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Bovine Serum Albumin in Aqueous Guanidine Hydrochloride Solutions. Preferential and Absolute Interactions and Comparison with Other Systems†

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ABSTRACT: The partial specific volume, $\bar{v}_2^0$, of bovine serum albumin at 25 °C was found to be 0.728 ± 0.001 ml/g in solutions of guanidine hydrochloride (GuHCl), 0.01 M dithioerythritol (DTE), independent of GuHCl concentration (3–6 M). The volume decrease upon denaturation is about 400 ml/mol ($\bar{v}_2^0$ in water at the same temperature was found to be 0.734). From the reduced density increments at constant chemical potential of diffusible solutes, the apparent volumes, ϕ', were found to increase from 0.693 ml/g at 3 M GuHCl to about 0.725 ml/g at 7 M GuHCl. The phenomenological interaction parameter, ξ_3 (grams of GuHCl "bound" per gram of protein), was found to decrease from about 0.2 at 3 M GuHCl to about 0.07 at 6.4 M GuHCl. The phenomenological interaction parameter, ξ_1 (grams of water "bound" per gram of protein), is negative and become less negative with increase in GuHCl concentration. The relation between ξ_3 and ξ_1 and physical binding and exclusion of low-molecular-weight components are discussed in terms of simple model consideration. It is concluded that over the range of GuHCl concentrations studied about 0.2 g of water as well as 0.28 g of GuHCl are bound per gram of protein. This corresponds on the average to 1.3 molecules of water and 0.35 molecule of GuHCl per amino acid residue. Similar results were found by recalculating some previous results for aldolase. These results on proteins in GuHCl solution are in marked contrast to the behavior of DNA at high concentrations of NaCl and CsCl, which is analyzed on the basis of earlier work.

In a previous publication (Reisler and Eisenberg, 1969), we have determined density increments, partial specific volumes, apparent volumes, and interaction parameters of aldolase with low-molecular-weight components in the three component system water–salt–protein. Whereas the primary purpose of that work related to the correct interpretation of the subunit composition of aldolase from equilibrium sedimentation, interesting results with respect to interaction parameters of protein with the low-molecular-weight salt were also derived. Bovine serum albumin was also investigated by us in that work, but in less detail. In the present work, we have extended the measurements on this protein to cover a wide range of concentrations of the denaturing solvent GuHCl.[1] Though bovine serum albumin solutions have been extensively studied in the past, we believe that the new data to be presented below will clarify some basic points relating to the behavior of proteins upon denaturation in GuHCl solutions. The experimental section follows closely the work of Reisler and Eisenberg (1969); the precise description of experiments leading to density increments at constant chemical potential of diffusible solutes and correct partial specific volumes has also been given by Cohen and Eisenberg (1968) in an investigation concerned

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[1] Abbreviations used are: GuHCl, guanidine hydrochloride; DTE, dithioerythritol.

with the study of DNA solutions. We also present here a theoretical discussion which clarifies certain problems in the analysis of three-component systems, emphasizes symmetry in multicomponent systems, and aims at achieving a clear separation between, so-called, "preferential" interaction parameters, and quantities which may be associated with distinct physical phenomena relating to the low-molecular-weight components, namely, binding and Donnan exclusion.

Materials and Methods

Materials and methods were as described by Reisler and Eisenberg (1969) with a slight modification of the solvent system. It was found that 0.1 M β-mercaptoethanol could be successfully replaced by 0.01 M DTE (dithioerythritol), described by Cleland (1964). The use of DTE improved the accuracy of spectroscopic concentration determinations, both because of lower absorbance in the ultraviolet at the specified concentration of the reagent as well as because of its low volatility when contrasted to β-mercaptoethanol. Concentrations of bovine serum albumin (in milligrams per milliliter) in solutions in the presence of GuHCl were obtained from readings at 278 nm by multiplying the observed absorbance values by 1.63. This value was found to be independent of GuHCl concentration, in the range 3–7 M. This is identical with the value reported by Noelken and Timasheff (1967).

Theoretical

To obtain an understanding of quantities which may be derived from density determinations of multicomponent systems, we first consider a three-component system composed of a principal solvent (water) component 1; a macromolecular (protein or nucleic acid) component 2; and a low-molecular-weight salt or nonionic component 3. The presentation here is based on a number of previous studies (Reisler and Eisenberg, 1969; Eisenberg, 1976) and can be extended to include, with a certain loss in simplicity, further high-molecular or low-molecular-weight components (Casassa and Eisenberg, 1964). Closely related information can also be derived from distribution of solutes in equilibrium dialysis (Scatchard et al., 1946), buoyant weights (Ifft and Vinograd, 1966), isopiestic distillation (Hade and Tanford, 1967; Hearst, 1965), electromotive force (emf) measurements (Imai and Eisenberg, 1966) and refractive index increments of multicomponent solution (Timasheff and Inoue, 1968), but these topics will not be dealt with within the scope of the present discussion.

We may write for the density increment $(\partial\rho/\partial c_2)_\mu$ the exact expression

$$\left(\frac{\partial\rho}{\partial c_2}\right)_\mu = \frac{(1-\bar{v}_2\rho)+\xi_3(1-\bar{v}_3\rho)}{1-(\bar{v}_2+\xi_3\bar{v}_3)c_2} + \kappa\rho\left(\frac{\partial\Pi}{\partial c_2}\right)_\mu \quad (1)$$

where c_2 is the volume concentration of component 2 in g/ml, subscript μ signifies constancy of all chemical potentials of components diffusible through a semipermeable membrane (components 1 and 3), subscript T is not indicated but constant temperature is assumed throughout. The $\bar{v}_i$'s are partial specific volumes, κ is the isothermal compressibility $\mathrm{d}\ln\rho/\mathrm{d}P$ at constant composition, and the interaction parameters, ξ_i,

$$\xi_i \equiv \left(\frac{\partial w_i}{\partial w_2}\right)_\mu \quad (2)$$

indicate the change in molality w_i (in units of grams of component i per gram of component 1) with change in molality w_2 in order to maintain μ_1 and μ_3 constant; Π is the osmotic pressure.

The density increment $(\partial\rho/\partial c_2)_\mu$ is of particular interest, since it is required for the evaluation of equilibrium and velocity sedimentation and small angle x-ray scattering (Timasheff, 1963; Cohen and Eisenberg, 1968) experiments in multicomponent systems. For these methods, $(\partial\rho/\partial c_2)_\mu$ may, as a rule, be obtained as an experimental quantity and a decomposition into the contributions appearing on the right-hand side of eq 1 is not required. Our main interest though in the present work centers on such decomposition of $(\partial\rho/\partial c_2)_\mu$ into the constituting quantities and their interpretation.

For the subsequent discussion, we shall make two common approximations which are inconsequential in relation to the principles involved, but lead to significant simplification in presentation. We make the reasonable assumption that the density of the solution, at low polymer concentration, is linear in c_2

$$\rho = \rho^0 + (\partial\rho/\partial c_2)_\mu c_2$$

where ρ^0 is the density of the solvent mixture (components 1 and 3) in the absence of component 2. We, furthermore, neglect the compressibility term in eq 3, which is due to the fact that the equilibrium osmotic pressure, Π, has to be maintained (this term is equal to $\kappa\rho RT/M_2$ at low protein concentration, and is negligible in aqueous solutions, in most cases of practical interest). With the above assumptions, eq 1 simplifies to the more attractive and frequently encountered form

$$(\partial\rho/\partial c_2)_\mu = (1-\bar{v}_2{}^0\rho^0) + \xi_3(1-\bar{v}_3\rho^0) \quad (3)$$

Here $\bar{v}_2{}^0$ is the partial specific volume at vanishing concentration, c_2, whereas the value of $\bar{v}_3$ corresponds to the finite concentration c_3. The value of $(\partial\rho/\partial c_2)_\mu$ is often expressed, in analogy to simple two-component systems, by introducing (Casassa and Eisenberg, 1964) an apparent quantity, ϕ', defined by

$$(\partial\rho/\partial c_2)_\mu \equiv 1 - \phi'\rho^0 \quad (4)$$

To determine ξ_3 from eq 3, it is necessary to evaluate the density increment $(\partial\rho/\partial c_2)_\mu$ and the partial volume $\bar{v}_2{}^0$.[2]

$$\bar{v}_2{}^0 = [1-(\partial\rho/\partial c_2)_{\mathrm{P,m}}]/\rho^0 \quad (5)$$

where the subscript m indicates constant composition on the molality scale; the values for $\bar{v}_3$ may be derived from the literature. Highly accurate data are required throughout as $(\partial\rho/\partial c_2)_\mu$ is quite close to $(1-\bar{v}_2{}^0\rho^0)$ and we are therefore calculating the difference between two large numbers.

Whereas the basis for the molality in the definition of ξ_3 was component 1, water, the symmetry of the system specifies that the reduced density increment, due to addition of component 2, may be expressed to within the same assumptions as above, in the equivalent form

$$(\partial\rho/\partial c_2)_\mu = (1-\bar{v}_2{}^0\rho) + \xi_1'(1-\bar{v}_1\rho) \quad (6)$$

where $\xi_1' = (\partial w_1'/\partial w_2')_\mu$ and the primed quantities represent weight molalities, w_i', expressed in gram per gram of component 3. No basic advantage accrues from the use of eq 6 over eq 3. Sometimes though a given parameter may be preferable to another one under conditions of a specific experiment. The connection between ξ_3 and ξ_1' is easily established by use of procedure suggested by Hade and Tanford (1967), derived from the fact that, independent of any definition of the molality

[2] Because of the practical difficulty of adding "dry" component 2 to a specified solvent mixture a method was devised (Cohen and Eisenberg, 1968) based on a weighing procedure, first applied to DNA solutions and more recently also (Reisler and Eisenberg, 1969) to proteins, which simplifies the determination of correct values of $\bar{v}_2{}^0$ of biological macromolecules.

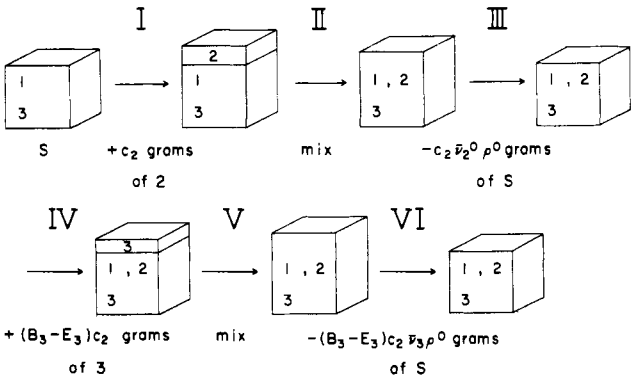

FIGURE 1: Schematic representation of addition (I) of component 2 to 1 ml of solvent mixture S, mixing (II); restoration of original volume by removal (III) of solvent mixture S; addition (IV) of component 3; mixing (V) and removal (VI) of solvent mixture S to restore original volume of 1 ml. Steps IV–VI are designed to restore original value μ_3 in solvent mixture S; μ_3 was disturbed by addition of component 2 in steps I–III.

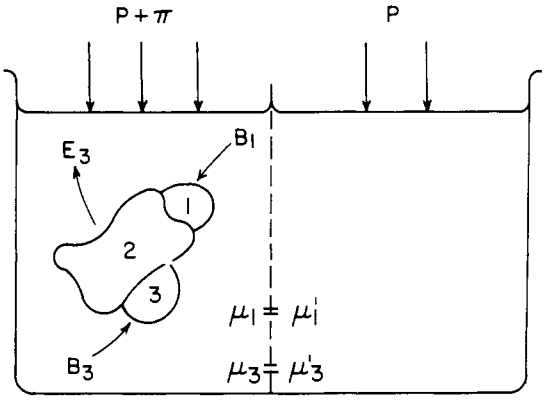

FIGURE 2: Schematic representation of binding, B_1, of component 1 and, B_3, of component 3 and Donnan exclusion E_3 of the latter, to the macromolecular component 2. The right-hand compartment, which does not contain component 2, is separated from the left-hand compartment by a membrane permeable to components 1 and 3; Π is the osmotic pressure required for complete thermodynamic equilibrium.

scale, the proportionality $1:w_2:w_3 = w_1':w_2':1$ must hold. More simply, we use, as before (Cohen and Eisenberg, 1968), for the case of vanishing concentration, c_2

$$c_1 + c_3 = \rho^0$$

and

$$c_1\bar{v}_1 + c_3\bar{v}_3 = 1$$

to give

$$c_1(1 - \bar{v}_1\rho^0) + c_3(1 - \bar{v}_3\rho^0) = 0 \qquad (7)$$

Comparison of eq 6 and 3 substitution of eq 7 yields

$$\xi_1' = -\xi_3/w_3 \qquad (8)$$

The weight molalities are related to the volume concentrations by $w_i = c_i/c_1$ and $w_i' = c_i/c_3$.

The interaction parameter ξ_3 as well as ξ_1' are thermodynamic operational quantities and do not reflect any specific molecular mechanism per se. They are sometimes called preferential interaction parameters but this may be a misleading designation because of the intuitive tendency to associate a positive value of the parameter with physical binding of either component 1 or 3 to the protein entity. Positive ξ_3, for instance, may indicate binding of salt but this does not lead (compare Gordon and Warren, 1968, for instance) to "partial or complete exclusion of other solute components from the surface of the protein". Negative values of ξ_3 (with the sign as defined here) may indicate salt rejection, which usually results from an electrostatic Donnan mechanism. This may be, as we shall show below, equivalent to water "binding" (positive ξ_1') and cannot easily be distinguished from it. This is sometimes called net hydration (cf., for instance, Ifft and Vinograd, 1966). The connection between interdependent parameters has been given before (Lauffer, 1964; Cohen and Eisenberg, 1968; Bull and Breese, 1970a,b; Timasheff, 1970; Inoue and Timasheff, 1972) but we feel that the present exposition is simple and rigorous, and can easily be extended to concentrated systems and to systems containing any number of independent components. For a detailed discussion and the consequences deriving from the addition of an additional diffusible nonionic low-molecular-weight component 4 to the system discussed above, see Eisenberg (1976).

It is now possible to proceed one step further and to attempt to evaluate the phenomena described in terms of a "realistic" physical model. To do this, we perform the following Gedanken experiment (Figure 1). To 1 ml of a mixed solvent, containing components 1 and 3, we add c_2 grams of component 2 (step I); we let counterions dissociate and physical binding of components 1 and 3 to component 2 (if appropriate) occur upon mixing (step II). At this stage, volume expansion or contraction may come about, but the total composition (on a weight molality basis) remains unchanged. The volume occupied by component 2 is $c_2\bar{v}_2^0$ and the partial specific volume $\bar{v}_2^0$ takes into account all processes having occurred on a molecular scale, without change in solvent composition. To restore the volume to 1 ml, we remove (step III) $c_2\bar{v}_2^0\rho^0$ grams of the original solvent mixture of density ρ^0.

In the next steps of our imaginary process, we aim to restore the values μ_1 and μ_3, disturbed by the addition of component 2, to their original values, by appropriate modification of solvent composition.[3] In doing so, we should, however, bear in mind that the following are model considerations and they, therefore, do not convey the same infallibility as the formally correct, but rather poorer in content, thermodynamic derivations.[4] Yet they make possible to rationalize some notions about physical binding of electroneutral components in nucleic acid and protein systems. Simple-minded interpretation of the preferential interaction parameter leads to seemingly inconsistent results.

We denote binding of component 3 (salt) and electrostatic (Donnan) exclusion of the same by the quantities B_3 and E_3 and binding of component 1 (water) by B_1. All these above quantities are expressed in grams per gram of the macromolecular component 2. Binding of components 3 and 1 to component 2 is not exclusive and may be located in different domains of the protein or nucleic acid (Figure 2). Positive B_3

[3] In the establishment of the original values for μ_1 and μ_3, we neglect the effect on the density of the system of the small pressure increase (of osmotic origin) following introduction of component 2; this neglect is inconsequential in dilute protein solutions in its effect on solution density.

[4] We quote here a statement by Kac (1969): "Models are for the most part, caricatures of reality, but if they are good, like good caricatures, they portray, though perhaps in a distorted manner, some of the features of the real world. The main role of models is not so much to explain and to predict—though ultimately these are the main functions of science—as to polarize thinking and to pose sharp questions. Above all, they are fun to invent and to play with, and they have a particular life of their own. The 'survival of the fittest' applies to models even more than it does to living creatures".

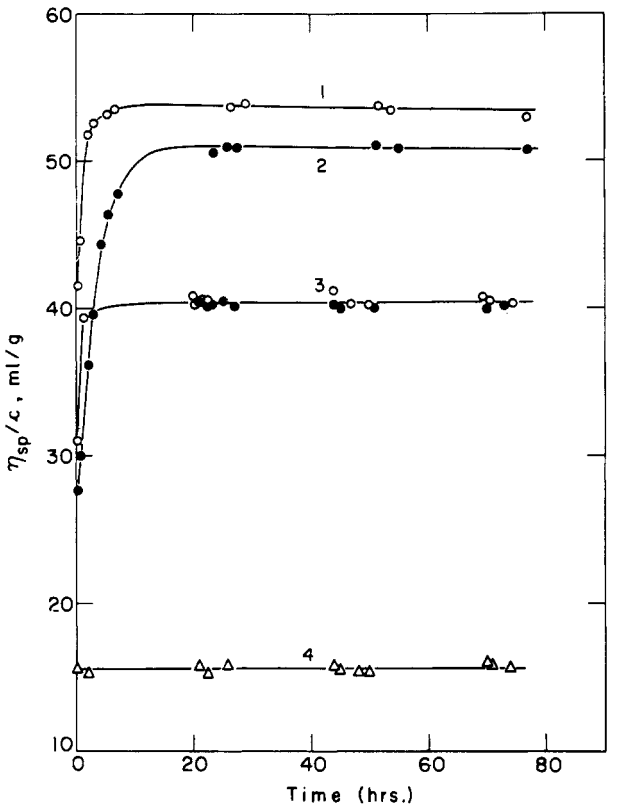

FIGURE 3: Viscosity of bovine serum albumin solutions at 25 °C, as a function of time; (1) 6 M GuHCl, 0.1 M β-mercaptoethanol; (2) 6 M GuHCl, 0.01 M DTE; (3) 3 M GuHCl, (○) 0.1 M β-mercaptoethanol, (●) 0.01 M DTE; (4) 3 M GuHCl.

values remove salt from the bulk of the solvent, which may be replaced by influx of component 3 across a semipermeable membrane from an "infinite" outer solvent compartment, which is free of component 2. Contrarily, binding of water, B_1, corresponds to an effective increase in the concentration of component 3 (with concomitant increase in μ_3) and, therefore, outflow of component 3 into the outer solvent compartment; the Donnan effect, E_3, acts in the same direction. We therefore add $(B_3 - E_3)c_2$ grams of component 3 in step IV of Figure 1, to allow for salt binding and Donnan exclusion, mix (step V), and remove in step VI $(B_3 - E_3)c_2\bar{v}_3\rho^0$ grams of the original solvent mixture, occupying the same volume; $\bar{v}_3$ corresponds to the value for the free solvent mixture (at the proper concentration of component 3 in the protein-free solvent). The change in volume inherent in the binding process has already been absorbed in the experimental value of $\bar{v}_2^0$. To account for binding of component 1, we proceed by a similar argument. We have assumed that consideration of binding and Donnan exclusion and application of the osmotic pressure (with inconsequential effects on the solution densities) restores the original values of the chemical potentials of the solvent components to the values in the solvent in dialysis equilibrium with the protein solution.

In mathematical terms, the total change in density is given by

$$\left(\frac{\partial\rho}{\partial c_2}\right)_\mu = (1 - \bar{v}_2^0\rho^0) + (B_3 - E_3)(1 - \bar{v}_3\rho^0) + B_1(1 - \bar{v}_1\rho^0) \quad (9)$$

Substitution of $(1 - \bar{v}_1\rho^0)$ from eq 7 into eq 9 yields

$$\left(\frac{\partial\rho}{\partial c_2}\right)_\mu = (1 - \bar{v}_2^0\rho) + (B_3 - E_3 - w_3B_1)(1 - \bar{v}_3\rho^0) \quad (10)$$

From a comparison of eq 10 and 3, we note that

$$\xi_3 = B_3 - E_3 - w_3B_1 \quad (11)$$

and the preferential interaction parameter, ξ_3, can be expressed in terms of physical interactions based on the above presented model considerations. Application of eq 8 to eq 11 immediately yields

$$\xi_1' = B_1 - \frac{B_3 - E_3}{w_3} \quad (12)$$

Both preferential interaction parameters can therefore be expressed in terms of the relevant model parameters. Applications to proteins in GuHCl and to nucleic acid solutions in high salt (NaCl and CsCl) will be discussed below. The Donnan term can, in favorable circumstances, be estimated from first principles by solving the Poisson–Boltzmann equation with appropriate boundary conditions. The binding terms B_1 and B_3 may be estimated from other types of experiments, yet one should keep in mind that operationally different concepts of binding from the ones postulated above may apply in other experiments. Reasonable estimates may, nevertheless, be derived. B_1 and B_3 may depend on w_3 and this should be kept in mind when analyzing the parameters in eq 11 and 12. An interesting experiment consists in locating an additional neutral component 4, in which case it can be shown (Eisenberg, 1976) that eq 11 remains unchanged, and an additional interaction parameter ξ_4 can be derived

$$\xi_4 = B_4 - w_4B_1 \quad (13)$$

If the binding, B_4, of component 4 to component 2 is negligibly small, then eq 11 and 13 can be solved for B_1 and B_3 at given w_3 and w_4; this can then be repeated for various values of these concentrations. An extensive study of this type has, to our mind, not been undertaken.[5] We shall, in our analysis below, restrict ourselves to the three-component system and attempt to determine the binding parameter from eq 11 by varying w_3; we will assume that, over the range of this study, the binding and exclusion parameters are insensitive to this change.

Results and Discussion

Viscosity Measurements. In order to determine conditions under which complete unfolding of the bovine serum albumin chains would result, the time dependence of the reduced specific viscosity, η_{sp}/c, at 25 °C, at a concentration c = 3.5 mg/ml, was determined under various experimental conditions. In our previous work (cf. Figure 2 of Reisler and Eisenberg, 1969), we have studied η_{sp}/c of bovine serum albumin (at c = 4.8 mg/ml) in 6 M GuHCl, at 25 °C, with varying amounts of β-mercaptoethanol. It was concluded that a concentration of 0.1 M of this reducing reagent was required for complete unfolding; the viscosity values corresponded closely to the values previously found by Tanford et al. (1967). In this work, we looked for conditions to substitute β-mercaptoethanol by lower concentrations of DTE. In Figure 3, we show the time dependence of η_{sp}/c under various conditions. Curves 3 and 4 refer to 3 M GuHCl; the data in curve 4 reveal that in the

[5] Stevens and Lauffer (1965) and Jaenicke and Lauffer (1969) have described a novel, if protracted, method to measure $(\partial\rho/\partial c_2)_\mu$ by determining the buoyant weight of a dialysis bag enclosing protein solution, in equilibrium with suitable reference solutions; they further added a fourth component, glycerol, to the equilibrating buffered solutions, although the individual interaction parameters ξ_3 and ξ_4 were not separately established.

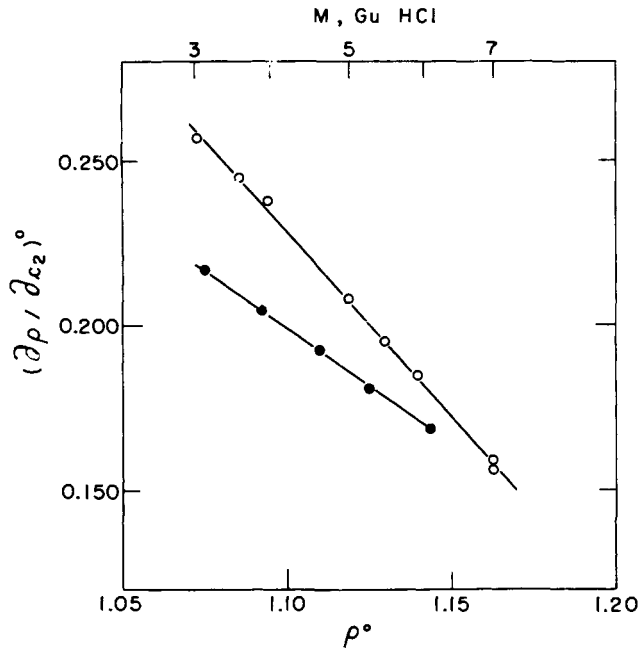

FIGURE 4: Reduced density increments $(\partial\rho/\partial c_2)$ in bovine serum albumin solutions at constant composition, m (●), and at constant chemical potential, μ, of diffusible solutes (○), against GuHCl concentration at 25 °C.

absence of either β-mercaptoethanol or DTE incomplete unfolding results. In curve 3, the open circles refer to 0.1 M β-mercaptoethanol and the filled circles to 0.01 M DTE; identical results are obtained, indicating that 0.01 M DTE is sufficient to produce the unfolding of the chains. Curves 1 and 2 refer to experiments in 6 M GuHCl, 0.1 M β-mercaptoethanol, and 0.01 M DTE, respectively. The data are close enough to justify density and partial volume measurements in the presence of the lower DTE concentrations only. The shape of the unfolded protein may depend to some extent on the interaction with the solvent system but this is of no consequence with respect to the measurements reported here. We therefore used 0.01 M DTE in all the density measurements reported in this work.

Partial Specific Volumes. A summary of the partial specific volume determinations at 25 °C in water and in a series of GuHCl concentrations extending from 3 to 6 M GuHCl is given in Table I. $\bar{v}_2$ was found to be independent of the protein concentration, which varied between 8 and 15 mg/ml; $\bar{v}_2^0$ of the enzyme in water is 0.734, in agreement with literature results (Hunter, 1967). In GuHCl, we find $\bar{v}_2^0$ to be somewhat lower (0.728 ± 0.001) but independent of GuHCl concentration. We conclude that the volume, ΔV, of unfolding is −0.006 ml/g or about 400 ml/mol of protein. This result is identical with the previous result (Reisler and Eisenberg, 1969) from our measurements of ϕ' and ξ_3, as given by Hade and Tanford (1967), in 6 M GuHCl only. More precise values for ΔV could, in principle, be obtained by proper dilatometric measurements but for the absolute value of $\bar{v}_2$ absolute density measurements are required. The dilatometric measurements of Katz (1968) in the GuHCl concentration range 2–3.8 M indicate a significantly smaller value for ΔV but no reducing agent is mentioned (compare Figure 3), and, furthermore, it was not established that equilibrium conditions were attained. Our experimental values for $(\partial\rho/\partial c_2)_{P,m_3}$ (Table I) have also been plotted as filled circles in Figure 4, as a function of ρ^0.

Density Increments at Constant μ. The density increments at constant chemical potentials of diffusible solutes are shown

TABLE I: Partial Specific Volumes in Aqueous Solutions of Bovine Serum Albumin at 25 °C.

Solvent System[a]	ρ^0	$\left(\frac{\partial\rho}{\partial c_2}\right)_{p,m_3}^0$	σ^b	$\bar{v}_2^0$
H_2O	0.997	0.267_8	0.0004	0.734
3 M GuHCl	1.0750	0.217_5	0.0012	0.728
3 M GuHCl	1.0749	0.216_4	0.0012	0.729
3.8 M GuHCl	1.0925	0.205_0	0.0003	0.728
4.5 M GuHCl	1.1085	0.193_1	0.0020	0.728
4.5 M GuHCl	1.1095	0.191_1	0.0016	0.729
5.2 M GuHCl	1.1252	0.180_4	0.0011	0.728
6 M GuHCl	1.1432	0.168_4	0.0005	0.727
6 M GuHCl	1.1432	0.168_5	0.0014	0.727

[a] All experiments in GuHCl solutions in the presence of 0.01 M DTE. [b] Standard deviation.

TABLE II: Reduced Density Increments at Constant Chemical Potentials of Diffusible Solutes and Apparent Volumes ϕ' in Aqueous Solutions of Bovine Serum Albumin at 25 °C.

Solvent[a]	ρ^0	$\left(\frac{\partial\rho}{\partial c_2}\right)_\mu^0$	σ^b	ϕ'
0.2 M NaCl	1.0053	0.263_7	0.0004	0.732
3 M GuHCl	1.0729	0.256_9	0.0013	0.693
3.5 M GuHCl	1.0853	0.244_7	0.0017	0.696
4 M GuHCl	1.0945	0.237_5	0.0015	0.697
5 M GuHCl	1.1185	0.208_1	0.0019	0.708
5 M GuHCl	1.1193	0.208_0	0.0025	0.708
5.5 M GuHCl	1.1302	0.194_8	0.0012	0.712
6 M GuHCl	1.1407	0.185_1	0.0012	0.714
6 M GuHCl	1.1413	0.185_0	0.0035	0.714
7 M GuHCl	1.1626	0.156_4	0.0005	0.726
7 M GuHCl	1.1628	0.159_4	0.0035	0.723
6 M GuHCl[c]	1.1412	0.178_4	0.0023	0.720
6 M GuHCl[d]	1.1407	0.178_5	0.0010	0.720
6 M GuHCl[d]	1.1415	0.175_9	0.0015	0.722

[a] In the presence of 0.01 M DTE, unless otherwise specified. [b] Standard deviation. [c] In the presence of 0.1 M β-mercaptoethanol, this work. [d] In the presence of 0.1 β-mercaptoethanol, Reisler and Eisenberg (1969).

in column 3 of Table II, and in Figure 4 (unfilled circles). In column 5 of Table II, we show that ϕ' (cf. eq 4) decreases upon denaturation (compare the result in 0.2 M NaCl and 3 M GuHCl) but again increases with increasing GuHCl concentration. A similar result was previously reported for aldolase (Reisler and Eisenberg, 1969) and points out the danger of using ϕ' as a constant, independent of experimental conditions. Examination of the last three rows of Table II reveals that we could successfully reproduce our previous results in 0.1 M β-mercaptoethanol, but that the corresponding results in 6 M GuHCl, in the presence of 0.01 M DTE, are not identical with the results in the presence of the higher concentrations of β-mercaptoethanol. Only results under strictly comparable experimental conditions can therefore be compared.

Interaction Parameters. The interaction parameter, ξ_3, as a function of GuHCl concentration has been derived from eq 3 for a three-component system.[6] We see (Figure 5) that,

[6] Strictly speaking, DTE represents an additional component, but its distribution was not determined. Therefore, an additional reason for lowering the concentration of the reducing agent to a minimum was to reduce as much as possible the influence on the density due to addition of more solvent components.

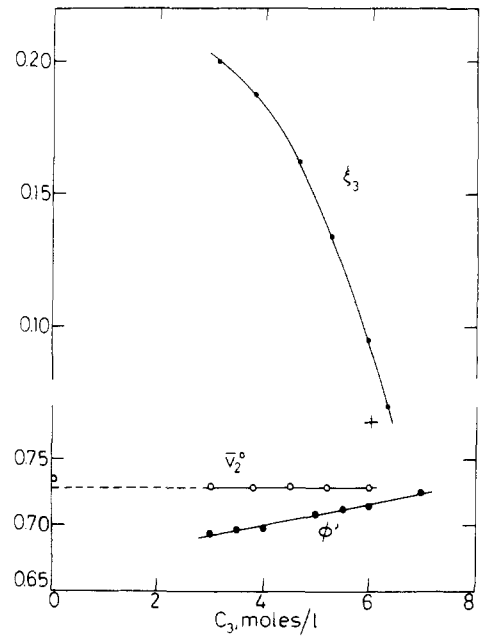

FIGURE 5: Interaction parameters, ξ_3, and partial, $\bar{v}_2^0$ and apparent, ϕ', volumes in bovine serum albumin solutions, as a function of GuHCl concentration, at 25 °C; (+) ξ_3 from Hade and Tanford (1967).

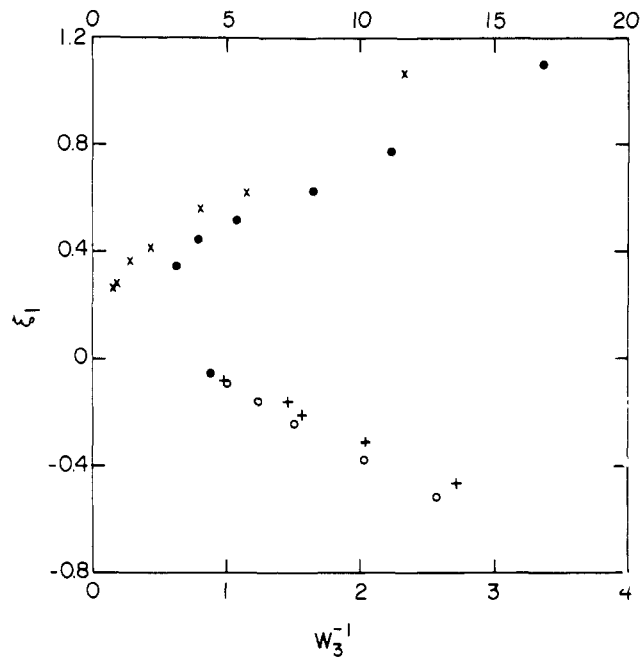

FIGURE 6: Preferential interaction (hydration) parameter ξ_1', as a function of reciprocal salt concentration, w_3 (g of salt/g of water), for (○) bovine serum albumin and (+) aldolase (Reisler and Eisenberg, 1969) solutions in GuHCl (lower scale); (●) NaDNA in NaCl and (x) CsDNA in CsCl solutions (upper scale; Cohen and Eisenberg, 1968).

similarly to the results for aldolase, ξ_3 decreases sharply with increasingly GuHCl concentration. Our result agrees well with the result of Hade and Tanford (1967) at 6 M GuHCl (cf. Figure 5). Similar agreement had been obtained for aldolase. In Figure 5, we also show graphically the result from Tables I and II that $\bar{v}_2^0$ is independent of GuHCl concentration, and ϕ' increases over the range of GuHCl concentrations studied.

In solutions of DNA in aqueous NaCl and CsCl (Cohen and Eisenberg, 1968; Eisenberg, 1974, 1976), both $\bar{v}_2^0$ and ϕ' increase with an increase concentration of the salts; the values of ξ_3 are negative at low concentration of salt and become more negative with increasing concentration of salts.

It is most intuitive, perhaps, to analyze the interaction with water and with salts in terms of the interaction parameters ξ_1' calculated from ξ_3 by eq 8 and plotted in Figure 6 against w_3^{-1} for bovine serum albumin (this work), aldolase (results of Reisler and Eisenberg, 1969), and DNA in NaCl and in CsCl (results of Cohen and Eisenberg, 1968). Consider bovine serum albumin first. ξ_1' is negative and further decreases with a decrease in w_3. So, apparently, water is not "bound". Yet consideration of eq 12, the derivation of which was based on the schematic model of Figure 2, provides a very reasonable explanation. For the type of behavior exhibited by bovine serum albumin in Figure 6, we must have $B_3 > E_3$; that is, whatever Donnan exclusion of salt may exist, it must be smaller than binding of component 3 to the protein moiety. If we now assume that, over the range of w_3 concentrations studied, the plot is linear, then we immediately deduce from eq 12[7] B_1 to be 0.2 g of water bound/g of protein and a minimum B_3 (because of possible Donnan exclusion E_3) of 0.28 g/g of protein. Taking the molecular weight of bovine serum albumin to be 69 000 and 579 amino acids per protein molecule (Dayhoff, 1972), we calculate 1.3 water molecules and 0.35 GuHCl molecule bound, on the average, per amino acid residue. The corresponding results for aldolase are 0.16 g of water/g of protein and 0.23 g of GuHCl/g of protein, which corresponds to (taking for the molecular weight of the aldolase subunit 40 000 and 362 amino acid residues per subunit; Harris et al. (1969)) 1 water and 0.27 GuHCl molecule per amino acid residue. Lee and Timasheff (1974) ascribe considerably higher values for water binding (based on known hydration of constituent amino acids) and estimate salt binding from measurements at a single GuHCl concentration (6 M). Their conclusion is based on formulation of interaction parameters (Inoue and Timasheff, 1972) very similar to our own, although the actual derivation of their model proceeds via a somewhat different route.

The behavior of the DNA solutions is radically different. Here (Figure 6) ξ_1 is positive and increases with decrease in w_3. For this type of behavior, we must have (cf, eq 12) $E_3 > B_3$; that is, Donnan exclusion, E_3, must outweigh any binding of, B_3, salt ($E_3 > B_3$). If we again take that over the range examined ξ_1 varies linearly with w_3^{-1}, then we find for NaDNA in NaCl, B_1 equal to 0.2 g of water/g of NaDNA and a minimum value E_3 (because of possible binding of salt B_3) equal to 0.054 g of NaCl/g of NaDNA. In molar units this corresponds to 3.7 mol of water bound and 0.3 mol of NaCl excluded per phosphate or base (of average molecular weight 331). These numbers are very reasonable, both in terms of water molecules, which can be accommodated in the wide and narrow grooves of double helical DNA, and the Donnan exclusion, which can be estimated (Gross and Strauss, 1966) from solution of the Poisson-Boltzmann equation for rod-like particles. For CsDNA in CsCl, we find from Figure 6 $B_3 \equiv 0.24$ g of water/g of CsDNA and $E_3 - B_3 \sim 0.07$ g of CsCl/g of CsDNA, or in molar terms 5.9 mol of water bound and 0.19 mol of CsCl excluded per mol of phosphate or base (of average molecular weight 441).

It is worthwhile, in closing, to state once more why our emphasis is not on a quantitative description of water and salt binding and exclusion to the macromolecular moieties but

[7] Linearity is assumed over a limited range of concentrations only, and extrapolation to infinite w_3 is not required. The parameters derived, therefore, do not necessarily apply beyond this range.

rather on the physical insight to be gained by the description presented—in support of this thesis we again point out the striking difference in behavior between the DNA and the protein solutions in the respective systems chosen for illustration (other types of behavior could well arise under different circumstances). Solvation and ion binding are operational concepts, and different results will result if definitions based on either thermodynamic (as above), spectroscopic, or hydrodynamic phenomena are considered. Yet we would certainly expect that a given class f phenomena should, as a rule, give compatible results, and it should, furthermore, be possible to find a connection between definitions deriving from different classes of physical phenomena. The density measurements described in this work are difficult and the interaction parameters are calculated as relatively small differences between large numbers. The model quantities B_1, B_3, and E_3 may not necessarily be independent on concentration of component 3—addition of a fourth noninteracting component, as proposed in the theoretical part to circumvent this ambiguity, would lead to more reliable interpretation of binding values. No doubt the model analysis satisfies the requirement in the quotation from Kac (1969), in that our thinking on this topic has now been polarized and sharper questions can be posed.

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