Solubilities of Amino Acids in Water and Aqueous Sodium Sulfate and Related Apparent Transfer Properties

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The solubilities of amino acids (glycine, β-alanine γ-amino butyric acid, DL-alanine, DL-amino butyric acid, and DL-valine) have been measured in water and aqueous solutions of 0.5, 1.0, and 1.5 M sodium sulfate at (288.15, 298.15, and 308.15) K. Related transfer parameters are also calculated. A model, with a maximum standard deviation of 1 g per l00 g of solvent, based on the experimental data, is given to predict solubilities of these amino acids under other conditions. The results have been interpreted in terms of interactions of the amino acids with the solvent, and a qualitative comparison has been done with other cosolvents.

Introduction

The solubility and stability of proteins have generated interest for a long time,[1] but because of various complications involved in dealing with these complex molecules, various low molecular weight model compounds[2−7] have been preferably taken for studies. The outcomes of these studies have been quite interesting and promising. In fact, the measurements of the solubility of model compounds in a solvent relative to that in water give the relative free energy of interaction of a group with the solvent, and thus quantitative predictions of the denaturating ability of the solvents can be made. Also, the entropy and enthalpy of transfer can provide a more detailed understanding because these thermodynamic parameters can be related more directly to the structural and interactional parts of the solvent effect. Guanidine hydrochloride, urea, alcohol, and polyol, solutions are among the most popular protein denaturants that have been used for various studies, and in most of the cases the temperature has been restricted to 298.15 K for studies.

Sodium sulfate has a slight salting in effect on the peptide group and a strong salting out effect on the nonpolar groups.[3,8] These outcomes lead to the fact that sodium sulfate stabilizes the native structure of proteins and hence distinguishes itself from the usual protein denaturants.

In view of the above, the present communication reports the solubilities of a series of (α and α,ω) amino acids (glycine (Gly), β-alanine (β-Ala), γ-amino butyric acid (Gaba), DL-alanine (DL-Ala), DL-amino butyric acid (DL-Aba), and DL-valine (DL-Val)) in water and aqueous solutions of (0.5, 1.0, and 1.5) M sodium sulfate at (288.15, 298.15, and 308.15) K. Related apparent thermodynamic parameters are reported. Mathematical relations predicting the solubilities of these amino acids under other conditions are given. The results have been interpreted in terms of interactions of the amino acids with the solvent, and a quantitative comparison has been done with other cosolvents studied.

Experimental Section

The amino acids were all procured from Sigma Chemical and were used without further purification, but they were dried at 373 K for 10 h in an oven. Sodium hydroxide (AR) and formaldehyde (GR) were obtained from S.D. Fine Chem. Pvt. Ltd., Boisar, and E.Merck Pvt. Ltd., India, respectively. Water used for the experiment was double distilled, deionized, and degassed by boiling.

Glass tubes of about 10 cm^3 capacity fitted with B-19 ground glass stoppers were used for the equilibration. The solvent (water or water + sodium sulfate) and an excess amount of amino acid were placed into the glass tubes, which were half filled to facilitate good mixing.

The stoppers were greased with silicone grease to prevent leakage. Glass tubes were clamped into a circular aluminum disk fixed in a brass frame. The unit with the glass tubes was immersed in the thermostat bath where the temperature was controlled within ±0.01 K with the help of an electronic thermoregulator (Science-Tech, India) and was read on a calibrated Beckmann thermometer. Solutions in the tubes were shaken by rotating the disk for (30 to 72) h. For analysis, the stopper of one of the tubes was removed while holding the rest of the tubes in the water bath. The solutions were filtered by using a glass syringe with a long stem tube containing a G-2 sintered disk which was preheated to a temperature higher than that of the experiment. During the filtration process, the solution was kept immersed in the bath. A known mass of the filtered solution was transferred quickly to a stoppered conical flask for estimating the amino acid by titration. Three solutions were equilibrated for each amino acid−solvent pair, and the measured solubility values found to disagree beyond ±0.1% were rejected. The means of the retained values are reported in this paper. Equilibration was found to be achieved within 48 h.

Results and Discussion

Solubilities of the amino acids in water, $S_{A,W}$, are reported in Table 1. To test the procedure and experimental setup, solubilities of the amino acids in water were compared with literature values, and the agreement is within 1.2%. Solubilities of the amino acids in mixed solvent, $S_{A,SS}$, are reported in Table 2.

When an amino acid, A, is dissolved in a solvent, the chemical potential of the amino acid can be expressed as

$$\mu_A = \mu_A^\circ + RT \ln x_A + RT \ln \gamma_A \qquad (1)$$

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Table 1. Solubilities, $S_{A,W}$, of Amino Acids in Water at 288.15, 298.15, and 308.15 K

amino acid	$S_{A,W}$/g·(100 g of H_2O)$^{-1}$ 288.15 K	298.15 K	308.15 K
Gly	20.19	25.02	30.17
	19.95[10]	24.99[10]	30.16[10]
β-Ala	63.21	72.36	78.85
Gaba	91.29	97.08	106.19
DL-Ala	15.10	16.63	18.74
		16.66[5]	
Aba	21.85	23.30	26.59
Val	6.30	8.81	9.70
		8.84[5]	

Table 2. Solubilities, $S_{A,SS}$, of Amino Acids in Aqueous Sodium Sulfate at 288.15, 298.15, and 308.15 K

amino acid	m/mol·kg^{-1}	$S_{A,SS}$/g·(100 g of Na_2SO_4(aq))$^{-1}$ 288.15 K	298.15 K	308.15 K
Gly	0.5	20.07	24.66	29.58
	1.0	19.86	24.38	28.37
	1.5	18.69	22.84	26.83
β-Ala	0.5	59.79	69.96	76.25
	1.0	55.94	64.57	73.78
	1.5	50.85	59.14	68.01
Gaba	0.5	86.33	93.28	101.08
	1.0	79.63	85.69	95.91
	1.5	72.54	78.48	87.87
DL-Ala	0.5	14.11	15.82	17.35
	1.0	12.34	13.98	15.38
	1.5	10.65	11.95	13.45
Aba	0.5	19.92	21.72	24.80
	1.0	16.77	18.40	21.51
	1.5	13.42	14.76	17.12
Val	0.5	5.05	7.05	8.17
	1.0	3.94	5.52	6.50
	1.5	2.93	4.47	5.12

where x_A is the mole fraction of the amino acid, μ_A° is the standard chemical potential of the amino acid in the solvent, and γ_A is the activity coefficient. The standard free energy of transfer, ΔG_t, of the amino acid can be defined as $\mu_{A,SS}^\circ - \mu_{A,W}^\circ$, where $\mu_{A,W}^\circ$ and $\mu_{A,SS}^\circ$ are the standard chemical potentials of the amino acid in water and sodium sulfate solution of a particular concentration. It can be shown that ΔG_t can be written as

$$\Delta G_t = RT \ln\left(\frac{S_{A,W}}{S_{A,SS}}\right) + RT \ln\left(\frac{\gamma_{A,W}}{\gamma_{A,SS}}\right) \tag{2}$$

However, since $\gamma_{A,SS}$ is not available, ΔG_t is better termed as $\Delta G_t'$, the apparent standard free energy of transfer. These are calculated with all corresponding solubility data on a mole fraction scale and are reported in Table 3. The $\Delta G_t'$ values were regressed by least-squares to the equation

$$\Delta G_t' = a + bT + cT^2 \tag{3}$$

where T is the temperature in kelvin.

The apparent standard entropy of transfer $\Delta S_t'$ and apparent standard enthalpy of transfer $\Delta H_t'$ are calculated as

$$\frac{d(\Delta G_t')}{dT} = -\Delta S_t' \tag{4}$$

$$\Delta H_t' = \Delta G_t' + T\Delta S_t' \tag{5}$$

These parameters are given at 298.15 K in Table 4.

Table 3. Apparent Free Energies of Transfer, $\Delta G_t'$, of Amino Acids from Water to Aqueous Sodium Sulfate at 288.15, 298.15, and 308.15 K

amino acid	m/mol·kg^{-1}	$\Delta G_t'$/kJ·mol^{-1} 288.15 K	298.15 K	308.15 K
Gly	0.5	−0.133 (0.002)[a]	−0.116 (0.001)	−0.106 (0.002)
	1.0	−0.265 (0.001)	−0.249 (0.002)	−0.170 (0.003)
	1.5	−0.293 (0.003)	−0.268 (0.002)	−0.212 (0.002)
β-Ala	0.5	−0.018 (0.002)	−0.065 (0.000)	−0.067 (0.001)
	1.0	−0.022 (0.001)	−0.040 (0.009)	−0.145 (0.001)
	1.5	0.025 (0.001)	−0.009 (0.001)	−0.128 (0.001)
Gaba	0.5	−0.017 (0.001)	−0.051 (0.001)	−0.032 (0.002)
	1.0	0.009 (0.000)	−0.016 (0.001)	−0.066 (0.001)
	1.5	0.050 (0.001)	0.015 (0.001)	−0.036 (0.001)
DL-Ala	0.5	0.009 (0.004)	−0.082 (0.005)	0.032 (0.003)
	1.0	0.162 (0.006)	0.051 (0.005)	0.161 (0.005)
	1.5	0.334 (0.003)	0.251 (0.004)	0.311 (0.004)
Aba	0.5	0.066 (0.003)	0.015 (0.001)	0.014 (0.003)
	1.0	0.306 (0.003)	0.247 (0.002)	0.195 (0.002)
	1.5	0.653 (0.003)	0.600 (0.003)	0.577 (0.002)
Val	0.5	0.373 (0.007)	0.389 (0.002)	0.272 (0.003)
	1.0	0.801 (0.002)	0.821 (0.007)	0.678 (0.002)
	1.5	1.331 (0.001)	1.159 (0.004)	1.096 (0.005)

[a] Entries in parentheses are the standard errors on each coefficient.

Table 4. Entropies, $\Delta S_t'$, and Enthalpies, $\Delta H_t'$, of Transfer of Amino Acids from Water to Aqueous Sodium Sulfate at 298.15 K

amino acid	m/mol·kg^{-1}	$\Delta\Delta S_t'$/J·mol^{-1}·K^{-1}	$\Delta H_t'$/kJ·mol^{-1}
Gly	0.5	−1.3 (0.1)[a]	−0.5 (0.0)
	1.0	−4.8 (0.2)	−1.7 (0.1)
	1.5	−4.1 (0.2)	−1.5 (0.1)
β-Ala	0.5	2.5 (0.0)	0.7 (0.0)
	1.0	6.2 (0.0)	1.8 (0.0)
	1.5	7.6 (0.1)	2.3 (0.0)
Gaba	0.5	0.7 (0.1)	0.2 (0.0)
	1.0	3.7 (0.0)	1.1 (0.0)
	1.5	4.3 (0.0)	1.3 (0.0)
DL-Ala	0.5	−1.2 (0.1)	−0.4 (0.0)
	1.0	0.0 (0.1)	0.0 (0.0)
	1.5	1.1 (0.1)	0.6 (0.0)
Aba	0.5	2.6 (0.1)	0.8 (0.0)
	1.0	5.5 (0.1)	1.9 (0.0)
	1.5	3.8 (0.2)	1.7 (0.0)
Val	0.5	5.1 (0.1)	1.9 (0.0)
	1.0	6.2 (0.0)	2.7 (0.0)
	1.5	11.7 (0.2)	4.6 (0.1)

[a] Entries in parentheses are the standard errors on each coefficient.

More interestingly, the contribution of an amino acid alkyl side chain to $\Delta G_t'$ gives better insight into the mechanism of protein denaturation. This contribution, defined as $\Delta G_{t,r}'(H \rightarrow R)$, can be calculated assuming the additivity of different group effects. This is, in fact, better termed as the replacement contribution, as the contribution due to the hydrogen atom has not been taken into account. $\Delta G_{t,r}'$ values are reported in Table 5.

Similarly, as given above, the replacement entropy of transfer, $\Delta S_{t,r}'$, and the replacement enthalpy of transfer, $\Delta H_{t,r}'$, are given in Table 6.

The solubilities of an amino acid are fitted to a general equation:

$$S/\text{g}\cdot(100\ \text{g of } Na_2SO_4(\text{aq}))^{-1} = a + bm + cm^2 + (T/\text{K} - 298.15)(d + em) \tag{6}$$

where m is the molality of sodium sulfate solution in moles per kilogram and the regressed coefficients are given in Table 7.

The solubility of α,ω amino acids increases with the increase in the number of CH_2 groups in the backbone, and

Table 5. Replacement (H→R) Contribution $\Delta G'_{t,r}$ to $\Delta G'_t(H_2O\rightarrow H_2O+Na_2SO_4)$ at Different Temperatures and Sodium Sulfate Concentrations

amino acid	m / mol·kg^{-1}	$\Delta G'_{t,r}$/kJ·mol^{-1} 288.15 K	298.15 K	308.15 K
		Method Ala−Gly		
CH_3	0.5	0.142 (0.004)[a]	0.034 (0.003)	0.138 (0.002)
	1.0	0.427 (0.004)	0.300 (0.004)	0.331 (0.004)
	1.5	0.627 (0.004)	0.519 (0.002)	0.523 (0.003)
		Method Aba−Gly		
CH_3CH_2	0.5	0.199 (0.003)	0.131 (0.002)	0.120 (0.001)
	1.0	0.571 (0.003)	0.496 (0.002)	0.365 (0.002)
	1.5	0.946 (0.004)	0.868 (0.005)	0.789 (0.001)
		Method Val−Gly		
$(CH_3)_2CH$	0.5	0.506 (0.005)	0.505 (0.003)	0.378 (0.002)
	1.0	1.066 (0.002)	1.070 (0.008)	0.848 (0.002)
	1.5	1.624 (0.004)	1.427 (0.002)	1.308 (0.007)

[a] Entries in parentheses are the standard errors on each coefficient.

Table 6. Replacement Entropies, $\Delta S'_{t,r}$, and Enthalpies, $\Delta H'_{t,r}$, of Amino Acid Alkyl Chains from Water to Aqueous Sodium Sulfate at 298.15 K

amino acid	m / mol·kg^{-1}	$\Delta S'_{t,r}$ / J·mol^{-1}·K^{-1}	$\Delta H'_{t,r}$ / kJ·mol^{-1}
	Method Ala−Gly		
CH_3	0.5	0.1 (0.1)[a]	0.1 (0.0)
	1.0	4.8 (0.2)	1.7 (0.1)
	1.5	5.2 (0.2)	2.1 (0.1)
	Method Aba−Gly		
CH_3CH_2	0.5	3.9 (0.1)	1.3 (0.0)
	1.0	10.3 (0.2)	3.6 (0.1)
	1.5	7.9 (0.1)	3.2 (0.1)
	Method Val−Gly		
$(CH_3)_2CH$	0.5	6.4 (0.1)	2.4 (0.1)
	1.0	10.9 (0.2)	4.4 (0.1)
	1.5	15.8 (0.4)	6.1 (0.2)

[a] Entries in parentheses are the standard errors on each coefficient.

Table 7. Regression Coefficients for Eq 6

	a/g	b/g·mol^{-1}·kg	c/g·mol^{-2}·kg^2	d/g	e/g·mol^{-1}·kg
Gly (0.1)[a]	25.02	0.24	−1.14	0.50	−0.07
β-Ala (0.9)	72.36	−6.14	−1.67	0.84	
Gaba (1.0)	97.08	−5.26	−4.31	0.78	
DL-Ala (0.2)	16.63	−1.51	−1.06	0.18	−0.03
Aba (0.5)	23.30	−1.44	−2.72	0.23	
Val (0.4)	8.81	−4.47	0.92	0.17	−0.04

[a] Values in parentheses represent the standard deviation of the experimental solubility data of the respective amino acid using the model.

this is due to the increase in dipole moment. At all temperatures DL-Aba is more soluble than DL-Ala, although this is not in accordance with their hydrophobic nature. However, this is because the former has a lower crystal lattice energy. The same trend is observed for the solubilities of the amino acids in various water + sodium sulfate solutions. In addition, an increase in salt concentration renders the amino acids less soluble. The $\Delta G'_{t,r}$ for the amino acids is positive and increases with the sodium sulfate concentration The same parameter increases with an increase in the hydrophobility of the amino acid. This is due to the effect of sodium sulfate on the nonpolar parts of the amino acids. The contribution of the CH_2 groups to $\Delta G'_{t,r}$, obtained from two successive amino acids of a series, decreases with the increase in chain length. This is in agreement with the results of other workers,[4,5] and it may be due to the accommodation of sodium and sulfate ions around the nonpolar groups with larger accessible surface area, resulting in a decrease in the contribution of the CH_2 for the higher amino acids.

The positive $\Delta G'_{t,r}$ indicates that sodium sulfate has a salting out effect[8,9] on the hydrophobic group. This parameter increases with the hydrophobicity of the amino acid and sodium sulfate concentration precisely because of the disruption of the hydrophobic hydration sphere around the nonpolar moiety by sodium sulfate. As the sodium sulfate concentration and the surface area of the nonpolar group are increased, there is more interaction, resulting in more positive $\Delta G'_{t,r}$. This disruption of hydrophobic hydration cospheres favors the hydrophobic interactions among the nonpolar groups. It can, therefore, be concluded that sodium sulfate tends to stabilize the hydrophobic interactions among the nonpolar groups with the proteins, unlike urea[10] and guanidine hydrochloride,[8,11] which tend to unfold proteins.

The transfer of the amino acids from water to aqueous sodium sulfate is primarily enthalpic, but for glycine it is entropic. The positive $\Delta S'_{t,r}$ and $\Delta H'_{t,r}$ values of the alkyl chains reveal that the hydrogen bonds around them are weaker in aqueous sodium sulfate than in water.

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Literature Cited

(1) Hvidt, A.; Westh, P. Different Views on the Stability of Protein Conformations and Hydrophobic Effects. *J. Solution Chem.* **1998**, *27*, 395−402.
(2) Nozaki, Y.; Tanford, C. The Solubility of Amino Acids and Two Glycine Peptides in Aqueous Ethanol and Dioxane Solutions. *J. Biol. Chem.* **1971**, *246*, 2211−2217.
(3) Bull, B. H.; Breese, K.; Swenson, A. C. Solubilities of Amino Acids. *Biopolymers* **1978**, *17*, 1091−1100.
(4) Gekko, K. Enthalpy and Entropy of Transfer of Amino acids and Diglycine from Water to Aqueous Polyol Solutions. *J. Biochem.* **1981**, *90*, 1643−1652.
(5) Lahiri, S. C.; Dey, B. P. Solubilities of Amino Acids in Methanol + Water Mixtures at Different Temperatures. *Indian J. Chem.* **1988**, *27A*, 297−302.
(6) Kirwan, J. D.; Orella, C. J. Correlation of Amino Acids Solubilities in Aqueous Alcohol Solutions. *Ind. Eng. Chem. Res.* **1991**, *30*, 1040−1045.
(7) Chao, K. C.; Jin, X. Z. Solubility of Four Amino Acids in Water and of Four Pairs of Amino Acids in Their Water Solutions. *J. Chem. Eng. Data.* **1992**, *37*, 199−203.
(8) Kumar, D. Apparent molar volume of some ω-amino acids in aqueous electrolytes systems. *Can. J. Chem.* **1999**, *77*, 1288−1294.
(9) Ramasami, P.; Wadi, R. K. Partial Molal Volumes and Adiabatic Compressibilities of Transfer of Glycine and DL-Alanine from Water to Aqueous Sodium Sulphate at 288.15, 298.15 and 308.15 K. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 243−247.
(10) Talukdar, H.; Rudra, S.; Kundu, K. K. Thermodynamics of Transfer of Glycine, Diglycine, and Triglycine from Water to Aqueous Solutions of Urea, Glycerol, and Sodium nitrate. *Can. J. Chem.* **1988**, *66*, 461−468.
(11) Yan, Z.; Wang, J.; Zheng, H.; Liu, D. Volumetric Properties of Some α-Amino Acids in Aqueous Guanidine Hydrochloride at 5, 15, 25 and 35 °C. *J. Solution Chem.* **1998**, *27*, 473−483.

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