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# Quantitative Characterization of the Interaction Between Sucrose and Native Proteins via Static Light Scattering

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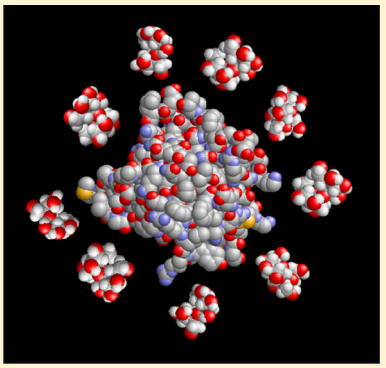
**ABSTRACT:** The composition-dependent static light scattering of binary mixtures of each of four dilute globular proteins and sucrose were measured over a broad range of sucrose concentrations. A conventional analysis of the dependence of excess scattering of a single macrosolute in a continuum solvent yields unphysical results. The data are reanalyzed in the context of multicomponent light scattering theory to yield the dependence of the free energy of solvation of each protein upon the concentration of sucrose. The results could be satisfactorily accounted for by an effective hard particle model that indicates the nature of the underlying interactions between sucrose and each protein.



## INTRODUCTION

Compatible osmolytes are small organic compounds that are synthesized in cells to protect proteins and other macromolecules from the effect of osmotic stress.[1] Other small organic compounds, such as sucrose, are known to have similarly protective effects. The mechanism of this protective effect has been the object of extensive study in several laboratories.[2−4]

The thermodynamic interaction between one osmolyte, trimethylamine oxide (TMAO), and proteins has been studied in our laboratory via the measurement of the dependence of light scattering upon the total solute concentration and weight fraction of each solute in a binary solution mixture of each of three proteins (bovine serum albumin, ovalbumin, and ovomucoid) and TMAO. The results were analyzed in the context of multicomponent scattering theory.[5] The measured scattering intensity was shown to be quantitatively accounted for over a wide range of compositions and total solute concentrations by a simple approximate model, according to which each scattering species is represented by an equivalent hard spherical particle with a molar mass equal to that of the scattering molecule and an effective specific volume that is a measure of solute−solute interaction in solution.[6] According to the best fit of this model, the net interaction between each protein and the osmolyte was approximately equal to that expected on the basis of steric repulsion alone, indicating that longer-ranged attractive and repulsive interactions, if present, approximately canceled out under the conditions of those experiments (pH 7.4 phosphate buffered saline, 25 °C). This conclusion agrees semiquantitatively with the results of prior experimental and theoretical studies of the interaction of TMAO with other proteins.[7,8]

The aim of the present work is to extend the light-scattering methodology to characterize interactions between globular proteins and another small organic molecule, sucrose, which has long been known to stabilize proteins against denaturation by heat or chaotropes and has been shown to attenuate structural fluctuations in native proteins.[9,10] The stabilizing effect has been attributed to preferential hydration, or exclusion of sucrose from the immediate vicinity of the protein surface, so that the addition of sucrose to a solution of protein promotes formation of those conformations minimizing solvent-accessible surface area.[4,11]

In many biological fluids, no single macromolecular solute may be present at high concentration, but all species taken together occupy a significant fraction of the fluid volume.[12] A high concentration of small space-filling solutes may therefore influence the behavior of proteins and nucleic acids present at low concentration. To investigate the behavior of dilute proteins in high concentrations of a small molecule cosolute, we measured the composition-dependent static light scattering of binary mixtures of sucrose at concentrations up to 200 g/L and each of four proteins at concentrations up to 2 g/L. The four proteins selected, bovine serum albumin (BSA), ovalbumin, ovomucoid, and soybean trypsin inhibitor (STI), have been shown previously not to self-associate to a significant extent under the conditions of the present experiments.[13,14] The experimental data were obtained using the recently developed method of automated sequential dilution.[5,14] The experimental protocol was modified as described below to simplify the analysis and reduce the number of unknown variables required to fully characterize the system.

The resulting data was subjected to two different analyses. The first analysis follows the conventional treatment of the dependence of excess scattering of a single macromolecular

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solute in a continuum solvent, summarized below. This analysis will be shown to lead to an unphysical result, indicating its unsuitability for use in mixed solvents. The second analysis is based upon multicomponent scattering theory, treating sucrose as well as protein as a scattering species, and will be shown to yield reliable information about the nature and magnitude of solute−solute interactions in protein−sucrose mixtures.

## MATERIALS

Sucrose was obtained from Invitrogen (15503−022). Monomeric BSA was obtained from Sigma-Aldrich (A1900). Ovalbumin, ovomucoid, and STI were obtained from Worthington Biochemical Corporation (LS003048, LS003086, and LS003571, respectively). Sucrose and proteins were used without additional purification. The molecular weights of the four proteins are: BSA, 66.4 kD;[15] ovalbumin, 45 kD;[16] ovomucoid, 28 kD;[17] STI, 21.5 kD.[18]

BSA, ovalbumin, ovomucoid, and STI were dialyzed against phosphate buffer saline (PBS, 0.15 M NaCl, 0.0066 M phosphate) at pH 7.4 overnight before use. Pierce 10000MWCO Slide-A-Lyzer Dialysis cassettes (Thermo Scientific) were used for the buffer exchange dialysis. Final protein concentrations were determined by absorbance at 280 nm. The standard values of absorbance of each protein in optical density units per centimeter path length for 1 g/L are: BSA, 0.65;[19] ovalbumin, 0.75;[19] ovomucoid, 0.41;[20] STI, 0.94.[21]

A stock solution of sucrose in PBS at 400 g/L was prepared, and the final pH of the solution was adjusted to 7.4.

All of the proteins, sucrose, and buffer were prefiltered through 0.1 $\mu$m Whatman Anotop filters (Whatman). Immediately before the experiments, all samples were centrifuged at 5000*g* for 10 min at 20 °C for degassing and removal of residual particulates.

## METHODS

**Refractive Increments of Sucrose and Protein.** The refractive increment of sucrose in PBS was measured at 685 nm and 20 °C in an Optilab rEX refractometer (Wyatt Technology, Santa Barbara CA) and determined to be 0.140 cm$^3$/g. The refractive increment of ovalbumin in sucrose solutions of varying concentrations measured under the same conditions was found to be dependent upon the concentration of sucrose (Table 1). In accordance with previous findings,[22] it is assumed that the refractive increments of all four proteins at each sucrose concentration are identical to within experimental uncertainty.

**Light Scattering Measurement.** Concentration-dependent light scattering at 90° was measured via automated sequential dilution as described by Fernandez and Minton.[14] A Variomag Mini cuvette stirrer (Variomag-USA, Daytona Beach, FL) was fixed in the base of the MiniDAWN Tristar light scattering detector (Wyatt Technology, Santa Barbara, CA). The standard flow cell was replaced by a custom-built thermostatted cuvette holder to permit measurement of scattering at a constant temperature of 20 °C.

**Table 1. Refractive Index of Sucrose Solutions in PBS and Refractive Increment of Proteins in Each Sucrose Solution (20°C, 685 nm)**

| $w_{sucrose}$ (g/L) | $n$ | $dn/dw_{prot}$ |
|---|---|---|
| 0 | 1.3311 | 0.186 |
| 50 | 1.3381 | 0.182 |
| 100 | 1.3451 | 0.179 |
| 150 | 1.3521 | 0.175 |
| 200 | 1.3591 | 0.171 |

Light scattering was measured in a square fluorescence cuvette containing 1.5 mL of a solution initially containing approximately 1.5 g/L protein in PBS plus various concentrations of sucrose (0, 50, 100, 150, and 200 g/L). Protein concentration was progressively decreased at constant sucrose concentration by sequential dilution with a sucrose solution of identical concentration in PBS.[5] The intensity of light scattering was measured at 90° following each dilution and recorded using ASTRA (v4.9, Wyatt Technology, Santa Barbara, CA). Raw data were subsequently exported and analyzed with user-written scripts in MATLAB (R2009a, Mathworks, Natick, MA).

**Interpretation of the Composition Dependence of Light Scattering.** *Excess Scattering of a Single Macromolecular Solute in a Continuum Solvent.* In the limit of low macromolecular concentration, the scattering intensity of a solution of a single macromolecular solute, represented by a point scatterer, is conventionally given by[23]

$$\frac{R}{K'} = Mw \tag{1}$$

where $R$ denotes the excess Rayleigh ratio, proportional to scattering intensity over and above that of the solvent, $M$ and $w$ denote the molecular weight and w/v concentration of the macromolecular solute, and $K'$ is an optical constant given by

$$K' = \frac{4\pi^2 n^2 (dn/dw)^2}{\lambda_0^4 N_A} \tag{2}$$

where $\lambda_0$ denotes the vacuum wavelength of incident light, $N_A$ Avogadro's number, $n$ the refractive index of solvent, and $dn/dw$ the refractive increment of macromolecular solute in the solvent.

*Excess Scattering of Two Scattering Solute Species in a Continuum Solvent.* According to multicomponent scattering theory,[5,23−25] the excess Rayleigh scattering for two species (1 for protein, 2 for sucrose) can be written as below

$$\frac{R}{K_0} = \left(\frac{n}{n_0}\right)^2 \frac{G_1^2 M_1^2 c_1\left(1 + c_2\frac{\partial \ln \gamma_2}{\partial c_2}\right) + G_2^2 M_2^2 c_2\left(1 + c_1\frac{\partial \ln \gamma_1}{\partial c_1}\right) - 2G_1 G_2 M_1 M_2 c_1 c_2 \frac{\partial \ln \gamma_1}{\partial c_2}}{\left(1 + c_1\frac{\partial \ln \gamma_1}{\partial c_1}\right)\left(1 + c_2\frac{\partial \ln \gamma_2}{\partial c_2}\right) - c_1 c_2\left(\frac{\partial \ln \gamma_1}{\partial c_2}\right)^2} \tag{3}$$

where $R$ denotes the excess Rayleigh ratio, proportional to the difference between the scattering intensity of solution and that of cosolute-free solvent, $c_i$, $M_i$, $\gamma_i$ the molar concentration, molar mass, and thermodynamic activity coefficient of the $i$th solute species respectively, and $G_i \equiv (dn/dw_i)/(dn/dw)^*$, where $(dn/dw)^*$ denotes a "standard" refractive increment for most proteins in buffer (0.185 cm$^3$/g).[5] The optical constant $K_0$ is given by

$$K_0 = \frac{4\pi^2 n_0^2 [(dn/dw)^*]^2}{\lambda_0^4 N_A} \tag{4}$$


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Since the value of $R$ calculated according to eq 3 corresponds to the scattering in excess of that of cosolute-free buffer, the value of $n_0$ is set equal to that of the aqueous buffer, 1.3311. Since the concentration of protein is low, its contribution to the refractive increment is negligible, and $n = n_0 + (dn/dw_2)w_2$, $G_1 = 1.0$, and $G_2 = 0.74$.

To model the experimental dependence of $R/K_0$ upon $c_1$ and $c_2$ it is additionally necessary to specify the dependence of the thermodynamic activity coefficients of both species and their concentration derivatives upon solution composition. Below we present two alternative approaches.

*1. General Statistical–Thermodynamic Formulation (Virial Expansion).* According to the statistical–mechanical solution theory of McMillan and Mayer,[26] the natural logarithm of thermodynamic activity coefficient of the $i$th species in a multiple mixture can be written as

$$\ln \gamma_i = \sum_j B_{ij}c_j + \sum_j \sum_k B_{ijk}c_j c_k + \ldots \quad (5)$$

where $B_{ij}$, $B_{ijk}$, ..., denote interaction (or Virial) coefficients. In a binary mixture of dilute solute species 1 (here protein) and a second solute species 2 at arbitrary concentration (here sucrose), the activity coefficient of species 2 will be independent of the concentration of the dilute species, and eq 5 reduces to

$$\ln \gamma_1 = B_{11}c_1 + B_{12}c_2 + B_{112}c_1c_2 + B_{122}c_2^2 + \ldots \quad (6)$$

$$\ln \gamma_2 = B_{22}c_2 + B_{222}c_2^2 + \ldots \quad (7)$$

from which the concentration derivatives appearing in eq 3 may be readily obtained. Since the value of $G_2$ has been independently determined, the value of $R/K_0$ may thus be calculated as a function of $c_1$ and $c_2$ given the values of $M_1$, $M_2$, $B_{11}$, $B_{12}$, $B_{112}$, $B_{122}$, $B_{22}$, and $B_{222}$. It follows that, according to this model, the dependence of scattering intensity upon the concentrations of sucrose and each of four proteins should be globally describable by a total of 23 parameters: the molecular weights of sucrose and each of the four proteins, the values of $B_{22}$ and $B_{222}$, and the values of $B_{11}$, $B_{12}$, $B_{112}$ and $B_{122}$ for each of the four proteins.

*2. Equivalent Hard Sphere Model.* While the Virial expansion approach described above is rigorous, it becomes rapidly computationally intractable when treating systems containing solutes at concentrations such that two-body interactions alone are insufficient to provide a complete thermodynamic description. This will be documented in the Results and Discussion section below. It has been shown elsewhere that the concentration dependence of several colligative properties of solutions of individual proteins and binary mixtures of proteins may be accounted for quantitatively over a broad range of concentrations—up to hundreds of grams per liter—by modeling the solution as a fluid of equivalent hard convex particles, the sizes and shapes of which parametrize the interactions between protein molecules.[25,27−30] The thermodynamic activity coefficients and the concentration derivatives of the activity coefficients of each protein are estimated using the scaled particle theory of hard convex particle fluids.[31,32] More recently, it has been demonstrated that the composition dependence of the light scattering of mixtures of proteins and a small molecule osmolyte, trimethylamine oxide, may also be modeled as a mixture of large and small effective hard spheres.[5] Although approximate, the effective hard particle model provides results that are identical to the virial expansion at concentrations sufficiently low to be described by first-order deviations from ideal behavior, remains computationally tractable at all compositions, and will be shown to provide a parsimonious description of all of our data to within experimental uncertainty.

By assumption that we can model sucrose and each of the four proteins as equivalent hard spheres, the dependence of scattering intensity upon the concentrations of the four proteins and sucrose should be globally describable by a total of 10 parameters: the molecular weight of the sucrose and each of the four proteins, and the values of the effective specific volumes of sucrose and each of the four proteins. Given these parameters, the values of $d \ln \gamma_i/dc_j$ are calculated using relations obtained from scaled particle theory presented in the appendix to ref 25.

## RESULTS AND DISCUSSION

**Single Scattering Species Analysis.** The results of experiments in which the concentration of protein was varied in a solution containing a constant concentration of sucrose were analyzed according to eqs 1 and 2. For each sucrose concentration, the value of $K'$ was evaluated using the values of $n$ and $dn/dw$ given in Table 1 and the value of $R$ calculated from the difference between total scattering intensity and the baseline scattering intensity of the sucrose solution. Equation 1 was fit to the dependence of $R/K'$ upon $w$, allowing only the value of $M$ to vary. The dependence of $R/K'$ upon $w$ calculated using eq 1 together with the best-fit value of $M$ obtained for each data set is plotted together with the data in parts A–D of Figure 1.

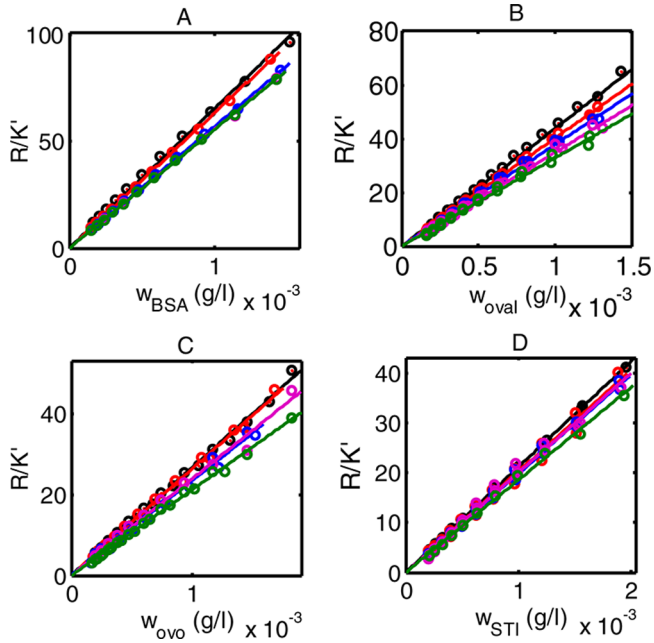


**Figure 1.** Light scattering of proteins in excess of that of a "solvent" containing different concentrations of sucrose, plotted as a function of protein concentration. Sucrose concentrations: 0 (black), 50 g/L (red), 100 g/L (blue), 150 g/L (magenta), and 200 g/L (green). The optical constant $K'$ is calculated for each sucrose concentration according to eq 2. Straight lines represent the best fit of eq 1 to each data set. Panel A, BSA; panel B, ovalbumin (oval); panel C, ovomucoid (ovo); panel D, STI.

The corresponding best-fit values of $M$ are plotted as a function of sucrose concentration in parts A–D of Figure 2.


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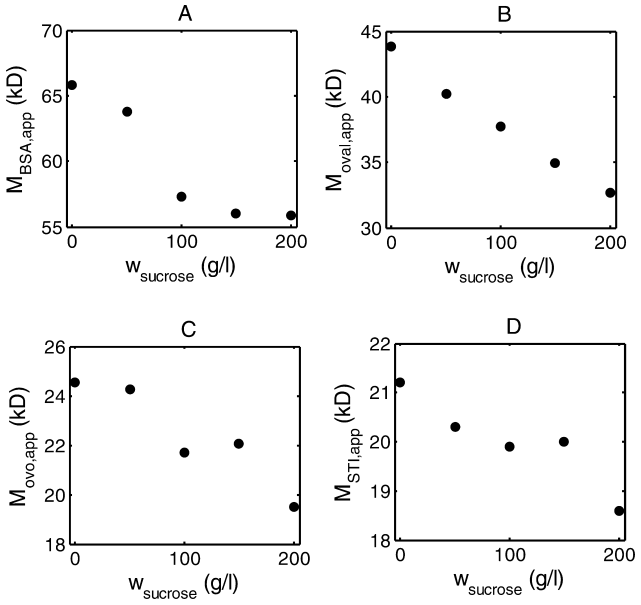


**Figure 2.** Apparent molecular weight of proteins, evaluated by fitting eq 1 to the data plotted in Figure1, plotted as a function of sucrose concentration. Panels refer to the same proteins as in Figure 1.

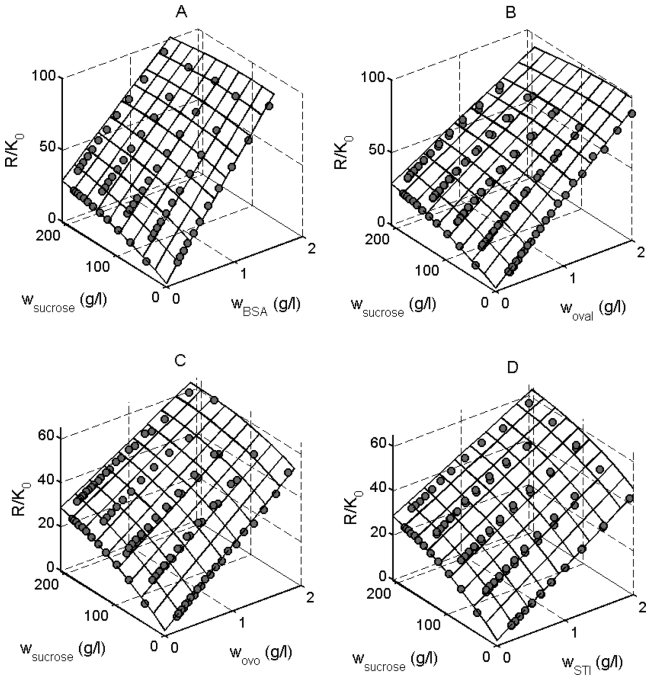


**Figure 3.** Scattering of binary mixtures of sucrose and BSA (A), ovalbumin (B), ovomucoid (C), and STI (D) normalized to the optical constant calculated according to eq 4, plotted as functions of the concentrations of protein and sucrose. Symbols: experimental data. Mesh plots calculated using eq 3 with the corresponding best-fit parameter values appearing in Table 2.

From the results shown in Figures 1 and 2 it is evident that the conventional analysis, which fails to take into account differential interactions between components of the mixed solvent and the single macromolecular solute, may lead to a qualitatively incorrect estimate of the molecular weight of the macrosolute. Such differential interactions may be taken into account explicitly through application of multicomponent scattering theory, as described below.

**Two Scattering Species Analysis.** The measured dependence of scattering intensity of mixtures of each protein and sucrose upon the concentrations of protein and sucrose are plotted in parts A–D of Figure 3. These data were globally modeled using eq 3 with two different calculations of the derivatives of the logarithms of activity coefficients with respect to concentrations. (The term "globally modeled" means that the value of scattering intensity was simultaneously calculated as a function of the concentrations of both solutes for all four data sets with a single set of parameters. In this fashion, a best-fit of the model to each data set may be sought subject to the condition that values of parameters pertaining only to sucrose, such as molecular weight or effective HS volume, are "global", or common to all data sets.) The first calculation utilizes eqs 6 and 7, and the second method utilizes the equivalent hard sphere model eq 27 of ref 25. In both cases, eq 3 together with the respective description of the composition dependence of the activity coefficients of both solutes upon the concentrations of each solute was fit via nonlinear least-squares to the combined experimentally measured dependence of $R/K$ upon the w/v concentrations of protein and sucrose for all four protein–sucrose combinations. It was found that both methods for calculating the activity coefficient derivatives provided equally good fits to the combined data. However, over twice as many adjustable parameters were required to fit the data using the Virial expansion model, and unique values of the two- and three-body interaction coefficients appearing in eqs 6 and 7 could not be determined due to the high degree of correlation between them. In contrast, fits obtained using activity coefficient derivatives calculated using the equivalent hard sphere model yielded reasonably well determined values of all best fit parameters, presented in Table 2. Subsequent analysis was therefore limited to results obtained using the equivalent hard sphere model.

**Table 2. Best Fit Values and Uncertainties of Parameters Appearing in the Equivalent Hard Sphere Model for Scattering of Protein–Sucrose Mixtures**[a]

| | molecular weight (kD) | $v_{eff}$ ($cm^3/g$) |
|---|---|---|
| sucrose | 0.35 (+0.4, −0.2) | 0.32 (+0.06, −0.04) |
| BSA | 66.7 (+2.8, −2.1) | 0.87 (+ 0.16, −0.15) |
| ovalbumin | 44.3 (+1.7, −1.2) | 1.04 (+0.15, −0.13) |
| ovomucoid | 25.3 (+1.3, −1.0) | 0.82 (+0.18, −0.17) |
| STI | 21.4 (+1.2, −1.0) | 0.55 (+0.21,-0.18) |

[a]Indicated uncertainties correspond to 1 standard error of estimate (lower and upper bounds) as determined from distributions of the F-statistic.

The best fit of eq 3 obtained with the equivalent hard sphere model to data from each of the protein–sucrose mixtures are plotted together with the corresponding data sets in Figure 3. Fits of eq 3 obtained with the Virial expansion model are indistinguishable and therefore not plotted.

The best-fit values of the molecular weights of sucrose and each of the four proteins are in excellent agreement with values obtained from the literature.[15−18] The significance of the effective specific volumes of each of the species obtained by modeling the data with the effective hard sphere model is as follows.

Within the context of the effective hard sphere model, the two-body coefficient of interaction is equal to the volume excluded by species $i$ to the center of species $j$ (and vice versa)[33]


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$$B_{ij} = \frac{4\pi}{3}(r_{\mathrm{eff},i} + r_{\mathrm{eff},j})^3 \quad (8)$$

where $r_{\mathrm{eff},i}$ denotes the effective spherical radius of species $i$, given by

$$r_{\mathrm{eff},i}(\text{Å}) = \frac{4M_i v_{\mathrm{eff},i}}{3\pi N_A} = 0.735(v_{\mathrm{eff},i}M_i)^{1/3} \quad (9)$$

The contact distance $r_{\mathrm{eff},i} + r_{\mathrm{eff},j}$ is thus a measure of the total interaction between the two solute species. We can compare the interaction observed experimentally with that expected on the basis of a purely steric interaction by defining a reference "steric radius" equal to

$$r_{\mathrm{steric},i}(\text{Å}) = 0.735(\bar{v}_i M_i)^{1/3} \quad (10)$$

where $\bar{v}_i$ denotes the experimentally measured partial specific volume of solute species i, (i.e., the volume excluded to solvent), which is equal to approximately 0.73 $cm^3/g$ for proteins[22] and 0.618 $cm^3/g$ for sucrose.[34] It follows that

$$\frac{B_{ij}}{B_{ij}^{(\mathrm{steric})}} = \left[\frac{(v_{\mathrm{eff},i}M_i)^{1/3} + (v_{\mathrm{eff},j}M_j)^{1/3}}{(\bar{v}_i M_i)^{1/3} + (\bar{v}_j M_j)^{1/3}}\right]^3 \quad (11)$$

**Table 3. $B_{ij}/B_{ij}^{(\mathrm{steric})}$ for Each Pairwise Interaction[a]**

| interacting pair | $B_{ij}/B_{ij}^{(\mathrm{steric})}$ |
|---|---|
| sucrose−sucrose | 0.52 ± 0.07 |
| BSA−sucrose | 1.07 ± 0.17 |
| ovalbumin−sucrose | 1.24 ± 0.16 |
| ovomucoid−sucrose | 0.99 ± 0.19 |
| STI−sucrose | 0.70 ± 0.21 |
| BSA−BSA[b] | 2.45 ± 0.09 |
| ovalbumin−ovalbumin[b] | 2.08 ± 0.08 |
| ovomucoid−ovomucoid[b] | 2.27 ± 0.10 |

[a]Indicated uncertainties correspond to 1 standard error determined from distributions of the F-statistic. [b]Calculated according to the data from ref 5.

Values of $B_{ij}/B_{ij}^{(\mathrm{steric})}$, calculated for each pairwise interaction according to eq 11, are presented in Table 3. The physical significance of these values for each pairwise interaction is discussed below.

*Sucrose−Sucrose.* The observation that $B_{ij}$ is only about half of what would be expected for a purely steric interaction between equivalent spheres indicates either that there exists a significant attractive interaction between sucrose molecules or interpenetration of sucrose molecules, which is physically possible since sucrose is a disaccharide rather than a solid sphere, or some combination of the two effects. An identical conclusion may be drawn from an effective hard sphere analysis of the concentration dependence of the osmotic pressure of sucrose solutions (Appendix).

*Sucrose−BSA and Sucrose−Ovomucoid.* In both cases, the value of $B_{ij}$ calculated from the best-fit effective volumes is approximately equal to that expected for a purely steric excluded volume interaction.

*Sucrose−Ovalbumin.* The value of $B_{ij}$ calculated from the best-fit effective volumes is somewhat larger than that expected for a purely steric excluded volume interaction, indicating the presence of a soft repulsive interaction in addition to the steric interaction.

*Sucrose−STI.* The value of $B_{ij}$ calculated from the best-fit effective volumes is somewhat smaller than that expected for a purely steric excluded volume interaction, indicating the presence of a soft attractive interaction in addition to the steric interaction.

*Protein−Protein Self-Interactions.* Utilizing best-fit values of the effective HS volume describing the self-interaction of BSA, ovalbumin, and ovomucoid obtained by Fernández and Minton,[14] the value of $B_{ij}$ calculated from the best-fit effective volumes is at least twice as large as that expected for a purely steric excluded volume interaction, indicating the presence of a strong repulsive interaction in addition to the steric interaction. This is in qualitative accord with expectation, since the isoelectric point of all three proteins is between pH 4 and 5, and the present experiments are carried out at pH 7, so each is expected to carry a

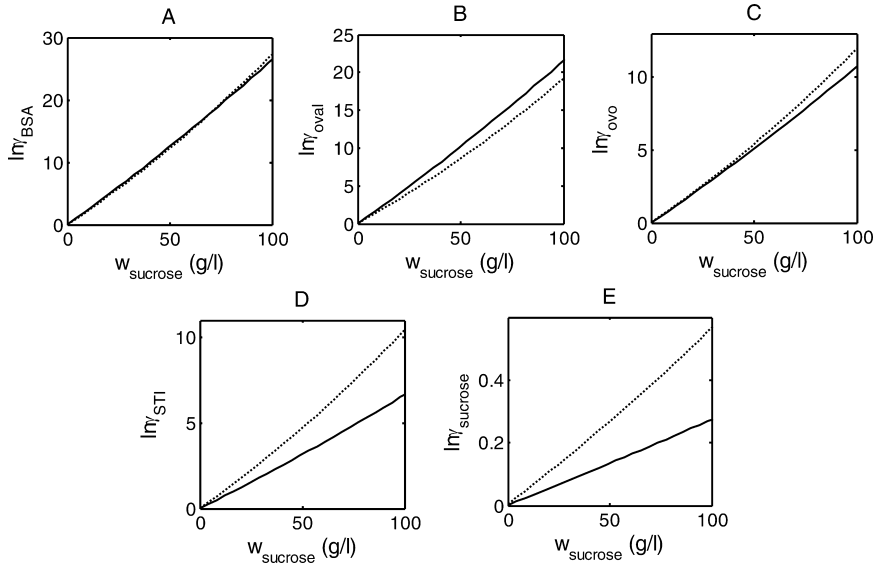


**Figure 4.** Natural logarithm of thermodynamic activity coefficient of each protein at limiting low concentration and sucrose plotted as a function of sucrose concentration according to the effective hard sphere model, using best-fit effective hard sphere exclusion volumes given in Table 2 (solid lines) and experimentally determined partial specific volumes (dashed lines).


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significant negative net charge, leading to significant electrostatic repulsion.

Given the best-fit values of molecular weight and effective volumes of the equivalent spheres, scaled particle theory may be used to estimate the dependence of ln $\gamma_{protein}$ in the limit of high protein dilution (proportional to the excess free energy of solvation) upon the concentration of sucrose.[25,32] The results are plotted in Figure 4. Also plotted for comparison is the dependence of ln $\gamma_{protein}$ calculated using the steric exclusion volumes of each equivalent sphere, which approximates the excess free energy of solvation expected on the basis of pure excluded volume. It should be noted that the difference between ln $\gamma_{protein}$ calculated using best-fit effective exclusion volumes and those calculated using steric exclusion volumes is in qualitative accord with that expected on the basis of comparing the respective values of $B_{ij}$, as discussed above. The results plotted in Figure 4 indicate that the interactions between sucrose and BSA, ovalbumin, and ovomucoid are primarily or entirely steric, subject only to small perturbations at most from "soft" interaction. This finding is in qualitative accord with conclusions of Lee and Timasheff[4] based upon the results of their studies of the stabilization of chymotrypsin and ribonuclease by sucrose. Only in the case of STI do "soft" interactions contribute substantially to the overall interaction between sucrose and protein. The physical basis of the attraction between sucrose and STI remains to be established. This result is of interest as it demonstrates that one cannot automatically assume the absence of significant attractive (or soft repulsive) interactions when considering the effects of nominally unreactive cosolutes upon the behavior of nominally "well-behaved" proteins. Finally, the thermodynamic interaction between sucrose molecules has been clearly shown to reflect soft attraction between and/or partial interpenetration of the disaccharides.

## APPENDIX: HARD SPHERE MODEL FOR THE CONCENTRATION-DEPENDENT OSMOTIC PRESSURE OF SUCROSE

The osmotic pressure of a solution of solute of molecular weight $M$ and w/v concentration $w$, modeled as equivalent hard spheres, may be calculated using the following relation[28]

$$\Pi(w) = \frac{RT}{M}\left[w + \frac{4\phi^2 + 10\phi^3 + 18.4\phi^4 + 28.2\phi^5 + 39.8\phi^6 + 56.5\phi^7 + \ldots}{v_{eff}}\right] \quad \text{(A1)}$$

where $R$ denotes the molar gas constant, $T$ the absolute temperature, $v_{eff}$ the effective specific volume of the equivalent hard spherical particle representing solute, and $\phi$ the fraction of volume occupied by equivalent hard spherical particles, given by $\phi = v_{eff}w$.

Equation A1 was fitted by the method of nonlinear least-squares minimization to the data of Frazer and Myrick[35] for fixed values of $T$ (303 K) and $M$ (340). The best fit of eq A1 to the data, plotted together with the data in Figure A1, was achieved with a value of $v_{eff} = 0.29$ cm$^3$/g. It was verified that the expansion in powers of $\phi$ on the rhs of eq A1 converged at the highest solute concentration in the data set.

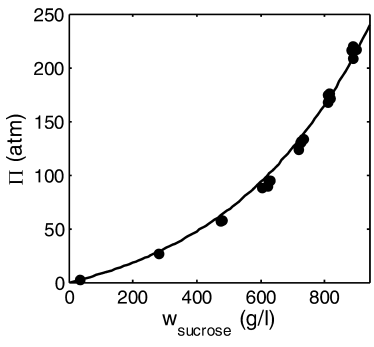


**Figure A1.** Osmotic pressure of sucrose solutions at 30 °C as a function of w/v concentration. Points: data of Frazer and Myrick.[35] Curve: best-fit of eq A1.

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

(1) Yancey, P. H. *Integrative Comparative Biol.* **2001**, *41*, 11.
(2) Carpenter, J. F.; Crowe, J. H. *Cryobiology* **1988**, *25*, 244−255.
(3) Kendrick, B. S.; Chang, B. S.; Arakawa, T.; Peterson, B.; Randolph, T. W.; Manning, M. C.; Carpenter, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11917−11922.
(4) Lee, J. C.; Timasheff, S. N. *J. Biol. Chem.* **1981**, *256*, 7193−7201.
(5) Fernandez, C.; Minton, A. P. *J. Phys. Chem. B* **2011**, *115*, 1289−1293.
(6) Hall, D.; Minton, A. P. *Biophys. Biochim. Acta* **2003**, *1649*, 127−139.
(7) Nagarajan, S.; Amir, D.; Grupi, A.; Goldenberg, D. P.; Minton, A. P.; Haas, E. *Biophys. J.* **2010**, *100*, 2991−2999.
(8) Rosgen, J.; Pettitt, B. M.; Bolen, D. W. *Protein Sci.* **2007**, *16*, 733−743.
(9) Borowitzka, L. J. *Transport processes, iono-and osmoregulation*; Springer-Verlag: Berlin, 1985.
(10) Yancey, P. H.; Clark, M. E.; Hand, S. C.; Bowlus, R. D.; Somero, G. N. *Science* **1982**, *217*, 1214−1222.
(11) Cioni, P.; Bramanti, E.; Strambini, G. B. *Biophys. J.* **2005**, *88*, 4213−4222.
(12) Minton, A. P.; Wilf, J. *Biochemistry* **1981**, *20*, 4821−4826.
(13) Attri, A. K.; Minton, A. P. *Anal. Biochem.* **2005**, *346*, 132−138.
(14) Fernández, C.; Minton, A. P. *Anal. Biochem.* **2008**, *381*, 254−257.
(15) Hirayama, K.; Akashi, S.; Furuya, M.; Fukuhara, K. *Biochem. Biophys. Res. Commun.* **1990**, *173* (2), 639−646.
(16) Stein, P. E.; Leslie, A. G.; Finch, J. T.; Carrell, R. W. *J. Mol. Biol.* **1991**, *221*, 941−959.
(17) Feeney, R. E.; Stevens, F. C.; Osuga, D. T. *J. Biol. Chem.* **1963**, *238*, 1415−1418.
(18) Wu, Y. V.; Scheraga, H. A. *Biochemistry* **1962**, *1*, 905−911.
(19) Kirschenbaum, D. M. In *Handbook of Biochemistry and Molecular Biology*; Fasman, G. D., Ed.; CRC Press: Cleveland, 1976, p 383−545.
(20) Donovan, J. W. *Biochemistry* **1967**, *6*, 3918−3927.
(21) Zahnley, J. C.; Davis, J. G. *Biochemistry* **1970**, *9*, 1428−1433.
(22) Zhao, H.; Brown, P. H.; Schuck, P. *Biophys. J.* **2011**, *100*, 2309−2317.
(23) Stacey, K. A. *Light-scattering in physical chemistry*; Academic Press: New York, 1956.
(24) Stockmayer, W. H. J. *Chem. Phys.* **1950**, *18*, 58−61.
(25) Minton, A. P. *Biophys. J.* **2007**, *93*, 1321−1328.
(26) McMillan, W. G., Jr.; Mayer, J. E. *J. Chem. Phys.* **1945**, *13*, 276−305.

(27) Fernandez, C.; Minton, A. P. *Biophys. J.* **2009**, *96*, 1992−1998.
(28) Minton, A. P. *Biophys Chem* **1995**, *57*, 65−70.
(29) Minton, A. P. *Biophys. J.* **2008**, *94*, L57−L59.
(30) Minton, A. P. *Biophys. J.* **1983**, *55*, 119−140.
(31) Boublík, T. *Mol. Phys.* **1974**, *27*, 1415−1427.
(32) Lebowitz, J. L.; Helfand, E.; Praestgaard, E. *J. Chem. Phys.* **1965**, *43*, 774−779.
(33) Minton, A. P. *Methods Enzymol.* **1998**, *295*, 127−149.
(34) Gosting, J. L.; Morris, S. M. *J. Am. Chem. Soc.* **1949**, 71.
(35) Frazer, J. C. W.; Myrick, R. T. *J. Am. Chem. Soc.* **1916**, *38*, 1907−1922.