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Interaction of homologous series of amino acids with sarcosine in presence of denaturant: Volumetric and calorimetric approach

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ABSTRACT

Densities (ρ) and speeds of sound (u) of homologous serious of five amino acids: glycine, L-alanine, DL-α-amino-n-butyric acid, L-valine, and L-leucine were measured in aqueous 1.0 mol · dm^{-3} sarcosine and (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solutions. The values of corresponding apparent molar volume ($V_{2,\phi}$), apparent molar compressibility ($K_{S,2,\phi}$) were calculated from the density and speed of sound data at $T = 298.15$ K. Enthalpies of dilution (q) of amino acids from water to 1.0 mol · dm^{-3} sarcosine and (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solution were also measured. By linear regression fitting, the values of standard partial molar volume ($V^0_{2,m}$) and partial molar compressibility ($K^0_{S,2,m}$) and standard enthalpy of dilution ($\Delta_{tr}\Delta_{dil}H^0$) were determined. The contribution of zwitterionic and hydrophobic groups of amino acids to $V^0_{2,m}$ were also calculated from linear regression fitting of $V_{2,\phi}$ values. The different cosolvent interactions were interpreted on the basis of cosphere overlap model. The results suggest the dominance of ionic–ionic and hydrophilic–ionic group interactions over hydrophobic–hydrophilic and ionic–hydrophobic interactions.

1. Intoduction

Protein stability and its functions depend on the myriad of environmental conditions such as ion concentrations, pH, osmotic pressure, and solvent conditions. Small naturally occurring organic molecules known as osmolytes, can affect functions and integrity of proteins significantly [1,2]. Osmolytes can be categorized as stabilizing and destabilizing osmolytes. Methylamines, polyols, and amino acids are the naturally occurring stabilizing osmolytes and urea is well known naturally occurring destabilizing osmolyte which is accumulated at high concentration in many species such as marine elasmobranch, amphibians, and mammalian kidneys [2–4]. Some of the osmolytes such as trimethylamide-N-oxide (TMAO), sarcosine (N-methylglycine), glycine betaine, and glycerophosphocholine are, despite stabilizing proteins, known to counteract the effect of urea on protein stability [3,5–12].

Sarcosine is a well known osmolyte which is known to stabilize proteins and counteract the effect of denaturants. There have been many studies on its affect on protein folding, stability and counteraction of the effect of urea [3,9,11,13,14]. Sarcosine is also used in the treatment of Schizophrenia [15] and its role in prostate cancer progression as bio-marker has recently been reported [16]. Understanding the interactions of sarcosine and (sarcosine + urea) with amino acids can provide insights of its role in protein stabilization as well as on its mechanism to counteract the denaturing effect of urea.

Standard partial molar volume ($V^0_{2,m}$), partial molar compressibility ($K^0_{S,2,m}$) and limiting enthalpy of dilution ($\Delta_{dil}H^0$) of amino acids are important thermodynamic properties which provide the information about solute–solvent interactions. To the best of our knowledge, no report is available on interactions of combined presence of sarcosine and urea with amino acids. Therefore, in this study the interactions of a homologous series of five amino acids: glycine, L-alanine, DL-α-amino-n-butyric acid, L-valine, L-leucine with sarcosine and (sarcosine + urea) has been studied.

2. Experimental

2.1. Materials

Glycine, L-alanine, DL-α-amino butyric acid, L-valine, L-leucine and sarcosine were obtained from Sigma–Aldrich Chemical Co., USA. Urea was procured from Loba Chemie, Mumbai (table 1). All the five amino acids were dried over P_2O_5 and used without further purification. The solutions were prepared in doubly distilled water which was deionized by using a Cole-Parmer Barnstead mixed-bed ion exchange resin column and then degassed to remove any dissolved air. All the mass determinations were done on a Sartorius BP 211D digital balance which has the readability of 0.01 mg.

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TABLE 1
The compounds used in the study with their empirical formula, and the mole fraction purity (x) as reported by the vendor.

Compound	Empirical Formula	x	Source
Glycine	$C_2H_5NO_2$	>0.99	Sigma Aldrich
L-Alanine	$C_3H_7NO_2$	>0.99	Sigma Aldrich
DL-α-Amino-n-butyric acid	$C_3H_9NO_2$	>0.98	Sigma Aldrich
L-Valine	$C_5H_{11}NO_2$	>0.99	Sigma Aldrich
L-Leucine	$C_6H_{13}NO_2$	>0.99	Sigma Aldrich
Sarcosine	$C_3H_7NO_2$	>0.99	Sigma Aldrich
Urea	CH_4N_2O	>0.99	Loba Chemie, Mumbai

2.2. Density and speed of sound measurements

Density and speed of sound measurements were done on DSA 5000 digital density and sound velocity analyser from Anton Paar GmbH, Austria. Accuracy of the instrument was tested by measuring the values of apparent molar volume ($V_{2,\phi}$) and apparent adiabatic compressibility ($K_{S,2,\phi}$) of aqueous sodium chloride at different molalities and comparing the results with literature values [17] which were in excellent agreement. $V_{2,\phi}$ and $K_{S,2,\phi}$ of amino acids were determined from density (ρ) and speed of sound (u) measurements by using following equations

$$V_{2,\phi} = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0} \tag{1}$$

and,

$$K_{S,2,\phi} = \frac{k_s M}{\rho} - \frac{1000(k_s^0\rho - k_s\rho_0)}{m\rho\rho_0}, \tag{2}$$

where ρ_0 is the density of the reference solvent, m is the molality of the solutions in mol · kg^{-1}, and M is the molar mass of the amino acids in g · mol^{-1}. The units of density and sound (u) were in g · cm^{-3} and m · s^{-1}, respectively. The maximum uncertainties in density and velocity measurements were observed to be $3 \cdot 10^{-6}$ g · cm^{-3} and 0.03 m · s^{-1}, respectively. k_s is the adiabatic compressibility of solution, k_s^0 is the adiabatic compressibility of reference solvent. Adiabatic compressibility (k_s) of the solution was calculated by using following equation

$$k_s = \frac{1}{u^2\rho}. \tag{3}$$

2.3. Isothermal titration calorimetry

To calculate limiting enthalpy of dilution ($\Delta_{dil}H^0$), isothermal titration calorimetric (ITC) measurements were performed at $T = 298.15$ K on isothermal titration calorimeter (Nano ITC from TA Instruments). All the solutions were thoroughly degassed using Barnstead Thermolyne degassing stirrer to avoid any bubble formation during the experiment. In all titrations, aqueous amino acid solutions were filled in a syringe of 0.250 cm^3 capacity and were titrated into 0.950 cm^3 sample cell filled with 1.0 mol · dm^{-3} sarcosine or (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solutions with stirring speed of 250 rpm. Each titration consisted 25 consecutive injections of 10 μL volume with the interval of 240 s. A typical ITC experiment provided heat (q) liberated or absorbed during each injection. Control experiments were performed and appropriate corrections were made to the main experiment. The limiting enthalpy of dilution ($\Delta_{dil}H^0$) of aqueous amino acid solution were calculated by linear regression fitting to the following equation

$$q = \Delta_{dil}H^0 + S_v m, \tag{4}$$

where S_v is the empirical slope and m is the molality of the amino acid solution in the cell.

3. Results and discussion

3.1. Volumetric properties of amino acids in the presence of aqueous sarcosine and (sarcosine + urea) solutions

Results of the density measurements of amino acids in aqueous 1.0 mol · dm^{-3} sarcosine and (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solution at $T = 298.15$ K are given in tables 2 and 3. By definition, the standard partial molar volume ($V_{2,m}^0$) will be equal to apparent molar volume ($V_{2,\phi}$) at infinite dilution. Therefore, $V_{2,m}^0$ was calculated by linear regression fitting of the data to equation 5 and extrapolating the fitted line to zero molality (m),

$$V_{2,\phi} = V_{2,m}^0 + S_v m. \tag{5}$$

Here, S_v is the slope representing the solute–solute interactions, also known as the volumetric pairwise interaction coefficient ν_{AA} [18]. It was found that for all the amino acids, the values of $V_{2,\phi}$ and $K_{S,2,\phi}$ increased with the increase in molality. This molality dependence of $V_{2,\phi}$ and $K_{S,2,\phi}$ indicates the occurrence of solute–solute interactions in the solution. The values of $V_{2,m}^0$ were found to be increasing as hydrophobic chain length of amino acids increased. The $V_{2,m}^0$ and transfer partial molar volume ($\Delta_{tr}V_{2,m}^0$) from water to 1.0 mol · dm^{-3} sarcosine or (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solution of all the five amino acids for both the solutions are given in table 4. The value of $\Delta_{tr}V_{2,m}^0$ from water to 1.0 mol · kg^{-1} urea for glycine, L-alanine and L-valine has been reported [19] to be 0.69, 0.40 and 0.25 cm^3 · mol^{-1}, respectively. To understand the effect of urea on interaction of sarcosine with different amino acids, the value of $\Delta_{tr}V_{2,m}^0$ of glycine, L-alanine and L-valine from water to 1.0 mol · kg^{-1} urea system were subtracted with corresponding value of $\Delta_{tr}V_{2,m}^0$ for the (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) system to get the $\Delta_{tr}V_{2,m}^0$ of these amino acids from water to sarcosine in the presence of urea. This transfer volume for glycine, L-alanine, and L-valine from water to sarcosine in the presence of urea was calculated to be 1.44, 1.26 and 0.18 cm^3 · mol^{-1}, respectively (from table 4 and reference [19]). And, the values of $\Delta_{tr}V_{2,m}^0$ for glycine, L-alanine and L-valine from water to 1.0 mol · dm^{-3} sarcosine solution are 1.29, 0.91 and 0.72 cm^3 · mol^{-1}, respectively (from table 4). Here, the larger value of $\Delta_{tr}V_{2,m}^0$ of glycine and L-alanine in presence of urea indicate the increase in the favourable hydrophilic–hydrophilic and ionic–hydrophilic interaction between glycine and L-alanine with sarcosine. However, the value of $\Delta_{tr}V_{2,m}^0$ is decreased for valine in the presence of urea which might be due to unfavourable hydrophilic–hydrophobic and hydrophobic–ionic interactions.

The individual contribution of zwitterionic groups (NH_3^+, COO^-) and hydrophobic group (CH_2) of the amino acids to $V_{2,m}^0$ were calculated through the following relationship [21,22].

$$V_{2,m}^0 = V_{2,m}^0(NH_3^+, COO^-) + n_c V_{2,m}^0(CH_2), \tag{6}$$

where n_c is the number of carbon atoms in the hydrophobic chain of amino acids, and $V_{2,m}^0(NH_3^+, COO^-)$ and $V_{2,m}^0(CH_2)$ are the zwitterionic and hydrophobic CH_2 group contribution to $V_{2,m}^0$, respectively. The value of $V_{2,m}^0(NH_3^+, COO^-)$ and $V_{2,m}^0(CH_2)$ were calculated by least square regression analysis. According to method given elsewhere [23], the alkyl chain contribution to $V_{2,m}^0$ of five amino acids in 1.0 mol · dm^{-3} sarcosine and (1.0 mol · dm^{-3} sarcosine + 1.0 mol · dm^{-3} urea) solution has been provided in table 5. The values of alkyl chain contribution of amino acids to $V_{2,m}^0$ in water were calculated from the values of $V_{2,m}^0$ reported in earlier studies [20,21].