FORCES BETWEEN PROTEIN MOLECULES IN SOLUTION ARISING FROM FLUCTUATIONS IN PROTON CHARGE AND CONFIGURATION*

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The forces between protein molecules in solution are in large part electrostatic in origin. Evidence for the dominant role of electrostatic forces is provided by the marked effect of ionic strength on the thermodynamic interaction of proteins. Thus, the commonly observed reduction in interaction produced by the screening action of the statistical space charge of the ions of an electrolyte would only be effective on that part of the force between protein molecules, which is electrostatic in origin. At values of pH departing from the isoionic points of the molecules in question, a simple Coulomb force, determined by their net electric charges, is predominant. This force is non-specific and is sensitive to molecular structure only in so far as structure influences the net charges. When one or both of a pair of protein molecules are at their isoionic points, structure sensitive electrostatic forces come into play. These forces have been customarily attributed to fixed constellations of electric charge, which impart to the molecules permanent electric multipole moments characteristic of their structure.

We shall investigate here another type of electrostatic interaction between protein molecules, which arises from fluctuations in charge and charge distribution associated with fluctuations in number and configuration of the protons bound to the molecules. Proteins, considered as ampholytes, contain a large number of neutral and negatively charged basic groups, such as $-NH_2$ and $-COO^-$, to which protons are attached. Except in highly acid solutions, the number of basic sites generally exceeds the average number of protons bound to the molecule, so that there exist many possible configurations of the protons, differing little in free energy among which fluctuations may occur. Fluctuations in the number and configuration of the mobile protons impart to the molecules fluctuating charges and fluctuating electric multipole moments. We have demonstrated in a previous investigation[1] that the dipole moment fluctuations, arising from configurational fluctuations of the protons, are sufficient to account for the dielectric constant increments of many proteins without postulating the existence of permanent dipole moments. Linderstrøm-Lang[2] has shown that fluctuations in net charge contribute to interaction between proteins and the small ions of an electrolyte. In the present investigation, we shall demonstrate that the interaction between the

fluctuating charges and multipole moments of two protein molecules makes a significant contribution to the intermolecular force, by a relatively simple mechanism. The fluctuating electric field of each molecule alters the distribution of fluctuations in the charge and constellation of the mobile protons of the other in such a manner as to produce at the isoionic point a long range attractive force between them with a potential diminishing asymptotically as $1/R^2$, in the absence of screening by the statistical space charge of an electrolytic environment. With screening, the range is reduced by an exponential factor, $e^{-\kappa R}$, depending upon ionic strength through the Debye-Hückel parameter, κ.

We consider two protein molecules in aqueous solution, the centers of mass of which are separated by a distance R. We suppose that molecule 1 contains ν_1 basic groups of intrinsic charge $e_i^{(1)}$ and that molecule 2 contains ν_2 basic groups of intrinsic charge $e_k^{(2)}$. We define proton occupation variables $x_i^{(1)}$ and $x_k^{(2)}$ to be unity when the respective basic groups are occupied by one proton and zero otherwise. For specified proton configurations, $x_1^{(1)} \ldots . x_{\nu_1}^{(1)}$ and $x_1^{(2)} \ldots . x_{\nu_2}^{(2)}$, the electric charges of the several groups, $q_i^{(1)}$ and $q_k^{(2)}$ are $e_i^{(1)} + ex_i^{(1)}$ and $e_k^{(2)} + ex_k^{(2)}$, where e is the protonic charge. The mutual electrostatic energy V of the two molecules, in specified orientations and proton configurations is given by,

$$V = \sum_{i=1}^{\nu_1} \sum_{k=1}^{\nu_2} \frac{q_i^{(1)} q_k^{(2)}}{D R_{ik}^{(12)}}$$

$$\begin{aligned} q_i^{(1)} &= e_i^{(1)} + ex_i^{(1)} \\ q_k^{(2)} &= e_k^{(2)} + ex_k^{(2)} \end{aligned} \qquad (1)$$

where D is the dielectric constant of the solvent and $R_{ik}^{(12)}$ is the scalar distance between basic group i of molecule 1 and basic group k of molecule 2. Electrolytic screening is not included in equation (1), but will be taken into account for special models later. For generality, the sum of equation (1) may be regarded as extending over other charged groups of the molecules, as well as basic groups, with the convention the proton occupation variable is always zero for a non-basic group.

The potential, $W(R)$, of average force between the two molecules is related to the potential V of the force in fixed orientation and proton configuration in the following manner,

$$\begin{aligned} e^{-\beta W(R)} &= \langle e^{-\beta V} \rangle_{\text{av.}} \\ W(R) &= \langle V \rangle_{\text{av.}} - \frac{\beta}{2} \langle V^2 \rangle_{\text{av.}} + O(\beta^2) \\ \beta &= 1/kT \end{aligned} \qquad (2)$$

where the average is to be taken over all orientations and over all proton configurations of the two molecules, with uncorrelated distribution functions. For simplicity in the present argument, we shall suppose that at distances not permitting overlap of the excluded volumes of the two molecules, their only interaction is electrostatic, with the potential, equation (1), and we shall retain only the first two terms in the expansion, equation (2) of $W(R)$ in powers of β. With these simplifications, we may express $W(R)$ as follows,

$$W(R) = \frac{\langle q^{(1)}\rangle_{\text{av.}}\langle q^{(2)}\rangle_{\text{av.}}}{DR} - \frac{\beta}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}\langle q_i^{(1)}q_l^{(1)}\rangle_{\text{av.}}\langle q_k^{(2)}q_s^{(2)}\rangle_{\text{av.}} f_{ikls}$$

$$f_{ikls} = \left\langle \frac{R^2}{R_{ik}^{(12)}R_{ls}^{(12)}} \right\rangle_{\text{av.}} \tag{3}$$

where $\langle q^{(1)}\rangle_{\text{av.}}$ and $\langle q^{(2)}\rangle_{\text{av.}}$ are the average total charges of the two molecules, equal to zero at their respective isoionic points, and the functions f_{ikls}, averages over all orientations of both molecules, approach unity asymptotically as the distance R between their centers of mass becomes large relative to the linear dimensions of both molecules. We now write,

$$\begin{aligned} q_i^{(1)} &= \langle q_i^{(1)}\rangle_{\text{av.}} + \Delta q_i^{(1)} \\ q_k^{(2)} &= \langle q_k^{(2)}\rangle_{\text{av.}} + \Delta q_k^{(2)} \\ \Delta q_i^{(1)} &= e[x_i^{(1)} - \langle x_i^{(1)}\rangle_{\text{av.}}] \\ \Delta q_k^{(2)} &= e[x_k^{(2)} - \langle x_k^{(2)}\rangle_{\text{av.}}] \end{aligned}$$

$$q^{(1)} = \sum_{i=1}^{\nu_1} q_i^{(1)};\quad q^{(2)} = \sum_{k=1}^{\nu_2} q_k^{(2)} \tag{4}$$

in order to separate the contributions of the mean and fluctuating charge distributions to $W(R)$ in the following manner,

$$W(R) = \frac{\langle q^{(1)}\rangle_{\text{av.}}\langle q^{(2)}\rangle_{\text{av.}}}{DR} + W^{(00)}(R) + W^{(10)}(R) + W^{(01)}(R) + W^{(11)}(R)$$

$$W^{(00)}(R) = -\frac{\beta}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}\langle q_i^{(1)}\rangle_{\text{av.}}\langle q_l^{(1)}\rangle_{\text{av.}}\langle q_k^{(2)}\rangle_{\text{av.}}\langle q_s^{(2)}\rangle_{\text{av.}} f_{ikls}$$

$$W^{(10)}(R) = -\frac{\beta}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}\langle \Delta q_i^{(1)}\Delta q_l^{(1)}\rangle_{\text{av.}}\langle q_k^{(2)}\rangle_{\text{av.}}\langle q_s^{(2)}\rangle_{\text{av.}} f_{ikls}$$

$$W^{(01)}(R) = -\frac{\beta}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}\langle q_i^{(1)}\rangle_{\text{av.}}\langle q_l^{(1)}\rangle_{\text{av.}}\langle \Delta q_k^{(2)}\Delta q_s^{(2)}\rangle_{\text{av.}} f_{ikls}$$

$$W^{(11)}(R) = -\frac{\beta}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}\langle \Delta q_i^{(1)}\Delta q_l^{(1)}\rangle_{\text{av.}}\langle \Delta q_k^{(2)}\Delta q_s^{(2)}\rangle_{\text{av.}} f_{ikls} \tag{5}$$

By expansion of the functions f_{ikls} in powers of $(1/R)$, each of the terms $W^{(\alpha\beta)}(R)$ may be expressed as a sum of terms corresponding to the interaction of electric multipoles of various orders. The term $W^{(00)}(R)$ represents the interaction of the permanent electric multipoles of the molecules, the terms $W^{(01)}(R)$ and $W^{(10)}(R)$ the interaction of the permanent multipoles of the one molecule with the fluctuating multipoles of the other, and the term $W^{(11)}(R)$ the interaction of the fluctuating charges and fluctuating multipoles of both.

We shall now limit our considerations to values of pH corresponding to the isoionic point of one of the proteins. Under this condition, $W(R)$ becomes asymptotically equal to the fluctuation term, $W^{(11)}(R)$, at large intermolecular distances, as each of the functions f_{ikls} approaches unity. Among the several contributions to $W(R)$, we shall therefore undertake a detailed analysis only of this term. With the use of equations (4) and (5), it may be expressed in the form,

$$W^{(11)}(R) = -\frac{\beta e^4}{2D^2R^2}\sum_{i,l=1}^{\nu_1}\sum_{k,s=1}^{\nu_2}[\langle x_i^{(1)}x_l^{(1)}\rangle_{\text{av.}} - \langle x_i^{(1)}\rangle_{\text{av.}}\langle x_l^{(1)}\rangle_{\text{av.}}][\langle x_k^{(2)}x_s^{(2)}\rangle_{\text{av.}} - \langle x_k^{(2)}\rangle_{\text{av.}}\langle x_s^{(2)}\rangle_{\text{av.}}]f_{ikls} \quad (6)$$

The pertinent mean values of the products of the proton occupation variables are to be taken over distributions, $x_1^{(1)}\ldots.x_{\nu_1}^{(1)}$ and $x_1^{(2)}\ldots.x_{\nu_2}^{(2)}$ of the protons among the basic sites of the two molecules. For molecule 1, we have,

$$\langle x_i\rangle_{\text{av.}} = \sum_{x_1\ldots x_{\nu 1}=0}^{1} x_i e^{\beta[A_c^{(1)} - W_c^{(1)}(x_1\ldots x_{\nu 1})]}$$

$$\langle x_i x_l\rangle_{\text{av.}} = \sum_{x_1\ldots x_{\nu 1}=0}^{1} x_i x_l e^{\beta[A_c^{(1)} - W_c^{(1)}(x_1\ldots x_{\nu 1})]}$$

$$e^{-\beta A_c^{(1)}} = \sum_{x_1\ldots x_{\nu 1}=0}^{1} e^{-\beta W_c^{(1)}(x_1\ldots x_{\nu 1})} \quad (7)$$

where $W_c^{(1)}$ is the local free energy of proton configuration $x_1^{(1)}\ldots.x_{\nu_1}^{(1)}$, and $A_c^{(1)}$ is the total configurational free energy of molecule 1. The configurational mean values for molecule 2 may be calculated from exactly similar relations. Methods developed by Kirkwood[3] in the theory of acid-base equilibrium of ampholytes may be employed for the explicit calculation of the mean values of the occupation variable products.

$$\langle x_i\rangle_{\text{av.}} = \frac{\partial \log G^{(1)}}{\partial \log \lambda_i^{(1)}}$$

$$\langle x_i x_l\rangle_{\text{av.}} = \frac{\lambda_i^{(1)}\lambda_l^{(1)}}{G^{(1)}}\frac{\partial^2 G^{(1)}}{\partial\lambda_i^{(1)}\partial\lambda_l^{(1)}}$$

$$G^{(1)} = \sum_{x_1 \ldots x_{\nu_1} = 0}^{1} B^{(1)}(x_1 \ldots . x_{\nu_1})[H^+]_i^{\sum_{i=1}^{\nu_1} x}$$

$$B^{(1)}(x_1 \ldots . x_{\nu_1}) = \left[\prod_{i=1}^{\nu_1} (\lambda_i^{(1)})^{x_i}\right] e^{-\alpha^{(1)}\left[\sum_{i=1}^{\nu_1} (e_i^{(1)}/e + x_i)\right]^2}$$

$$\lambda_i^{(1)} = [K_i^{(1)} e^{\alpha^{(1)}}]^{-1}$$

$$\alpha^{(1)} = \frac{e^2}{2b_1 DkT} \frac{1}{1 + \kappa b_1} \tag{8}$$

where b_1 is the radius of molecule 1 considered as a sphere, $K_i^{(1)}$ is the intrinsic dissociation constant of the acid conjugate to the basic group i, $[H^+]$ is the hydrogen ion activity, and κ is the ionic strength parameter of the Debye-Hückel theory. The averages for molecule 2 may be calculated by means of similar relations. If intramolecular electrostatic interaction between the protons of each molecule is neglected, the following result is obtained,

$$\langle x_i^{(1)} x_l^{(1)} \rangle_{\text{av.}} - \langle x_i^{(1)} \rangle_{\text{av.}} \langle x_l^{(1)} \rangle_{\text{av.}} = \frac{\delta_{il}}{2 + K_i^{(1)}/[H^+] + [H^+]/K_i^{(1)}}$$

$$\langle x_k^{(2)} x_s^{(2)} \rangle_{\text{av.}} - \langle x_k^{(2)} \rangle_{\text{av.}} \langle x_s^{(2)} \rangle_{\text{av.}} = \frac{\delta_{ks}}{2 + K_k^{(2)}/[H^+] + [H^+]/K_k^{(2)}} \tag{9}$$

where δ_{il}, the Kronecker delta, is equal to unity of $i = l$ and zero otherwise. In this approximation the potential $W^{(11)}(R)$ becomes

$$W^{(11)}(R) = -\frac{\beta e^4}{2D^2R^2} \sum_{i=1}^{\nu_1} \sum_{k=1}^{\nu_2} [2 + K_i^{(1)}/[H^+] + [H^+]/K_i^{(1)}]^{-1}[2 + K_k^{(2)}/[H^+] + [H^+]/K_k^{(2)}]^{-1} f_{ik} \tag{10}$$

$$f_{ik} = \frac{1}{4u_i u_k}\left\{\log\left[\frac{1 - (u_i + u_k)^2}{1 - (u_i - u_k)^2}\right] + u_i \log\left[\frac{(1 + u_i + u_k)(1 + u_k - u_i)}{(1 + u_i - u_k)(1 - u_i - u_k)}\right] + u_k \log\left[\frac{(1 + u_i + u_k)(1 + u_i - u_k)}{(1 + u_k - u_i)(1 - u_i - u_k)}\right]\right\}$$

$$u_i = b_i^{(1)}/R; \quad u_k = b_k^{(2)}/R$$

where $b_i^{(1)}$ and $b_k^{(2)}$ are the distances of the basic groups i and k from the centers of mass of the respective molecules 1 and 2, and the symbol for the function f_{ikik} has been abbreviated to f_{ik}. The factor f_{ik} does not depart greatly from unity. For a pair of identical spherical molecules with

basic groups located on their surfaces, f_{ik} diminishes from 1.39 on contact to its asymptotic value, unity, at large separations. The asymptotic form of $W^{(11)}(R)$ is obtained by approximating all f_{ik} by unity.

$$W^{(11)}(R) = -\frac{\langle\Delta q^{(1)^2}\rangle_{av.}\langle\Delta q^{(2)^2}\rangle_{av.}}{2D^2R^2kT}$$

$$\langle\Delta q^{(1)2}\rangle_{av.} = e^2\sum_{i=1}^{\nu_1}[2 + K_i^{(1)}/[H^+] + [H^+]/K_i^{(2)}]^{-1}$$

$$\langle\Delta q^{(2)^2}\rangle_{av.} = e^2\sum_{k=1}^{\nu_2}[2 + K_k^{(2)}/[H^+] + [H^+]/K_k^{(2)}]^{-1} \quad (11)$$

where $\langle\Delta q^{(1)^2}\rangle_{av.}$ and $\langle\Delta q^{(2)^2}\rangle_{av.}$ are the total charge fluctuations of the two molecules.

The potential $W^{(11)}(R)$ of equation (11) leads to a long-range attractive force between the two molecules, diminishing as the inverse cube of the distance. A force of this range fails to yield convergent expressions for the thermodynamic functions. Properly, a Debye-Hückel factor should be included to provide for screening of the fluctuating electrostatic interaction by the statistical space charge arising from counterions to the proteins and from the ions of other electrolytes present in the solution. If the molecules are spherical, the asymptotic potential, equation (11), is modified by screening as follows,

$$W^{(11)}(R) = -\frac{\langle\Delta q^{(1)^2}\rangle_{av.}\langle\Delta q^{(2)^2}\rangle_{av.}}{2D^2R^2kT}\frac{e^{-2\kappa(R-a_{12})}}{(1+\kappa a_{12})^2}$$

$$\kappa^2 = \kappa_0^2 + \kappa_1^2$$

$$\kappa_1^2 = \frac{4\pi N}{1000DkT}\left[\frac{\langle\Delta q^{(1)^2}\rangle_{av.}C_1}{M_1} + \frac{\langle\Delta q^{(2)^2}\rangle_{av.}C_2}{M_2}\right]$$

$$\kappa_0^2 = \frac{4\pi Ne^2}{1000DkT}\sum_j c_jZ_j^2 \quad (12)$$

where a_{12} is the sum of the radii of the two molecules, κ_0^2 is proportional to the ionic strength of other electrolytes present in the solution and the second term represents the contribution of the fluctuating charges of the proteins to κ^2, their concentrations C_1 and C_2 being expressed in grams per 100 ml. of solution. Here M_1 and M_2 are the molecular weights of the proteins and N is Avogadro's number. If either of the proteins is not at its isoionic point, $(\Delta q^2)_{av.}$ should be replaced by the mean square $<q^2>_{av.}$ to its total charge. When either of the proteins is isoionic, $W^{(11)}(R)$, of equation (12), is the dominant asymptotic term in the total potential of average force, $W(R)$, at large intermolecular distances, since then the first term of equation (3), appropriately modified with a Debye-

Hückel screening factor vanishes. A more exact calculation of $W^{(11)}(R)$, based on equations (6), (7) and (8), taking into account the influence of intramolecular electrostatic interaction between the protons on the mean values of the proton occupation variable products, may be carried out numerically. When both proteins are isoionic, the correction for proton interaction is not large.

According to the theory of Kirkwood and Shumaker,[1] $W^{(11)}(R)$ may also be expressed in terms of the dipole moment fluctuations $\Delta\mu_1^2$ and $\Delta\mu_2^2$ of the two molecules, through their relationship to the charge fluctuations. The expression is the following,

$$W^{(11)}(R) = -\frac{\Delta\mu_1^2\Delta\mu_2^2}{2D^2b_1^2b_2^2kT}\frac{e^{-2\kappa(R-A_{12})}}{R^2(1+\kappa a_{12})^2} \tag{13}$$

where b_1 and b_2 are the radii of the two spherical protein molecules. Equation (13) is convenient for estimating the order of magnitude of $W^{(11)}(R)$ from experimental values of the dielectric increments of the proteins.

We shall now investigate the influence of the interaction of protein molecules through charge fluctuations on the chemical potential of a single protein at its isoionic point. According to the theory of Kirkwood and Buff,[4] we may write at low concentrations, for a protein component 1 in a solvent 0, at constant κ_0 of other electrolytes present in the solution,

$$\begin{aligned}
\frac{100M_1}{NkT}\left(\frac{\partial\mu_1^e}{\partial c_1}\right)_{T,p,\kappa_0} &= G_{10} - G_{11} \\
G_{11} &= 4\pi\int_0^\infty R^2[g_{11}(R) - 1]\,dR \\
G_{10} &= 4\pi\int_0^\infty R^2[g_{10}(R) - 1]\,dR \\
\mu_1 &= kT\log C_1 + \mu_1^e + \mu_1^0(T,p) \\
\mu_1^0(T,p) &= \lim_{C\to 0}[\mu_1 - kT\log C_1]
\end{aligned} \tag{14}$$

where $g_{11}(R)$ and $g_{10}(R)$ are the radial distribution functions of a pair of protein molecules and a protein and solvent molecule, respectively, which are related to the potentials of average force $W_{11}(R)$ and $W_{10}(R)$ in the following manner,

$$\begin{aligned}
g_{11}(R) &= e^{-\beta W_{11}(R)} \\
g_{10}(R) &= e^{-\beta W_{10}(R)}
\end{aligned} \tag{15}$$

In each of the integrals of equation (14) there is a co-volume term arising from the region of overlap of the excluded volumes of the molecular pair.

If the dimensions of a solvent molecule are ignored in comparison with those of the protein, the co-volume contribution to G_{10} is one-eighth that of G_{11}. If, further, the solvent is assumed to play only the role of a dielectric continuum exterior to the excluded volume of the protein, and $W_{11}(R)$ is approximated by (12) exterior to this volume, we obtain with the neglect of powers of order β^3,

$$\frac{100M_1}{kT}\left(\frac{\partial\mu_1^e}{\partial c_1}\right)_{T,\,p,\,\kappa_0} = 2B_0 - \frac{\pi N\langle\Delta q^{(1)^2}\rangle_{\text{av.}}}{(DkT)^2\kappa(1+\kappa a)^2}$$

$$B_1 = 7\pi Na^3/12 \tag{16}$$

where a is the molecular diameter and $\langle\Delta q^{(1)^2}\rangle_{\text{av.}}$ is given by equation (11). If the contribution of the protein to the ionic strength is negligible in comparison to that of other electrolytes present in the solutions, we obtain by integration of equation (16) the following result for the excess chemical potential, μ_1^e, per molecule.

$$\mu_1^e/kT = 2BC_1$$

$$B = \left(\frac{1}{100M_1}\right)\left\{B_0 - \frac{\pi N\langle\Delta q^{(1)^2}\rangle_{\text{av.}}}{2(DkT)^2\kappa_0(1+\kappa_0 a)^2}\right\} \tag{17}$$

The coefficient of C_1 in the expansion of the osmotic coefficient in powers of C_1 is equal to B by conventional thermodynamic relationships. In the absence of other electrolytes with the ionic strength determined entirely by the fluctuating charge of the protein, integration of equation (16) yields the Debye-Hückel limiting law,

$$\mu_1^e/kT = -\frac{\langle\Delta q^{(1)^2}\rangle_{\text{av.}}}{2DkT}\kappa_1 + 0(\kappa_1^2)$$

$$\kappa_1^2 = \frac{4\pi\langle\Delta q^{(1)^2}\rangle_{\text{av.}}}{100M_1DkT}C_1 \tag{18}$$

characteristic of electrolytes.

At high ionic strengths, the co-volume contribution, B_0, to the interaction coefficient, B, of equation (17) is the dominant one leading to a positive value of the excess chemical potential. Only at quite low ionic strengths does the second term arising from proton fluctuations become dominant, leading to a negative excess chemical potential. For serum albumin in water at 300 K at its isoionic point, the coefficient B is estimated to be,

$$B = \left(4.3 - \frac{1.2}{\sqrt{\Gamma}}\right)\Big/1000 \tag{19}$$

where Γ is the ionic strength of other electrolyte. At ionic strength of

the order of 10^{-3}, the fluctuation term is approximately ten times as large as the co-volume term and gives rise to substantial negative values of the excess chemical potential at protein concentrations of the order of several per cent.

The present analysis of the attractive force between proteins arising from fluctuations in charge and configuration of mobile protons is necessarily schematic because of lack of knowledge of the details of protein structure. Although it is a force of long range at low ionic strength, it appears to exhibit specificity only through the influence of structure on the fluctuations. It is clear, however, that highly specific interactions might well arise from the mechanism of interaction, which has been described. In favorable orientations, 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 depending upon the local structural details of the complementary constellations. As an extreme example it is possible to imagine proton fluctuations to be frozen in such a manner as to produce an intermolecular zwitterion, with matching areas of positive and negative charge on complementary areas of the two molecules. Such considerations relating to specificity of the fluctuation force must necessarily remain speculative until detailed knowledge of structure is available.

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[1] Kirkwood, J. G., and Shumaker, J. B., these PROCEEDINGS **38**, 855–862 (1952).

[2] Linderstrøm-Lang, K., *Compt. Rend. trav. lab. Carlsberg*, **15**, No. 7 (1924). See also Scatchard, G., Chapter 3, Cohn and Edsall, *Proteins, Amino Acids and Peptides*, Reinhold, New York (1943).

[3] Kirkwood, J. G., Chapter 12, Cohn and Edsall, *Proteins, Amino Acids, and Peptides*, Reinhold, New York (1943).

[4] Kirkwood, J. G., and Buff, F. P., *J. Chem. Phys.*, **19**, 774 (1951).