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Interactions of Peptides and Lysozyme with Aqueous Tetraethylammonium Bromide at 298.15 K

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Abstract The apparent molar volumes, $V_{\phi,2}$, of gly-leu, gly-gly-leu and the partial specific volume ν° of hen-egg-white lysozyme have been determined in aqueous of TEAB solutions by density measurements at 298.15 K. These data have been used to calculate the infinite dilution apparent molar volumes $V^{o}_{2,m}$ for the peptides in aqueous TEAB solutions and the standard partial molar volumes of transfer $\Delta_{tr}V^{o}_{2,m}$ of the peptides from water to aqueous TEAB solutions. The results on $\Delta_{tr}V^{o}_{2,m}$ of peptides from water to aqueous TEAB solutions have been interpreted in terms of ion-ion, ion-polar, hydrophilic-hydrophilic and hydrophobic-hydrophobic group interactions. In order to supplement this information, enthalpies of transfer of aqueous peptides from water to TEAB solution have been determined at 298.15 K using a VP-ITC titration calorimeter. The data on partial molar volumes and enthalpies of transfer have been discussed in light of various interactions operating in the ternary system of peptides, water and TEAB.

The partial specific volume of transfer of lysozyme from water to aqueous TEAB solutions also indicates the predominance of hydrophobic interactions.

Keywords Standard partial molar volume · Peptides · Lysozyme · Tetraethylammonium bromide · Partial specific volume · Isothermal titration calorimetry · Enthalpies of transfer

1. Introduction

Interaction of proteins with their surrounding environment plays an important role in their conformational characteristics. The study of these interactions provides an important insight into the conformational stability and unfolding behavior of globular proteins. Various co-solutes/co-solvents, such as guanidine hydrochloride, sodium thiocyanate, magnesium chloride, urea and alcohols, affect proteins in different ways, acting as effective probes of their conformations in solutions [1–6].

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Tetraalkylammonium salts can give better insight into the effect of electrostatic and hydrophobic interactions on the stability of proteins as these salts are expected to influence the macromolecular conformation by weakening attractive or repulsive inter- and intra-chain, charge-charge interactions and by affecting hydrophobic interactions through the side chains of the alkyl groups. Tetraalkylammonim salts are bulky in nature and are known to orient water molecules around them depending on their alkyl chain. These salts undergo hydrophobic hydration in water that is usually understood as the formation of a more ordered and rigid structure of water surrounding the solute molecules. The effect of tetraalkylammonium salts on the stability of lysozyme and phycocyanin has been reported in the literature [7–9].

Tetraalkylammonium salts are also known to act as inhibitors of cholinesterases [10]. They are also known to reduce the temperature required for conversion of the DNA double-strand helix to a coil, which has been explained as being due to their preferential binding to the coil of DNA during heating and a preferential hydrophobic interaction with the adenine-thymine bases [11].

Reports on interactions of the constituent groups of proteins with TEAB in solution are rarely available in the literature. Due to the complex structural organization of proteins, interaction of the amino acids and peptides with TEAB can provide fine details of protein-salt interactions. Volumetric studies can characterize the properties of molecules as a function of solution conditions, including the role of solvation.

As a part of the continuation of our studies on the interaction of constituents of proteins with TEAB [12], in this work we report the standard partial molar volumes of transfer of some peptides and the partial specific volume of transfer of hen-egg-white lysozyme from water to aqueous TEAB solutions at 298.15 K. In order to supplement this information, enthalpies of transfer of aqueous peptides from water to a 0.25 $mol \cdot dm^{-3}$ TEAB solution have been determined at 298.15 K. The data on partial molar volumes and enthalpies of transfer have been discussed in light of various interactions operating in the ternary system of amino acid/peptides, water and TEAB.

2. Experimental

The peptides glycine-leucine (>99%), glycine-glycine-leucine (>99%) and protein hen-egg-white lysozyme (>95%) were procured from Sigma-Aldrich Company, USA. Lysozyme was further dialyzed and lyophilized. Tetraethylammonium bromide (TEAB) was of extra-pure analytical reagent grade (>99%) purchased from Sisco Research Laboratories, India. The numbers in parentheses represent the purity as reported by the vendors. The peptides were dried over P_2O_5 in a vacuum desiccator. The moisture content in the peptides was determined using a Karl Fisher Titrator (Systronics, India).

Appropriate corrections to the weights of the peptides were applied wherever necessary. Water used for preparing the solutions was double distilled and de-ionized by passing it through a Cole-Parmer Barnstead mixed-bed, ion-exchange resin column followed by degassing.

2.1. Densimetry

Solution densities were measured on a vibrating tube digital densimeter (model DA-210 from Kyoto Electronics, Japan). The temperature of the densimeter cell was maintained by circulating water from a Julabo constant temperature circulation bath. This arrangement gave a temperature stability of ±0.01 K. The vibrational period of the densimeter tube containing

the solution of interest was measured three times each. The reproducibility of the density measurements on the average was $\pm 3 \times 10^{-6}$ g·cm^{-3}. The calibration of the densimeter was performed every day and the accuracy was checked by measuring the densities of aqueous sodium chloride and the results were found to be in excellent agreement with those reported in the literature [13] with a maximum difference of 0.03 cm^3·mol^{-1} in the values of $V_{\phi,2}$.

2.2. Isothermal titration calorimetry

ITC measurements were performed at 25 °C on a VP-ITC titration calorimeter (MicroCal Northampton, MA, USA). All solutions were thoroughly degassed with the help of a Thermovac sample degassing and thermostat unit provided with the instrument. The sample cell was loaded with a solution of interest and the reference cell contained the corresponding solvent. Titrations were carried out using a 0.25 cm^3 syringe filled with peptide solution, stirred at 300 rpm. The injections were started after baseline stability had been achieved. A typical titration experiment consisted of 25 consecutive injections of 15 μL volume for 5 s duration at 4 min intervals. Control experiments were performed and appropriate corrections were applied to the main experiment. Titrations were performed of 0.08 mol·kg^{-1} gly-leu, 0.08 mol·kg^{-1} gly-gly-leu and 0.2 mol·kg^{-1} glycine with water and in presence of 0.25 mol·dm^{-3} TEAB solution. A typical ITC experiment provided a set of heat-liberated (q) data at different concentrations of amino acid/peptide in solution. The enthalpy of dilution (ΔH_{dil}) of the respective aqueous amino acid/peptide solution was calculated by fitting the values of heat liberated to the polynomial equation:

$$q = \Delta H^{\text{o}}_{\text{dil}} + B_1 X + B_2 X^2 + \cdots \tag{1}$$

where q is the amount of heat absorbed at molality, m, and $\Delta H^{\text{o}}_{\text{dil}}$ is the enthalpy change at infinite dilution. B_1 and B_2 are fitting coefficients and X is the molality of the amino acid/peptide in the cell after each injection. The units of B_1 and B_2 are J·mol^{-2}·kg and J·mol^{-3}·kg^2, respectively, and that of X are mol·kg^{-1}. ΔH_{dil} refers to the enthalpy of dilution of the respective aqueous amino acid or peptide taken in the syringe upon addition to the cell containing water or the desired concentration of TEAB.

3. Results and discussion

3.1. Volumetric properties of peptide in the presence of TEAB

The measured densities of aqueous amino acid and peptide solutions were used to calculate the value of apparent molar volume, $V_{\phi,2}$, using the following Eq. [14]:

$$V_{\phi,2} = (M/\rho) - (\rho - \rho_{\text{o}})10^3/(m\rho\rho_{\text{o}}) \tag{2}$$

where M (g·mol^{-1}) is the molar mass of the solute, m in (mol·kg^{-1}) is the molality of the peptide in TEAB-water mixtures, ρ and ρ_{o}, are the densities in (g·cm^{-3}) of the peptide-salt-water ternary system and reference solvent (desired concentration of aqueous TEAB), respectively. The results of the density measurements at 298.15 K are given in Table 1. In the cases where the molality dependence of $V_{\phi,2}$ was either found to be negligible or having no definite trend, the value of standard partial molar volume at infinite dilution, $V^{\text{o}}_{2,\text{m}}$, was evaluated by taking an average of all the data points. The values of $V_{\phi,2}$ at infinite dilution

provide information regarding solute-solvent interactions. The values of $V^{o}_{2,m}$ are positive for all the studied peptides in aqueous tetraethylammonium bromide solutions (Table 2). The values of $V^{o}_{2,m}$ for gly-leu in aqueous TEAB decrease with an increase in concentration of the salt, whereas for gly-gly-leu the decrease in the value of $V^{o}_{2,m}$ is less.

3.2. Standard partial molar volumes of transfer of peptides and amino acid from water to aqueous TEAB

Limiting thermodynamic properties of transfer yield qualitative and quantitative information on solute-solvent interactions without contributions from solute-solute interactions.

The values of standard partial molar volume of transfer, $\Delta_{tr}V^{o}_{2,m}$, were calculated using the following equation:

$$\Delta_{tr}V^{o}_{2,m}(\text{water to aqueous TEAB}) = V^{o}_{2,m}(\text{in aqueous TEAB}) - V^{o}_{2,m}(\text{in water}) \qquad (3)$$

With an increase in the concentration of TEAB, the value of $\Delta_{tr}V^{o}_{2,m}$ becomes more negative in the case of gly-leu, whereas in the case of gly-gly-leu it decreases, but to a lesser extent (Table 3). The following types of interactions can occur in the ternary system containing peptide, TEAB and water: (a) ion-ion interactions between Br^- of TEAB and the $-NH_3^+$ group of the peptide; (b) between $(C_2H_5)_4N^+$ of TEAB and the COO^- group of the peptide; and (c) hydrophobic-hydrophobic interactions between the ethyl group of TEAB and the hydrophobic group of the peptide. Taking the cosphere overlap model as the guideline, (a) and (b) types of interactions would lead to a positive $\Delta_{tr}V^{o}_{2,m}$ since there is a reduction in the electrostriction effect and the overall water structure is enhanced. Interactions of type (c) would lead to a negative $\Delta_{tr}V^{o}_{2,m}$ because the introduction of alkyl group provides an additional tendency of hydrophilic-hydrophobic and hydrophobic-hydrophobic groups to interact and as a result there will be a reduction in the structure of water formed due to overlap of their cospheres. In the case of gly-leu, the value of $\Delta_{tr}V^{o}_{2,m}$ becomes more negative with a rise in the concentration of the salt. These data indicate the predominance of type (c) interactions suggesting that the hydrophobic groups of gly-leu interact strongly with the hydrophobic groups of TEAB, thereby leading to a negative volume of transfer. The negative value of $\Delta_{tr}V^{o}_{2,m}$ increases with a rise in the concentration of TEAB due to enhanced interactions between the hydrophobic groups of gly-leu and TEAB. However, the value of $\Delta_{tr}V^{o}_{2,m}$ for gly-gly-leu from water to 0.25 mol·dm^{-3} TEAB is positive, which indicates that the presence of an additional peptide bond induces more polar interactions of type (a) and (b). With an increase in the concentration of TEAB, the value of $\Delta_{tr}V^{o}_{2,m}$ remains almost unchanged up to 1.0 mol·dm^{-3} TEAB concentration, beyond which it decreases indicating an enhancement in hydrophobic interactions at higher concentrations of TEAB.

3.3. Enthalpy of transfer of peptides and glycine from water to 0.25 mol·dm^{-3} TEAB

Titrations were performed of 0.08 mol·kg^{-1} gly-leu, gly-gly-leu and 0.2 mol·kg^{-1} glycine in water and in 0.25 mol·dm^{-3} aqueous TEAB. These concentrations of amino acid and peptides were chosen taking into account their solubility or the amount of heat liberated to avoid saturating the signal (Table 4). The enthalpies of transfer of aqueous gly-leu, gly-gly-leu and gly from water to 0.25 mol·dm^{-3} TEAB are presented in Table 5. The transfer

Table 1 Results of volumetric measurements on gly-gly-leu (relative molar mass, $M_r = 245.28$) and gly-leu ($M_r = 188.22$) in the presence of TEAB at 298.15 K

m/(mol·kg^{-1})	ρ/(g·cm^{-3})	$V_{\phi,2}$/(cm^3·mol^{-1})
0.25 mol·dm^{-3} TEAB with gly-gly-leu		
0.000000	1.006271	
0.022910	1.007685	182.52
0.032967	1.008298	182.65
0.040289	1.008769	182.06
0.045903	1.009092	182.55
0.052728	1.009526	182.19
0.5 mol·dm^{-3} TEAB with gly-gly-leu		
0.000000	1.015718	
0.020780	1.016986	182.06
0.018984	1.016876	182.15
0.030953	1.017605	182.04
0.032609	1.017703	182.12
0.035320	1.017863	182.23
0.050884	1.018843	181.40
0.75 mol·dm^{-3} TEAB with gly-gly-leu		
0.000000	1.025408	
0.020213	1.026620	181.93
0.025556	1.026953	181.41
0.028679	1.027144	181.31
0.039725	1.027780	181.98
0.049272	1.028346	181.91
1.0 mol·dm^{-3} TEAB with gly-gly-leu		
0.000000	1.035626	
0.018013	1.036682	182.02
0.019235	1.036757	181.84
0.029107	1.037329	182.01
0.037393	1.037827	181.59
0.046105	1.038354	181.20
1.5 mol·dm^{-3} TEAB with gly-gly-leu		
0.000000	1.056668	
0.017272	1.057662	180.43
0.018770	1.057742	180.74
0.029787	1.058354	181.16
0.028099	1.058286	180.30
0.042718	1.059110	180.53
0.046106	1.059301	180.54
0.25 mol·dm^{-3} TEAB with gly-leu		
0.000000	1.006259	
0.017726	1.007117	139.10
0.013549	1.006916	139.04
0.033664	1.007896	138.79
0.032526	1.007850	138.50
0.050285	1.008699	138.77

Table 1 Continued

m/(mol·kg^{-1})	ρ/(g cm^{-3})	$V_{\phi,2}$/(cm^3·mol^{-1})
0.50 mol·dm^{-3} TEAB with gly-leu		
0.000000	1.015564	
0.018404	1.016475	137.20
0.025427	1.016823	137.13
0.028578	1.016989	136.78
0.028480	1.016973	137.16
0.043109	1.017699	136.99
0.041696	1.017620	137.11
0.75 mol·dm^{-3} TEAB with gly-leu		
0.000000	1.025450	
0.023007	1.026608	135.55
0.019228	1.026426	135.18
0.025341	1.026735	135.17
0.029441	1.026932	135.49
0.040814	1.027485	135.87
0.050937	1.027986	135.86
1.0 mol·dm^{-3} TEAB with gly-leu		
0.000000	1.035342	
0.022653	1.036498	134.02
0.021649	1.036432	134.66
0.029631	1.036825	134.90
0.028850	1.036802	134.37
0.039876	1.037365	134.19
0.041402	1.037445	134.11
1.5 mol·dm^{-3} TEAB with gly-leu		
0.000000	1.056554	
0.018371	1.057487	132.51
0.024808	1.057788	133.40
0.023650	1.057732	133.36
0.036442	1.058391	132.73
0.047110	1.058928	132.67

parameters were calculated as follows:

$$\Delta\Delta H^{o}_{tr}(\text{water to aqueous TEAB}) = \Delta H^{o}(\text{in aqueous TEAB}) - \Delta H^{o}(\text{in water}) \quad (4)$$

The values of ΔH_{dil} for gly-leu and gly-gly-leu in water are negative. The exothermicity arises from interactions such as ion-dipole and H-bonding between the amino acids/peptide and water.

Table 2 Standard partial molar volumes of peptides in aqueous TEAB solutions at 298.15 K

	$V^{o}_{2,m}$/(cm^3·mol^{-1}) Aqueous tetraethylammonium bromide/mol·dm^{-3}					
Peptide		0.25	0.50	0.75	1.0	1.50
gly-leu	139.69 (0.07)	138.84 (0.24)	137.06 (0.16)	135.52 (0.31)	134.38 (0.34)	132.93 (0.41)
gly-gly-leu	177.80 (0.50)	182.44 (0.25)	182.12 (0.08)	181.70 (0.30)	181.73 (0.30)	180.62 (0.30)

Table 3 Transfer volumes of peptides from water to aqueous tetraethyl ammonium bromide at 298.15 K

$\Delta_{tr}V^{o}_{2,m}/cm^3\cdot mol^{-1}$					
Tetraethylammonium bromide/mol·dm^{-3}					
Amino acids Peptides	0.25	0.50	0.75	1.00	1.50
gly	−0.18 (0.09)	0.45 (0.08)	1.07 (0.07)	1.20 (0.08)	1.69(0.07)
gly-leu	−0.83 (0.25)	−2.63 (0.17)	−4.17 (0.32)	−5.31 (0.35)	−6.76 (0.42)
gly-gly-leu	4.64 (0.56)	4.27 (0.51)	3.99 (0.58)	3.93 (0.58)	2.82 (0.58)

In the ternary solutions containing (amino acid/peptide, TEAB and water), there are mainly three kinds of interactions that would contribute to the value of $\Delta\Delta H^{o}_{tr}$. Firstly, interactions of an ionic nature between the solvated amino acid/peptide zwitterion and TEAB give an exothermic contribution to $\Delta\Delta H^{o}_{tr}$. The second kind constitutes the hydrophobic interactions imparting an endothermic contribution to the value of $\Delta\Delta H^{o}_{tr}$. The third is the kind of interaction between amino acid/peptide and solvent molecule that may give an exothermic or endothermic contribution according to their nature. In an aqueous solution, the molecules

Table 4 Heat absorbed (q) upon titration of 0.2 mol·kg^{-1} glycine, 0.08 mol·kg^{-1} gly-leu and 0.08 mol·kg^{-1} gly-gly-leu against 0.25 mol·dm^{-3} TEAB and the molality of the solute in the cell after each injection at 298.15 K

Gly		gly-leu		gly-gly-leu	
Molality/(mol·kg^{-1})×10^3	q/(J·mol^{-1})	Molality/(mol·kg^{-1})×10^3	q/(J·mol^{-1})	Molality/(mol·kg^{-1})×10^3	q/(J·mol^{-1})
1.40	84.1	0.56	331.0	0.56	3030.9
2.10	92.9	0.84	241.0	0.84	2956.4
2.79	95.0	1.11	274.9	1.11	2893.2
3.48	101.3	1.39	226.8	1.39	2847.6
4.17	102.9	1.67	188.7	1.67	2811.6
4.86	107.5	1.94	171.5	1.94	2756.8
5.55	105.9	2.22	138.1	2.22	2734.2
6.23	106.3	2.49	133.1	2.49	2715.4
6.91	108.8	2.76	125.0	2.76	2701.2
7.59	106.7	3.03	107.0	3.03	2671.5
8.26	108.4	3.30	102.0	3.30	2660.6
8.94	110.0	3.57	97.1	3.57	2643.9
9.61	107.9	3.84	90.8	3.84	2627.1
10.28	107.5	4.11	84.5	4.11	2610.4
10.94	106.7	4.37	78.2	4.37	2594.5
11.60	105.4	4.64	73.2	4.64	2570.2
12.26	105.0	4.90	71.5	4.90	2564.8
12.92	105.4	5.17	69.9	5.17	2546.8
13.58	103.8	5.43	67.4	5.43	2530.9
14.23	104.6	5.69	64.4	5.69	2515.8
14.88	102.5	5.95	61.9	5.95	2501.6
15.53	102.9	6.21	59.0	6.21	2486.1
16.18	102.5	6.47	59.4	6.47	2479.4
16.82	100.8	6.72	55.6	6.72	2470.2

Table 5 ITC results for the interaction of 0.20 mol·kg^{-1} glycine and 0.08 mol·kg^{-1} peptides with 0.25 mol·dm^{-3} TEAB at 298.15 K

	ΔH^{o}_{dil}/(J·mol^{-1})		$\Delta\Delta H^{o}_{tr}$/(J·mol^{-1})
Amino acid/Peptides	Water	0.25 mol·dm^{-3}	Water to 0.25 mol·dm^{-3} TEAB
Glycine	86.6 ± 0.4	60.2 ± 7.1	−26.4 ± 7.1
gly-leu	−129.3 ± 0.8	497.9 ± 20.9	627.2 ± 20.9
gly-gly-leu	−94.1 ± 0.8	2820.0 ± 12.6	2914.1 ± 12.6

interact with each another with participation of the solvent molecules, which surround the polar and ionic groups with hydrating layers. For direct interaction to occur, a number of water molecules have to be removed from the hydration shells of the interacting molecules. The removal of a number of water molecules from the hydration shell of the polar head of the amino acid/peptides results in an endothermic effect. For amino acids/peptides with alkyl side chains, this process is superimposed on an additional effect of hydrophobic hydration, causing reinforcement of the hydrogen bonds between the water molecules surrounding these alkyl groups [15–16]. Studies on the structure of water in amino acid solutions [17–18] have indicated that the water-water hydrogen bonds in the zones surrounding the alkyl side chains are stronger than those in bulk water and, as the size of the side chain increases, the size of the clathrate around the hydrophobic side chains also increases. This effect gets transferred, due to the co-operation of hydrogen bonds, on to the water in the zwitterion hydration layers which reinforces the interaction between the water molecules and amino acids/peptides polar heads. As a result, the effect of partial dehydration of the reinforced hydration layers of the zwitterion can be endothermic. Consequently, the overall effect of the interaction is also endothermic in the case of gly-leu and gly-gly-leu. As for glycine with 0.25 mol·dm^{-3} TEAB, a negative enthalpy of transfer suggests that the ionic interaction of the zwitterions, giving an exothermic contribution to $\Delta\Delta H_{tr}$, is dominant over the other interactions.

3.4. Partial specific volume of protein in aqueous TEAB at 298.15 K

The main concepts and formalisms of the present work have been extended to the macromolecular system lysozyme. The partial specific volume is a well-defined thermodynamic quantity. It is a macroscopic observable that is particularly sensitive to the hydration properties of the solvent-exposed groups, as well as to the structure, dynamics and conformational properties of the solvent-inaccessible protein interior.

The apparent specific volume, ϕ, of hen-egg-white lysozyme was calculated from the density data using the following equation [19]:

$$\phi = (1/\rho_o)\{1 - (\rho - \rho_o)/c\} \tag{5}$$

where c is the concentration of the protein in 10^{-3} g · cm^{-3}, ρ is the density of the ternary solution and ρ_o is the density of the solvent in g·cm^{-3}. The calculated values of the apparent specific volume are presented in Table 6. Since the values of the apparent specific volume did not show any concentration dependence, the value of standard partial specific volume at infinite dilution, v°, was evaluated by taking the average of all the data points.

As seen in Table 7, the value of the partial specific volume of the protein decreases with an increase in the concentration of TEAB. Taking the value of the partial specific volume for

Table 6 Densities and apparent specific volumes of hen-egg-white lysozyme in aqueous TEAB solutions at 298.15 K

Protein/(g·c $^{-3}\times10^{-3}$)	ρ/(g·cm^{-3})	ϕ/(cm^3·g^{-1})
0.00	1.006294	
0.95	1.006548	0.728
1.71	1.006763	0.727
3.31	1.007152	0.736
3.91	1.007311	0.737
4.76	1.007549	0.732
7.08	1.008119	0.729
0.5 mol·dm^{-3} TEAB		
0.00	1.015833	
0.94	1.016076	0.730
2.21	1.016418	0.725
3.71	1.016792	0.730
4.21	1.016913	0.732
5.50	1.017272	0.726
6.42	1.017511	0.727
1.0 mol·dm^{-3} TEAB		
0.00	1.035641	
1.19	1.035943	0.722
2.93	1.036385	0.720
3.45	1.036511	0.723
5.11	1.036915	0.725
5.45	1.037008	0.724
1.5 mol·dm^{-3} TEAB		
1.74	1.057354	0.717
2.04	1.057417	0.722
2.49	1.057953	0.720
3.91	1.057879	0.718
5.62	1.058305	0.715
6.20	1.058415	0.720
7.10	1.058651	0.717

native lysozyme at infinite dilution as being 0.734 ± 0.001 cm^3·g^{-1} [20], the values of the partial specific volume of transfer for lysozyme from water to 0.25, 0.5, 1.0 and 1.5 mol·dm^{-3} TEAB are -0.003 ± 0.001, -0.006 ± 0.002, 0.011 ± 0.002 and 0.016 ± 0.002 cm^3·g^{-1}, respectively. Kauzmann [21] and Chalikian *et al*. [22] expressed a protein's limiting partial specific volume as a sum of two terms,

$$\nu^{o} = \nu_{int} + \nu_{h} \tag{6}$$

In this expression, ν_{int} is the specific intrinsic volume, which can be considered as a sum of two factors: (i) the geometric volume of the constituent atoms of the polypeptide chain V_M, and (ii) the void volume V_B due to imperfect packing in the protein interior. The term, ν_h,

Table 7 Partial specific volume of lysozyme at 298.15 K and different concentrations of aqueous TEAB

TEAB/(mol·dm^{-3})	0.25	0.5	1.0	1.5
v°/(g·cm^{-3})	0.732 ± 0.004	0.728 ± 0.003	0.723 ± 0.002	0.718 ± 0.002

is the change in volume of the solvent due to hydration. The hydration term, ν_h, contributes negatively to the partial specific volume of a globular protein, while the terms V_M and V_B are positive. Hydration induced changes in volume, ν_h, that occur due to solvent interactions with all the different solvent-accessible protein atomic groups can be expressed as follows [23]:

$$\nu_h = \sum n_{h,i}\left(V^o_{h,i} - V^o_0\right) \tag{7}$$

where $n_{h,i}$ is the number of water molecules in the hydration shell of the surface of the ith group on a protein surface, $V_{h,i}$ is the partial molar volume of the water solvating the ith accessible surface group and V_o is the partial molar volume of the bulk solvent. Using the X-ray coordinate data for 12 globular proteins, the average value of ν_{int} has been estimated to be $0.764\,cm^3{\cdot}g^{-1}$ [24]. By substituting this value and the experimental ν° value in Eq. (7), the change in the volume of solvent due to hydration comes to -0.031, -0.036, -0.041 and $-0.046\,cm^3{\cdot}mol^{-1}$ in the presence of 0.25, 0.5, 1.0 and $1.5\,mol{\cdot}dm^{-3}$ TEAB, respectively. The modulus of the hydration contribution, ΔV_h, to the partial specific volume of lysozyme at $1.5\,mol{\cdot}dm^{-3}$ TEAB is small and corresponds to 2% of the total value of V_o. It is generally known that the V_h contribution to the V_o values of globular proteins is about 3–7%. Negative values of volume changes could be explained on account of the elimination of intramolecular voids and the exposure of a large number of buried groups to the solvent, but the magnitude of the negative volume change is very small as it is compensated by an increase in V_T (V_T represents a layer of void volume around the solute molecule originating from thermally activated mutual vibrational motions of the solute and solvent molecules) that provides the positive contribution to ΔV_h. Therefore, large volume alterations are not expected for processes that involve hydration changes in globular proteins.

4. Conclusions

Introduction of glycine residues to the dipeptide, gly-leu, enhances the hydrophilic interactions between TEAB and the peptide as reflected by positive values of transfer for gly-gly-leu compared to that of gly-leu. Increase in the concentration of salt induces increased interactions with hydrophobic groups thereby leading to reduction in the values of $\Delta_{tr}V^o_{2,m}$ for peptides. The enthalpies of transfer of aqueous peptides from water to aqueous TEAB solutions have helped in understanding the interactions operating in the ternary system of peptides, acids, water and TEAB. An increase in TEAB concentration leads to a decrease in the partial specific volume of the protein. The modulus of hydration contribution, ΔV_h, to the partial specific volume of lysozyme at $1.5\,mol{\cdot}dm^{-3}$ TEAB is small and corresponds to 2% of the total value of V_o.

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References

1. Makhatadze, G.I., Privalov, P.L.: Protein interactions with urea and guanidinium chloride: a calorimetric study. J. Mol. Biol. **226**, 491–505 (1992)
2. Creighton, T.E.: Stability of folded conformations: Current opinion in structural biology. Curr. Opin. Struct. Biol. **1**, 5–16 (1991)

3. Arakawa, T., Timasheff, S.N.: Mechanism of protein salting in and salting out by divalent cation salts: balance between hydration and salt binding. Biochem. **3**, 5912–5923 (1984)
4. Velicelebi, G., Sturtevant, J.M.: Thermodynamics of the denaturation of lysozyme in alcohol-water mixtures. Biochem. **18**, 1180–1186 (1979)
5. Schellman, J.A.: Selective binding and solvent denaturation. Biopolymers **26**, 549–559 (1987)
6. Greene, Jr., Pace, C.N.: Urea and guanidine hydrochloride denaturation of ribonuclease, lysozyme, alpha-chymotrypsin, and beta-lactoglobulin. J. Biol. Chem. **249**, 5388–5393 (1974)
7. Chen, C.-H., Berns, D.S.: Thermodynamic studies of protein-salt interaction. Effects of geometrical configuration in alkyl-substituted quaternary ammonium salts on their interactions with phycocyanin. J. Phys. Chem. **82**, 2781–2785 (1978)
8. Chen, C.-H., Berns, D.S.: Thermodynamic studies of protein-salt interaction. Phycocyanin-tetrabutylammonium bromide and -tetraethylammonium bromide. J. Phys. Chem. **81**, 125–129 (1977)
9. Jain, S., Ahluwalia, J.C.: Differential scanning calorimetric studies on the effect of ammonium and tetraalkylammonium halides on the stability of lysozyme. Biophys. Chem. **59**, 171–177 (1996)
10. Stojan, J., Golicnik, M., Froment, M., Estour, F., Masson, P.: Concentration-dependent reversible activation-inhibition of human butyrylcholinesterase by tetraethylammonium ion. Eur. J. Biochem. **269**, 1154–1161 (2002)
11. Collins, J.M., Rogers, K.S.: Melting point depression of DNA by tetraalkylammonium bromides. Chem. Biol. Interactions **19**, 197–203 (1977)
12. Banerjee, T., Kishore, N.: Interactions of some amino acids with aqueous tetraethylammonium bromide at 298.15 K: a volumetric approach. J. Solution Chem. **34**, 137–153 (2005)
13. Archer, D.G.: Thermodynamic properties of the NaCl+H_2O system. II. Thermodynamic properties of NaCl(aq), NaCl·$2H_2O$(cr), and phase equilibria. J. Phys. Chem. Ref. Data **21**, 793–829 (1992)
14. Hoiland, H.: Partial molar volumes of biochemical model compounds in aqueous solutions. In: Hinz, H.-J. (ed.) Thermodynamic Data for Biochemistry and Biotechnology, Springer-Verlag, Berlin (1986) pp. 18
15. James, D.W., Armishaw, R.F., Foost, R.L.: Structure of aqueous solutions. Librational band studies of hydrophobic and hydrophilic effects in solutions of electrolytes and nonelectrolytes. J. Phys. Chem. **80**, 1346–1350 (1976)
16. Hallenga, K., Grigera, I.R., Berenelson, H.J.: Influence of hydrophobic solutes on the dynamic behavior of water. J. Phys. Chem. **84**, 2381–2390 (1980)
17. Hechte, D., Tadesse, F., Walters, L.: Correlating hydration shell structure with amino acid hydrophobicity. J. Am. Chem. Soc. **115**, 3336–3337 (1993)
18. Ide, M., Maeda, Y., Kitano, H.: Effect of hydrophobicity of amino acids on the structure of water. J. Phys. Chem. B **101**, 7022–7026 (1997)
19. Lee, J.C., Timasheff, S.N.: Partial specific volumes and interactions with solvent components of proteins in guanidine hydrochloride. Biochem. **13**, 257–265 (1974)
20. Sasahara, K., Sakurai, M., Nitta, K.: The volume and compressibility changes of lysozyme associated with guanidinium chloride and pressure-assisted unfolding. J. Mol. Biol. **291**, 693–701 (1999)
21. Kauzmann, W.: Some factors in the interpretation of protein denaturation. Adv. Protein. Chem. **14**, 1–63 (1959)
22. Chalikian, T.V., Gindikin, V.S., Breslauer, K.J.: Volumetric characterizations of the native, molten globule and unfolded states of cytochrome C at acidic pH. J. Mol. Biol. **250**, 291–306 (1995)
23. Chalikian, T.V., Totrov, M., Abagyan, R., Breslauer, K.J.: The hydration of globular proteins as derived from volume and compressibility measurements: cross correlating thermodynamic and structural data. J. Mol. Biol. **260**, 588–603 (1996)
24. Richards, F.M.: Areas, volumes, packing, and protein structure. Annu. Rev. Biophys. Bioeng. **6**, 151–176 (1977)