Volume and Adiabatic Compressibility of Amino Acids in Urea-Water Mixtures

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Apparent molal volumes (ϕ_V) and apparent molal adiabatic compressibilities (ϕ_{KS}) at 25 °C have been obtained for the following amino acids in water and in urea-water mixtures (up to 8 mol kg^{-1} urea for ϕ_V and at 3 mol kg^{-1} for ϕ_{KS}) from measurements of density and ultrasonic velocity: glycine, L-alanine, β-alanine, α-aminoisobutyric acid, L-valine, L-serine, and L-threonine. The limiting values ϕ_V° and ϕ_{KS}° were calculated by a linear extrapolation using the least-squares method. These data were used to derive the volumes of transfer and the adiabatic compressibilities of transfer of the amino acids from water to aqueous urea solutions. Both of these transfer functions were positive. The results were interpreted in terms of structure-breaking interaction of urea upon water.

In recent years, mixed aqueous solvents are used extensively in chemistry and other fields to control factors, *e.g.*, solubility, reactivity, and stability of systems. Due to the high solubility of urea in water and its protein-denaturing ability in aqueous solutions urea-water mixtures have been employed as the solvents for extensive experimental investigations. The theoretical treatment of the mixtures also has been carried out by Frank and Franks.[1] It is generally accepted that urea does not appreciably interact with either hydrophobic or hydrophilic molecules or groups, and acts mainly to disrupt hydrogen bonding among water molecules in aqueous medium. Thus, as noted by Mathieson and Conway, thermodynamic and transport properties of aqueous urea solutions with added electrolytes or nonelectrolytes have been interpreted in terms of nonspecific interaction of urea with the solute.[2] The values of the thermodynamic functions are consistent with the hypothesis that urea solutions are similar to water but with less structure.

For a better understanding of an increased interest in the states of water in the living cells it will be necessary to carry out systematic investigations of model systems under various conditions. In the present work densities and ultrasonic velocities were measured at 25 °C for aqueous amino acid solutions and for amino acid–urea–water ternary systems at various concentrations. Apparent molal volumes and apparent molal adiabatic compressibilities and their limiting values were calculated from these data.

Experimental

The amino acids and urea used in this study were guaranteed reagents from Wako Chemical Industries, Ltd., and were used without further purification. As seen later, their limiting apparent molal volumes in aqueous solutions measured here were all in good agreement with the values reported recently by other workers, except α-aminoisobutyric acid, for which there is no previous value reported. The densities of the amino acids solutions were measured in water and in 1, 3, 5, and 8 mol kg^{-1} aqueous urea solutions and the ultrasonic velocities in water and in 3 mol kg^{-1} urea solution.

All solutions were prepared by weight in the concentration range 0.2—1 mol kg^{-1} (the number of moles of amino acid per kg of mixed solvent whenever the mixed solvent was used); the highest concentration depends on the solubility of the amino acids. The experimental errors were greatly reduced by employing the rather high amino acid concentration range.[3-5] Fresh stock solutions of urea were prepared for each series of measurements; the densities and ultrasonic velocities were determined for each stock solution.

The densities were measured to $\pm 2\times10^{-6}$ g cm^{-3} with an Anton Paar DMA 02D Densimeter. The system was calibrated with dry air and distilled water; their densities were taken from the literatures.[6,7]

The ultrasonic velocities were measured at 5 MHz to a precision of ±0.1 m s^{-1} using an improved ultrasonic interferometer designed by Nomura and Kawaizumi,[8] and the principle of the system is identical with the one used by Nomura *et al.*[5] Since there is an error due to the temperature variation, a relative error is estimated to be $\delta u/u=\pm1.0\times10^{-4}$. The temperature control of the thermostated bath systems regulating the densimeter and ultrasonic interferometer was better than ±0.005 °C.

Results

The densities and ultrasonic velocities were determined at 25 °C for the following amino acids; glycine (Gly), L-alanine (Ala); β-alanine (β-Ala), α-aminoisobutyric acid (Me-ala), L-valine (Val), L-serine (Ser), and L-threonine (Thr). The apparent molal volumes were calculated from the relation:

$$\phi_V = \frac{M}{d} + \frac{1000(d_0-d)}{md_0d}, \quad (1)$$

where d_0 and d are the densities of a solvent and a solution respectively and m is the molal concentration; M, the molecular weight of the amino acid. The adiabatic compressibilities β_S and the apparent molal adiabatic compressibilities ϕ_{KS} were calculated using Eqs. 2 and 3 respectively, where β_S is expressed in bar^{-1}.

$$\beta_S = \frac{100}{du^2}, \quad (2)$$

$$\phi_{KS} = \frac{\beta_S M}{d} + \frac{1000(\beta_S d_0 - \beta_S^\circ d)}{md_0d}, \quad (3)$$

where u is the velocity of sound (m s^{-1}) and β_S° is the adiabatic compressibility of the solvent. The limiting values ϕ_V° and ϕ_{KS}° were obtained by linear extrapolation using the least-squares fit to the equations:

Table 1. Limiting apparent molal volumes of amino acids in water

Amino acid	ϕ_V° / cm^3 mol^{-1}	S_V / cm^3 mol^{-2} kg	σ[a] / cm^3 mol^{-1}	ϕ_V° / cm^3 mol^{-1}
Glycine	43.23	0.82	0.01	43.19[b]
L-Alanine	60.50	0.59	0.02	60.47[b]
β-Alanine	58.25	0.95	0.02	58.28[c]
α-Aminoisobutyric acid	77.54	0.21	0.01	—
L-Valine	90.78	0.16	0.01	90.78[b]
L-Serine	60.72	1.14	0.02	60.62[d]
L-Threonine	76.94	0.89	0.01	76.83[e]

a) Standard deviation. b) Ref. 9. c) Ref. 11. d) Ref. 12. e) Ref. 13.

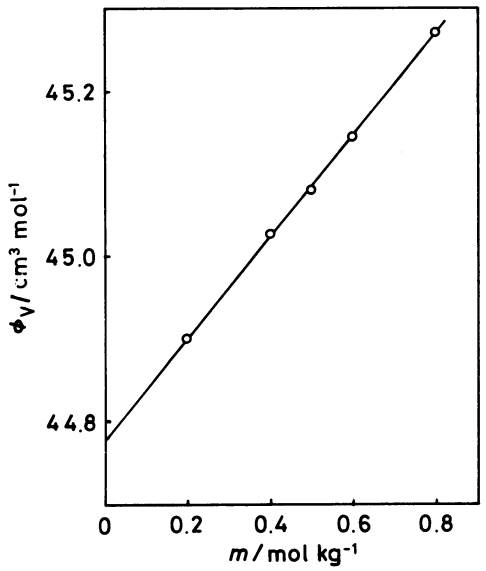

Fig. 1. Plot of ϕ_V *vs.* m for glycine in 3 mol kg^{-1} urea solution.

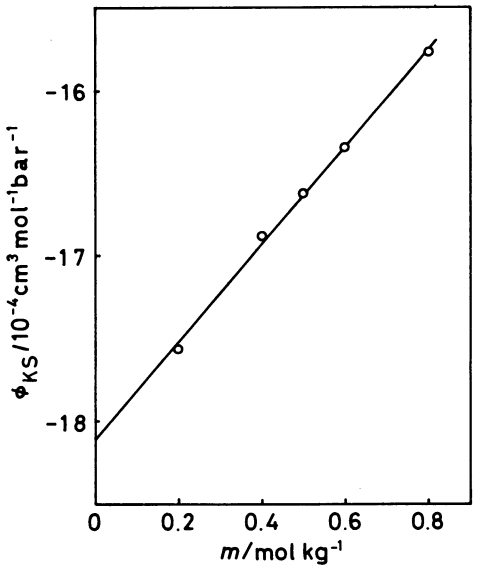

Fig. 2. Plot of ϕ_{KS} *vs.* m for glycine in 3 mol kg^{-1} urea solution.

$$\phi_V = \phi_V^\circ + S_V m, \tag{4}$$

and

$$\phi_{KS} = \phi_{KS}^\circ + S_K m, \tag{5}$$

where $\phi_V^\circ = \overline{V}^\circ$ and $\phi_{KS}^\circ = \overline{K}^\circ$ are the infinite dilution partial molal volume and partial molal adiabatic compressibility, respectively, and S_V and S_K are the experimental slopes. The ϕ_V and ϕ_{KS} of glycine in 3 mol kg^{-1} aqueous urea solutions are plotted against the amino acid molality in Figs. 1 and 2 respectively. The values of ϕ_V°, S_V, and standard deviations, σ, obtained in water are listed in Table 1 along with literature values reported recently. The apparent molal volumes at infinite dilution in 1, 3, 5, and 8 mol kg^{-1} aqueous urea solutions are given in Table 2, and the apparent molal adiabatic compressibilities at infinite dilution and standard deviations, σ, in water and in 3 mol kg^{-1} aqueous urea solutions are given in Table 3.

As seen from Table 1, our results are in good agreement with the literature values. As with the apparent molal adiabatic compressibilities our results are in good agreement with the values reported by Millero *et al.*,[9] but Cabani *et al.* obtained rather larger (less negative) values.[10] For example, the $10^4 \times \phi_{KS}^\circ$ value of glycine is reported as −27.00 by Millero *et al.* and as −25.0 by Cabani *et al.* The main reason for the discrepancy is probably due to the difference in the extrapolation procedure, thus Cabani *et al.* fitted to the equation

$$\phi_{KS} = \phi_{KS}^\circ + Bm + Cm^2. \tag{6}$$

Table 2. Limiting apparent molal volumes of amino acids in urea-water mixtures

Amino acid	ϕ_V° / cm^3 mol^{-1}			
	1[a]	3[a]	5[a]	8[a]
Glycine	43.92	44.78	45.39	46.08
L-Alanine	60.90	61.75	62.33	63.10
β-Alanine	58.77	59.65	60.28	60.89
α-Aminoisobutyric acid	77.92	78.50	78.99	79.80
L-Valine	91.03	91.94	92.47	93.08
L-Serine	61.37	62.41	63.05	64.06
L-Threonine	77.46	78.38	79.06	79.79

a) Urea concentration in mol kg^{-1}.

Table 3. Limiting apparent molal adiabatic compressibilities

Amino acid	Water		Urea-Water[a)]	
	ϕ_{KS}° / 10^{-4} cm^3 mol^{-1} bar^{-1}	σ / 10^{-4} cm^3 mol^{-1} bar^{-1}	ϕ_{KS}° / 10^{-4} cm^3 mol^{-1} bar^{-1}	σ / 10^{-4} cm^3 mol^{-1} bar^{-1}
Gly	−27.16	0.36	−18.11	0.05
Ala	−24.74	0.18	−15.30	0.14
β-Ala	−26.36	0.11	−16.67	0.09
Mc-ala	−23.46	0.27	−12.55	0.10
Val	−30.43	0.10	−15.91	0.45
Ser	−29.88	0.10	−19.49	0.16
Thr	−31.23	0.10	−19.01	0.18

a) Urea concentration is 3 mol kg^{-1}.

In our experiment no systematic deviations at higher concentrations from the straight line expressed by Eq. 5 were observed up to the highest concentration studied, and so our data were extrapolated using Eq. 5 following Millero *et al.*[9]

Discussion

The volume and compressibility behaviour of solutes in solution can provide information concerning solute-solvent and solute-solute interactions. The infinite dilution partial molal volumes and adiabatic compressibilities are, by definition, independent of solute-solute interactions and thus determined only by the respective intrinsic value and the solute-solvent interactions. Accordingly, they can be used to examine solute-solvent interactions. The slopes, S_V and S_K, can be assumed to be an indication of solute-solute interactions. The volumes of transfer, $\Delta\overline{V}_{tr}^{\circ}=\overline{V}^{\circ}$(urea)$-\overline{V}^{\circ}$(water), for some amino acids studied are plotted *versus* the urea concentration (Fig. 3). The magnitudes of the transfer functions increase continuously with the urea concentration. The same trend was observed by Enea *et al.*[14] for the transfer functions of the heat capacities and volumes of three amino acids (Gly, Ala, and Ser) and several oligopeptides in urea-water systems. Detailed results are, however, presented only for 7 mol dm^{-3} urea concentration, and we cannot compare our volume data with their data directly, though the sequence of increment of $\Delta\overline{V}_{tr}^{\circ}$ for these three amino acids are identical with ours. Desrosiers *et al.*[15] also observed the same trend for the transfer functions of the volume, expansibility, compressibility and heat capacity of alkali halides in urea-water mixtures. The transfer functions of volume for Ala and β-Ala are very similar up to 8 mol kg^{-1} urea concentration, though their $\overline{V}^{\circ}$ values differ by *ca.* 2 cm^3 mol^{-1} in urea-water mixtures as well as in water. This fact indicates the more effective hydration of β-Ala in aqueous urea solutions in comparison with Ala to almost the same amount as in water. In Fig. 4 $\overline{V}^{\circ}$'s are plotted *versus* V_W; V_W is the van der Waals volume, which was calculated using the data reported by Bondi[16] and by Edward.[17] As seen from Fig. 4, the amino acids having no OH group lie almost on a straight line both in water and in 8 mol kg^{-1} urea. Positions of

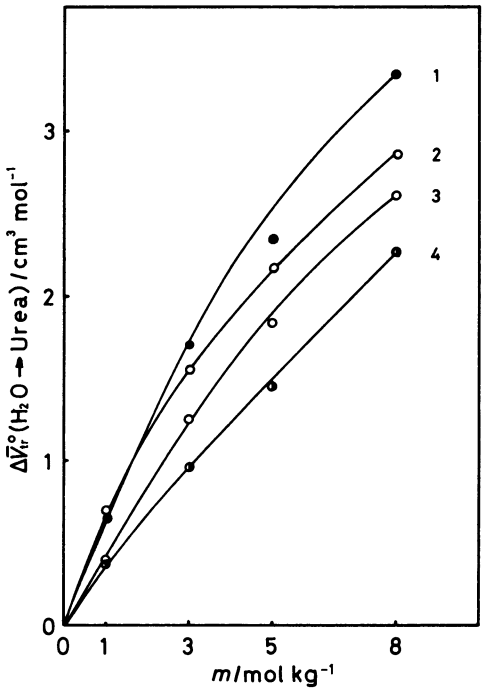

Fig. 3. Plot of $\Delta\overline{V}_{tr}^{\circ}$ *vs.* urea concentration.
(1) Ser, (2) Gly, (3) Ala, (4) Me-ala.

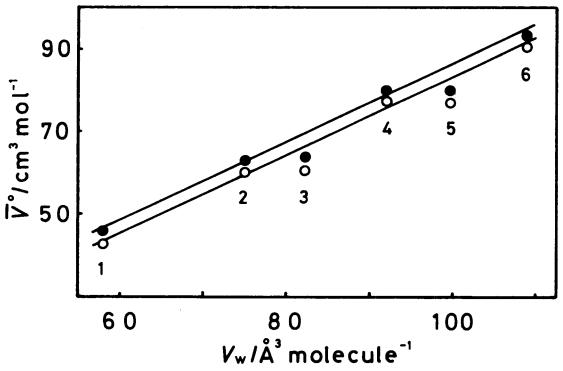

Fig. 4. Plot of $\overline{V}^{\circ}$ of α-amino acids *vs.* van der Waals volume.
(1) Gly, (2) Ala, (3) Ser, (4) Me-ala, (5) Thr, (6) Val.
○: in water, ●: in 8 mol kg^{-1} urea.

serine and threonine which have OH groups are below the straight line in urea solution as in water. This fact shows that even in 8 mol kg^{-1} urea solution there is a rather large volume decrease brought about by hydrogen bonding of the OH group with water.

Millero *et al.*[9] estimated the intrinsic partial molal volume $\overline{V}°$(int) of amino acids from the crystal volume and also from the partial molal volume of uncharged isomers. The intrinsic volume is made up of two terms, the van der Waals volume and the volume due to packing effects. They obtained the electrostriction partial molal volume, $\overline{V}°$(elect), due to the hydration of amino acid from the experimentaly measured $\overline{V}°$ and the reasonably estimated $\overline{V}°$(int):

$$\overline{V}°(\text{elect}) = \overline{V}° - \overline{V}°(\text{int}). \quad (7)$$

Using the corresponding equation for the adiabatic compressibility

$$\overline{K}°(\text{elect}) = \overline{K}° - \overline{K}°(\text{int}), \quad (8)$$

they also estimated the electrostriction partial molal compressibilities of the amino acids. From both $\overline{V}°$(elect) and $\overline{K}°$(elect) they estimated the number of water molecules hydrated to the amino acids as 4.1±0.4. Recently, Shahidi and Farrell[13] also calculated the electrostriction volumes and the number of hydrated water for the amino acids from a somewhat different standpoint using Eq. 9 and consideration of the branching effects

$$\overline{V}° = 4\pi(r_w + 0.57)^3 N_A/3 - \sum_{i=1}^{m} n_i\sigma_i - E, \quad (9)$$

where r_W is the van der Waals radius of the amino acid, n_i is the number of interacting groups of type i in the solute, which contains m different interacting groups with the solvent; σ_i is the volume decrease due to the specific group-solvent interaction (*e.g.*, hydrogen-bonding),[18] and N_A is the Avogadro constant. The van der Waals radius is calculated from the van der Waals volume V_W assuming the amino acid to be spherical *i.e.*, $r_W=(3V_W/4\pi)^{1/3}$. The value 0.57 in Eq. 9 is added to the van der Waals radius because there is an empty volume between the solute and the solvent, which is assumed to be a spherical shell of constant thickness of 0.57 Å. An average of 13.0±1.2 cm^3 mol^{-1} was obtained for the electrostriction E for the α-amino acids. Since the volume of water is decreased by 3 cm^3 per mole when taken from the bulk phase to the region near the amino acid,[9] the number of water molecules involved in hydration of the α-amino acid is calculated as to be 4.33±0.39.

As noted by Millero *et al.*,[9] $\overline{K}°$(int) is small and as a first approximation one can assume $\overline{K}°$(int)=0. This makes $\overline{K}°=\overline{K}°$(elect). Thus the adiabatic compressibilities are the better measure for the hydration of the solute. The partial molal adiabatic compressibilities of the seven amino acids studied increase (*i.e.*, less negative) in the following order in water:

Thr<Val<Ser<Gly<β-Ala<Ala<Me-ala,

and in the following order in 3 mol kg^{-1} urea solution:

Ser<Thr<Gly<β-Ala<Val<Ala<Me-ala.

From these two series it is seen that the most hydrophobic solute studied, valine, is most affected by the transfer from water to 3 mol kg^{-1} urea solution. Of the solutes having an OH group threonine is more hydrophobic than serine and has a larger value of $\Delta\overline{K}_{tr}°$. The sequence Gly<β-Ala<Ala<Me-ala is the same both in water and in urea solution. By the introduction of the methyl group in glycine, *i.e.*, Gly→Ala and Ala→Me-ala, cospheres of the hydrophilic COO^- and NH_3^+ groups overlap with that of the hydrophobic group, thus decreasing their hydration ability in that order both in water and in aqueous urea solution. The decrease in hydration is reflected in the increase in compressibility. Concerning alanines, β-alanine with a larger charge separation has a larger negative value of adiabatic compressibility, indicating that β-alanine has a larger number of hydrated water molecules. According to Millero *et al.*[9] the number of water molecules hydrated to the amino acid, n_H, is given by

$$n_H = -\overline{K}°(\text{elect})/\beta_B°\overline{V}_B°, \quad (10)$$

where $\beta_B°$ is the compressibility of bulk water and $\overline{V}_B°$ is the molar volume of bulk water. As shown by Desrosiers *et al.*,[15] $\beta_B°$ and $\overline{V}_B°$ values in 3 mol kg^{-1} urea solution differ only slightly from those in pure water. Thus the ratio $\overline{K}°$(urea)/$\overline{K}°$(water) represents the ratio of hydration number of the solute in aqueous urea solution to that in water. The ratios for valine and α-aminoisobutyric acid are 0.52 and 0.53 respectively and those for the other amino acids studied are 0.61—0.67. The results show that more hydrophobic amino acids undergo more dehydration effect of urea.

As noted by Enea and Jolicoeur,[14] urea-water mixtures exhibit a dielectric constant similar to water (8% higher at 3 mol dm^{-3} urea), so the electrostatic hydration effects of amino acids should be comparable in both solvents; further, it may be improbable that the pK values of amino acids in urea-water mixtures differ appreciably from those in pure water. The pH values of 0.3 mol kg^{-1} amino acid aqueous solutions studied here, *e.g.*, glycine; 6.0, β-alanine: 6.8, were very close to the isoelectric point of the respective amino acid. Those of the corresponding aqueous urea solutions were only *ca.* 0.4 pH unit higher than those in water, *e.g.*, glycine: 6.4, β-alanine: 7.2. Accordingly, all amino acid molecules can be considered to exist virtually as zwitter ions in both solvents; hence, association or dissociation effects of ionic groups to $\phi_V°$ or $\phi_{KS}°$ must be trivial for water→aqueous urea transfer.

As noted above, the amino acids have *ca.* 4 molecules of hydrated water on an average, then how many water molecules hydrate the amino acids in 3 mol kg^{-1} urea-water mixtures? Since, for example, the value of $\overline{K}°$(urea)/$\overline{K}°$(water) for alanine is 0.6, it is estimated that it has 2.4 hydrated water molecules in the solvent. On the other hand, the value of $\Delta\overline{V}_{tr}°$ of alanine from water to the solvent was 1.25 cm^3 mol^{-1}; if it is supposed that this increment of volume is entirely due to the decrease in hydration in the solvent, the decrease in hydrated water molecules is 1.25/3=0.42, thus the alanine molecule has 3.6 water molecules in the solvent. Accordingly, the compressibility data gave the smaller hydration number than that derived from the vol-

ume data. Of these two values, 2.4 and 3.6, we consider that the former may be more correct, because the compressibility data are more directly related to hydration; the same relationship as for alanine was seen for the other amino acids studied. Thus, using the compressibility data as a measure of hydration, we tried to estimate the thickness of the empty volume between the amino acid and the solvent in 3 mol kg^{-1} urea solution, which is 0.57 Å in aqueous solution, from Eq. 9. The decrease in the thickness brings about the decrease in $\Delta\overline{V}_{tr}^{\circ}$, so seemingly the increase in hydration number in the solvent. The electrostriction E in Eq. 9 was modified by a factor $\overline{K}^{\circ}$(urea)/$\overline{K}^{\circ}$(water) of respective amino acid; the resulting electrostriction E' was used, together with σ_i and $\overline{V}^{\circ}$(urea), to calculate the thickness for each amino acid in the mixed solvent. As with σ_i values, the hydrogen-bonding effect of OH group of serine and threonine is still apparent in even 8 mol kg^{-1} urea solution as seen from Fig. 4, so the values in water were used. The results show that the thickness is 0.54±0.02 Å instead of 0.57 Å in water. Though more experimental studies must be necessary to draw a firm conclusion, the decrease in thickness by 0.03 Å on the average might be due to the lower structure in the mixed solvent broken by the presence of urea. In order to clarify the thermodynamics of amino acid solutions, similar studies in other mixed solvent media are in progress.

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