Effective Hard Particle Model for the Osmotic Pressure of Highly Concentrated Binary Protein Solutions

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ABSTRACT The experimentally measured concentration dependence of the osmotic pressure of an equimolar mixture of hen egg ovalbumin and bovine serum albumin at pH 7.0 and 25°C in the presence of 0.15 M NaCl is shown to be quantitatively accounted for by a model in which each protein species is represented by an effective hard sphere. The size of this sphere is determined by analysis of the concentration dependence of the osmotic pressure of the isolated protein.

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Analysis of the dependence of the colligative properties of protein solutions upon solute composition is a classical approach to the detection and quantitation of protein-protein interactions at high dilution (1–3). Textbook theories of the three colligative properties—osmotic pressure, static light scattering, sedimentation equilibrium—are generally limited to descriptions of the behavior of dilute solutions that are either thermodynamically ideal (with respect to macrosolute) or nearly ideal, so that residual deviations from thermodynamic ideality may be adequately described by so-called second virial coefficients (4). More recently, it has been demonstrated that the composition dependence of each of these colligative properties in highly concentrated solutions (50–500 g/l) of a single non-self-associating globular protein may be quantitatively accounted for by an effective hard-sphere model in which the protein molecule is represented by a hard spherical particle whose apparent size reflects not only steric repulsion, but also short-ranged electrostatic repulsion (5–7). The magnitude of thermodynamic activity coefficients and concentration derivatives of activity coefficients required to describe colligative properties in concentrated solution may then be calculated from approximate equations of state describing hard sphere fluids of arbitrary composition (8).

It has been recognized that the application of the effective hard sphere model to solutions containing high concentrations of multiple solute species may be problematic, because of the possible nonadditivity of interactions between like and unlike pairs of protein molecules (5,6). A recent report of the measurement of the osmotic pressure of equimolar mixtures of ovalbumin and bovine serum albumin (BSA) (9) at pH 7.0 and 25°C in the presence of 0.15 M NaCl over a broad range of concentration (up to 400 g/l total protein), in combination with earlier measurements of the osmotic pressures of separate solutions of concentrated ovalbumin (10) and BSA (11) under very similar conditions, provides a unique opportunity to test the applicability of the effective hard sphere model to mixtures of concentrated proteins.

In the absence of Donnan effect (i.e., under conditions of moderate salinity), the concentration dependence of the osmotic pressure of a single macromolecular (nondiffusible) solute species is given by (4)

$$\Pi(c^*) = \frac{RT}{v}\left[c^* + \int_0^{c^*} c\left(\frac{d\ln\gamma}{dc}\right)dc\right], \tag{1}$$

where R denotes the molar gas constant, T the absolute temperature, v the specific volume of solution, and γ the thermodynamic activity coefficient of nondiffusible solute. Jiménez et al. (12) extended the derivation of osmotic pressure to the case of multiple nondiffusible solute species. For two nondiffusible solute species, the result is

$$\Pi(c_1^*, c_2^*) = \frac{RT}{v}\left[c_1^* + c_2^* + \int_0^{c_1^*} c_1\left(\frac{\partial\ln\gamma_1}{\partial c_1}\right)_{c_2=0} dc_1 + \int_0^{c_2^*} c_2\left(\frac{\partial\ln\gamma_2}{\partial c_2}\right)_{c_1=c_1^*} dc_2 + c_1^*\int_0^{c_2^*}\left(\frac{\partial\ln\gamma_1}{\partial c_2}\right)_{c_1=c_1^*} dc_2\right]. \tag{2}$$

The specific volume of solution, in units of cm^3/g, is calculated according to

$$v(c_1^*, c_2^*) = [\rho_o + \Delta(c_1^* M_1 + c_2^* M_2)]^{-1}, \tag{3}$$

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where ρ_o denotes the density of pure solvent in g/cm^3 and Δ the density increment of protein, assumed to be the same for both proteins and equal to 2.7×10^{-4} (13).

According to the effective hard sphere model of protein solutions (5,12,14,15), the thermodynamic properties of a solution of globular proteins may be estimated via approximate equations of state for hard spheres or hard sphere mixtures (8). Each species of protein molecule is represented as a sphere whose interactions with other protein molecules under a particular set of experimental conditions is parameterized as an effective specific volume, denoted by v_{eff} (16). Explicit expressions used to evaluate $\partial \ln \gamma_i/\partial c_j$ as a function of the concentrations of all species are derived from the scaled particle theory of fluid mixtures of convex hard particles (17) and are presented in the Appendix to Minton (14).

The dependence of the osmotic pressure of BSA (MW 69,000) upon concentration, measured at pH 7.4 and 25°C in 150 mM NaCl, at concentrations up to 400 g/L (11), was modeled using Eq. 1 together with Eq. 27 of Minton (14). In Fig. 1 the data are plotted as a function of molar concentration together with the function calculated using the best-fit value of $v_{\text{eff}} = 1.46$ cm^3/g (7). The dependence of the osmotic pressure of ovalbumin (MW 45,000), measured at pH 7.0 and 25°C in 150 mM NaCl at concentrations up to 450 g/L (10), was similarly modeled, and the data are plotted together with the function calculated using the best-fit value of $v_{\text{eff}} = 1.03$ cm^3/g. Given these two values of v_{eff}, Eq. 2 together with Eq. 27 of Minton (14) was used to calculate the osmotic pressure of an equimolar mixture of BSA and ovalbumin, which is plotted as a function of the total molar concentration of protein (i.e., twice the molar concentration of each protein), together with the experimental data of Yousef et al. (9).

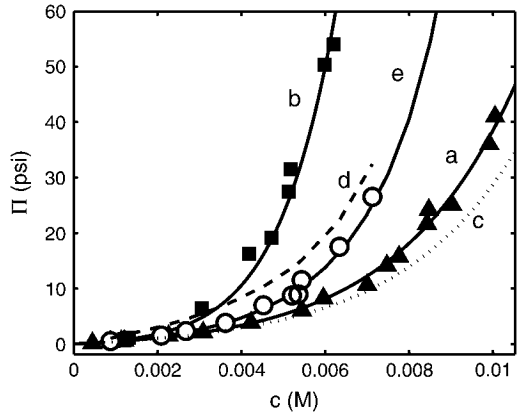

FIGURE 1 Concentration dependence of osmotic pressure of protein solutions. (*Triangles*) Ovalbumin (10); (*squares*) BSA (11); and (*circles*) equimolar mixture of ovalbumin and BSA (11). Calculated: (*a*) Eq. 1 with M = 45,000, $v_{\text{eff}} = 1.03$ cm^3/g; (*b*) Eq. 1 with M = 69,000, $v_{\text{eff}} = 1.46$ cm^3/g; (*c*) osmotic pressure of equimolar mixture calculated by simple summation of osmotic pressures of individual proteins (neglecting nonideal interaction between proteins); (*d*) osmotic pressure of equimolar mixture, calculated according to the free-solvent model of Yousef et al. (9); and (*e*) osmotic pressure of equimolar mixture, calculated according to Eq. 2 with same parameter values used to calculate curves *a* and *b*.

It was previously shown (7), and confirmed here, that the osmotic behavior of BSA in saline solution may be quantitatively accounted for over the entire experimentally accessible range of concentrations by the effective hard sphere model for nonassociating proteins. It is shown here that the osmotic behavior of ovalbumin in saline solution may likewise be quantitatively accounted for over the entire experimentally accessible range of concentrations by the same model. Finally, and most importantly, it is shown here that the osmotic behavior of an equimolar mixture of BSA and ovalbumin is correctly predicted by the same effective hard sphere model applied to binary mixtures of hard spheres, using only the two size parameters obtained from prior analysis of the osmotic behaviors of the individual protein solutions. The evident validity of the hard particle approximation under the conditions of the experiments considered here is probably due to two factors:

1. The isoelectric pH values of both BSA and ovalbumin are $\sim 4.7 \pm 0.2$ (18,19), and hence both proteins bear net negative charge at pH 7.0.
2. At a concentration of 150 mM NaCl, the repulsive electrostatic interaction between all protein molecules is short-ranged relative to their actual sizes (5).

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