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A molecular model for the dependence of the osmotic pressure of bovine serum albumin upon concentration and pH

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Abstract

Expressions derived from the effective hard particle model of Minton and Edelhoch (Biopolymers, 21 (1982) 451) account quantitatively for the combined data of Kanal et al. (Biophys. J., 66 (1994) 153) describing the osmotic pressure of bovine serum albumin as a function of protein concentration ($\leq$ ca. 100 g/l) and pH (3–8) in buffered 0.1 M NaCl. The best fit of the model yields a molar mass of 68360 and a pH-dependent value of the effective specific volume ranging from a minimum of -0.17 cm^3/g at pH 4.6 (the isoelectric point) to maxima of 3.1 cm^3/g at pH 3.0 and 2.2 cm^3/g at pH 8.0. These values are shown to be consistent with the magnitude of known attractive and repulsive electrostatic interactions between proteins in solution.

Keywords: Bovine serum albumin; Osmotic pressure; Proteins

1. Introduction

Classical thermodynamics permits the concentration dependence of a colligative property of a protein solution to be unambiguously interpreted as a manifestation of the concentration dependence of the chemical potential or thermodynamic activity of the solute [1]. But thermodynamic analysis yields no information regarding the molecular basis of the observed dependence. However, one can construct statistical–thermodynamic models based upon postulated intermolecular interactions of various functional forms, and calculate the concentration-dependent colligative properties of each of these models. If the calculated colligative properties of a particular model resemble those of a real protein solution, one may argue that the intermolecular interactions postulated in that model likewise resemble those operative in the actual solution.

Ross and Minton [2] demonstrated that the osmotic pressure of solutions of hemoglobin at concentrations up to 350 g/l, and the sedimentation equilibrium of hemoglobin solutions at concentrations of up to 300 g/l, could be quantitatively accounted for by a very simple model in which the hemoglobin solution was treated as a suspension of hard spherical particles with a diameter very close to the average diameter of a hemoglobin molecule as determined by X-ray diffraction. It was inferred from the success of that model that the potential of average force acting between hemoglobin molecules in solution (under conditions of the experiments being mod-

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eled) is dominated by steric repulsion between the quasispherical protein molecules, and that deviations from the hard particle approximation introduced by the quantal nature of the solvent are negligible so long as the solute is much larger than the solvent.

Minton and Edelhoch [3] subsequently analyzed the concentration and pH dependence of the Rayleigh light scattering of bovine serum albumin (BSA) solutions in buffered 0.15 M NaCl. They found that at each pH the concentration dependence of the light scattering could be well accounted for by a hard particle model similar to that employed in the analysis of the colligative properties of hemoglobin solutions. However, the size of the hard particle best fitting the experimental data at each pH value varied significantly with pH. Thus Minton and Edelhoch introduced an 'effective hard particle' model, according to which molecules interacting by a repulsive electrostatic potential are mimicked by an effective hard particle having a larger radius than the actual molecule. The net charge on the BSA molecule was calculated as a function of pH, and by means of approximate calculations of electrostatic potential, a reasonable (although far from rigorous) correlation between net charge and the radius of the effective hard particle established.

Recently Kanal et al. [4] presented a study of the osmotic pressure of BSA solutions in buffered 0.1 M NaCl as a function of concentration and pH. It was therefore of interest to determine whether the new data can, like the earlier light scattering data, be satisfactorily interpreted in the context of the effective hard particle model. In the following section it is shown that the effective hard particle model is consistent with the new data to within the uncertainty of experimental measurement, and, moreover, parameters derived from fitting the new data are qualitatively in accord with expectations based upon the prior analysis. One new feature is the observation that at a pH close to the isoionic point, the second virial coefficient and hence the volume of the effective hard sphere become negative. In the Appendix a simple example is presented to demonstrate how the addition of a longer-range attractive intermolecular potential to the intrinsic hard repulsive core deriving from mutual impenetrability of protein molecules can result in a negative second virial coefficient and an effective hard sphere of negative volume.

2. Effective hard sphere model for osmotic pressure of protein solutions

According to the statistical–mechanical theory of MacMillan and Mayer [5], the osmotic pressure of a binary solution (solvent + one solute) may be expressed as a power series in the molar concentration of solute:

$$\Pi(c) = RT\left(c + \frac{1}{2}B_2c^2 + \frac{2}{3}B_3c^3 + \frac{3}{4}B_4c^4 + \ldots\right) \quad (1)$$

where the B_i called virial coefficients, are analytical functions of the potential of average force acting between i solute molecules in a bath of solvent. For example, for an angle-independent potential, the second virial coefficient is given by

$$B_2 = 4\pi N_a \int_0^\infty \left[1 - \exp(-U(r)/kT)\right] r^2\,dr \quad (2)$$

where N_a is Avogadro's number, r is the distance between the centers of the interacting particles, $U(r)$ is the potential of average force, k is the Boltzmann constant and T is the absolute temperature. Although it is possible in theory to calculate the value of any virial coefficient, given a functional form for the potential of average force between solute molecules, in reality it is prohibitively difficult to calculate the values of higher order virial coefficients ($i > 2$) for all but the simplest potential forms.

The simplest non-zero interaction potential is the hard or impenetrable sphere potential, defined by a single parameter, namely the radius (or equivalently, the volume) of the hard sphere:

$$U(r) = \begin{cases} \infty & r \le 2r_h \\ 0 & r > 2r_h \end{cases} \quad (3)$$

where r_h denotes the radius of the hard sphere, and $2r_h$ is the distance of closest approach of two spheres. Using this potential, the values of the first six virial coefficients have been computed [6]: $B_2 = 8\ V$, $B_3 = 15\ V^2$, $B_4 = 24.48\ V^3$, $B_5 = 35.3\ V^4$, $B_6 = 47.7\ V^5$, and $B_7 = 65.9\ V^6$, where V is the molar volume

of the hard sphere ($V = 4\pi N_a r_h/3$). Substituting these coefficients into Eq. (1), one obtains

$$\Pi(c) = RTc\left[1 + 4Vc + 10(Vc)^2 + 18.36(Vc)^3 + \ldots\right] \quad (4)$$

Note that all terms within the power series expansion are functions of the unitless product Vc. Eq. (3) may be rewritten as a function of the w/v concentration w:

$$\Pi(w) = \frac{RT}{M} w\left[1 + 4\upsilon w + 10(\upsilon w)^2 + 18.36(\upsilon w)^3 + \ldots\right] \quad (5)$$

where M is the molar mass and υ is the specific volume of the hard sphere ($\upsilon = V/M$). Thus the osmotic pressure of a solution of molecules that may be modeled by hard spheres is completely determined at all concentrations by the two parameters M and υ, provided that a sufficient number of terms in the power series is specified to attain convergence [2].

In order to account for colligative properties in solutions of proteins interacting via long range (electrostatic) forces, Minton and Edelhoch [3] proposed as a first approximation that the parameter υ may treated as a variable, the value of which is defined by the second virial coefficient using the inverse of the relationship given above for an actual hard sphere, i.e.,

$$\upsilon = \upsilon_{eff} \equiv B_2/8M \quad (6)$$

According to this model, υ_{eff} is a calculable function of the mass of the protein molecule and potential of average force acting between two protein molecules in a bath of solvent. υ_{eff} may therefore be treated as a variable parameter, the value of which can vary with conditions, such as pH or ionic strength, that affect the interaction between protein molecules in solution, while M remains constant.

Table 1
Best-fit values of independently variable parameters obtained by simultaneous fitting of Eq. (5) to data of Kanal et al. [4] at all pH values

M = 68360 g/mol	
pH	υ_{eff} (ml/g)
3.0	3.1
4.0	0.88
4.6	−0.17
5.0	0.26
5.4	0.67
6.0	1.4
6.9	2.0
7.3	1.9
8.0	2.2

3. Non-linear least squares analysis of the data of Kanal et al. [4]

The original data of Kanal et al. [4] are presented as plots of osmotic pressure as a function of pH at fixed w/w concentration. w/w concentrations were converted to w/v concentrations using a value of 0.734 ml/g for the partial specific volume of BSA [7] [1]. In Fig. 1 these data are replotted as a function of w/v protein concentration at fixed pH.

The effective hard particle model Eq. (5), with all terms up to and including seventh order in concentration (i.e., far more than necessary for convergence at the maximum protein concentration [2]), was fitted to the data by the method of nonlinear least squares as follows. A single value of M was assumed to characterize the data at all pH values, while the value of υ_{eff}, the volume of the effective hard sphere representing the BSA molecule, was allowed to vary for each different pH value to achieve a best fit. The best fit was obtained by minimizing the sum of squared residuals using the Nelder–Mead modified simplex algorithm [9]. Best-fit parameter values are presented in Table 1. The dependence of osmotic pressure on concentration at each pH value calculated using Eq. (5) with the best-fit value of υ_{eff} at each pH value is plotted in Fig. 1 together with the data. It may be seen that the calculated dependence

[1] The partial specific volume of BSA, assumed constant in the present work, is distinct from υ_{eff}, which will be allowed to vary. The distinction between the two quantities is discussed in the Appendix to Ref. [8].

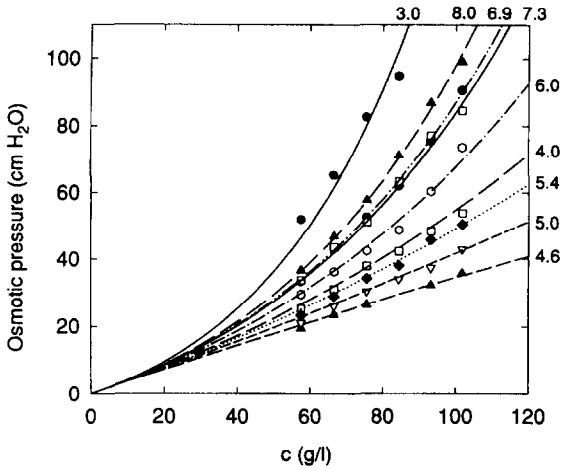

Fig. 1. Osmotic pressure of BSA in 0.1 osmolal solution (primarily NaCl) versus w/v concentration at various pH values. Data of Kanal et al. [4], transformed as described in the text. Curves are calculated using Eq. (5) with the best-fit parameter values given in Table 1. pH values are indicated along the figure border.

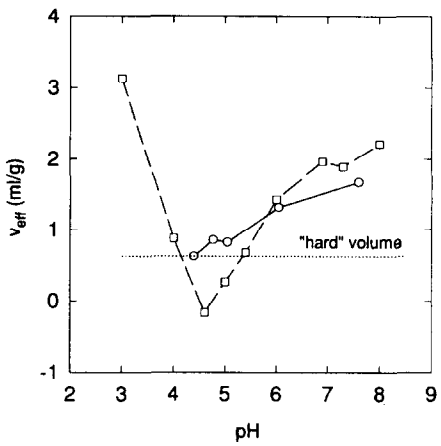

Fig. 2. Best-fit values of v_{eff} plotted as a function of pH. (□) Values derived from present analysis of osmotic pressure of BSA in ca. 0.1 M salt solution. (○) Values derived from analysis of light scattering of BSA in 0.15 M salt solution.

seems to agree with the observed dependence to within the uncertainty of measurement at all pH values, with the possible exception of pH 3.0 [2]. The best-fit values of v_{eff} are plotted as a function of pH in Fig. 2.

4. Significance of the pH dependence of v_{eff}

According to the effective hard particle model of Minton and Edelhoch [3], the volume of the effective hard particle represents a sum of contributions from the hard impenetrable core of the protein molecule (the 'hard' interaction), and from long range (primarily electrostatic) interactions between protein molecules (the 'soft' interaction). The contribution of the hard interaction will always be positive, while the contribution of the soft interaction may be either positive or negative, depending upon whether the soft interaction is predominantly repulsive or attractive. A concrete example is given in the Appendix for two spherical particles interacting via a square well potential. To the extent that the BSA molecule maintains its native globular tertiary structure, the dependence of v_{eff} upon pH indicated in Fig. 2 reflects the pH dependence of the primarily electrostatic soft intermolecular interaction, presumably due to the titration of ionizable side chains [3].

Using the value of M (68360 g/mol) obtained from the least-square fit to the combined data of Kanal et al. [4] together with the estimate of the hard core diameter of BSA (51.5×10^{-8} cm) obtained from the analysis of Minton and Edelhoch [3], we calculate the hard contribution to v_{eff} to be ca. 0.63 cm^3/g. Between ca. pH 4 and pH 5.5, $v_{eff} < v_{hard}$, indicating that in this range of pH values the soft interaction is predominantly attractive.

We suggest that the total electrostatic interaction between two protein molecules may be treated, at least qualitatively, as a combination of repulsive monopole–monopole interactions between two bodies bearing like net charges and attractive dipole–dipole interactions between two bodies bearing like dipole moments. At pH values in the vicinity of the isoelectric point, the net charge on the protein will be

[2] No information regarding uncertainty of the raw data were provided by Kanal et al. [4].

[3] BSA is known to undergo an alteration in tertiary structure as pH decreases below 4, presumably associated with partial denaturation [10]. Thus the difference between v_{eff} at pH 3.0 and 4.0 may reflect changes in the hard as well as in the soft part of the intermolecular interaction.

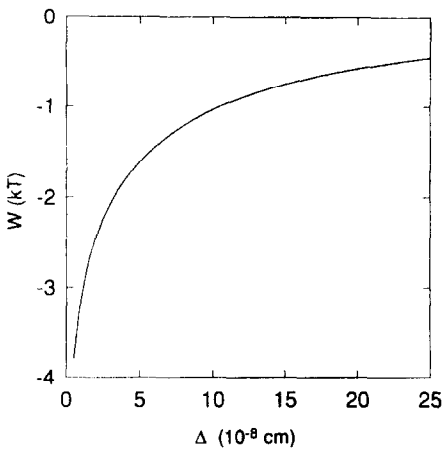

Fig. 3. Points on the line represent pairs of values of the two square well potential parameters (W, Δ) consistent with the negative best-fit value of υ_{eff} at pH 4.6, calculated using Eq. (A3) as described in the text.

small, and the soft contribution to υ_{eff} will be dominated by the attractive dipole–dipole interaction. As the pH moves away from the isoelectric point, the net charge of the protein molecule will increase, and eventually the soft contribution to υ_{eff} will be dominated by the repulsive monopole–monopole interaction.

Although we lack quantitative information about the nature of the attractive interaction between BSA molecules near the isoelectric point we can obtain a rough estimate of the depth of the potential well using the square well approximation (Appendix). Using Eq. (A3) one can calculate the value of the well depth W for a given value of the well width Δ that satisfies a particular ratio $\upsilon_{eff}/\upsilon_{hard}$. This functional relationship is plotted in Fig. 3 for the minimum value of υ_{eff} obtained from the data analysis described (-0.17 ml/g at pH 4.6). It may be seen that even the shortest-range interaction, which is probably shorter than any realistic electrostatic potential, has an attractive potential of less than 4 kT (2.5 kcal/mol at room temperature). Hence the negative value of υ_{eff} obtained from the present analysis of osmotic pressure data obtained near the isoelectric point of BSA is clearly consistent with known magnitudes of weak nonspecific attraction between proteins in solution [11].

The dependence of υ_{eff} upon pH for BSA obtained from the present analysis of the osmotic pressure data of Kanal et al. [4] may be compared with the dependence of υ_{eff} upon pH obtained from analysis of light scattering data of Edsall et al. [12] by Minton and Edelhoch [3], plotted in Fig. 2. In both studies, υ_{eff} increases as the pH moves away from the isoelectric point of BSA, but in the light-scattering analysis the dependence is less marked. The difference may be attributed to the fact that the light scattering study was carried out in 0.15 M NaCl solutions, whereas the osmotic pressure study was carried out in ca. 0.10 M NaCl. Since the pH dependence of υ_{eff} is postulated to reflect the pH dependence of electrostatic interactions between BSA molecules (see above), one would expect that dependence to be more marked in the lower ionic strength medium, where both attractive and repulsive electrostatic interactions are considerably stronger [13].

Appendix A

The effective specific volume of a model protein with self-interaction described by a square well potential

Let the potential of average force between two identical protein molecules in solution be approximated by the following square well potential:

$$U(r) = \begin{cases} \infty & r \le d^* \\ Wkt & d^* \le r < d^* + \Delta \\ 0 & r > d^* + \Delta \end{cases} \qquad \text{(A1)}$$

where d^* is the diameter of the impenetrable hard core of the protein molecule, and Δ is the thickness or 'range' of the soft interaction, which is attractive if W is negative and repulsive if W is positive. This potential is depicted schematically in Fig. 4. Using Eqs. (2) and (6) of the text, the specific volume of the effective hard particle is calculated to be

$$\upsilon_{eff} = \frac{B_2}{8M} = \frac{\pi N_a}{2M}\left[\int_0^{d^*} r^2\,dr + \int_{d^*}^{d^*+\Delta}(1 - e^{-W})\,r^2\,dr\right] \qquad \text{(A2)}$$

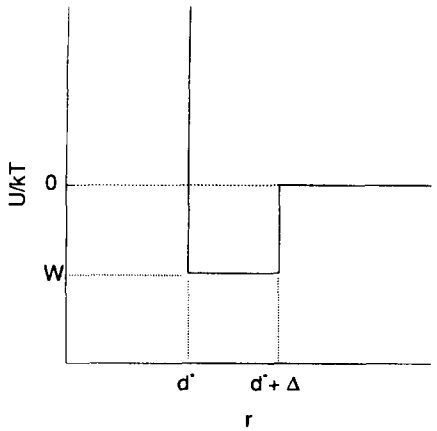

Fig. 4. Schematic diagram of a square well potential, defining the parameters d^*, Δ, and W.

The first term in the bracketed sum on the right hand side of Eq. (A2) represents the contribution of the hard interaction, and the second term represents the contribution of the soft interaction. Evaluation of the integrals in Eq. (A2) and simplification of the resulting expressions leads to

$$v_{\mathrm{eff}} = v_{\mathrm{hard}}\left[(1+x)^3 - \mathrm{e}^{-W}\left\{(1+x)^3 - 1\right\}\right] \tag{A3a}$$

where $x \equiv \Delta/d^*$ and

$$v_{\mathrm{hard}} = \frac{\pi N_{\mathrm{a}} d^{*3}}{6M} \tag{A3b}$$

It follows from Eq. (A3) that v_{eff} will be equal to zero when W is equal to some critical value given by

$$W^* = \ln\left[(1+x)^3 - 1\right] - \ln\left[(1+x)^3\right] \tag{A4}$$

and will be negative when $W < W^*$.

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