Partial Specific Volume, Expansibility, Compressibility, and Heat Capacity of Aqueous Lysozyme Solutions*

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Density measurements have been made on aqueous lysozyme solutions at 20, 25, and 30°. The apparent specific volumes, \( \phi_a \), and expansibilities, \( \alpha \), have been determined from the density measurements and fitted to a function of concentration (weight per cent). Sound velocities and heat capacities have also been measured for various concentrations of lysozyme-water solutions at 25°. From the density, expansibility, heat capacity, and sound velocity data at 25°, the isothermal compressibility, \( \beta_T \), and the apparent specific compressibility, \( \phi_a \), for lysozyme solutions have been calculated over a range of concentrations. All of the physicochemical properties measured were found to be a linear function of the weight per cent of lysozyme. The number of water molecules hydrated to 1 mol of lysozyme was estimated from the volume and compressibility and found to be 162 at 25°.

Most biological macromolecules are physiologically important when they become associated with water. To understand proteins as a chemical species, basic information is needed on their physicochemical properties and a quantitative characterization of their aqueous solutions. A useful insight into the chemistry of these substances is provided by accurate quantitative measurements of such properties as: density, \( d \); compressibility, \( \beta = -(1/V)(\partial V/\partial p)_T \); expansibility, \( \alpha = (1/V)(\partial V/\partial T)_p \); and heat capacity, \( c_p \). Although some data exist for these properties, most are quite uncertain and little has been done on this aspect of protein chemistry (1). Up to now, the major application of thermodynamic methods to proteins has been the study of folding processes by measuring the changes in heat capacities (2). Other properties, however, can be used to enlighten our knowledge of protein-water interactions.

In this paper, we present data on the aqueous solutions of the conjugated protein, lysozyme, at 25°. Various thermodynamic properties were determined at various concentrations of lysozyme in order to provide basic physicochemical information that hopefully can be used to obtain a better understanding of protein chemistry.

EXPERIMENTAL PROCEDURE

The lysozyme was obtained from Worthington Biochemical Co. and was used without further purification. The densities were measured to a precision of ±3 ppm at 20, 25, and 30° on a flow vibrating densimeter (3) manufactured by SODEV, Inc. The system was calibrated with Copenhagen standard seawater using the densities from Kell (5) and the water heat capacities described in detail elsewhere (6). The system was calibrated at 25° with Copenhagen standard seawater and pure water using the seawater heat capacities from Miller et al. (7) and the water heat capacities tabulated by Stimson (8). The sound velocities were measured to a precision of ±0.02 ms⁻¹ on a single transducer sonic solution monitor manufactured by NuSonics, Inc. The system was calibrated with pure water using the sound velocities for pure water of Kell (9).

All of the solutions were made up by weight using Millipore ion-exchanged water. The pH of the solutions was 6.65 at 25°. The temperature of the bath was set to 20.002° with a platinum resistance thermometer (traceable to the National Bureau of Standards) and a G-2 Mueller Bridge.

RESULTS AND DISCUSSION

The densities of aqueous lysozyme solutions have been measured at 20, 25, and 30° and are given in Table I. The densities are shown as a linear function of weight fraction, \( X_w \), in Fig. 1 and have been fitted by a least squares method to the equation

\[
d = d^0 + A X_w \tag{1}
\]

where \( d^0 \) is the density of pure water and \( A \) is a temperature dependent parameter. The coefficients for Equation 1 determined by a least squares method are: \( d^0 = 0.996677, 0.997075, \) and 0.998237 g ml⁻¹ (5) and \( A = 0.25939, 0.25983, \) and 0.25878 g ml⁻¹, respectively, at 30, 25, and 20°. The standard deviations are 2, 3, and 2 ppm at 30, 25, and 20°.

The partial specific volume of lysozyme, \( \phi_s \), is related to the specific volume (\( \nu = 1/d \)) by the equation

\[
\nu = \bar{\phi} X_w + \bar{\phi} X_s \tag{2}
\]

where \( X_w \) and \( X_s \) are the weight fraction of water 1 and lysozyme 2 and \( \bar{\phi} \) is the partial specific volume of water in the solution. To determine \( \phi_a \) from density data, it is convenient to use the apparent specific volume, \( \phi_a \), concept. The apparent

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Table I

<table>
<thead>
<tr>
<th>$X(10^3)$</th>
<th>$d$</th>
<th>$10^3\omega$</th>
<th>$c$</th>
<th>$c_p$</th>
<th>$10^3\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.07682</td>
<td>0.999874</td>
<td>262.91</td>
<td>1499.23</td>
<td>4.1531</td>
<td>44.992</td>
</tr>
<tr>
<td>0.80723</td>
<td>0.999162</td>
<td>261.38</td>
<td>1498.60</td>
<td>4.1595</td>
<td>45.055</td>
</tr>
<tr>
<td>0.53710</td>
<td>0.998477</td>
<td>259.83</td>
<td>1497.91</td>
<td>4.1662</td>
<td>45.120</td>
</tr>
<tr>
<td>0.36854</td>
<td>0.997769</td>
<td>258.29</td>
<td>1497.30</td>
<td>4.1760</td>
<td>45.182</td>
</tr>
<tr>
<td>0.10777</td>
<td>0.997355</td>
<td>257.37</td>
<td>1496.89</td>
<td>4.1764</td>
<td>45.237</td>
</tr>
<tr>
<td>0.05391</td>
<td>0.997215</td>
<td>257.06</td>
<td>1496.78</td>
<td>4.1777</td>
<td>45.233</td>
</tr>
</tbody>
</table>

The specific volume is defined in terms of specific volumes as

$$\phi_v = (\nu - \phi_v^0) X_1/X_2$$

which can be calculated directly from the density of the solutions.

The apparent specific volumes are shown plotted as a function of $X_2$ in Fig. 2 at various temperatures and were fitted to the equation

$$\phi_v = \phi_v^0 + S_v^* X_2$$

where $\phi_v^0$ is the apparent specific volume at infinite dilution, which is equal to $\phi_v^0$, and $S_v^*$ is the experimental slope. The $\phi_v^0$, $S_v^*$, and standard deviation at various temperatures are given in Table II. The values of $\phi_v^0$ increase with increasing temperature which is similar to the behavior of most electrolytes (10, 11) and hydrated nonelectrolytes (12). This increase in volume can be attributed to the loss of hydrated water molecules at higher temperatures (10-12). The slope, $S_v^*$, is indicative of protein-protein interactions which yield positive volume changes at 20° and negative changes at 30°. The greater loss of the water molecules at 30° appear to make it easier for the protein to overlap with a conservation of space (the negative value, $S_v^*$, is found for a number of hydrophobic electrolytes like the tetraalkylammonium ions) (13).

The expansibility ($\alpha$) of the solutions can be calculated from the effect of temperature on the density data

$$\alpha = -\left(\frac{1}{d}\left(\frac{d\rho}{dT}\right)\right) = \left(\frac{1}{u}\left(\frac{du}{dT}\right)\right)$$

The values of $d\rho$ and $A$ in Equation 1 have been fitted to the equations

$$d\rho = a_1 + b_1 t + c_1 t^2$$

$$A = a_2 + b_2 t + c_2 t^3$$

where $a_1 = 1.000525$, $b_1 = -2.0 \times 10^{-3}$, $c_1 = -4.72 \times 10^{-4}$, $a_2 = 1.7448$, $b_2 = 7.451 \times 10^{-3}$, and $c_2 = -1.618 \times 10^{-3}$ (with a standard deviation of 4 ppm). The $\alpha$ is thus given by

$$\alpha = \left[\frac{(b_1 + 2c_1 t) + (b_2 + 2c_2 t X_2)}{(a_1 + b_1 t + c_1 t^2)}(a_2 + b_2 t + c_2 t^3)\right] X_2$$

The values of $\alpha$ at 20, 25, and 30° determined from Equation 8 are given in Table I. These values should be reliable to $\pm 0.5 \times 10^{-6}$ deg$^{-1}$.

The apparent specific expansibility, $\psi_v$, of the lysozyme solutions were determined from

$$\psi_v = \frac{\partial \phi_v}{\partial T} = \left(\frac{\partial \phi_v^0}{\partial T}\right) + \left(\frac{\partial S_v^*}{\partial T}\right) X_2$$

where $\phi_v^0$ is the apparent specific expansibility at infinite dilution and $S_v^*$ is the experimental slope. The values for $\phi_v^0$ and $S_v^*$ at 20, 25, and 30° were obtained from the temperature fits of $\phi_v^0 = 0.664 + 3.01 \times 10^{-3} t + 2.0 \times 10^{-6} t^2$ and $S_v^* = 0.591 - 6.346 t + 9.943 \times 10^{-4} t^2$. The $\psi_v$ is shown as a function of concentration and temperature in Fig. 2. The $\psi_v^0$ and $S_v^*$ at various temperatures are given in Table II. The infinite dilution apparent specific expansibility is equal to the infinite dilution partial specific expansibility, $\psi_v^0$, and increases with increasing temperature. Using the reasoning of Hepler (14), the positive sign of $\partial \psi_v^0/\partial T$ can be attributed to the protein behaving as a "structure making" solute similar to the behavior of most nonelectrolytes and the tetraalkylammonium salts (10).
The specific heats of the lysozyme solutions were measured at 25° and the results are given in Table I. Similar to the specific volumes, the specific heats were found to be a linear function of the weight fraction (X,). The values of c, were fitted by a least squares method to the equation

\[ c_p = 4.1791 - 2.4194 X, \tag{11} \]

to a standard deviation of 1 x 10^-4 J g^-1 K^-1. The addition of lysozyme decreases the heat capacity of the solutions similar to most electrolytes (15).

The apparent specific heat capacity \( \phi_a \) can be determined from the heat capacity by the equation

\[ \phi_a - (c_p - \phi_a^o) X, / X, \tag{12} \]

where \( c_p \) and \( \phi_a^o \) are the specific heats of the solution and pure water, respectively. The \( \phi_a \) values for lysozyme were fitted to

\[ \phi_a = 1.681 + 6.377 X, \tag{13} \]

and are shown plotted versus \( X, \) in Fig. 2. The \( \phi_a \) increases only slightly with concentration at 25° similar to nonelectrolytes such as sucrose (15).

The sound velocities, c, of the lysozyme solutions were measured at 25° and the results are given in Table I. The sound velocities were fitted to the equation

\[ c = 1496.63 + 241.5 X, \tag{14} \]

to a standard deviation of 0.02 ms^-1 at 25°.

The adiabatic compressibilities, \( \beta_a \), of the solution were determined from the sound speeds and specific volumes using the equation

\[ \beta_a = v/c^2 \tag{15} \]

The adiabatic compressibilities have been converted to isothermal values \( \beta_T = -(1/v) (\partial v/\partial T)_T \) using

\[ \beta_T = \beta_a + 0.1 \alpha T v/c^2 \tag{16} \]

where \( T \) is the temperature (K). The values of \( \beta_T \) determined from Equation 16 are given in Table I and are plotted versus \( X, \) in Fig. 1. The \( \beta_T \) values were fitted to the equation

\[ 10^3 \beta_T = 45.245 - 23.83 X, \tag{17} \]

to a standard deviation of \( 7 \times 10^{-4} \) bar^-1. This decrease in compressibility can be attributed to the solvated water molecules on lysozyme being less compressible than the bulk water (16). As the concentration of lysozyme increases, a large number of water molecules are electrostricted by the protein, thereby decreasing the bulk water molecules and the compressibility of the solution.

The apparent specific compressibility, \( \phi_c \), is related to the apparent specific volume by the relation

\[ \phi_c = -(\partial \phi_a / \partial X,)_T = (\beta_T - \phi_a^o) X, / X, \tag{18} \]

where \( \beta_T \) and \( \beta_a^o \) are the compressibilities of the solution and pure water, respectively. The \( \phi_c \) values of the lysozyme solutions, calculated from the isothermal compressibilities, \( \beta_T \), are a linear function of concentration (see Fig. 2) and were fitted to the equation

\[ \phi_c = \phi_c^o + S_c X, \tag{19} \]

where \( \phi_c^o = 9.0917 \times 10^{-4} \) and \( S_c = 7.305 \times 10^{-4} \) at 25°. The \( \phi_c \) increases with increasing concentration at 25° similar to the behavior of many electrolytes (16).

The infinite dilution partial molal properties of lysozyme can be determined from the apparent specific values by multiplying by the molecular weight (e.g., \( \psi^o = \phi_c^o M \), where \( M \) is the molecular weight). Using a molecular weight of 14,600 estimated by Blake et al. (17), we obtain values for the partial molal properties given in Table III.

The infinite dilution partial molal properties of proteins can be examined by using a simple model for hydration (16)

\[ V^o(protein) = V^o(int) + V^o(elect) \tag{20} \]

where \( V^o(protein) \) is the measured partial molal volume of
lysozyme, $\bar{V}(\text{int})$ is the intrinsic partial molal volume, and $\bar{V}(\text{elect})$ is the electrostriction partial molal volume. The $\bar{V}(\text{elect})$ is the decrease in volume due to the hydration of water. The $\bar{V}(\text{int})$ is equal to $\bar{V}(\text{crystal})$, the crystal or actual volume of the protein, plus $\bar{V}(\text{disord})$, the void space or packing volume (10, 11). Some workers (16, 18) have related the $\bar{V}(\text{elect})$ to the hydration number, $n_H$, by the relation

$$\bar{V}(\text{elect}) = n_H (\bar{V}_k^b - \bar{V}_B^b)$$  \hspace{1cm} (21)

where $\bar{V}_k^b$ is the partial molal volume of electrostricted water molecules and $\bar{V}_B^b$ is the partial molal volume of the bulk water. This model assumes that for every water molecule ($n_H$) taken from the bulk phase to the region near the protein, the volume is decreased by ($\bar{V}_k^b - \bar{V}_B^b$). If we assume the effect of pressure on the volume of protein is negligible ($\partial \bar{V}(\text{int})/\partial P = 0$), we obtain upon substituting Equation 21 into Equation 20 and differentiating with respect to pressure,

$$-K^*(\text{protein}) = \frac{\partial \bar{V}(\text{protein})}{\partial P} = n_H (\frac{\partial \bar{V}_k^b}{\partial P} - \frac{\partial \bar{V}_B^b}{\partial P})$$  \hspace{1cm} (22)

Since the electrostricted water molecules are already compressed to their maximum extent (16), $\partial \bar{V}_k^b/\partial P$ is nearly zero. Upon substituting $\beta_B^b = -1/\bar{V}_B^b$ ($\partial \bar{V}_B^b/\partial P$) and rearranging, Equation 22 becomes

$$n_H = \frac{-K^*(\text{protein})}{\beta_B^b \bar{V}_B^b}$$  \hspace{1cm} (23)

This equation gives $n_H = 162$. Since we have assumed $\partial \bar{V}(\text{int})/\partial P = 0$ in this calculation, which may not be correct for a protein, the hydration number should be considered as only an approximation. By combining our estimated hydration number with a value of $\bar{V}_k^b - \bar{V}_B^b = -2.7 \text{ cm}^3 \text{ mol}^{-1}$ found for electrolytes (16), we obtain

$$\bar{V}(\text{elect}) = 162 \times -2.7 = -438 \text{ cm}^3 \text{ mol}^{-1}$$  \hspace{1cm} (24)

and

$$\bar{V}(\text{int}) = \bar{V}(\text{protein}) - \bar{V}(\text{elect}) = 10,833 + 438 - 11,271 \text{ cm}^3 \text{ mol}^{-1}$$  \hspace{1cm} (25)

By assuming the $\bar{V}(\text{int}) = 4.48 r^2$ for the protein (similar to electrolytes) (10, 11), we obtain a value of $r = 13.6 \text{ Å}$. If we use $\bar{V}(\text{int}) = 2.02 r^2$, the crystal molal volume, a higher volume of $r$ is found (16.5 Å). Both estimates for the mean radius of lysozyme are in reasonable agreement with the values of 15 Å estimated by Blake et al. (17).

Since little is known about the heat capacity change that occurs when a water molecule is taken from the bulk solution to the electrostriction region of an electrolyte (as well as $\bar{C}_p^*(\text{int})$, the intrinsic molal heat capacity), it is not possible, at present, to use this simple model on the thermal data.

To summarize, volume and compressibility data have been determined for lysozyme solutions and the results have been used to estimate the number of hydrated water molecules and the intrinsic size of lysozyme in solution. Further studies on other protein systems should prove useful in understanding the physical chemistry of aqueous protein solutions.

Acknowledgment—The authors wish to thank Dr. R. Lumry for supplying the sample.

REFERENCES

Additions and Corrections

Vol. 251 (1976) 1708–1711

Properties of succinyl-CoA:3-ketoacid coenzyme A transferase.

Howard White and William P. Jencks

Page 1710, Column 1, Paragraph 2, replace the last sentence by:

The specific activity refers to the rate of acetoacetyl-CoA synthesis measured by the initial rate of appearance of absorption at 310 nm of the enolate of acetoacetyl-CoA in the presence of 0.24 mM succinyl-CoA, 67 mM acetoacetate, 5 mM magnesium sulfate, 67 mM Tris sulfate, 6 to 12 pgiml of CoA transferase, pH 8.1, 25°. Tris sulfate was found to give more reproducible assays than Tris chloride (9); however, the assay in the direction of AcAcCoA disappearance (10) is more satisfactory for most purposes.

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Partial specific volume, expansibility, compressibility, and heat capacity of aqueous lysozyme solutions.

Frank J. Millero, Gary K. Ward, and Peter Chetirkin

The hydration number \( n_h \) calculated for lysozyme in our recent paper (1) is in error. The partial molal compressibility, \( K^p \), for lysozyme is positive; thus, the calculated value for \( n_h \) is negative which has no physical meaning. Since the intrinsic specific volumes for some solid proteins (2) have been determined, it is possible to use Equation 21 to estimate \( n_h \). From the proteins studied (2) we estimate specific volumes of \( v = 0.785 \pm 0.01 \text{ cm}^3 \text{ g}^{-1} \) or \( \psi_0 \) for lysozyme. The electrostriction partial molal volume for lysozyme is given by Equation 1

\[
\psi_0(\text{elect}) = \psi_0(\text{meas}) - \psi_0(\text{int}) - 10,833 = (11,461 \pm 146) \text{ cm}^3 \text{ mol}^{-1}
\]

This value of \( \psi_0(\text{elect}) \) gives Equation 1

\[
n_h = \psi_0(\text{elect})/(\psi_0^* - \psi_0^*) = (-1,628 \pm 146)/-2.7 = 233 \pm 72
\]

This value of \( n_h \) calculated from molal volume data is in agreement with the value (235 ± 40) obtained by other methods (3).

The electrostriction partial molal compressibility, \( K^p(\text{elect}) \) of lysozyme can be determined from Equation 1

\[
K^p(\text{elect}) = -n_h \beta \psi_0 = -233 \times 45,248 \times 18.015 \times 10^{-6} = -0.1899 \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1}
\]

This value of \( K^p(\text{elect}) \) can be combined with the experimental value of \( K^p \) to obtain \( K^p(\text{int}) = K^p(\text{meas}) - K^p(\text{elect}) = 0.1327 + 0.1899 = 0.3226 \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1} \) or \( \phi_h(\text{int}) = 2.2 \times 10^3 \text{ cm}^3 \text{ g}^{-1} \text{ bar}^{-1} \). This value of \( \phi_h(\text{int}) \) is two times larger than our measured value of \( \phi_h^0 \). This value of \( \phi_h(\text{int}) \) is also about three times larger than the value of \( -7 \times 10^2 \text{ cm}^3 \text{ g}^{-1} \text{ bar}^{-1} \) for solid proteins recently measured by Murphy and Libby at high pressures.

This discrepancy between the calculated and measured \( \phi_h \) for proteins is probably related to hydrophobic or structural hydration (4). The decrease in volume of transferring soluble organic solutes into water is related to the structuring of water around the hydrocarbon groups or packing effects and is not due to electrostriction. Our results, as well as the work of Fahey et al. (5), does indicate that this structured water is not strongly affected by pressure. Further studies of the volume and compressibility properties of other proteins and amino acids can, hopefully, clear up this discrepancy.

REFERENCES


\[1\] R. B. Murphy and W. F. Libby, personal communication.

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