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INVESTIGATION OF THERMAL AND CHEMICALSTABILITY OF INTERRELATIONSHIP BETWEEN FOLDING AND UNFOLDING OF OVALBUMIN INDUCED BY UREA AND β- ME BY ULTRASONIC STUDIES

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ABSTRACT

This study attempts to assess the level of denaturation of ovalbumin in a chicken egg. The intensity of denaturation induced by urea and βME(β-mercaptoethanol) was measured at pH 7.0. Ovalbumin is identified as a **Serpin** class of protein. The denaturation by "ultrasonics" and "densitometry" in terms of partial specific volume, $\overline{v}^0$, and partial specific adiabatic compressibility, $\overline{\beta}_S$, of ovalbumin molecule in buffer solution and mixture of denaturant-buffer solution. The average hydration contribution to $\overline{v}^0$ and $\overline{\beta}_S$ was determined as functions of temperature using measured and calculated parameters by application of linear regression analysis. The negative values of $\overline{v}^0$ were obtained due to the low concentration of ovalbumin and high value of hydration effect, while positive value of $\overline{\beta}_S$ showed a significant contribution of the cavity effect in case of compressibility of protein molecule. The maxima obtained for the partial specific volume of ovalbumin in buffer versus temperature was shifted from 315 K to 323 K on addition of βME in ovalbumin-buffer solution, which reflects conformational changes due to breakage of disulfide bridges. The magnitude of $\overline{\beta}_S$ of ovalbumin increase with increase of concentration of protein and on addition of βME. These changes support the idea of conformational change of denatured protein. The partial specific adiabatic compressibility of ovalbumin decrease with increase of temperature upto 323 K and then increases, the extent of decrease is higher in presence of βME indicating the flexibility of ovalbumin increases on addition of βME, so it becomes more susceptible to digestion. In general, our results emphasize the conformational fluctuation, changes in free energy of denaturation and the hydration properties of ovalbumin in presence of denaturants urea and βME. The partial specific adiabatic compressibility of ovalbumin decrease with increase of temperature upto323K and then increase, the extent of decrease is high in presence of βME indicating the flexibility of ovalbumin increase on addition of βME, so it becomes susceptible to digestion.

KEYWORDS: Ultrasonic Interferometer, Interrelationship, Denaturation, Ovalbumin, Urea, β-BME, Partial Specific Adiabatic Compressibility

INTRODUCTION

Numerous studies have been made to investigate new dimensions of chemical science with biological systems. The positive results of the investigations in the field of physico-chemical studies of protein have attracted the interests of many researchers globally. In view of the fundamental interest in the studies of structural dynamics of proteins, efforts were made to reveal the molecular mechanism of biological activity related to a "structure-function" relationship and in understanding protein recognition.

Numerous indirect techniques helped to examine the method of protein folding, which enabled the understanding of thermal stability of proteins. Some of the techniques employed were amino acid determination and its association with structure, using denaturants to understand the methods of protein unfolding, and thermodynamic analysis of the conformations that were part of the unfolding process. The thermodynamic properties of proteins during denaturation were reviewed with better precision by Huntington *et al.* (1995) and Anfinsen *et al.* (1975)[1-3]. Contradictory and complicated outcomes were obtained on conducting thermal unfolding experiments by chemical denaturation.

Ultrasonic velocity of solutions has been proved to be a significant physical property that provides useful informations regarding the nature and the extent of intermolecular-interionic interactions occurring in solutions. Different workers have proposed different theories for the calculation of ultrasonic velocity [7-14]. The ultrasonic velocities of aqueous solution of electrolytes and non-electrolytes have also been studied [14-17]. The ultrasonic velocities of proteins have also been measured experimentally in aqueous as well as mixed aqueous solvents [18-22]. The ultrasonic velocity, u, and density, ρ, of the solutions [1-3] facilitated the calculation of various ultrasonic parameters. Some of the calculated parameters were specific acoustic impedance, Z, and adiabatic compressibility, β.

In present study, we want to compare the stability of ovalbumin in the presence of different denaturants in terms of their unfolding free energy changes, with the help of partial specific adaiabatic compressibility, which was determined by partial specific volume ultrasonic studies. Contradictory and complicated outcomes were obtained on conducting thermal unfolding experiments by chemical denaturation [23, 24, and 25]. The secondary, tertiary and quaternary protein structures were denatured by heat and chemical denaturants, such as urea, sodium dodecyl sulphate (SDS), guanidium hydrochloride (GdnHCl) and β-mercaptoethanol (βME). These denaturing elements targeted the bonds of the proteins and broke them. The ultrasonic velocity, u, and density, ρ, of the solutions [1-3] facilitated the calculation of various ultrasonic parameters. Some of the calculated parameters were specific acoustic impedance, Z, adiabatic compressibility,β, and compressibility lowering, Δβ [23, 25, 26]. The effects of the hydrophobic interaction, hydrogen bonding [27], electrostriction [28], ionization [29], and zwitterions formation on the partial molal volumes of the solutes can be estimated. Recently, many researchers have investigated volumetric properties of aqueous solution of protein constituents' viz. the amino acids and peptides [27, 30].

Protein-chains of amino acids fold into functional structures in water. Although, those folded structures are relatively compact, they invariably contain packing defects or cavities. The role of internal cavities in protein stability and function has been highlighted through several experimental studies. More recently, molecular modeling and simulations of proteins in solution have allowed for systematic studies of the presence of cavities in different parts of the heterogeneous protein interior [31]. These studies suggest that analysis of cavity formation provides an excellent probe of the properties (e.g. stiffness and compressibility) of the protein interior [32].

This study aims to identify the stability of ovalbumin in the presence of various denaturants. It attempts to do so by studying the pattern of unfolding. The values of adiabatic compressibility, relative lowering of adiabatic compressibility, partial specific volume, and partial specific adiabatic compressibility of ovalbumin in phosphate buffer at pH 7, the change in magnitude of above parameters during thermal denaturation and the change in magnitude of above parameters for chemical denaturation in presence of βME.

EXPERIMENTAL PROCEDURES

Materials

Ovalbumin, from chicken egg, was obtained from Sigma in a purified, crystal form. Chemicals, Urea, and β-mercaptethanol (βME) of analytical grade were obtained from Merck chemicals. Double distilled water was used to prepare all solutions. The solutions' concentrations were determined spectrophotometrically in sodium phosphate buffer of pH 7. Sodium azide was added to the buffer to inhibit the growth of microbes.

Ultrasonic Interferometry

The ultrasonic velocities of liquids were measured by ultrasonic interferometer. The multiple frequency ultrasonic interferometers (M-82 by Mittals and Co.) were used for the measurement of the ultrasonic velocity of the test solutions of protein in the temperature range (30 – 65^{o}C). Measuring cell is a double-walled cell. It is specially designed to maintain a steady temperature of the liquid with the help of a thermostat water bath. For all measurements, the protein concentration of all solutions was between (3.6 and19.7) $\times 10^{-4}$g.ml^{-1}. The working principle is based on the accurate measurement of wavelength,λ, of the medium. The ultrasonic waves of known frequency,ν, are produced by a quartz plate fixed at the bottom of the cell filled with test solution and are reflected by a movable metallic plate.

Ultrasonic velocity can be obtained from the equation.

$u = \nu \text{ X } \lambda$

THEORY

According to the Laplace equation, the coefficient of adiabatic compressibility (β) was found to be associated with the sound velocity (u), and the density of solution (ρ).

$$\beta = 1/ (\rho u^2) \quad \textbf{(1)}$$

The level of compressibility lowering is estimated by the difference in the compressibility between the solvent and solution.

$$\Delta\beta = \beta^o - \beta \quad \textbf{(2)}$$

where β and β^o are the coefficients of adiabatic compressibility of the solution and solvent, respectively.

Equation [3] determines the relative change in adiabatic compressibility.

$$= \Delta\beta/\beta^o \quad \textbf{(3)}$$

The experimental technique of Gekko*et al*. (1986), and Taulier*et al*. (2002) was applied to find the partial specific volume $\overline{\upsilon}^0$. The partial specific volume of a protein is defined as follows:

$$\overline{\upsilon}^o = \frac{\lim}{C \to 0}[(1 - V_o)/C] \quad \textbf{(4)}$$

where $V_o = (\rho - \mathbf{C})/ \rho_o$ **(5)**

The following equation estimates the partial specific adiabatic compressibility of ovalbumin:

$$\bar{\beta}_S = \left(\beta_o / \bar{\upsilon}^0\right) \lim_{C \to 0} (\beta / \beta_o - V_o) / C \quad \textbf{(6)}$$

where $\bar{\upsilon}^0$ is the partial specific volume of solute; β and β_o are the coefficients of the adiabatic compressibility of the solution and solvent, respectively; V_o is the apparent volume fraction of the solvent in solution; and C is the concentration of the solute.

Consequently, $\bar{\beta}_S$ of a protein, which was estimated experimentally, would be primarily because of involvement of cavity and hydration.

$$\bar{\beta}_S = -\left(1/\bar{\upsilon}^0\right)\left(\partial V_{cav} / \partial P + \partial \Delta V_{sol} / \partial P\right) \quad \textbf{(7)}$$

Thermal Unfolding

Temperature-induced denaturation of protein, under given conditions was performed as an increasing temperature. The actual temperature of the sample in the cell was obtained with a thermostat. Occasionally, samples were also checked by any possible aggregation due to heat, by light scattering measurement.

RESULTS

The Laplace equation ($\beta = 1/\rho U^2$) was employed to determine the rate of adiabatic compressibility, β, of the solutions and solvent, for protein-buffer and protein-βME-buffer systems, respectively. This equation helped to analyze the thermal stability by ultrasonic studies, using ultrasonic velocity, u, as shown in Figure 1 and Figure 2 and density of the solution, ρ. Adiabatic compressibility,β, is shown to decline with increasing temperature and concentration of ovalbumin and ovalbumin-βME, respectively. The decline in adiabatic compressibility in represented in Figure 3 and Figure 4, Figure 5 and Figure 6.

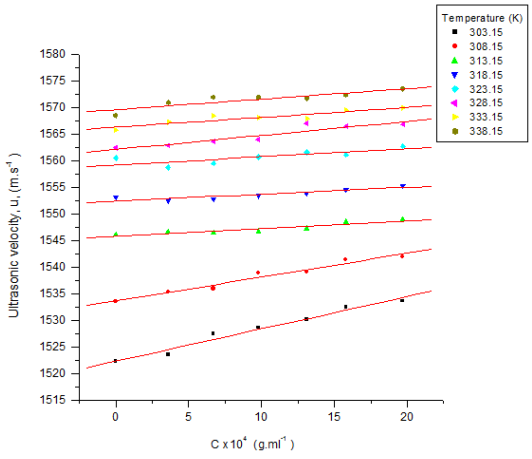

Figure 1: U Itrasonic Velocity, U, vs. Concentration, C, of Ovalbumin in Sodium Phosphate Buffer at pH 7.0

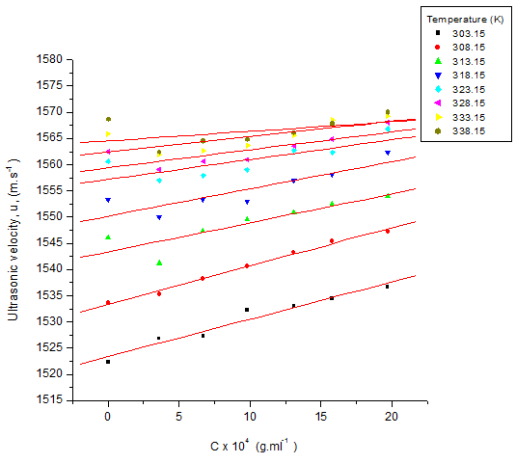

Figure 2: U Itrasonic Velocity, U, vs. Concentration, C, of Ovalbumin -βME Sodium Phosphate Buffer at pH 7.0

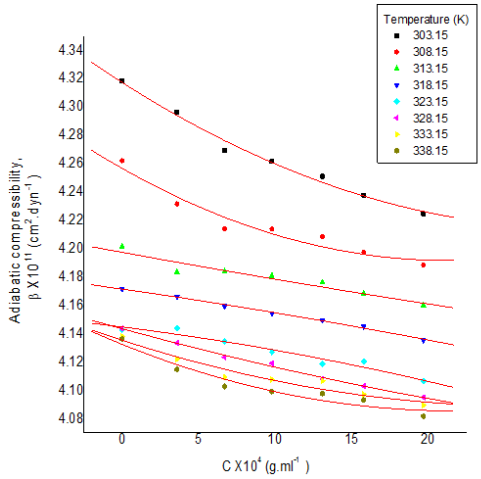

Figure 3: Adiabatic Compressibility, β vs, Concentration, C, of Ovalbumin in Sodium Phosphate Buffer at pH 7.0

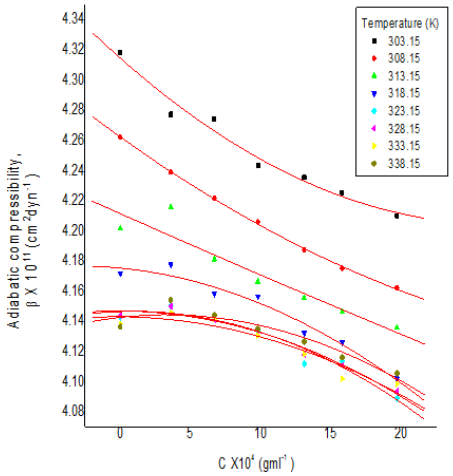

Figure 4: Adiabatic Compressibility, β, vs. Concentration, C, of Ovalbumin in -βME in Sodium Phosphate Buffer at pH 7.0

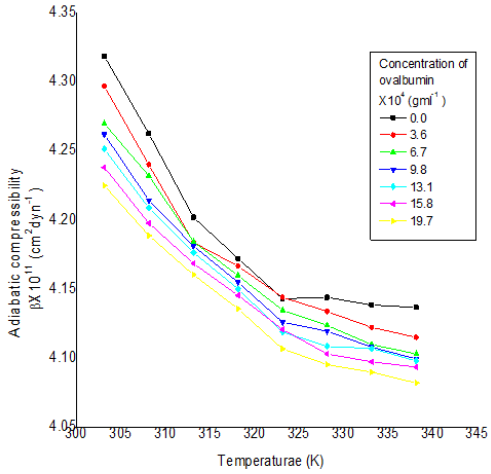

Figure 5: Adiabatic Compressibility, β, vs. Temprature of Ovabumin in Sodium Phosphate Buffer at pH 7.0

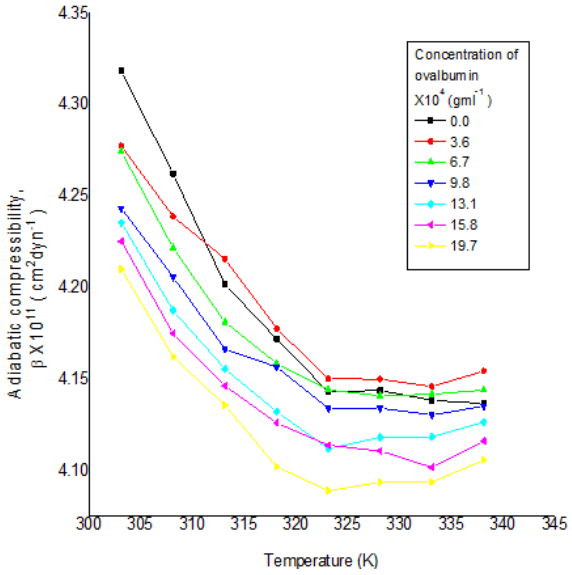

Figure 6: Adiabatic Compressibility,β, vs Temprature of Ovabumin in -βME in Sodium Phosphate Buffer at pH 7.0

The values of partial specific volume, $\overline{\upsilon}^0$, of ovalbumin and ovalbumin-βME system were determined by linear extrapolation of the apparent specific volume, $(1-V_o)/C$ to infinite dilution of protein (i.e. zero concentration of ovalbumin) obtained from equation 4. The apparent volume fraction of solvent of ovalbumin solution, V_o, the apparent specific volume $(1-V_o)/C$ and the partial specific volume of ovalbumin and ovalbumin-βME are listed in Tables 1 and 2 respectively. On analyzing the data, the difference between $\overline{\upsilon}^0$ of native and that of the thermally denatured ovalbumin is –2.965 $ml.g^{-1}$ and the value obtained after both thermal and chemical (0.01 N βME) denaturation of the protein was found to be +0.216 mlg^{-1}. As shown in Figure 7 in case of thermal denaturation, the partial specific volume increases with temperature steadily upto 323 K and then decreases while for protein undergoing both thermal as well as chemical denaturation, partial specific volume increases upto 313 K and then decreases until 338 K.

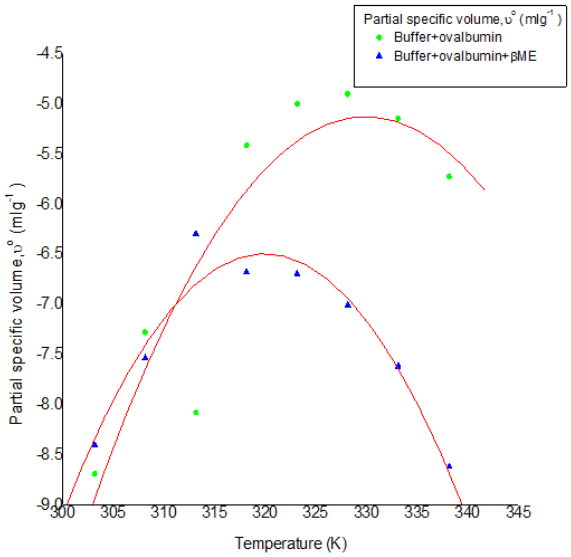

Figure 7: Partial Specific Volume υ^{o}, of Ovaibumin and Ovalbumin-βME vs Temperature in Phosphate Buffer at pH 7.0

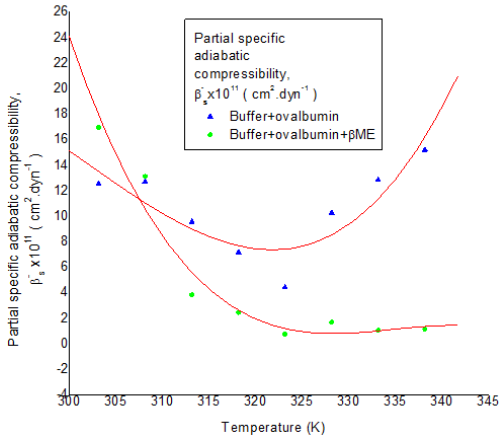

Figure 8: Partial Specific Adiabatic Compressibility, β_s, of Ovaibumin and Ovalbumin-βME vs. Temperature in Phosphate Buffer at pH 7.0

The linear extrapolation of $(\beta/\beta^{o}-V_o)/C$ to zero protein concentration was employed to estimate the partial specific adiabatic compressibility, $\overline{\beta}_S$, for ovalbumin and ovalbumin-βME systems, respectively, as is represented by the Figures. Equation 6 generated the values of (β/β_o-V_o) and $\overline{\beta}_S$, which are listed in Table 3 and 4, respectively. During thermal denaturation, there is regular reduction in the partial specific adiabatic compressibility of ovalbumin up to 323 K; this reduction is observed till 338 K. However, when thermal and chemical denaturation are employed, the $\overline{\beta}_S$ of ovalbumin reduced till 323 K; beyond 323 K, $\overline{\beta}_S$ reported further increases. Nevertheless, the increases beyond 323 K were not significant. This observation is shown in Figure 8.

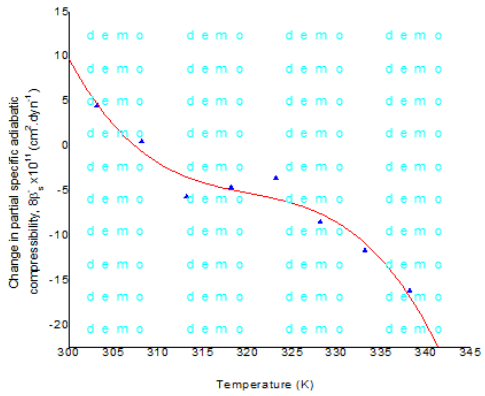

Figure 9: Change in Partial Specific Adiabatic, β_s, of Ovalbumin-βME and Ovalbumin vs Temperature in Sodium Phosphate

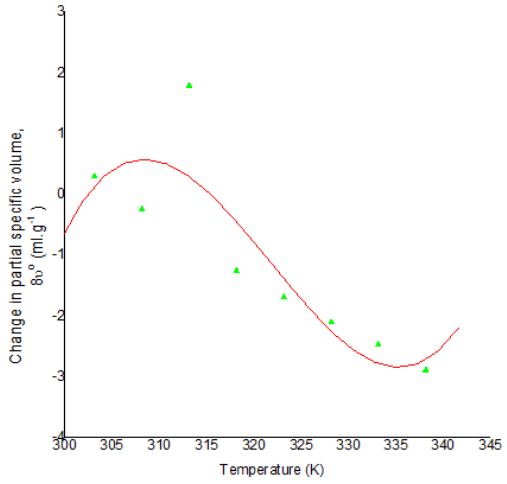

Figure 10: Change in Partial Specific Volume, $\delta\upsilon^o$, of Ovalbumin-βME and Ovalbumin vs Temperature in Sodium Phosphate Buffer at pH 7.0

For the ovalbumin-βMEin buffer and ovalbumin in buffer systems, respectively, there was reduction in the change in partial specific volume, $\Delta\upsilon^o$, and partial specific adiabatic compressibility; $\Delta\overline{\beta}_S$. The decrease with increase in temperature shows a slightly sigmoidal curve. This reduction is represented in Figures (9 and 10), respectively.

DISCUSSIONS

This study reports the presence of a complex chemical interaction on the ovalbumin–water interface. This interaction is the cause for the compressibility of globular protein hydration shell chemical as well as thermal denaturation. There is variation in the compressibility values between water around the atomic groups in proteins and bulk water. The compressibility value for water surrounding the charged atomic groups in proteins is lower than bulk water; however, the order of magnitude is similar as protein intrinsic compressibility. This observation is in spite of the fact that the compressibility of water surrounding non-polar atomic groups is higher than that of the bulk water [33-35].

A decrease in compressibility values was noticed as the concentration of solution increased; in addition, a subsequent increase in the number of incompressible solute molecules was also noticed [36]. As mentioned in theory, in equation (7), the term ($\partial V_{cav}/\partial P$) has a positive contribution and the term ($\partial\Delta V_{sol}/\partial P$) has a negative contribution to $\overline{\beta}_S$.

Thus, the values are indicative of the fact that the cavity effect incapacitates the solvation effect to result in $\overline{\beta}_S$ positive [37]. Reported value of $\overline{\upsilon}^0$ at 35°C, pH 7.0 was 0.740 ml.g^{-1} [38]. In this study, $\overline{\upsilon}^0$ of ovalbumin was –7.281 ml.g^{-1}, as shown in Table 3 and Figure 7. WhenβME was added, $\overline{\upsilon}^0$ of ovalbumin was –7.54 mlg^{-1}, as shown in Table 4 and Figure 9. Values of $\overline{\beta}_S$ were reported in the range of 23 X 10^{-12} to 28X10^{-12}cm^2.dyne^{-1} [37]. The range of $\overline{\beta}_S$ for globular proteins was from –2.5 X 10^{-12} to 11X10^{-12}cm^2.dyne^{-1} [37, 39]. However, in this study, the values of $\overline{\beta}_S$ for ovalbumin were in the range of –4.4X10^{-11} to 15.1X10^{-11}cm^2.dyne^{-1}. This result is shown in Figure 9. On addition of βME, the values of $\overline{\beta}_S$ for ovalbumin ranged from –1.1 X 10^{-11} to 17 X 10^{-11}cm^2.dyne^{-1}, as shown in Table 5.

Upto 323 K, there was decrease in the partial specific adiabatic compressibility, $\overline{\beta}_S$. Beyond 323 K, the partial specific adiabatic compressibility increases sharply upto 338 K. However, when denatured by βME, there was rapid decrease in $\overline{\beta}_S$ upto 323K, with a slow increase beyond this temperature in comparison to denaturation in the previous case (Figure 8). The disulfide bridges of inter- and intra-peptide chains are broken because of the denaturant βME. This led to reduced number of disulfide bonds. Furthermore, ovalbumin underwent a partial denaturation. This denaturation resulted in formation of a transition state, which had exposed cysteine residues that are usually buried in the native state [40, 41].

As per statistical analysis, irregular packing of the atoms and the dynamic–domain quality of α-helix were the predominant cause for the volume fluctuations. This observation has been reported by Gekko*et al.* (1986) [35, 42, 37, and 43]. In this study as well, the value of concentration of ovalbumin is noticed to be 0.03–0.2% lesser than reported value (0.5–1.0%) because of this reason. The factor, V_{cav}, i.e., volume as a result of cavity effect has increased efficiency than electrostriction in the ionic groups, hydrogen-bonded hydration of polar groups and hydrophobic hydration, and volume change due to solvation (ΔV_{sol}) (equation 8). Therefore, to make $\overline{\beta}_S$ positive, cavity contribution played a greater role when compared to the reported value [35, 37-39, 43].

These results indicate the breakdown of the regularly folded segments of α-helix and β-sheet, which results in loss of their specific configurations. As a result of these changes, the polypeptide chain forms a random coil, which is a flexible chain that is folded in a disordered pattern. These changes in the structure cause some noticeable alterations in the molecular properties of protein. The molecular properties that are impacted are partial specific volume, $\overline{\upsilon}^0$, and partial specific adiabatic compressibility, $\overline{\beta}_S$. Upto 310 K, with the addition of βME, the trend in the curve denotes increase in $\overline{\upsilon}^0$. Further, a decreases till 335 K was noticed, that once more increases at 338 K to a small extent (Table 3). Therefore, the curve in Figure 10 represents the following three states: natured, intermediate, and completely denatured. Figure 9 shows a slightly S-shaped curve. The initial decrease in compressibility difference is caused by the addition of βME, which directs the conformational changes that cause the solvation effect. The addition of βME also causes the slight increase at 312 K (dominance of cavity effect) and further decrease at 327 K (dominance of solvation effect). Therefore, it is concluded that compressibility reduces on addingβME in the protein–buffer solution.

Enzymes tend to act more easily on denatured proteins [44, 45]. During the hydrolysis of peptide bonds, random

coil structures were found to be easily hydrolyzed. Denaturation by cooking (e.g. chicken egg ovalbumin) results in proteins that could be easily digested by the proteolytic enzymes. Essentially, denaturation causes a few non-polar groups that were hitherto buried inside the protein to become exposed. After the proteins were exposed by denaturation, the proteins reported reduced or no biological activity.

CONCLUSIONS

Ovalbumin in buffer solution had an elevation in the partial specific volume up to 328 K; however, further increase in temperature led to a decrease of partial specific volume. Nevertheless, after the addition of βME, partial specific volume increased only until 313 K; beyond this temperature, there was a similar decrease in partial specific volume as aforementioned. These observations are suggestive of a dominant cavity effect primarily upto 313 K; beyond this temperature, a dominant solvation effect was reported. The shift of maxima from 313 K to 323 K on addition of βME reveals that conformational changes that were a result of breakage of disulfide bonds result in improved solvation at even low temperatures. With increase in concentration of protein, the magnitude of partial specific adiabatic compressibility of ovalbumin increases.

With the addition of denaturant βME, there was increase in the magnitude of partial specific adiabatic compressibility. This alteration in compressibility of protein corroborates the concept of conformational change in denatured protein. Upto 323 K, the partial specific adiabatic compressibility of ovalbumin decreases with temperature; beyond this temperature, there was increase in partial specific adiabatic compressibility. However, with the addition of the denaturant βME, partial specific adiabatic compressibility decreased till 325 K. The degree of decrease is higher whenβME was added. This indicates that flexibility of ovalbumin increases in the latter case, and thereby it has become more prone to digestion.

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APPENDICES

Table 1: (i) $(1–V_o)/C$, and (ii) Partial Specific Volume, $\bar{\upsilon}^o$ (ml.g^{-1}) as Functions of Concentration and Temperature of Ovalbumin in Phosphate Buffer pH 7.0 System

Temp. (K)	Concentration of Ovalbumin 1 X 10^4 g. ml^{-1}						Partial Specific Volume $\bar{\upsilon}^o$ (ml.g^{-1})
	3.6	6.7	9.8	13.1	15.8	19.7	
303.15	-8.7222	-5.8358	-3.9388	-3.0840	-2.4810	-2.5685	-8.6924
308.15	-7.2222	-5.0597	-3.4490	-2.7176	-2.1835	2.3198	-7.2806
313.15	-8.8333	-4.4925	-3.1225	-2.4657	-1.9937	-2.1472	-8.0809
318.15	-5.1667	-4.1194	-2.9790	-2.3359	-1.9051	-2.0558	-5.4154
323.15	-4.6667	-3.9702	-2.9388	-2.3282	-1.9177	-2.0508	-5.0039
328.15	-4.4722	-4.0149	-3.1020	-2.4504	-2.0317	-2.1269	-4.9045
333.15	-4.6111	-4.2836	-3.4286	-2.6947	-2.2532	-2.2792	-5.1482
338.15	-5.0833	-4.7612	-3.9286	-3.0611	-2.5760	-2.5178	-5.7275

Table 2: (i) $(1–V_o)/C$, and (ii) Partial Specific Volume, $\bar{v}^o$ (ml.g^{-1}) as Functions of Concentration and Temperature of Ovalbumin-βME in Phosphate Buffer pH 7.0 System

Temp (K)	Concentration of Ovalbumin 1 X 10^4 g. ml^{-1}						Partial Specific Volume $\bar{v}^o$ (ml.g^{-1})
	3.6	6.7	9.8	13.1	15.8	19.7	
303.15	-9.1667	-4.7761	-3.5102	-3.1221	-2.7025	-2.4112	-8.4071
308.15	-8.0833	-4.3284	-3.2755	-2.8779	-2.3861	-2.0914	-7.5381
313.15	-7.3611	-4.000	-3.1531	-2.7099	-2.1772	-1.8528	-6.2987
318.15	-7.000	-3.7910	-3.1429	-2.6412	-2.0760	-1.6904	-6.6842
323.15	-7.000	-3.7015	-3.2551	-2.6641	-2.0823	-1.6193	-6.6992
328.15	-7.3611	-3.7313	-3.4898	-2.7786	-2.2025	-1.6244	-7.0126
333.15	-8.0833	-3.8955	-3.8367	-2.9771	-2.4367	-1.7210	-7.6227
338.15	-9.1667	-4.1642	-4.3061	-3.2748	-2.7785	-1.8985	-8.6227

Table 3: (i) $(\beta/\beta^o–V_o)/C$, and (ii) Partial Specific Adiabatic Compressibility ($\bar{\beta}_S$ X 10^{11} cm^2.dyn^{-1}) as Functions of Concentration and Temperature of Ovalbumin in Phosphate Buffer pH 7.0 System

Temp. (K)	Concentration of ovalbumin 1 X 10^4 g. ml^{-1}						Partial specific adiabatic compressibility ($\bar{\beta}_S \times 10^{11}$ cm^2.dyn^{-1})
	3.6	6.7	9.8	13.1	15.8	19.7	
303.15	-22.809	-22.738	-17.337	-14.981	-14.294	-13.583	12.5139
308.15	-21.887	-15.741	-14.989	-12.336	-11.792	-11.098	12.6731
313.15	-20.8	-10.745	-8.15	-7.099	-7.010	-7.173	9.5438
318.15	-8.829	-8.484	-7.156	-6.362	-5.941	-6.473	7.1340
323.15	-3.929	-6.997	-7.126	-6.769	-5.309	-6.511	-4.4040
328.15	-11.310	-11.29	-9.135	-8.990	-8.264	-8.068	10.2332
333.15	-15.553	-14.67	-10.974	-8.504	-8.554	-8.25	12.8128
338.15	-19.589	-16.921	-13.105	-10.203	-9.186	-9.218	15.1709

Table 4: (i) $(\beta/\beta^o–V_o)/C$ and (ii) Partial Specific Adiabatic Compressibility ($\bar{\beta}_S$ X 10^{11} cm^2.dyn^{-1}) as Functions of Concentration and Temperature of Ovalbuminin-βME in Phosphate Buffer pH 7.0 system

Temp.(K)	Concentration of Ovalbumin 1 X 10^4 g. ml^{-1}						Partial Specific Adiabatic Compressibility ($\bar{\beta}_S \times 10^{11}$ cm^2.dyn^{-1})
	3.6	6.7	9.8	13.1	15.8	19.7	
303.15	-35.605	-20.053	-21.233	-17.795	-16.348	-15.142	16.9416
308.15	-23.269	-18.546	-16.755	-16.257	-15.335	-14.002	13.0910
313.15	1.895	-11.318	-11.751	-11.104	-10.523	-9.815	3.8039
318.15	-3.205	-8.585	-6.885	-9.887	-9.025	-10.184	2.448
323.15	-2.172	-3.233	-5.423	-8.358	-6.528	-8.224	0.7445
328.15	-3.205	-4.812	-5.878	-7.513	-7.243	-7.774	1.6593
333.15	-2.982	-2.669	-5.809	-6.666	-8.035	-6.627	1.0326
338.15	2.719	-1.494	-4.652	-5.120	-5.899	-5.691	-1.1156

Table 5: Change in Partial Specific Volume, $\Delta\overline{\upsilon}^{o}$ (ml.g^{-1}) and Partial Specific Adiabatic Compressibility, $\Delta\overline{\beta}_S$ (cm^2.dyn^{-1}) as a Function of Temperature of Ovalbumin-βME and Ovalbumin in Phosphate Buffer at pH 7.0 Systems

Temp (K)	Change in Partial Specific Volume, $\Delta\overline{\upsilon}^{o}$ (ml.g^{-1}) on Denaturation	Change in Partial Specific Adiabatic Compressibility, $\Delta\overline{\beta}_S$ (cm^2.dyn^{-1}) on Denaturation
303.15	0.2853	4.4277
308.15	-0.2575	0.4179
313.15	1.7822	-5.7399
318.15	-1.2688	-4.6860
323.15	-1.6953	-3.6595
328.18	-2.1081	-8.5739
333.15	-2.4745	-11.7802
338.15	-2.8952	-16.2865