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Light-Scattering Study of the Interaction of β -Lactoglobulin Solvent Components in the System Water-2-Chloroethanol

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The preferential binding of solvent components to the macromolecular solute has been investigated by light scattering for the system 2-chloroethanol-water- β -lactoglobulin A. In the presence of 0.02M NaCl and 0.01M HCl, 2-chloroethanol is bound preferentially to the protein up to ca. 60 volume % of that component. Above this concentration of the organic solvent, water is bound preferentially in the immediate domain of the β -lactoglobulin. The necessary working equations are developed.

Light scattering is a powerful tool for studying interactions of biological macromolecules in solution. Since the first theoretical analysis of light scattering in multicomponent systems by Zernike (28) in 1918, this theory has been extended in great detail by several investigators. Thus, at present, the thermodynamic interactions which take place between macromolecules and between macromolecules and solvent components can be rigorously characterized. Some of the more important contributions to this field can be found in the work of Ewart, Roe, Debye, and McCartney (8), Brinkman and Hermans (5), Kirkwood and Goldberg (10), Stockmayer (18), Shoenji (14), Ooi (12), Vrij and Overbeek (26, 27), Stigter (16, 17), Casassa and Eisenberg (6, 7), Strazielle and Benoit (19), Timasheff and Kronman (21), and others.

Using the combined notation of Stockmayer (18) and of Kirkwood and Goldberg (10), and expressing the chemical potential functions

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according to Scatchard (13), we obtain the light-scattering equation for a three-component system:

$$\frac{H''V\Psi_2^2}{\Delta\tau} = \frac{a_{22} - a_{12}^2/a_{11}}{\left[1 + \frac{\Psi_1}{\Psi_2} \left(\frac{\partial m_1}{\partial m_2}\right)_{T,p,\mu_1}\right]^2} \quad (1)$$

$$H'' = \frac{32\pi^3 n^2}{3N\lambda^4} \quad \Psi_i = \left(\frac{\partial n}{\partial m_i}\right)_{T,p,m_j}$$

$$a_{ij} = \left(\frac{\partial \ln a_i}{\partial m_j}\right)_{T,p,m}$$

where n is the refractive index of the solution, N is Avogadro's number, λ is the wavelength of the light *in vacuo*, V is the volume of solution in milliliters containing 1000 grams of principal solvent, $\Delta\tau$ is the excess turbidity of the solution over that of the solvent, m_i is the molality of component i (moles per 1000 grams of principal solvent), and a_i is the activity of component i . The principal solvent is component 0, the added solvent or electrolyte is component 1, and the macromolecular solute is component 2. Subscript m denotes constancy of all the m_i not indicated as variables in the corresponding derivative.

Expressing activity a_2 in terms of the excess chemical potential (13), we obtain from Equation 1:

$$\frac{H''V\Psi_2^2 m_2}{\Delta\tau} = \left[1 + \frac{\Psi_1}{\Psi_2} \left(\frac{\partial m_1}{\partial m_2}\right)_{T,p,\mu_1}\right]^{-2} \left[1 + \left\{\beta_{22} - \frac{(a_{12})^2}{a_{11}}\right\} m_2\right]$$

$$\beta_{ij} = \frac{1}{RT} \left(\frac{\partial \mu_i^{(e)}}{\partial m_j}\right)_{T,p,m} \quad (2)$$

where $\mu_i^{(e)}$ is the excess chemical potential of component i , defined by $\mu_i = RT\Sigma\nu_i \ln m_i + \mu_i^{(e)} + \mu_i^\circ(T,p)$, and $\Sigma\nu_i$ is the number of particles into which component i dissociates.

In practice, concentration measurements are done more easily on a volume basis than on a weight basis. Changing the concentration units from molality, m_i , to grams per milliliter of solution, C_i , results in

$$\frac{H''C_2}{\Delta\tau} \left(\frac{\partial n}{\partial C_2}\right)_{m_1}^2 = \frac{1}{M_2(1+D)^2} \times$$

$$\left[1 + \frac{C_2}{RT} \left\{\left(\frac{\partial \mu_2^{(e)}}{\partial C_2}\right)_{m_1} + \left(\frac{\partial \mu_1^{(e)}}{\partial C_2}\right)_{m_1} \left(\frac{\partial m_1}{\partial m_2}\right)_{T,p,\mu_1} + 2RT\bar{V}_2\right\}\right] + O(C_2^2)$$

$$\begin{aligned}
D &= \frac{M_1}{M_2} \frac{(1 - C_1 \bar{V}_1)_{m_2} (\partial n / \partial C_1)_{m_2}}{(1 - C_2 \bar{V}_2)_{m_1} (\partial n / \partial C_2)_{m_1}} \left(\frac{\partial m_1}{\partial m_2} \right)_{T, p, \mu_1} \\
\left(\frac{\partial m_1}{\partial m_2} \right)_{T, p, \mu_1} &= - \frac{\beta_{12}}{\frac{\sum v_1}{m_1} + \beta_{11}} = \frac{M_2 (1 - C_2 \bar{V}_2)_{\mu_1} (\partial C_1)}{M_1 (1 - C_1 \bar{V}_1)_{\mu_2} (\partial C_2)_{T, p, \mu_1}} \\
&= \frac{[\mu_2^{(e)} / \partial m_2]_{T, p, \mu_1} - [\partial \mu_2^{(e)} / \partial m_2]_{m_1}}{(\partial \mu_2^{(e)} / \partial m_1)_{m_2}}
\end{aligned} \tag{3}$$

On extrapolation to zero concentration of the macromolecular component, the last of Equations 3 reduces to

$$\left(\frac{\partial m_1}{\partial m_2} \right)_{T, p, \mu_1}^{\circ} = \frac{M_2}{1000} \frac{m_1}{C_1 (\bar{V}_o)_{m_1, m_2}} \left[\left(\frac{\partial C_1}{\partial C_2} \right)_{T, p, \mu_1}^{\circ} + C_1 (\bar{V}_2)_{m_1} \right] \tag{3'}$$

where M_i is the molecular weight of component i , \bar{V}_i is its partial specific volume measured at equal molality of component i in the solution and the reference solvent, R is the gas constant, and T is the thermodynamic temperature. The conditions for applicability of Equation 3 are that the molality, m_1 , of component 1 be kept identical in solvent and solution in all the light-scattering and differential refractometric measurements. On the other hand, if both measurements are carried out at conditions at which the chemical potential of component 1 and the pressure are held fixed in the solvent and the protein solution, the light-scattering equation assumes a pseudo-two-component form (7, 12, 16, 26):

$$\begin{aligned}
\frac{H'' C_2 (\partial n)^2}{\Delta \tau (\partial C_2)_{\mu_1}} &= \\
\frac{1}{M_2} \left[1 + \frac{C_2}{RT} \left\{ \left(\frac{\partial \mu_2^{(e)}}{\partial C_2} \right)_{T, p, \mu_1} + 2RT \left[\bar{V}_2 + \frac{M_1}{M_2} \bar{V}_1 \left(\frac{\partial m_1}{\partial m_2} \right)_{T, p, \mu_1} \right] \right\} \right] &+ O(C_2^2)
\end{aligned} \tag{4}$$

While applicability of Equation 4 requires that all measurements be done at constant pressure, in practice it is much simpler to do the measurements in a state of dialysis equilibrium—*i.e.*, at constant T , μ_0 , and μ_1 . The error introduced by the practical approximation $(\partial m_1 / \partial m_2)_{T, p, \mu_1} = (\partial m_1 / \partial m_2)_{T, \mu_0, \mu_1}$ is minor (16). In this manner, light-scattering measurements on protein solutions in a mixed solvent, with and without dialysis, make possible the determination of both the molecular weight and the extent of preferential interaction of the protein with one of the solvent components (21).

Procedure. The molecular weight, M_2 , of component 2, is obtained by Equation 4 from light-scattering and refractive index measurements

on protein solutions which have been dialyzed against the solvent. The extent of aggregation of the protein can be determined from this molecular weight. Next, the measurements are repeated on undialyzed protein solutions, and the left side of Equation 3 is plotted *vs.* the concentration, C_2 . The deviation from $1/M_2$ of the intercept at $C_2 = 0$ makes it possible to evaluate D and, thus, $(\partial m_1/\partial m_2)_{T,p,\mu_1}$. The nonideality terms β_{ij} can be obtained then by combining D with the initial slope of the plot. If β_{11} is not known from other measurements, it can usually be assumed to be zero, as a first approximation.

A light-scattering study of this nature has been carried out on solutions of β -lactoglobulin A (β -A) dissolved in mixtures of water with 2-chloroethanol in the presence of 0.02M NaCl and 0.01M HCl. 2-Chloroethanol is known to be a structure-forming protein denaturant and can be expected to interact with proteins; freshly distilled, its refractive index at 436 $m\mu$ is 1.447, different from that of water, 1.340. This results in a large value of $\partial n/\partial C_1$.

Experimental

β -Lactoglobulin A was prepared by standard techniques (1) and recrystallized before use. Solvents were double-distilled in all-borosilicate glass stills and used immediately. Light-scattering measurements were performed in the Brice photometer (4) at 25°C. with the 436- $m\mu$ mercury arc line. Light-scattering measurements at constant concentration of 2-chloroethanol were carried out according to a modified Dintzis technique (20), as described previously (23). Solutions for operations at constant chemical potential of chloroethanol were prepared as follows. In each series, solutions (about 5 ml.) of β -A at different concentrations were made in a given water-chloroethanol mixture, dialyzed overnight against a large excess of the same solvent, and passed through an ultra-fine sintered-glass filter (2) after centrifuging. Refractive index increments were measured on the Brice differential refractometer (3) at 25°C. and 436 $m\mu$. Protein concentrations were measured on a Zeiss PMQ II spectrophotometer at 278 $m\mu$. An absorptivity value of 0.96 liter/cm.-gram (25) was used.

Results

Light Scattering at Constant Chemical Potential. In these measurements the concentration of chloroethanol was varied from 5 to 60 volume %. The intensity of scattering of β -A dissolved in these media remained constant for at least 24 hours if the β -A concentration were less than 2 grams per liter. Typical results of experiments at constant chemical

potential are shown in Figure 1, where the values of $H''(\partial n/\partial C_2)_{T,\mu_0,\mu_1} C_2/\Delta\tau$ are plotted against the protein concentration, C_2 ; the straight lines were calculated by the method of least squares using the data below 2 grams per liter. It is evident that all the data extrapolate essentially to the same intercept. The average value of the molecular weight found in these experiments is 18,700. It is known that at pH below 3.5, native β -A dissociates to a monomer with a molecular weight of *ca.* 18,000 (22); this dissociation is enhanced by low salt concentration and a low dielectric constant of the medium (24). The present results extend these previous findings to β -A in water-2-chloroethanol mixtures in the presence of 0.02M NaCl and 0.01M HCl; in these solvents β -A exists in monomeric form.

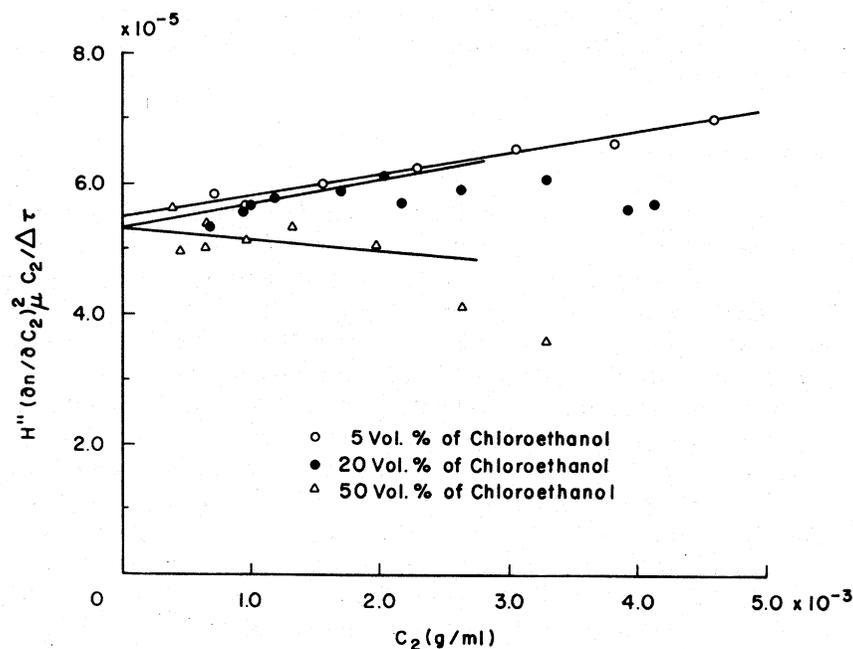


Figure 1. Light-scattering data on β -lactoglobulin A in various concentrations of 2-chloroethanol at constant chemical potential of 2-chloroethanol

Solvent components are water, 2-chloroethanol, 0.02M NaCl, and 0.01M HCl

Light Scattering at Constant Concentration. Measurements were carried out without previous dialysis in solvent mixtures containing up to 80 volume % of 2-chloroethanol. The scattered intensity showed no time dependence up to 24 hours at β -A concentrations below 6 grams per liter, except for the highest concentration of chloroethanol (80%), at which slow changes were seen at protein concentrations above 2 grams

per liter. Typical results are shown in Figure 2, where it can be seen that the intercept is a function of chloroethanol concentration. The variation of M_{app}/M_w , the ratio of the apparent molecular weight to the true molecular weight, with increasing chloroethanol concentration can be calculated from such data. This parameter first rises, then after passing a maximum at *ca.* 40% chloroethanol ($M_{app}/M_w = 1.7$), it drops, becoming less than unity above *ca.* 65% chloroethanol.

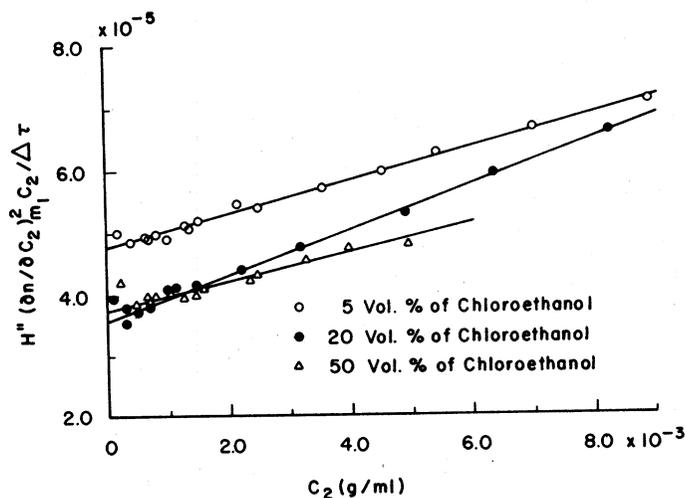


Figure 2. Light-scattering data on β -lactoglobulin A in various concentrations of 2-chloroethanol

Each series of experiments was carried out at constant concentration of 2-chloroethanol. Solvent components. Water, 2-chloroethanol, 0.02M NaCl, and 0.01M HCl

Preferential Solvation. Examination of the data of Figure 2 in terms of Equation 3 shows that below 65% chloroethanol, $(\partial m_1 / \partial m_2)_{T, \mu_0, \mu_1}$ is positive, while above this point it becomes negative. Negative values of this interaction parameter indicate a deficiency of component 1 in the immediate vicinity of molecules of component 2—*i.e.*, preferential hydration of component 2. The extent of hydration is given by Equation 5.

$$\left(\frac{\partial m_0}{\partial m_2}\right)_{T, p, \mu_1} = -\frac{m_0}{m_1} \left(\frac{\partial m_1}{\partial m_2}\right)_{T, p, \mu_1} \quad (5)$$

The interaction between solvent components and the macromolecule is reflected also in the difference between the refractive index increments measured at constant concentration and those found at constant chemical

potential of component 1. The relation between these is given in Equation 6 (6, 7, 12, 14, 16, 17, 26, 27).

$$\left(\frac{\partial n}{\partial m_2}\right)_{\mu_1} = \left(\frac{\partial n}{\partial m_2}\right)_{m_1} + \left(\frac{\partial n}{\partial m_1}\right)_{m_2} \left(\frac{\partial m_1}{\partial m_2}\right)_{\mu_1} \quad (6)$$

Converting from molal to grams per milliliter concentration units, and extrapolating to $C_2 = 0$, gives:

$$\left(\frac{\partial m_1}{\partial m_2}\right)_{T,p,\mu_1}^0 = \frac{M_2}{M_1(1 - \bar{V}_1 C_1)} \left\{ \left(\frac{\partial n}{\partial C_2}\right)_{T,p,\mu_1} - \left(\frac{\partial n}{\partial C_2}\right)_{T,p,m_1} \right\} / \left(\frac{\partial n}{\partial C_1}\right)_{T,p,m_2} \quad (7)$$

where superscript 0 indicates this extrapolation.

The values of the preferential binding of 2-chloroethanol to protein, obtained from light scattering and expressed in terms of moles of this component per mole of protein, are plotted in Figure 3 as a function of chloroethanol concentration. At low concentrations of chloroethanol, β -A has a higher affinity for this component than for water. This affinity

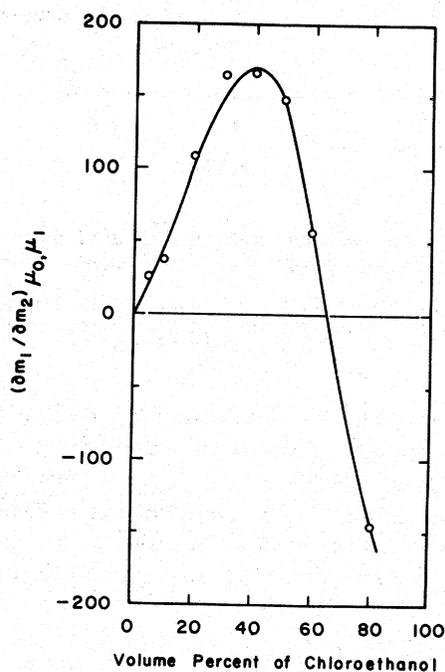


Figure 3. Variation of solvation of β -lactoglobulin A with increasing 2-chloroethanol concentration in water-2-chloroethanol mixtures

increases with an increase in C_2 , reaching a maximum at *ca.* 40 volume %. Above this chloroethanol concentration, it decreases monotonically to negative apparent absorption of chloroethanol after passing through zero at *ca.* 65 volume %. Above this concentration, β -A is preferentially hydrated. Thus, the point at 80 volume % chloroethanol corresponds to the preferential binding of 140 moles of water per mole of protein. These results are similar to those obtained in the serum albumin–water–2-chloroethanol system (15).

Optical rotatory dispersion and circular dichroism measurements have been carried out on β -A in this solvent system; the details are published elsewhere (9). A progressive change in solvent composition from aqueous to 2-chloroethanol induces a gradual change from the native globular structure to one rich in α -helix. This conformational change, however, does not seem to be related in any simple fashion to the variation in the thermodynamic interaction parameters. Further investigations on the effects of such solvent components on this and other proteins are in progress (11).

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