Charge Distribution in Protein Molecules. II[1]

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In part I, the linearized Poisson–Boltzmann equation was set up and solved numerically for a spherical protein molecule with uniformly distributed dissociable groups and penetrating electrolyte. In the present paper, the same problem is considered without linearizing the Poisson–Boltzmann equation.

In part I[2] we set up and solved numerically, for a special case, a non-linear integral equation, essentially in the electrostatic potential, for the following model. A spherical protein molecule is immersed in an electrolyte solution; the protein is rigid but swollen (to a fixed extent) by the electrolyte solution so that the volume fraction of protein is $1 - \alpha$ and the radius is b; the protein contains $\rho(r)$ sites per unit volume for the binding of ions of charge $Z\epsilon$ (ϵ = protonic charge); $\theta(r)$ is the fraction of sites (a vacant site has no charge) at r which are occupied by bound ions; all sites have the same intrinsic dissociation constant for bound ions K^0; and the concentration of bound ions far from the protein molecule is c. The essential way in which this problem differs from other recent work on spherical polyelectrolytes[3,4] is that the charge on the protein arises from a binding equilibrium and is therefore *not* fixed—in fact, the charge at r depends on $\theta(r)$ which in turn depends on $\psi(r)$, the electrostatic potential.

In part I the Poisson–Boltzmann equation was linearized (still leading to a non-linear integral equation). The present paper is a brief supplement to I which differs from I only in that the Poisson–Boltzmann equation is *not* linearized. It turns out that, for the numerical case of I, linearization is not too serious so only a single numerical example is included below. As might be expected, the numerical work required here[5] for each solution is rather more formidable than in I.

In eq. I-6 we use a 1-1 electrolyte with concentration m. The ions being bound (concentration c), for example H^+, are assumed to be present at sufficient dilution so that they make a negligible contribution to the ionic strength. Then eq. I-7 for the potential inside the sphere becomes

$$\nabla^2 \psi = -\frac{4\pi \rho(r)\theta(r)Z\epsilon}{\alpha D} + \frac{8\pi m\epsilon}{D} \sinh \frac{\epsilon \psi(r)}{kT} \quad (1)$$

where $\psi(r)$ and $\theta(r)$ are related to c by eq. I-5. We now follow the procedure of eq. I-7 to I-14 except that we do not use the "outside" potential to eliminate $\psi(b)$. That is, from the "inside" eq. 1 only, we find

$$F(s) = F(1) - B \int_0^1 \rho(x)\theta(x)x^2 \, dx +$$
$$\kappa^2 b^2 \int_0^1 x^2 \sinh F(x) \, dx + \frac{B}{s} \int_0^s \rho(x)\theta(x)x^2 \, dx +$$
$$B \int_s^1 \rho(x)\theta(x)x \, dx - \frac{\kappa^2 b^2}{s} \int_0^s x^2 \sinh F(x) \, dx -$$

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

(2) T. L. Hill, THIS JOURNAL, 78, 1577 (1956).

(3) F. T. Wall and J. Berkowitz, *J. Chem. Phys.*, 26, 114 (1957).

(4) S. Lifson, *ibid.*, 27, 700 (1957).

(5) Carried out, for the most part, by the Computation Laboratory of the National Bureau of Standards.

$$\kappa^2 b^2 \int_s^1 x \sinh F(x) \, dx \quad 0 \leqslant s \leqslant 1 \quad (2)$$

where

$$F(s) = \frac{\epsilon \psi(s)}{kT} \quad s = r/b$$

$$B = \frac{4\pi Z\epsilon^2 b^2}{\alpha DkT}$$

$$\kappa^2 = \frac{8\pi m\epsilon^2}{DkT}$$

Similarly, from the "outside" differential equation ($\rho = 0$ in eq. 1), we find

$$\psi(r) = \psi(b) + b^2\psi'(b)\left(\frac{1}{b} - \frac{1}{r}\right) +$$
$$\frac{8\pi m\epsilon}{D} \int_b^r y \sinh F(y) \, dy - \frac{8\pi m\epsilon}{Dr} \int_b^r y^2 \sinh F(y) \, dy \quad (3)$$

and

$$\psi(b) = \frac{8\pi m\epsilon}{Db} \int_b^\infty y^2 \sinh F(y) \, dy -$$
$$\frac{8\pi m\epsilon}{D} \int_b^\infty y \sinh F(y) \, dy \quad (4)$$

$$\psi'(b) = -\frac{8\pi m\epsilon}{Db^2} \int_b^\infty y^2 \sinh F(y) \, dy \quad (5)$$

The boundary condition $D\psi'_{out}(b) = \alpha D\psi'_{in}(b)$ gives us also

$$\frac{8\pi m\epsilon}{D} \int_b^\infty y^2 \sinh F(y) \, dy = \frac{4\pi Z\epsilon}{D} \int_0^b \rho(y)\theta(y)y^2 dy -$$
$$\frac{8\pi m\epsilon\alpha}{D} \int_0^b y^2 \sinh F(y) \, dy \quad (6)$$

If now we use eq. 4–6 in eq. 3, we obtain

$$F(s) = \frac{\alpha}{s}\left[B\int_0^1 \rho(x)\theta(x)x^2 dx - \kappa^2 b^2 \int_0^1 x^2 \sinh F(x) \, dx\right] -$$
$$\kappa^2 b^2 \int_s^\infty x \sinh F(x)dx - \frac{\kappa^2 b^2}{s} \int_1^s x^2 \sinh F(x) \, dx$$
$$s \geqslant 1 \quad (7)$$

Finally, if we employ eq. I-5 in the form

$$\theta(x) = [1 + (K^0/c)e^{F(x)}]^{-1} \quad (8)$$

to replace $\theta(x)$ by $F(x)$ in eq. 2 and 7, we have the required non-linear integral equation in $F(x)$, which of course must be solved numerically.

In the single numerical solution of eq. 2 and 7 which we have carried out, the choice of parameters was the same as in I: $T = 310.1°K.$, $D = 74.31$, $\alpha = 1/3$, $1/\kappa = 7.79$ Å. (0.15 M NaCl), $b = 31.16$ Å. ($\kappa b = 4$), $Z = 1$ and $\rho = $ constant (total of 25 binding sites).

Figure 1 shows $F(s)$ calculated from two of the $\theta(s)$ curves of I, using eq. I-15. In the solution of eq. 2 and 7 referred to above, we forced each successive approximation to pass through $F(1) = 0.340$, the same value of $F(1)$ as in the $\theta(0.5) = 0.5$ case of I. The iteration procedure was as described: Guess a first approximation $F_1(x)$. Guess a

value of K^0/c and hence calculate $\theta(x)$ from $F_1(x)$ and eq. 8. Put $F_1(x)$ and this $\theta(x)$ in eq. 7 and evaluate the right-hand side for $s = 1$. Compare the result with $F(1)$, preassigned. Adjust the value of K^0/c until the RHS of eq. 7 agrees with the preassigned $F(1)$. Call the adjusted value of K^0/c, $(K^0/c)_1$, and the $\theta(x)$ calculated therefrom, $\theta_1(x)$. Use $\theta_1(x)$ and $F_1(x)$ to calculate $F_2(s)$ from eq. 2 and 7. Repeat the procedure, starting with $F_2(x)$; etc.

The convergent $F(s)$ and $\theta(s)$ curves (labeled II) are given in Figs. 1 and 2, respectively. Also, we
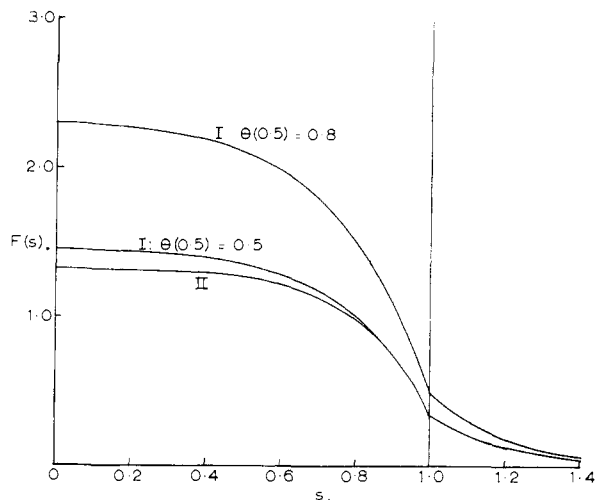

Fig. 1.—Electrostatic potential as a function of distance from center of protein molecule: I, paper I; II, this paper.
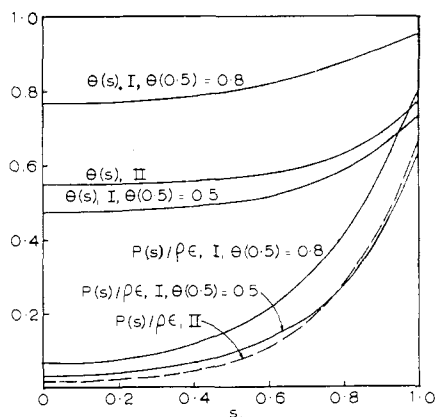

Fig. 2.—Fraction θ of sites occupied by bound ions, and normalized net charge density $P/\rho\epsilon$, both as a function of distance from center of protein molecule: I, paper I; II, this paper.
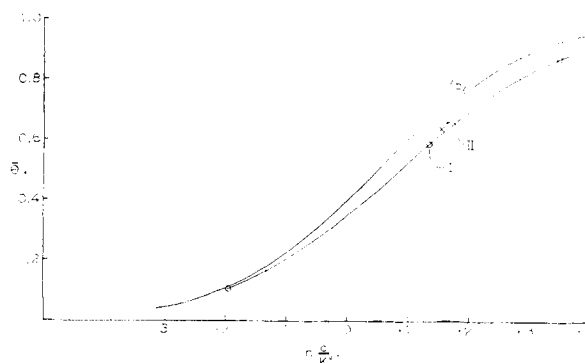

Fig. 3.—Titration curves: (b), charges on surface, no electrolyte penetration (see I); I, from solution of linearized PB equation in I; II, from solution of nonlinearized PB equation in present paper.

find $\ln (c/K^0) = 1.525$ and the average values $\bar{\theta} = 0.639$ and $\bar{P}/\rho\epsilon = 0.294$, where $\bar{P}$ is the average of the net charge density (see eq. 1)

$$P(r) = \rho(r)\theta(r)Z\epsilon - 2\alpha m\epsilon^2 \sinh F(r) \qquad (9)$$

or

$$\frac{P(r)}{\rho Z\epsilon} = \theta(r) - \frac{\kappa^2 b^2}{B\rho} \sinh F(r) \qquad (10)$$

if $\rho = $ constant. $P(s)/\rho\epsilon$ is included in Fig. 2. Finally, in Fig. 3, we reproduce two titration curves from I: the curve marked (b) is calculated on the basis of $\kappa b = 4$, 25 sites distributed uniformly on the surface of the protein, and no electrolyte penetrating the protein molecule; and the curve marked I is based on the three solutions of the linearized Poisson–Boltzmann equation in I (the arrow indicates the $\theta(0.5) = 0.5$ point on the titration curve). The above solution (II) of the non-linearized PB equation gives one point on a titration curve, marked by an X in Fig. 3.

It is clear from Figs. 1–3 that in this particular example, with $F(s)$ never very large, the difference between the solutions of the linearized and non-linearized PB equation is significant but not great. However, with other possible choices of parameters (especially lower ionic strength) it would become imperative to use the non-linearized PB equation and the equations of this paper (rather than I) in order to get realistic results.[3],[4] Finally, it should be kept in mind that the non-linearized PB equation itself is not exact.[6]

(6) See, for example, T. L. Hill, J. Phys. Chem., 61, 548 (1957).