12}\left(\frac{lq}{kT}\right)^2 [\lambda] E \quad (9)$$

where

$$\xi = x_1 + (j_1 x_2 + j_2 x_3)\lambda + j_1 j_2 x_4 \lambda^2$$
$$[\lambda] = \frac{(j_1 x_2 + j_2 x_3)\lambda(x_1 + j_1 j_2 x_4 \lambda^2) + 4 j_1 j_2 x_1 x_4 \lambda^2}{\xi^2}$$

Also

$$\frac{P - P^0}{kT} = \frac{M}{12}\left(\frac{lq}{kT}\right)^2 \{[\lambda] - [\lambda]^0\} E \quad (10)$$

$$\frac{w(E)}{kT} = \ln \frac{\Xi}{\Xi^0} = -\frac{MW}{kT} + M \ln \frac{\xi}{\xi^0} + \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 \{[\lambda] - [\lambda]^0\} \quad (11)$$

$$\frac{w(0) - w(E)}{kT} = -\frac{M}{24}\left(\frac{lqE}{kT}\right)^2 \{[\lambda] - [\lambda]^0\} \quad (12)$$

where

$$[\lambda]^0 = \frac{(j_1 + j_2)\lambda(1 + j_1 j_2 \lambda^2) + 4 j_1 j_2 \lambda^2}{\xi^{0\,2}}$$
$$\xi^0 = 1 + (j_1 + j_2)\lambda + j_1 j_2 \lambda^2$$

We might digress to point out that eq. 9 (see also eq. 22) resembles the familiar expression for the polarization of a perfect gas of permanent dipoles, since lq has the dimensions of a dipole moment (see below).

If paired chains are assumed stable when $E = 0$, the various contributions to $w(0)$ must balance to make this quantity slightly positive. Further, if imposition of a field E reverses the stability, $w(E)$

must be slightly negative; the difference $w(0) - w(E)$ is then positive and is given eq. 12. According to eq. 10, when $w(0) - w(E)$ is positive, separated chains have a greater polarizability (P/E) than paired chains, as expected.

One specific possibility[6] is summarized below

1	2	1	2	1	2	1	2
B	B	A	B	B	A	A	A
0	−	+	−	0	0	+	0

This might arise,[7] for example, if site 1 (charge zero) is on one ring of a pair of hydrogen bonded bases and site 2 (charge −1) on the other ring. Then $x_1 = x_3 = x_4 = 1$ and $x_2 > 1$. As an illustration, suppose $j_1 j_2 \lambda^2 = 1$ (*i.e.*, the pH midway between pK_1 and pK_2). Then $\{\}$ in eq. 12 simplifies to

$$\{\} = \frac{2}{2 + (j_1 x_2 + j_2)\lambda} - \frac{2}{2 + (j_1 + j_2)\lambda} < 0 \quad (13)$$

If pK_1 and pK_2 differ by unity, for example, then the maximum possible value of $-\{\}$ in eq. 13 (put $x_2 >> 1$) is 0.365. If we take,[8] say, $\psi = lE = 100$ mv., we find $w(0) - w(E) = 0.13$ kcal. per mole of base pairs. This is then the order of magnitude, which seems adequate, of the "range of stability balance" (from $w(0) > 0$ to $w(E) < 0$), according to this model.

We digress to amplify this point. Consider first, for comparison, the effect of an external electric field D on an ordinary phase equilibrium (vaporization, fusion, sublimation) in a one component system. We have[9]

$$\mu_1 = \mu_2, \; d\mu_1 = d\mu_2$$

where, for either phase, we can write

$$d\mu = -PvD\left(\frac{dD}{D}\right) - Ts\left(\frac{dT}{T}\right) + pv\left(\frac{dp}{p}\right) \quad (a)$$

with P = polarization, v = volume per molecule and s = entropy per molecule. Ts is of the order of magnitude of kT; pv is also of order kT for a gas and, say, $10^{-3}\,kT$ for a liquid or solid. For gas *or* condensed phase, $Pv = DO(v_c)$, where v_c represents a volume per molecule in a condensed phase. Hence, in eq. a, $PvD = D^2O(v_c)$. If we take a field of, say, D = 10,000 volts/cm., PvD is then of order $10^{-6}\,kT$. Hence ordinary electric fields have a rather insignificant effect on μ and therefore on the phase equilibrium.

Paired and unpaired chains can also be considered "phases" in the above sense. At equilibrium

$$\varphi_1 = \varphi_2, \; d\varphi_1 = d\varphi_2$$

where $$d\varphi = \frac{PE}{M}\left(\frac{dE}{E}\right) + \frac{TS}{M}\left(\frac{dT}{T}\right) + \frac{N\mu}{M}\left(\frac{d\mu}{\mu}\right) \quad (b)$$

Since N is of order M, (TS/M) and $(N\mu/M)$ are of order kT. Now in eq. b, we have (eq. 9)

$$\frac{PE}{M} = E^2O\left(\frac{l^2q^2}{kT}\right) \quad (c)$$

whereas in eq. a (taking a perfect gas of permanent dipoles for comparison)

$$PvD = D^2O(v_c) = D^2O\left(\frac{\mu_0^2}{kT}\right) \quad (d)$$

where μ_0 is the permanent dipole moment. In eq. d the polarization arises through the displacement (dipole orientation) of a charge q through a distance of order 1 Å.; but in eq. c *the charge q can be displaced a distance of order l.* Since l can be of order 1000 Å. or more, l^2q^2/μ_0^2 can be of order 10^6. Thus, for a field of 10,000 volts/cm. (say, 100 mv./1000 Å.) our estimate of $10^{-6}\,kT$ above for the field term in eq. a becomes kT for eq. b. Hence we expect the field to have an appreciable effect on the "phase" equilibrium in the latter case. This is illustrated in the numerical example above.

Another possibility[6] is

1	2	1	2	1	2	1	2
B	B	A	B	B	A	A	A
−	−	+	−	−	+	+	+

This could be due to the binding of Mg^{++} (or Ca^{++}) on the pair of singly charged phosphate groups associated with each pair of nucleotides. Then $x_1^{-1} = x_2 = x_3 = x_4^{-1}$, $x_2 > 1$, and $j_1 = j_2$. For example, if $j_1\lambda = 1$ ($pK_1 = pK_2 = p\mathrm{Mg}$)

$$\{\} = \frac{1 + x_2^{-2}}{(x_2^{-1} + x_2)^2} - \frac{1}{2} < 0 \quad (14)$$

$$\longrightarrow -\frac{1}{2} \text{ as } x_2 \longrightarrow +\infty$$

The effect is thus of the same magnitude as above (an extra factor of four enters, through q, because of the double charge on Mg^{++}).

A complication we have not taken into account above (see Section III), which would increase $w(0) - w(E)$, is the likelihood that the electric field would straighten out the separated (but not the paired) chains, thus increasing the polarizability of separated chains.

The possibility of the participation of an electric field in mitosis has been discussed off and on for a long time. The above analysis shows, on a molecular level, one way in which such a field could conceivably play a crucial role in chromosome division.

III. Elasticity of a Protein Chain

To show the possible effect of an electric field in this problem we choose a simplified model for the α–β transition.[10] We consider an elastic fiber (say, in muscle) to be made up of independent parallel molecular chains. Each chain consists of B units linked together. A unit might contain, say, four amino acid residues and can exist in a short (helical) state (α) of length l_α or a long (extended) state (β) of length l_β.

Consider a single chain. Let B_α units be in state α and $B - B_\alpha$ in state β, so that the length of the chain is

$$l = l_\alpha B_\alpha + l_\beta(B - B_\alpha) \quad (15)$$

The length is thus essentially determined by the value of B_α, for given B. Let j_α and j_β be the partition functions of individual units in states α and β, respectively. Let j be the partition function (as in Section II) of an ion bound on either an α or a β unit (we assume one site per unit, with j independent of whether the state is α or β). Let N be the number of ions bound on the B sites. The sites are assumed (again for simplicity) uncharged but each bound ion carries a charge q. As in Section II, we choose $\psi(B/2) = 0$ (see Appendix III).

We now proceed to derive the thermodynamic properties of the model. We may anticipate that imposition of an electric field, keeping the tension

(7) S. Bernhard, private communication.

(8) This is conservative since l is probably much greater than the thickness of biological membranes, some with membrane potential of the order of 100 mv.

(9) T. L. Hill, *J. Chem. Phys.*, **28**, 61 (1958).

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

constant, will cause the chain to lengthen by conversion of some α units to β units. This is a consequence of the fact that bound charges can move (when the field is applied) over a greater distance on a chain of β units than on a chain of α units; that is, a long chain is more polarizable (see eq. 22 below) than a short chain (if the bound ions are bound with equal strength to both the long and short chains, as we are assuming here). This mechanism could conceivably be the basis of muscle contraction; it should be noted that there is no need for a pH gradient as used by Klotz and Horowitz.[2]

As in Section II, we are interested in the behavior of the system in the presence of a reservoir of bound ions at a fixed chemical potential μ. If $Q(B_\alpha, B, N, T, E)$ is the canonical ensemble partition function, then we see from eq. II. 3 that the appropriate partition function for our purposes is

$$Y(B_\alpha, B, T, \mu, E) = \sum_{N=0}^{B} e^{N\mu/kT}\, Q(B_\alpha, B, N, T, E) \tag{16}$$

$$\mathrm{d}(kT \ln Y) = S\mathrm{d}T + \tau(l_\beta - l_\alpha)\mathrm{d}B_\alpha + P\mathrm{d}E - (\mu' + \tau l_\beta)\mathrm{d}B + N\mathrm{d}\mu \tag{17}$$

In view of our assumptions, the distribution of α and β units along the chain is independent of N and E for given B_α and B so that we can write

$$Q(B_\alpha, B, N, T, E) = Q^*(N, T, E)\frac{B! j_\alpha^{B_\alpha} j_\beta^{B-B_\alpha}}{B_\alpha!(B - B_\alpha)!}$$

where Q^* refers to the bound ions only. Then eq. 16 becomes (compare eq. 3)

$$Y = \frac{B! j_\alpha^{B_\alpha} j_\beta^{B-B_\alpha}}{B_\alpha!(B - B_\alpha)!} \prod_{n=1}^{B} [1 + je^{\beta(n - \frac{1}{2}B)\lambda}] \tag{18}$$

where

$$\beta = -lqE/BkT$$

Then

$$\ln Y = B \ln B - B_\alpha \ln B_\alpha - (B - B_\alpha)\ln(B - B_\alpha) + B_\alpha \ln j_\alpha + (B - B_\alpha)\ln j_\beta + \int_{-B/2}^{+B/2} \ln(1 + j\lambda e^{\beta m})\mathrm{d}m \tag{19}$$

Again the integral cannot be expressed in closed form so we confine ourselves to the first terms in an expansion in powers of E. We find for the integral

$$\mathcal{J} = B \ln(1 + j\lambda) + \frac{B}{24}\frac{j\lambda}{(1 + j\lambda)^2}\left(\frac{lqE}{kT}\right)^2 \tag{20}$$

From eqs. 17, 19 and 20 we easily derive the thermodynamic properties

$$\frac{\tau(l_\beta - l_\alpha)}{kT} = \ln\frac{B - B_\alpha}{B_\alpha} + \ln\frac{j_\alpha}{j_\beta} - \frac{1}{12}\frac{j\lambda}{(1 + j\lambda)^2}\frac{B(l_\beta - l_\alpha)}{l}\left(\frac{lqE}{kT}\right)^2 \tag{21}$$

$$\frac{P}{kT} = \frac{B}{12}\frac{j\lambda}{(1 + j\lambda)^2}\left(\frac{lq}{kT}\right)^2 E \tag{22}$$

$$\frac{\bar{N}}{B} = \frac{j\lambda}{1 + j\lambda} + \frac{1}{24}\frac{j\lambda(1 - j\lambda)}{(1 + j\lambda)^3}\left(\frac{lqE}{kT}\right)^2 \tag{23}$$

Equations 21 and 23 show that application of an electric field (a) reduces the tension at constant length, (b) increases the length at constant tension and (c) causes the number of bound ions to approach $B/2$ (for which value of $\bar{N}$ the polarization is a maximum), from either side.

Figure 2 illustrates eq. 21. In this example we see that the electric field causes an appreciable change in length at constant tension. It should be emphasized that in the region of a coöperative phase transition, as indeed would be expected to occur in the α–β system,[10] the sensitivity of the length to an electric field would be very greatly enhanced. However, to avoid undue complications, we have not attempted to introduce coöperative effects (associated with hydrogen bonding[10]) in the above analysis.

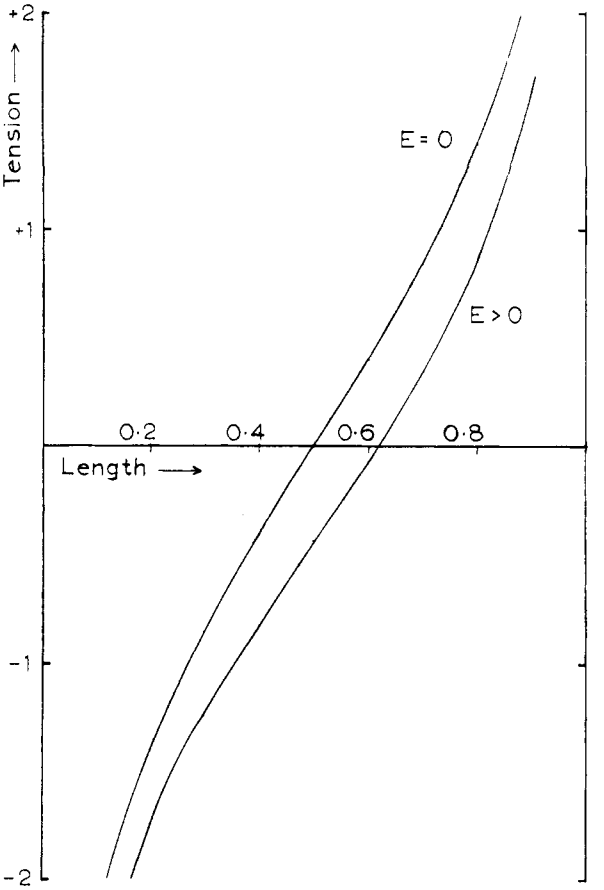

Fig. 2.—Plot of tension *vs.* length in the form

$$\frac{\tau(l_\beta - l_\alpha)}{kT} - \ln\frac{j_\alpha}{j_\beta} \text{ vs. } 1 - \frac{B_\alpha}{B}$$

for the special case $l\beta = 2l_\alpha$, $j\lambda = 1$, $B(l_\beta - l_\alpha)E = 100$ mv., q = charge on proton.

In earlier work[10] we have shown, on theoretical grounds, that an alteration in the concentration of bound ions (or of the ionic strength) could be the trigger for muscle contraction. The present discussion is adequate to demonstrate, alternatively, that application of an electric field (keeping the concentration of bound ions constant) might also serve as the trigger, especially if associated with a phase transition.

IV. Macromolecules in Solution

We wish to point out in this section that the general considerations in Sections I–III might be applied, in principle, both theoretically and experimentally, to any macromolecule which can exist

in solution in two or more forms. That is, introduction of an electric field will tend to shift the equilibrium to favor the more polarizable form. An obvious example is the α-helix-random coil transition in proteins and synthetic polypeptides, studied by P. Doty and co-workers. Others are possible structural changes or dissociations in nucleic acids, polynucleotides, serum albumin at low pH, etc. However, *in practice*, the fields required (see above) probably are unattainable.

It should be mentioned that the polarizability involved, for molecules in solution, is the total polarizability—including not only the Kirkwood–Shumaker proton migration polarizability but also a contribution associated with orientation of the permanent dipole moment. In the rigid structures contemplated in the preceding sections, there would be no orientation contribution.

Appendix I

A. Consider a spontaneous infinitesimal change in a thermodynamic system carried out in such a way that no work is done on or by the system. Then

$$\mathrm{d}U < T\mathrm{d}S + \sum_{i=1}^{\nu} \mu_i \mathrm{d}N_i (DW = 0)$$

$$\mathrm{d}A < 0 \quad (T, N_1, \ldots, N_\nu \text{ constant}; DW = 0)$$

For example, the volume of a gas will change in such a way (increase) as to decrease the Helmholtz free energy, if $T, N_1, \ldots N_\nu$ are constant and the external (resisting) pressure is zero. If the process is carried out, as it would be in many biological systems, in contact with a reservoir of molecules of species, say, 1,2, . . .,ν' with constant chemical potentials, then

$$\mathrm{d}\left(A - \sum_{s=1}^{\nu'} \mu_s N_s\right) < 0 \quad (T, \mu_1, \ldots, \mu_{\nu'}, N_{\nu'+1}, \ldots, N_\nu \text{ constant}; DW = 0) \quad \text{(I.1)}$$

determines the direction of a spontaneous change. If the process occurs at constant pressure but no non-pV work is done, then

$$\mathrm{d}\left(A + pV - \sum_{s=1}^{\nu'} \mu_s N_s\right) < 0 \quad (T, \mathrm{p}, \mu_1, \ldots, \mu_{\nu'}, N_{\nu'+1}, \ldots, N_\nu \text{ constant}; DW_{\text{non-pV}} = 0) \quad \text{(I.2)}$$

B. In the special case in Section II

$$\mathrm{d}U = T\mathrm{d}S - \varphi \mathrm{d}M - P\mathrm{d}E + \mu \mathrm{d}N \quad \text{(I.3)}$$

$$U = TS - \varphi M + \mu N \quad \text{(I.4)}$$

were φ is defined by (in adsorption problems it is called the "surface" or "spreading" pressure; it can also be considered the negative of a chemical potential)

$$-\varphi = (\partial U/\partial M)_{S.E.N}$$

and P by

$$-P = (\partial U/\partial E)_{S.M.N}$$

P might be called the total polarization. (In dielectric theory, the term analogous to $P\,\mathrm{d}E$ above is usually written[9] $PV\mathrm{d}D$.) P is an extensive quantity and E is intensive. P and E will always have the same sign.

Equation I.1 reads, for present purposes, $\mathrm{d}(A - N\mu) < 0$ (T, μ, M, E const.). That is, the two chains will tend (thermodynamically) to separate if $A - N\mu$ is lower for the separated chains than for the paired chains at the same T, μ, M and E and *vice versa*.

Also, for a reversible process

$$\mathrm{d}U = T\mathrm{d}S + DW_{\text{on}} + \mu \mathrm{d}N \ (M, E \text{ constant})$$

$$\mathrm{d}(A - N\mu) = DW_{\text{on}} \ (T, \mu, M, E \text{ constant})$$

where DW_{on} represents reversible work done on the system with M and E held fixed. Hence the difference in value of $A - N\mu$ in two states (T, μ, M, E fixed) is a measure of the work which must be done on the system to convert one state to the other.

Appendix II

The basic thermodynamic equation for the model in Section III is

$$\mathrm{d}U = T\mathrm{d}S + \tau \mathrm{d}l - P\mathrm{d}E + \mu' \mathrm{d}B + \mu \mathrm{d}N \quad \text{(II.1)}$$

where τ is the tension (force) and μ' and μ are chemical potentials. Using eq. 15 to change independent variables from l and B to B_α and B

$$\mathrm{d}U = T\mathrm{d}S - \tau(l_\beta - l_\alpha)\mathrm{d}B_\alpha - P\mathrm{d}E + (\mu' + \tau l_\beta)\mathrm{d}B + \mu \mathrm{d}N$$

$$U = TS - \tau(l_\beta - l_\alpha)B_\alpha + (\mu' + \tau l_\beta)B + \mu N = TS + \tau l + \mu' B + \mu N \quad \text{(II.2)}$$

$$\mathrm{d}(N\mu - A) = S\mathrm{d}T + \tau(l_\beta - l_\alpha)\mathrm{d}B_\alpha + P\mathrm{d}E - (\mu' + \tau l_\beta)\mathrm{d}B + N\mathrm{d}\mu \quad \text{(II.3)}$$

Appendix III

Section II.—Suppose that when the field E is applied the potential at $M/2$ is also changed from zero to ψ_0. ψ_0 may be regarded as a second intensive parameter (in addition to E) on which thermodynamic properties depend. The complete Ξ can then be written by multiplying each of Q_0, Q_1 and Q_2 in eq. 1 by

$$e^{\beta'(n - \frac{1}{2}M)} \, e^{-(q_1 + q_2)\psi_0/kT}$$

and replacing λ in eq. 3 by

$$\lambda' = \lambda e^{-q\psi_0/kT}$$

Then eq. 8 becomes

$$\ln \Xi = -\frac{MW}{kT} - \frac{M(q_1 + q_2)\psi_0}{kT} + M \ln \xi(\lambda') + \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 [\lambda'] \quad \text{(III.1)}$$

Eq. 9 is unchanged except for replacing λ by λ'. Equation 11 now reads

$$\frac{w(E,\psi_0)}{kT} = -\frac{MW}{kT} + M \ln \frac{\xi(\lambda')}{\xi^0(\lambda')} + \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 \{[\lambda'] - [\lambda']^0\} \quad \text{(III.2)}$$

and

$$\frac{w(0,0) - w(E,\psi_0)}{kT} = M \ln \frac{\xi(\lambda)\xi^0(\lambda')}{\xi^0(\lambda)\xi(\lambda')} - \frac{M}{24}\left(\frac{lqE}{kT}\right)^2 \{[\lambda'] - [\lambda']^0\} \quad \text{(III.3)}$$

That is, even when $E = 0$ there is an effect, if $\psi_0 \neq 0$, arising from the fact that the number of ions bound depends on ψ_0.

Section III.—We replace λ by λ' in eq. 18. Equations 21–23 are all unaffected except for replacement of λ by λ'. We note, incidentally, that

$$kT\left(\frac{\partial \ln Y}{\partial \psi_0}\right)_{T,B\alpha,B,\mu,E} =$$

$$kT\left(\frac{\partial \ln Y}{\partial \lambda}\right)_{T,B\alpha,B,E}\left(\frac{\partial \lambda'}{\partial \psi_0}\right)_{\lambda,T}\left(\frac{\partial \lambda}{\partial \lambda'}\right)_{\psi_0 T} =$$

$$kT \times \frac{\bar{N}}{\lambda} \times -\frac{\lambda' q}{kT} \times \frac{\lambda}{\lambda'} = -\bar{N}q$$

as would be expected on thermodynamic grounds. A similar result follows above (for Section II).

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Reversible Uptake of Oxygen by Vitamin B_{12a}

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The apparent specific volumes of vitamins B_{12} and B_{12a} have been measured, the values obtained being: for B_{12} 0.665 (independent of the presence or absence of oxygen); for B_{12a} 0.650 (in the absence of oxygen) and 0.713 (in the presence of oxygen). An amperometric titration of B_{12a} with a standard solution of oxygen confirmed the earlier finding that vitamin B_{12a} when placed in solution dimerizes through the agency of oxygen. It was further established that the combining ratio is two molecules of B_{12a} to one molecule of oxygen. The amperometric titration method further showed that vitamin B_{12} does not combine with oxygen. This is also in agreement with the earlier measurements of diffusion coefficients and apparent specific volumes. An amperometric titration of vitamin B_{12r} with oxygen gave results in accord with the concept that B_{12r} is a bivalent cobalt compound which is easily oxidized to B_{12a}. The titration showed two end-points corresponding first to the oxidation of the cobalt and second to the dimerization of B_{12a}. Vitamin B_{12a} combines reversibly with oxygen gas, this being the first case of a trivalent cobalt compound to exhibit such behavior.

A repetition[1] of the measurements of the diffusion coefficients of vitamins B_{12} and B_{12a} confirmed the earlier report[2] that the molecular weight of vitamin B_{12a} in solution is twice that of B_{12}. The newer measurements of the diffusion coefficients were made by a free diffusion method using the Tiselius electrophoresis apparatus (without applied potential); the results probably are accurate to within 5%. The molecular weights were calculated by both the Stokes–Einstein and the Stokes–Einstein–Longsworth[3] equations, the latter giving values for the molecular weight of B_{12} in reasonable agreement with that calculated on the cobalt content. Calculated by either method, however, the molecular weight of B_{12a} appeared to be twice that of B_{12}. Moreover, these results were confirmed by a measurement of the sedimentation coefficients and of the apparent specific volumes; these values together with the diffusion coefficients make possible a calculation of molecular weight by the Svedberg equation.

These studies showed in a gross way that vitamin B_{12a} dimerizes in water solution but offered no mechanism by which the dimerization might occur. A clue to this was obtained during the course of the density measurements. Erratic results were obtained in the initial measurements on B_{12a} although no difficulty was experienced with B_{12}. The variation was traced to the time of contact of the solutions with the atmosphere and ultimately to oxygen. That oxygen and vitamin B_{12a} do interact was shown then by density measurements and by amperometric titrations of B_{12a} with oxygen. The combining ratio is two molecules of B_{12a} to one molecule of oxygen. This is apparently the first record of a trivalent cobalt compound combining reversibly with molecular oxygen.

(1) B. Jaselskis, J. F. Foster and H. Diehl, *Iowa State Coll. J. Sci.*, **31**, 1 (1956).

(2) H. Diehl, R. R. Sealock and J. Morrison, *ibid.*, **24**, 433 (1950).

(3) L. G. Longsworth, THIS JOURNAL, **75**, 5705 (1953).

A. Apparent Specific Volumes of B_{12} and B_{12a}

The density measurements were made by the pycnometer method and the calculations made using the usual relationship

$$v_s = \frac{1}{\rho_0} - \left(\frac{\rho_0 - \rho_s}{\rho_0}\right)\left(\frac{V}{g}\right)$$

in which v_s is the apparent specific volume, ρ_0 and ρ_s the densities of water and solution, respectively, V the volume of the pycnometer, and g the weight of the solute.

Materials.—Vitamin B_{12}, obtained from the Squibb Institute for Medical Research, New Brunswick, N. J., was recrystallized from carbon dioxide-free water. Oxygen-free nitrogen was prepared by passing tank nitrogen through two scrubbers of vanadous sulfate, one scrubber of sodium hydroxide, and one of water.

Vitamin B_{12a} was prepared from crystalline vitamin B_{12} by the hydrogenation procedure.[4]

Apparatus and Procedure.—A 5.0-ml. pycnometer was used. Weighings were made using tares of identical weight and volume. Solutions were kept in a water-bath at 25.00 ± 0.01°. The balance room was maintained at slightly below 25°.

The pycnometer was charged with liquid already brought to equilibrium with oxygen-free nitrogen, air or oxygen. Water was placed in a small conical flask bearing a two-holed rubber stopper carrying lengths of glass tubing one of which reached the bottom of the flask and the second of which served as a gas outlet. The gas was bubbled through the solution for several minutes. The crystalline vitamin was added through the gas outlet tube and thus dissolved in the water without the stopper having been removed. The gas stream was then continued an additional 30 minutes. The solution was then transferred to the pycnometer with a hypodermic syringe. In the oxygen-free experiments the pycnometer and syringe were well flushed with nitrogen and

(4) E. Kaczka, D. E. Wolf and K. Folkers, *ibid.*, **71**, 1514 (1949).