tenth that of the hydrolysis. This somewhat complicates calculation of the rate coefficients for the hydrolysis since it becomes desirable (although not essential, since the decarboxylation is relatively slow) to use a rate law for successive first-order reactions of the type

$$\text{ester} \xrightarrow{k_1} \text{acid} \xrightarrow{k_D} \text{hydrocarbon} + CO_2$$

The applicable equation for calculation of k_1 is

$$C_{\text{acid}} = \frac{ak_1}{k_D - k_1}\left(e^{-k_1t} - e^{-k_Dt}\right)$$

where a is initial concentration of ester. The value of k_D is measured either by following the decarboxylation of the mesitoic acid which remains after the ester has reacted or by separate experiments with the acid. Incidentally, the decarboxylation is acid catalyzed, as is to be expected from the experiments of Schubert[18,19] at higher acidities, and the rate varies with the H_0 acidity function.

ITHACA, N. Y.

[CONTRIBUTION FROM THE NAVAL MEDICAL RESEARCH INSTITUTE]

On Intermolecular and Intramolecular Interactions between Independent Pairs of Binding Sites in Proteins and Other Molecules

BY TERRELL L. HILL

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The Kirkwood–Shumaker suggestion, that matching constellations of dissociable groups on two different protein molecules might lead to a significant intermolecular attractive force, is examined in terms of two models. The general conclusion reached is that in many cases the Kirkwood–Shumaker proposal is a very reasonable one. Two related topics also discussed are: (1) the intermolecular or intramolecular potential of average force between two groups whose charges are not permanent but fluctuate because of binding equilibria with ions in solution; and (2) the effect of pairs of interacting groups on titration curves of proteins and other molecules.

I. Introduction

At the end of their paper on the force between two protein molecules, Kirkwood and Shumaker[1] suggest that ". . . steric matching of a constellation of basic groups on one molecule with a complementary constellation on the other could conceivably produce a redistribution of protons leading to a strong and specific attraction . . . ." The primary purpose of this paper is to examine this suggestion quantitatively for perhaps the simplest possible model (described in Section II). Specifically, we assume that matching constellations on two neighboring protein molecules exist, and then calculate the extent to which the protons would, in fact, take advantage of these matching constellations by redistributing themselves in such a way as to produce an attractive force. The most extreme situation would arise when the protons are frozen in the particular distribution corresponding to the maximum possible attractive force between the protein molecules. Roughly speaking, such redistributions of protons would be favored as far as the energy of the systems is concerned, but opposed by configurational entropy considerations. The outcome of this energy–entropy competition is not particularly obvious in advance.

The treatment to be given applies also to intramolecular interactions and therefore to titration curves of proteins and other molecules. The results suggest that the usual discussion of electrostatic effects on titration curves of proteins in terms only of the net charge of the entire molecule may overlook important local electrostatic interactions.

Some of the equations derived below are new only[2] in their manner of derivation (*via* the grand partition function) and in their particular application to the problem outlined above.

II. The Model and General Relations

Figure 1a represents two protein or other molecules close together and with matching constellations of two types of sites or groups, 1 and 2. Both sites of a pair are capable of binding the same kind of ion (or molecule), for example, a proton. The distance between each pair of sites is r. The effects we are interested in here arise only when r is rather small (see Section III); hence, as a first approximation, we neglect (1) interactions between sites on the same protein molecule, (2) second and higher neighbor interactions between sites on different protein molecules, and (3) interactions between the sites shown and other types of sites. Thus we are concerned here with a group of independent pairs of binding sites.

With this model the discussion will apply also to Fig. 1b, in which several equivalent but independent pairs of sites are distributed through a single large molecule.

Because of the assumed independence of pairs, the average properties per pair will not depend on the number of pairs under consideration; hence this number need not be specified. For example, Figs. 1c and 1d, each with only one pair of sites, are included.

The most straightforward way to derive the required equations is to consider each pair of sites as a system in a grand ensemble. The ensemble consists of a very large number of systems (pairs). Let j_1 be the partition function (including the binding energy) of a molecule or ion bound at site 1 of a pair, and similarly let j_2 refer to a molecule or ion bound at site 2. Let $W_{AA}(r)$, $W_{AB}(r)$, $W_{BA}(r)$ and $W_{BB}(r)$ be the free energies[3] of interaction between the sites, separated by a distance r, when both sites are occupied (AA), when site 1 is occupied and site 2 unoccupied (AB), etc., respectively. That is, the

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[1] J. G. Kirkwood and J. B. Shumaker, *Proc. Nat. Acad. Sci.*, **38**, 863 (1952).

[2] See, for example, E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

[3] The corresponding energies are $W_{AA} - T(\partial W_{AA}/\partial T)$, etc.

W's are the potentials of average force[4] for the pair of sites occupied in each of the four ways possible; the potential zero is chosen at $r = \infty$ in each case. Then the canonical ensemble partition functions Q_n (n is the number of molecules or ions bound on a pair) are

$$\begin{aligned} Q_0 &= e^{-W_{\mathrm{BB}}/kT} \\ Q_1 &= j_1 e^{-W_{\mathrm{AB}}/kT} + j_2 e^{-W_{\mathrm{BA}}/kT} \qquad (1) \\ Q_2 &= j_1 j_2 e^{-W_{\mathrm{AA}}/kT} \end{aligned}$$

and the grand partition function is

$$\Xi = Q_0 + Q_1\lambda + Q_2\lambda^2 \qquad (2)$$

where

$$\lambda = e^{\mu/kT} \qquad (3)$$

and μ is the chemical potential of the bound molecules or ions. If c is the concentration (or activity) of these molecules or ions in solution, then

$$\mu = \mu_{\mathrm{soln}} = \mu^* + kT \ln c \qquad (4)$$

where μ^* is a standard ($c = 1$) or reference chemical potential, independent of c.

From well-known properties of the grand partition function,[5] the numbers n_{AA} of AA pairs, n_{AB} of AB pairs, etc., out of a total of, say, M pairs in the ensemble ($M \rightarrow \infty$), are

$$\begin{aligned} n_{\mathrm{BB}} &= \frac{MQ_0}{\Xi},\ n_{\mathrm{AB}} = \frac{Mj_1\lambda e^{-W_{\mathrm{AB}}/kT}}{\Xi} \\ n_{\mathrm{BA}} &= \frac{Mj_2\lambda e^{-W_{\mathrm{BA}}/kT}}{\Xi},\ n_{\mathrm{AA}} = \frac{MQ_2\lambda^2}{\Xi} \qquad (5) \end{aligned}$$

The probability θ_1 that a given site of type 1 is occupied is

$$\theta_1 = \frac{n_{\mathrm{AA}} + n_{\mathrm{AB}}}{M} = \frac{j_1\lambda e^{-W_{\mathrm{AB}}/kT} + Q_2\lambda^2}{\Xi} \qquad (6)$$

and, for a type 2 site

$$\theta_2 = \frac{n_{\mathrm{AA}} + n_{\mathrm{BA}}}{M} = \frac{j_2\lambda e^{-W_{\mathrm{BA}}/kT} + Q_2\lambda^2}{\Xi} \qquad (7)$$

The fraction of all sites occupied, θ, is given by[6]

$$\bar{n} = 2\theta = \theta_1 + \theta_2 = \frac{\lambda}{\Xi}\frac{\partial \Xi}{\partial \lambda} = \frac{Q_1\lambda + 2Q_2\lambda^2}{\Xi} \qquad (8)$$

For an AA pair, the interaction force is $f_{\mathrm{AA}} = -\partial W_{\mathrm{AA}}/\partial r$, and similarly for other pairs. The average force f between a pair of sites (*i.e.*, averaged over the four different manners of occupation) is then

$$f = \frac{f_{\mathrm{AA}}n_{\mathrm{AA}} + f_{\mathrm{AB}}n_{\mathrm{AB}} + f_{\mathrm{BA}}n_{\mathrm{BA}} + f_{\mathrm{BB}}n_{\mathrm{BB}}}{M} \qquad (9)$$

It is easy to verify by differentiation that the potential $\mathfrak{W}$ of this average force[7] (*i.e.*, $f = -\partial\mathfrak{W}/\partial r$) is

$$e^{-\mathfrak{W}/kT} = \frac{\Xi(r)}{\Xi(\infty)} = \frac{Q_0 + Q_1\lambda + Q_2\lambda^2}{1 + (j_1 + j_2)\lambda + j_1j_2\lambda^2} \qquad (10)$$

with the potential zero at $r = \infty$. is also the isothermal, reversible work, at constant μ, required to bring a pair of sites from $r = \infty$ up to a separation r.

For use below, let us define intrinsic *dissociation* constants for the two sites as

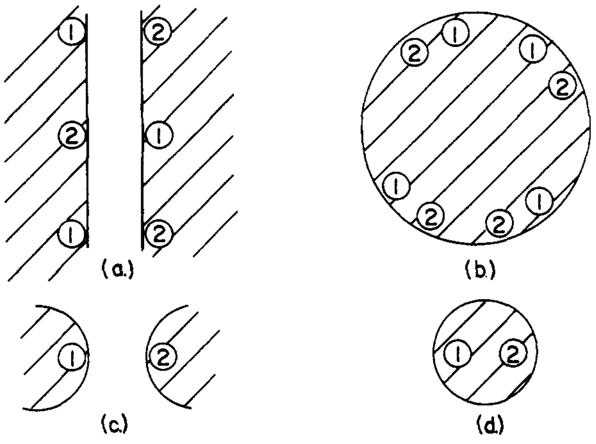

Fig. 1.—(a) Matching pairs of interacting sites on two large molecules; (b) pairs of interacting sites in the same large molecule; (c) pair of interacting sites on two small molecules; (d) pair of interacting sites in the same small molecule.

$$K_1 = \frac{c(1 - \theta_1{}^\circ)}{\theta_1{}^\circ},\ K_2 = \frac{c(1 - \theta_2{}^\circ)}{\theta_2{}^\circ} \qquad (11)$$

where the superscripts refer to $r = \infty$ (W's $= 0$). From eq. 6 and 7

$$\frac{1 - \theta_1{}^\circ}{\theta_1{}^\circ} = \frac{1}{j_1\lambda},\ \frac{1 - \theta_2{}^\circ}{\theta_2{}^\circ} = \frac{1}{j_2\lambda} \qquad (12)$$

Hence, using eq. 4, eq. 11 becomes

$$K_1 = \frac{e^{-\mu^*/kT}}{j_1{}^\circ},\ K_2 = \frac{e^{-\mu^*/kT}}{j_2} \qquad (13)$$

and

$$\frac{K_1}{K_2} = \frac{j_2}{j_1} \qquad (14)$$

III. Redistribution of Bound Ions

Special Case I.—In this special case the unoccupied (B) sites are equivalent ($j_1 = j_2 \equiv j$, $K_1 = K_2 \equiv K$) and have a charge of -1. The ion being bound has a charge of $+2$. This situation might arise, for example, in the binding of a doubly charged metallic ion on negatively charged groups (say, $-\mathrm{COO}^-$) of a protein. Hence we have the symmetrical situation in Fig. 2, with

B B	A B	B A	A A
− −	+ −	− +	+ +

Fig. 2.—Pairs of charges in Special Case I.

$$W_{\mathrm{AB}} = W_{\mathrm{BA}} = -W_{\mathrm{AA}} = -W_{\mathrm{BB}} < 0 \qquad (15)$$

When r is small ($-W_{\mathrm{AB}}$ large), the bound ions will tend to redistribute themselves to favor AB or BA over AA or BB pairs. To discuss this tendency quantitatively, let us define a correlation coefficient (or order parameter) ξ_1 by the relation

$$\xi_1 = \frac{n_{\mathrm{AB}} + n_{\mathrm{BA}} - n_{\mathrm{AA}} - n_{\mathrm{BB}}}{M} \qquad (16)$$

That is, each AB or BA pair contributes $+1$ and each AA or BB pair contributes -1; ξ_1 is the average contribution per pair. With perfect positive correlation or order ($\xi_1 = +1$) all pairs are AB or BA and we have the maximum possible attractive

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(4) The "average" here is over possible configurations of solvent molecules, electrolyte, etc., for a fixed manner of occupation of the pair of sites. We encounter the further average over manners of occupation in eq. 10.

(5) See, for example, T. L. Hill, "Statistical Mechanics," McGraw-Hill Book Co., New York, N. Y., 1956.

(6) Compare E_2/C_2 in eq. 67 of T. L. Hill, *J. Chem. Phys.*, **23**, 623 (1955).

(7) Compare eq. 2 of Kirkwood and Shumaker,[1] and eq. 87 of Hill.[6] See also eq. 37.69 of Hill,[5] with r for ξ.

force between the matching constellations in Fig. 1a. In fact, from eq. 9 and 15

$$f = \xi_1 f_{AB} = -\xi_1 \frac{\partial W_{AB}}{\partial r} \qquad (17)$$

Thus, the attractive force f falls off with increasing r not only because f_{AB} falls off with r but also because of ξ_1. Since f_{AB} is negative, f and ξ_1 have opposite signs.

We obtain from Section II and eq. 15 and 16

$$\xi_1 = \frac{2x - (1 + x^2)e^{2W_{AB}/kT}}{2x + (1 + x^2)e^{2W_{AB}/kT}} \qquad (18)$$

where

$$x = j\lambda = \frac{c}{K} \qquad (19)$$

Also

$$\theta = \theta_1 = \theta_2 = \frac{x + x^2e^{2W_{AB}/kT}}{2x + (1 + x^2)e^{2W_{AB}/kT}} \qquad (20)$$

Note that x is defined in such a way that $\theta = {}^1/_2$ when $x = 1$. At $r = \infty$ ($W_{AB} = 0$), these equations reduce to

$$\theta^\circ = \frac{x}{1 + x} \qquad (21)$$

$$\xi_1{}^\circ = -\left(\frac{1 - x}{1 + x}\right)^2 = -(1 - 2\theta^\circ)^2 \qquad (22)$$

Equation 18 and 20 can be used to calculate ξ_1 and θ as functions of r or W_{AB} at constant c. This corresponds physically to varying the distance between the two protein surfaces in Fig. 1a in the presence of a fixed concentration of ions in solution. On the other hand, θ as a function of x at constant r or W_{AB} would correspond to the binding isotherm for Figs. 1b and 1d (see Section V).

Figures 3 and 4 give ξ_1 and θ, respectively, as functions of W_{AB}/kT for several choices of x (which is proportional to c) or $\theta^\circ(x)$. These figures show that ξ_1 approaches +1 for moderate values of $-W_{AB}/kT$, and that this is accomplished by an adsorption or desorption of ions to give $\theta \rightarrow {}^1/_2$ together with a redistribution of the ions which are bound to favor AB and BA over AA and BB pairs.

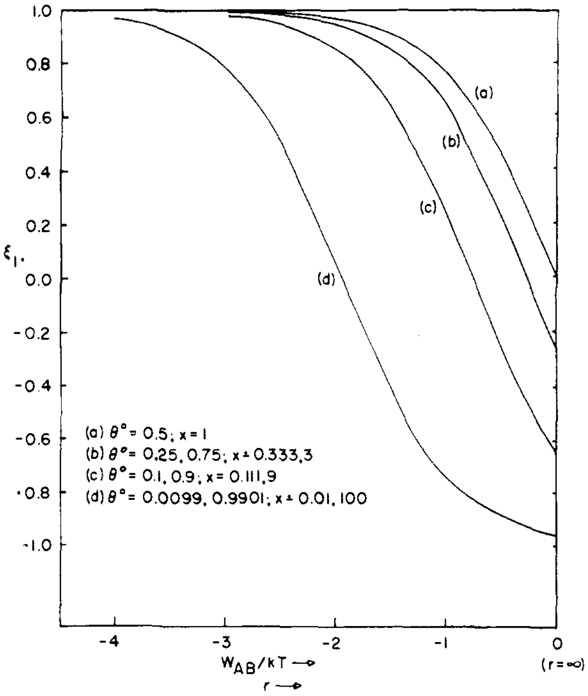

Fig. 3.—Correlation coefficient ξ_1 *versus* interaction free energy W_{AB}/kT in Special Case I.

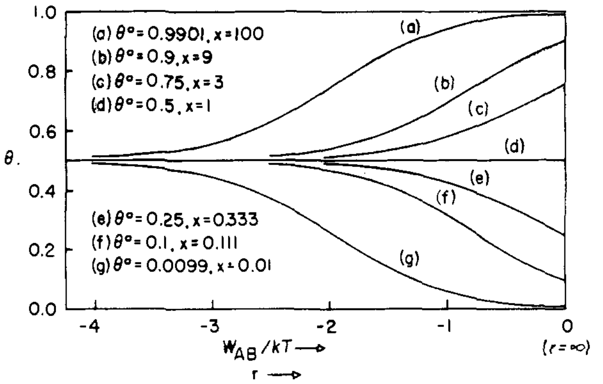

Fig. 4.—Fraction of sites occupied θ *versus* interaction free energy W_{AB}/kT in Special Case I.

To examine the dependence of ξ_1 on r, we write

$$-\frac{W_{AB}}{kT} = \frac{e^2}{D_E r k T} \qquad (23)$$

where D_E is defined by this equation and is therefore an "effective" dielectric constant. In Fig. 3, we see that, for values of x which are not too extreme, $-W_{AB}/kT > 3$ is a rough criterion for ξ_1 near +1. At 300°K., this is equivalent to $D_E r$ (in Å.) < 185.7. For example, if r = 5 Å., we have $D_E < 37$, or if r = 4 Å., $D_E < 46$. These inequalities could very well be satisfied in Fig. 1a, because of dielectric saturation, despite the possible presence of electrolyte ions; they are almost certainly satisfied in Figs. 1b and 1d, because in this case the medium between the charges has a relatively low dielectric constant.[8]

We can conclude that, in this example, the Kirkwood–Shumaker proposal that matching constellations (as in Fig. 1a) might lead to an essentially "frozen" optimal bound ion distribution is certainly a reasonable one.

Special Case II.—In this case unoccupied site 1 has zero charge (*e.g.*, $-NH_2$ or $-N=$), unoccupied site 2 has a charge −1 (*e.g.*, $-COO^-$ or $-O^-$), $j_1 \neq j_2$ (or $K_1 \neq K_2$) in general, and the ion being bound has a charge + 1 (*e.g.*, H^+). The charge distributions are shown in Fig. 5, with

$$W_{AB} < 0$$

$$W_{BB} = W_{BA} = W_{AA} = 0 \qquad (24)$$

Here we define a correlation coefficient ξ_2 by

$$\xi_2 = n_{AB}/M \qquad (25)$$

That is, each AB pair contributes +1 and all other pairs make no contribution; ξ_2 is the average con-

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

Fig. 5.—Pairs of charges in Special Case II.

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[8] J. G. Kirkwood and F. H. Westheimer, *J. Chem. Phys.*, **6**, 506 (1938).

tribution per pair. In the limit $r \rightarrow 0$, all pairs will be of the AB type, $\xi_2 = 1$, and we will have the maximum possible attractive force between the matching constellations of Fig. 1a. From eq. 9 and 24

$$f = \xi_2 f_{AB} = -\xi_2 \frac{\partial W_{AB}}{\partial r} \qquad (26)$$

ξ_2 is always positive and f always negative.

From Section II and eqs. 24 and 25

$$\xi_2 = \frac{\left(\frac{K_2}{K_1}\right)^{1/2} x e^{-W_{AB}/kT}}{\Xi} \qquad (27)$$

$$\theta_1 = \frac{\left(\frac{K_2}{K_1}\right)^{1/2} x e^{-W_{AB}/kT} + x^2}{\Xi} \qquad (28)$$

$$\theta_2 = \frac{\left(\frac{K_1}{K_2}\right)^{1/2} x + x^2}{\Xi} \qquad (29)$$

$$2\theta = \theta_1 + \theta_2$$

where

$$\Xi = 1 + \left[\left(\frac{K_2}{K_1}\right)^{1/2} e^{-W_{AB}/kT} + \left(\frac{K_1}{K_2}\right)^{1/2}\right] x + x^2 \qquad (30)$$

and

$$x^2 = j_1 j_2 \lambda^2 = \frac{c^2}{K_1 K_2} \qquad (31)$$

Figures 6, 7 and 8 show ξ_2, θ_1, θ_2 and θ as functions of W_{AB}/kT. The general tendency as $r \rightarrow 0$ ($-W_{AB}/kT \rightarrow \infty$) is for $\theta_1 \rightarrow 1$ and $\theta_2 \rightarrow 0$ so that AB (or + −) pairs may be formed ($\xi_2 \rightarrow 1$). Figure 6 ($K_2/K_1 = 100$) represents a case of the type site 1 = $-NH_2$ or $-N=$ and site 2 = $-COO^-$. Figure 8 ($K_1/K_2 = 100$) might apply to site 1 = $-N=$ and site 2 = $-O^-$ or $-S^-$. Figure 7 ($K_1 = K_2$) is intermediate; for example, site 1 = $-NH_2$ and site 2 = $-S^-$.

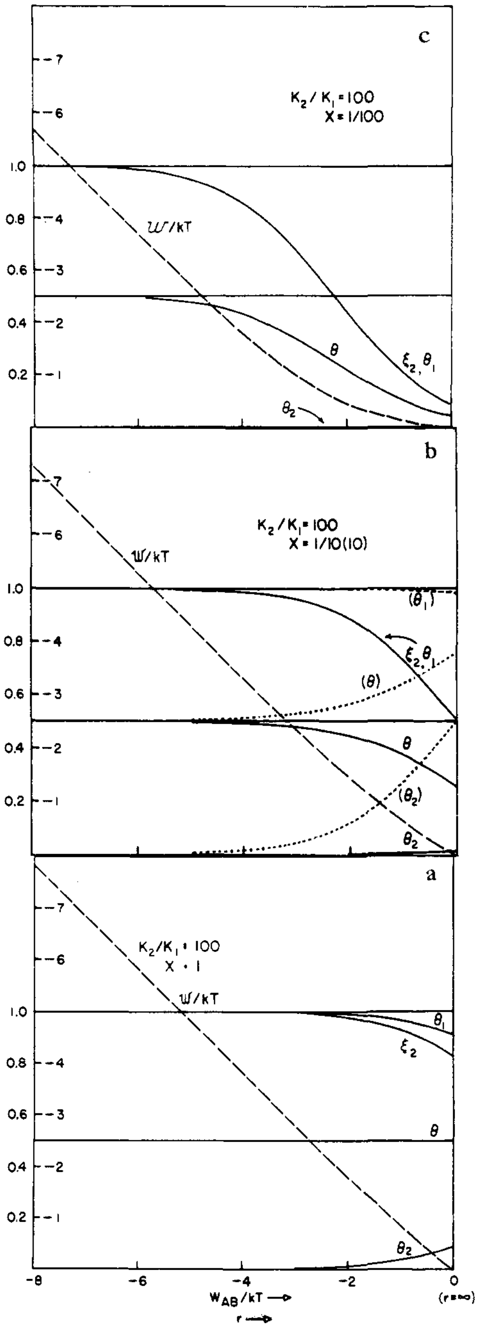

Fig. 6.—Special Case II with $K_2/K_1 = 100$. The inner ordinate refers to $\mathfrak{W}/kT$: (a) $x = 1$; (b) $x = 1/10$; the dotted curves and (θ), (θ_1), (θ_2) refer, however, to $x = 10$; (c) $x = 1/100$.

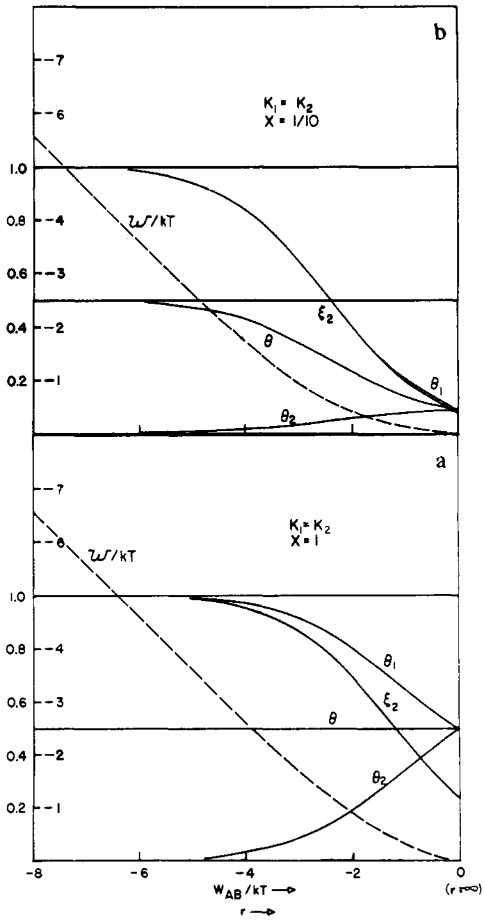

Fig. 7.—Special Case II with $K_1 = K_2$. The inner ordinate refers to $\mathfrak{W}/kT$: (a) $x = 1$; (b) $x = 1/10$.

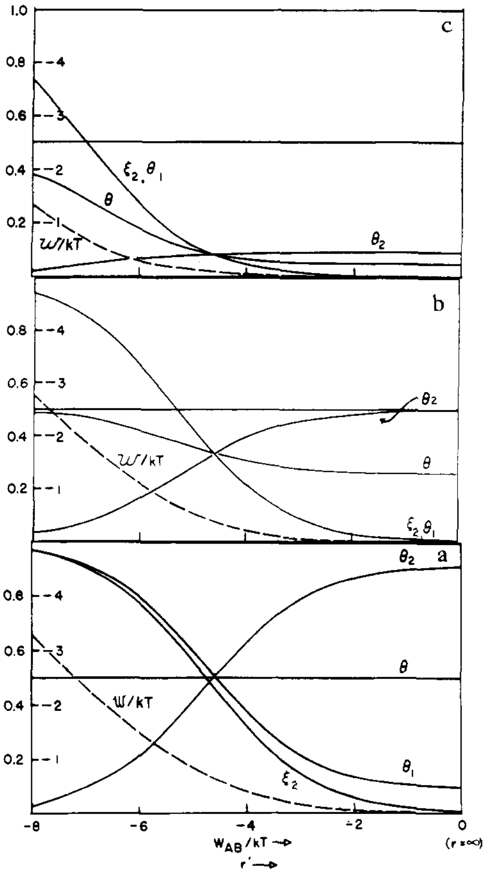

Fig. 8.—Specia lCase II with $K_1/K_2 = 100$. The inner ordinate refers to $\mathfrak{W}/kT$: (a) $x = 1$; (b) $x = 1/10$; (c) $x = 1/100$.

The magnitude of $-W_{AB}/kT$ necessary to give, say, $\xi_2 > 0.9$ varies rather widely depending on the particular choice of parameters. Table I lists the range in D_E associated with difference minimum values of $-W_{AB}/kT$, for $T = 300°K$. and $r = 4$ or 5 Å. The Kirkwood–Shumaker suggestion of a "frozen distribution" is seen to be reasonable in some cases but not in others. However, in Figs. 1b and 1d, values of $D_E < 14$ (see Table I) might be anticipated in general so that practically complete "freezing" would probably be the rule rather than the exception for r of the order of 5 Å. or less.

Incidentally, the curves in Figs. 6, 7 and 8 for $x < 1$ also apply, because of symmetry, to $x > 1$. That is

$$\begin{aligned} \xi_2(x) &= \xi_2(1/x) \\ \theta_2(x) &= 1 - \theta_1(1/x) \\ \theta_1(x) &= 1 - \theta_2(1/x) \\ \theta(x) &= 1 - \theta(1/x) \end{aligned} \qquad (32)$$

This is illustrated by the dotted curves in Fig. 6b.

IV. Potential of Average Force

The potential (of average force) between two permanent charges, as, for example, in eq. 23, is a familiar concept. Much less familiar but equally important in biology (proteins, nucleic acids, etc.) is the potential of average force between two groups whose charges are not permanent but fluctuate because of binding (or dissociation) equilibria with ions in solution. Some aspects of this subject have been discussed recently by Kirkwood and Shumaker[1] and by Hill.[6] The appropriate (intermolecular or intramolecular) potential of this latter type for the present model is given by eq. 10. We discuss the potential here instead of the force since this avoids any definite commitment concerning the dependence of D_E on r in eq. 23.

Special Case I.—Equations 10 and 15 give

$$e^{-\mathfrak{W}/kT} = \frac{2xe^{-W_{AB}/kT} + (1 + x^2)e^{W_{AB}/kT}}{(1 + x)^2} \qquad (33)$$

$\mathfrak{W}/kT$ is plotted against W_{AB}/kT in Fig. 9. $-\mathfrak{W}$ has a minimum where ξ_1 changes sign in Fig. 3, since $\mathfrak{W}$ is an integral of f, at constant μ, in eq. 17. Also, the slope in Fig. 9 approaches -1 as $\xi_1 \rightarrow +1$ in Fig. 3. After the slope in Fig. 9 has essentially attained the value -1 (as $r \rightarrow 0$), the residual constant difference between W_{AB} and $\mathfrak{W}$ is an accumulated (*i.e*, on integrating $f dr$ from ∞ to r) reflection of the lower values of the correlation coefficient ξ_1 at large r.

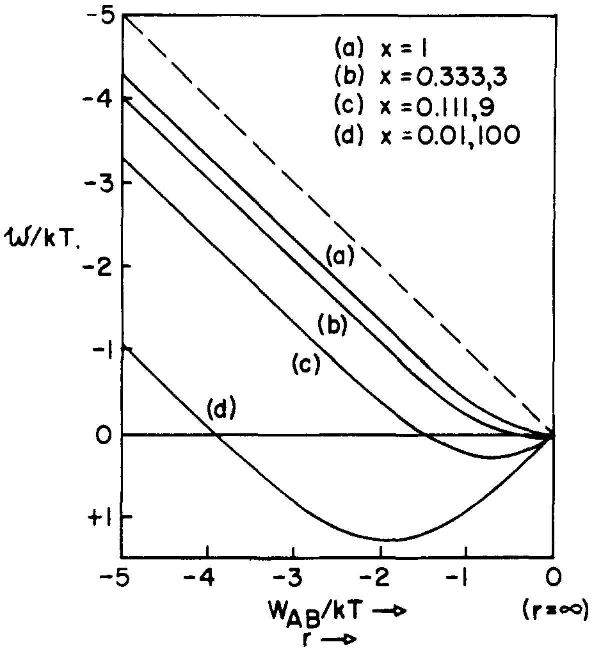

Fig. 9.—Potential of average force $\mathfrak{W}/kT$ *versus* interaction free energy W_{AB}/kT in Special Case I.

Special Case II.—We obtain from eq. 10 and 24

$$e^{-\mathfrak{W}/kT} = \frac{\Xi}{1 + \left[\left(\frac{K_2}{K_1}\right)^{1/2} + \left(\frac{K_1}{K_2}\right)^{1/2}\right] x + x^2} \qquad (34)$$

where Ξ is given by eq. 30. $\mathfrak{W}/kT$ is plotted against W_{AB}/kT as the dashed curves in Figs. 6, 7 and 8. There is exhibited a wide range in the magnitude of $\mathfrak{W}$ in these figures, as expected from the connection between $\mathfrak{W}$ and ξ_2 (eq. 26).

V. Titration Curves

Equation 8 is also of interest as a titration curve (*i.e.*, θ as a function of λ or c at constant r), when intramolecular interactions are under consideration (for example, Figs. 1b and 1d).

More explicitly, eq. 8 appears as

$$2\theta = \frac{\lambda(j_1e^{-W_{\mathrm{AB}}/kT} + j_2e^{-W_{\mathrm{BA}}/kT}) + 2\lambda^2 j_1j_2e^{-W_{\mathrm{AA}}/kT}}{e^{-W_{\mathrm{BB}}/kT} + \lambda(j_1e^{-W_{\mathrm{AB}}/kT} + j_2e^{-W_{\mathrm{BA}}/kT}) + \lambda^2 j_1j_2e^{-W_{\mathrm{AA}}/kT}} \tag{35}$$

Alternatively we have the equivalent thermodynamic expression which follows[2] from the conventional definition of successive dissociation constants $K_{(1)}$ and $K_{(2)}$

$$2\theta = \frac{\dfrac{c}{K_{(2)}} + \dfrac{2c^2}{K_{(1)}K_{(2)}}}{1 + \dfrac{c}{K_{(2)}} + \dfrac{c^2}{K_{(1)}K_{(2)}}} \tag{36}$$

Let us again (see eq. 19 and 31) define x so that $\theta = {}^1/_2$ when $x = 1$. Here

$$x^2 = \frac{c^2}{K_{(1)}K_{(2)}} \tag{37}$$

and

$$2\theta = \frac{\left(\dfrac{K_{(1)}}{K_{(2)}}\right)^{1/2} x + 2x^2}{1 + \left(\dfrac{K_{(1)}}{K_{(1)}}\right)^{1/2} x + x^2} \tag{38}$$

To put eq. 35 in the same form as eq. 38, we multiply numerator and denominator by $e^{W_{\mathrm{BB}}/kT}$. On equating the terms in λ^2 and x^2, using eq. 4 and 13, we find

$$K_{(1)}K_{(2)} = K_1K_2e^{(W_{\mathrm{AA}} - W_{\mathrm{BB}})/kT} \tag{39}$$

In eq. 19 and 31, $W_{\mathrm{AA}} = W_{\mathrm{BB}}$, and hence $K_{(1)}K_{(2)} = K_1K_2$. Also, from the terms in λ and x

$$\frac{K_{(1)}}{K_{(2)}} = e^{-2W/kT}\,(y + y^{-1})^2 \tag{40}$$

where

$$2W = W_{\mathrm{AB}} + W_{\mathrm{BA}} - W_{\mathrm{AA}} - W_{\mathrm{BB}} \tag{41}$$

and

$$y = \left(\frac{K_2}{K_1}\right)^{1/2} e^{(-W_{\mathrm{AB}} + W_{\mathrm{BA}})/2kT} \tag{42}$$

Incidentally, in applications one will in general have a set of "conservation" equations

$$W_{\mathrm{AB}} = W_{\mathrm{BB}} + W_{\mathrm{XB}}$$
$$W_{\mathrm{BA}} = W_{\mathrm{BB}} + W_{\mathrm{BX}}$$
$$W_{\mathrm{AA}} = W_{\mathrm{BB}} + W_{\mathrm{BX}} + W_{\mathrm{XB}} + W_{\mathrm{XX}}$$

where W_{XX} is the potential between two bound ions (or molecules) at a distance r apart, W_{XB} is the potential between an ion at site 1 and unoccupied site 2, etc. Then

$$-2W = W_{\mathrm{XX}} \tag{44}$$

Ordinarily, of course, $W_{\mathrm{XX}} \geqslant 0$. In this case, since $y + y^{-1} \geqslant 2$, $K_{(1)}/K_{(2)} \geqslant 4$. However, if $W_{\mathrm{XX}} < 0$ (*e.g.*, van der Waals attraction between uncharged molecules), we can have $K_{(1)}/K_{(2)} < 4$. The complete range possible is $0 \leqslant K_{(1)}/K_{(2)} \leqslant \infty$.

By differentiation of eq. 38 it is easy to show that there are three (symmetrically distributed) inflection points in a θ *versus* $\ln x$ curve (the central one being at $x = 1$) when $K_{(1)}/K_{(2)} > 16$. There is only one inflection point (at $x = 1$) when $K_{(1)}/K_{(2)} \leqslant 16$. The slope at $x = 1$ is

$$s = \left(\frac{\mathrm{d}\theta}{\mathrm{d}\ln x}\right)_{x=1} = \frac{1}{2 + \left(\dfrac{K_{(1)}}{K_{(2)}}\right)^{1/2}} \tag{45}$$

or

$$\frac{K_{(1)}}{K_{(2)}} = \left(\frac{1 - 2s}{s}\right)^2 \tag{46}$$

The latter relation might be useful in estimating $K_{(1)}/K_{(2)}$ from an experimental curve when $K_{(1)}/K_{(2)}$ is not large. Note that $0 \leqslant s \leqslant {}^1/_2$.

If $y >> 1$

$$\frac{K_{(1)}}{K_{(2)}} \cong e^{-2W/kT}\,y^2 \tag{47}$$

and site 1 fills (predominantly) first as x is gradually increased from $x = 0$. If $y << 1$

$$\frac{K_{(1)}}{K_{(2)}} \cong e^{-2W/kT}\,y^{-2} \tag{48}$$

and site 2 fills first. It is of course possible for, say, site 1 to fill first even though $K_1 >> K_2$ (see eq. 42 and Table II below).

The complete titration curve in the form θ *versus* $\ln x$ is determined by $K_{(1)}/K_{(2)}$ only, according to eq. 38. Hence, from eq. 40, there are an infinite number of combinations of K_1/K_2 and the W's which will lead to the same titration curve. As an illustration, consider Special Case II of Section IV, where

$$2W = W_{\mathrm{AB}}$$
$$y = \left(\frac{K_2}{K_1}\right)^{1/2} e^{-W_{\mathrm{AB}}/2kT} \tag{49}$$

The same titration curve ($\log K_{(1)}/K_{(2)} = 4.978$) is obtained, for example, in each of the cases shown in Table II. Only in the first case in Table II does site 2 fill first. Results of this type emphasize again the well recognized fact that the mutual interaction of close pairs of sites can modify profoundly the titration behavior which may be expected from the intrinsic constants alone. In some cases, because of such complications, association of a particular dissociable group with a given inflection point on a titration curve may be quite hazardous. Also, the usual treatment of electrostatic effects in large molecules in terms of the net charge of the entire molecule ignores possible local interactions of this sort. This might be particularly serious in well-organized molecules (*e.g.*, DNA) where many equivalent and hence reinforcing pairs might be present.

TABLE I

LIMITING EFFECTIVE DIELECTRIC CONSTANTS AT T = 300°K.

	$r = 4$ Å.	$r = 5$ Å.
$-\dfrac{W_{\mathrm{AB}}}{kT} > 2$	$D_{\mathrm{E}} < 70$	$D_{\mathrm{E}} < 56$
4	35	28
6	23	19
8	17	14

TABLE II

SETS OF PARAMETERS GIVING THE SAME TITRATION CURVE

$\log (K_1/K_2)$	$-(W_{AB}/kT)$
4.978	0.000
−4.978	0.000
2.000	8.000
−2.000	3.428
0.000	5.727

Finally, several additional special cases of eq. 40 should be noted

Special Case I of Section IV:

$$\frac{K_{(1)}}{K_{(2)}} = 4e^{-4W_{AB}/kT} \tag{50}$$

Symmetrical Dicarboxylic Acid:

$$\frac{K_{(1)}}{K_{(2)}} = 4e^{W_{BB}/kT} \tag{51}$$

Symmetrical Diamine:

$$\frac{K_{(1)}}{K_{(2)}} = 4e^{W_{AA}/kT} \tag{52}$$

The latter two examples are well-known,[2,8] of course.

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BETHESDA, MARYLAND