2064 © *1984 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, *57*, *2064—2068 (1984)* [Vol. 57, No. 8

The Volume, Adiabatic Compressibility, and Viscosity of Amino Acids in Aqueous Alkali-chloride Solutions

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(Received January 5, 1984)

The apparent molal volumes (ϕ_V), apparent molal adiabatic compressibilities (ϕ_{KS}), and relative viscosities (n_r) at 25 °C have been obtained for the following amino acids in aqueous alkali-chloride solutions from measurements of the density, the ultrasonic velocity, and the flow time: glycine, L-alanine, β-alanine, α-aminoisobutyric acid, L-serine, and L-threonine. Viscosity measurements were also carried out in water for reference. The alkali chlorides used were lithium, sodium, and potassium chlorides. The limiting values $\phi_V{}^\circ$, $\phi_{KS}{}^\circ$, and the extended Jones-Dole viscosity coefficients, *B* and *D*, were calculated by a linear extrapolation using the least-squares method. All the $\phi_V{}^\circ$, $\phi_{KS}{}^\circ$, and *B* values obtained in the mixed solvents were larger than the corresponding ones in water, except in a few cases of the *B* coefficient. The results are discussed in terms of the dehydration effect of the electrolytes upon the amino acids.

Much attention has been given recently to the effects of electrolytes and nonelectrolytes on the structure of water. A quantitative interpretation of the factors which govern the structure remains difficult notwithstanding numerous remarkable investigations. We ourselves carried out volume and compressibility measurements of several amino acids in urea–water mixtures in order to understand the effect of urea upon the hydration of the amino acids.[1] The results showed that both the apparent molal volumes and the apparent molal adiabatic compressibilities of the amino acids increased upon the addition of urea in aqueous amino-acid solutions. This was explained in terms of the dehydration effect of urea upon the amino-acid molecules. Although the influence of salts on the activity coefficients of nonelectrolytes in aqueous solutions and on kinetic problems was examined carefully for a long time, there was no systematic study of the influence of salts upon the volume, compressibility, and viscosity of nonelectrolytes. Recently, as with cyclic amino acids, proline and hydroxyproline, we carried out a volume, compressibility, and viscosity study in aqueous alkali-chloride solutions.[2] For a better understanding of the behavior of the amino acids in mixed aqueous solvents, the densities, ultrasonic velocities, and viscosities were measured at 25 °C for six amino acids in amino acid-water-alkali chloride ternary systems at various concentrations. The apparent molal volumes and apparent molal adiabatic compressibilities, and the viscosity *B*- and *D*-coefficients were calculated from these data.

Experimental

The amino acids and alkali chlorides used in this study were guaranteed reagents from Wako Chemical Industries, Ltd., and were used without further purification except the lithium chloride, which was used after having been dried for several hours at *ca.* 150 °C. The densities and efflux times were measured in 1 and 2 mol kg^{-1} aqueous alkali-chloride solutions. Viscosity measurements in water were also carried out. The ultrasonic velocities were determined in 2 mol kg^{-1} alkali-chloride solutions.

All the amino-acid solutions were prepared by weight in the concentration range of 0.2—1 mol kg^{-1} (the number of moles of amino acid per kg of the mixed solvent whenever the mixed solvents were used) ; the highest concentration of the respective amino acid depended on the solubility of the amino acid.

The density and ultrasonic velocity measurements have been described previously.[1] The viscosities were measured by means of two Cannon-Fenske capillary viscometers with efflux times of *ca.* 210 and 240 s for distilled water; the kinetic-energy correction needed was negligibly small. The temperature control of the thermostated bath systems regulating the densimeter, ultrasonic interferometer, and viscometers was better than ±0.005 °C.

The pH values of all the amino-acid solutions, both in water and in aqueous alkali-chloride solutions, were very close to the isoelectric point of the respective amino acid; thus, no appreciable change in the structure of dipolar molecules can be considered.

Results

The densities, ultrasonic velocities, and efflux times were measured at 25 °C for the following amino-acid solutions; glycine (Gly), L-alanine (Ala), β-alanine (β-Ala), α-aminoisobutyric acid (Me-ala), L-serine (Ser), and L-threonine (Thr). The apparent molal volumes were obtained from Eq. 1:

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

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

$$\beta_S = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_S = \frac{100}{d\,u^2}, \tag{2}$$

and:

$$\phi_{KS} = -\left(\frac{\partial \phi_V}{\partial P}\right)_s = \frac{\beta_S M}{d} + \frac{1000(\beta_S d_0 - \beta_S{}^\circ d)}{m\,d_0\,d}, \tag{3}$$

where *u* is the velocity of sound (m s^{-1}) and where $\beta_S{}^\circ$ is the adiabatic compressibility of the solvent. The limiting values of the apparent molal volumes and the apparent molal adiabatic compressibilities were calculated by a linear extrapolation using the least-squared fit to these equations:

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

and:

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

where $\phi_V{}^{\circ}$ and $\phi_{KS}{}^{\circ}$ are the infinite dilution apparent molal volume and the apparent molal adiabatic compressibility respectively, and where S_V and S_K are the experimental slopes. At an infinite dilution, $\phi_V{}^{\circ} = \bar{V}^{\circ}$ (the infinite dilution partial molal volume) and $\phi_{KS}{}^{\circ} = \bar{K}_S{}^{\circ}$ (infinite dilution partial molal adiabatic compressibility). The derived values for $\phi_V{}^{\circ}$ are summarized in Table 1, with the literature data on proline and hydroxyproline.[2] The standard deviations are mostly less than 0.02 $cm^3 mol^{-1}$. The values for $\phi_{KS}{}^{\circ}$ and the standard deviations, σ, in water and 2 $mol\,kg^{-1}$ aqueous alkali-chloride solutions are listed in Table 2.

The viscosity, η, of dilute solutions of nonelectrolytes is represented by:[3]

$$\eta = \eta_0(1 + Bc), \tag{6}$$

TABLE 1. LIMITING APPARENT MOLAL VOLUMES $\phi_V{}^{\circ}$

Amino acid	$\phi_V{}^{\circ}$ / $cm^3 mol^{-1}$						
	H_2O	LiCl		NaCl		KCl	
		1*m*	2*m*	1*m*	2*m*	1*m*	2*m*
Gly	43.23	44.68	45.63	45.05	46.24	44.89	46.12
Ala	60.50	61.59	62.48	62.08	63.19	61.97	63.14
β-Ala	58.25	59.91	60.67	60.12	61.36	60.13	61.21
Me-ala	77.54	78.45	79.18	78.86	79.97	78.78	79.98
Ser	60.72	62.15	63.27	62.74	64.12	62.76	64.08
Thr	76.94	78.10	79.00	78.73	80.11	78.73	79.95
Pro[a)]	82.87		84.37		85.30	84.22	85.00
Hyp[b)]	84.45		86.54		87.34	86.09	87.10

a) Ref. 2. b) Hyp: hydroxyproline, Ref. 2.

TABLE 2. LIMITING APPARENT MOLAL ADIABATIC COMPRESSIBILITIES WITH THEIR ESTIMATED STANDARD DEVIATIONS σ IN PARENTHESES

Amino acid	$\phi_{KS}{}^{\circ}$ / $10^{-4} cm^3 mol^{-1} bar^{-1}$			
	H_2O	2*m* LiCl	2*m* NaCl	2*m* KCl
Gly	−27.16(36)	−11.80(14)	−10.30(06)	−11.03(11)
Ala	−24.74(18)	−10.99(04)	−8.69(25)	−9.46(11)
β-Ala	−26.36(11)	−10.46(10)	−9.38(23)	−10.47(09)
Me-ala	−23.46(27)	−9.39(13)	−5.38(07)	−6.72(07)
Ser	−29.88(10)	−13.80(07)	−11.92(16)	−12.04(13)
Thr	−31.23(10)	−14.26(02)	−11.05(07)	−12.02(13)
Pro[a)]	−24.19(09)	−7.85(04)	−4.55(19)	−6.86(26)
Hyp[a)]	−22.27(08)	−7.23(12)	−4.74(09)	−6.70(13)

a) Ref. 2.

TABLE 3. VISCOSITY *B* AND *D* COEFFICIENTS

Amino acid		H_2O	LiCl		NaCl		KCl	
			1*m*	2*m*	1*m*	2*m*	1*m*	2*m*
Gly	$B/dm^3 mol^{-1}$	0.135	0.134	0.151	0.147	0.156	0.138	0.158
	$D/dm^6 mol^{-2}$	0.024	0.031	0.023	0.024	0.024	0.032	0.021
Ala	$B/dm^3 mol^{-1}$	0.247	0.255	0.262	0.256	0.263	0.248	0.253
	$D/dm^6 mol^{-2}$	0.050	0.055	0.060	0.056	0.059	0.061	0.065
β-Ala	$B/dm^3 mol^{-1}$	0.216	0.219	0.230	0.227	0.236	0.217	0.229
	$D/dm^6 mol^{-2}$	0.042	0.048	0.051	0.043	0.044	0.050	0.049
Me-ala	$B/dm^3 mol^{-1}$	0.346	0.359	0.369	0.354	0.366	0.352	0.354
	$D/dm^6 mol^{-2}$	0.112	0.111	0.115	0.114	0.113	0.112	0.118
Ser	$B/dm^3 mol^{-1}$	0.225	0.235	0.249	0.242	0.253	0.241	0.247
	$D/dm^6 mol^{-2}$	0.053	0.056	0.057	0.060	0.063	0.058	0.062
Thr	$B/dm^3 mol^{-1}$	0.335	0.337	0.348	0.336	0.352	0.324	0.343
	$D/dm^6 mol^{-2}$	0.084	0.106	0.113	0.111	0.108	0.124	0.108
Pro[a)]	$B/dm^3 mol^{-1}$	0.279		0.306		0.304	0.293	0.304
	$D/dm^6 mol^{-2}$	0.07		0.08		0.07	0.07	0.07
Hyp[a)]	$B/dm^3 mol^{-1}$	0.281		0.309		0.311	0.297	0.306
	$D/dm^6 mol^{-2}$	0.08		0.10		0.09	0.08	0.09

a) Ref. 2.

where η_0 is the viscosity of the solvent, c is the molar concentration of the solute, and B is an empirical constant which is called usually the Jones-Dole viscosity B-coefficient. This is known to depend on the size of the solute particle and also on the interaction between the solute and solvent molecules. The B-coefficient is expressed in molar concentration units; thus, the molar concentrations were calculated using the density data. For the more concentrated solutions of the amino acid used in this study, the relative viscosity is given by Eq. 7, in which D is an another empirical constant.[4] Desnoyers and Perron examined the D-coefficients and concluded that they cannot be interpreted unambiguously.[5]

$$\eta_r = 1 + Bc + Dc^2 \tag{7}$$

By rearranging this equation, we obtain:

$$(\eta_r - 1)/c = B + Dc. \tag{8}$$

The viscosity coefficients, B and D, were calculated by linear extrapolation using the least-squares fit to Eq. 8. Table 3 shows the viscosity in terms of the parameters of Eq. 7.

Discussion

Volume Results. The volume behavior of a solute in an infinite dilution is independent of the solute-solute interactions and thus can provide information concerning solute-solvent interactions. As may be seen from Table 1, the limiting apparent molal volumes of the amino acids in mixed solvents are larger than those of the corresponding ones in water. This was also observed for the data in aqueous urea solutions and was discussed in terms of the dehydration effect of urea upon the amino acids.[1] In order to ascertain the influence of the sodium-chloride concentration on the $\phi_V{}^\circ$ of the amino acids, the transfer volumes, $\Delta \bar{V}_{tr}{}^\circ$, are plotted *vs.* the concentration of sodium chloride in Fig. 1;

$$\Delta \bar{V}_{tr}{}^\circ = \phi_V{}^\circ(\text{NaCl}) - \phi_V{}^\circ(\text{water}). \tag{9}$$

The transfer functions increase with the concentration, as in aqueous urea solutions.[1,6]

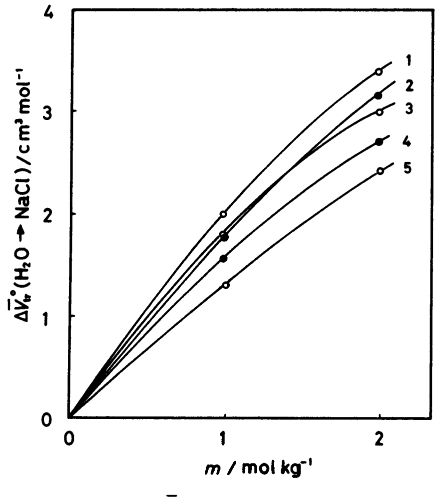

Fig. 1. Plot of $\Delta \bar{V}_{tr}{}^\circ$ *vs.* m for NaCl.
1: Ser, 2: Thr, 3: Gly, 4: Ala, 5: Me-ala.

The transfer volumes of the six amino acids studied decrease in the following order:

Ser > Thr > β-Ala > Gly > Ala > Me-ala,

for both water→aq NaCl and water→aq KCl transfers. The water→aq LiCl transfer volumes are in the same sequence except the Thr, which occurs between Gly and Ala. Our earlier study of the water→aqueous urea-transfer volumes gave a similar sequence:

Ser > Thr > Gly > β-Ala ≈ Ala > Me-ala.

The positive sign of the transfer volumes can be ascribed mainly to the fact that the hydration number of the amino acids is reduced by the addition of alkali chloride or urea: *i.e.*, the electrostriction effect which brings about the diminution in the volume of the solvent caused by the electrical fields of the dipolar solutes is reduced in the mixed solvents as compared with that in pure water. Ser and Thr, which contain an OH group, have larger transfer volumes; this was also observed in the case of hydroxyproline, *i.e.*, it has the larger transfer volume than proline, as may be seen from Table 1. In water-urea mixtures, the transfer volume of β-alanine was almost the same as that of alanine and less than that of glycine at a particular urea concentration.[1] In aqueous alkali-chloride solutions, β-Ala has a larger transfer volume than that of Gly. This may result from the fact that β-Ala has a larger dipole moment than that of Ala; thus, the former interacts more with the alkali and chloride ions than does Ala, which has a smaller dipole moment. The more the ion-dipole interaction, the more the dehydration effect. On the other hand, there may be no difference, or a very small one, as with the interaction of the amino acids with urea.

Compressibility Results. The infinite-dilution data for the compressibility can also give information concerning solute-solvent interactions. The limiting partial molal adiabatic compressibilities of the amino acids can be expressed by a simple model:[7]

$$\bar{K}_S{}^\circ = \bar{K}_S{}^\circ(\text{int}) + \bar{K}_S{}^\circ(\text{elect}), \tag{10}$$

where $\bar{K}_S{}^\circ$ (int) is the intrinsic partial molal adiabatic compressibility of the amino acid and where $\bar{K}_S{}^\circ$ (elect) is the electrostriction partial molal adiabatic compressibility due to the hydration of the amino acid. As has been noted by Millero *et al.*, as a first approximation, one can assume that $\bar{K}_S{}^\circ$ (int)≈0, since one would expect $\bar{K}_S{}^\circ$ (int) to be very small.[7] Thus, $\bar{K}_S{}^\circ$ may be thought to represent $\bar{K}_S{}^\circ$ (elect). The $\bar{K}_S{}^\circ$ values of the amino acids in water are all negative; this must come from the hydration of the charged centers of the amino acids, as the hydrated water molecules are already compressed and thus less compressible than that in the bulk. For the amino acids, the order of increasing (less negative) $\bar{K}_S{}^\circ$ values in water is:

Thr < Ser < Gly < β-Ala < Ala < Me-ala.

This sequence may be considered to show a decreasing order of hydration, as a first approximation, particularly for the amino acids without the OH group, as will be mentioned below. If all the amino acids were subjected to the dehydration effect by the addition of alkali chloride in the same proportion, the $\bar{K}_S°$ (MCl)/$\bar{K}_S°$ (water) ratios of the amino acids may be almost the same. The ratios decrease in the following order in $2m$ NaCl:

$$\text{Ser} > \text{Gly} > \beta\text{-Ala} \approx \text{Thr} > \text{Ala} > \text{Me–ala},$$

and in $2m$ KCl:

$$\text{Gly} \approx \text{Ser} > \beta\text{-Ala} > \text{Thr} \approx \text{Ala} > \text{Me–ala}.$$

The order in $2m$ LiCl differs a little compared with those in NaCl and in KCl, but the difference in ratios is rather small; therefore, the series is probably less reliable. The ratios in $3m$ aqueous urea show almost the same order as in NaCl and in the KCl solutions.[1] Thr and Ser are the amino acids with an OH group. As has been noted by Mathieson and Conway, ions which hydrogen-bond with water have unusual compressibilities.[8] Some have anomalously high compressibilities, while the OH^- ion exhibits a low compressibility. Thus, these two amino acids may be assumed to behave differently with the four other amino acids with no OH group. At any rate, however, Ser has a larger compressibility ratios than Thr in all the solvents studied. Of the four amino acids with no OH group, Gly has the largest value, and Me–ala , the smallest; β-Ala and Ala are in between in each solvent. This corresponds to the order of increasing absolute values of $\bar{K}_S°$ in water, which answers to the order of increasing hydration numbers. Thus, the less hydrated amino acid in water has the lower compressibility ratio in the mixed solvent and then loses the hydrated water molecules more easily in the transfer from water to the mixed solvent. In this connection, it is very interesting to note that the compressibility ratios of proline and hydroxyproline with cyclic secondary amine group are very small (Table 2). These two amino acids must be supposed to lose their hydrated water molecules very easily in the transfer from water to the mixed solvent. However, from another point of view, as seen from the volume data, the number of dehydrated water molecules involved in the transfer increase in the order of increasing hydration number in water; in general, the more hydrated amino acid loses the more hydrated water molecules are involved in the transfer.

Viscosity Results. The viscosity coefficients of several amino acids at 25 °C have been reported by Devine and Lowe;[4] the B-coefficients of Gly and β-Ala were 0.143 and 0.220 respectively, while the D-coefficients were 0.010 and 0.040 respectively. However, the viscosity datum for the lowest concentration for each amino acid appears to be rather erroneous. If it is omitted, the recalculated coefficients were $B=0.139$ and $D=0.020$ for Gly and $B=0.216$ and $D=0.051$ for β-Ala. These values are in good agreement with the respective data listed in Table 3. In Table 3, though the solvent concentration is indicated in the molal concentration, the viscosity coefficients were calculated in molar concentration units, as has been mentioned above. The B-coefficients in the mixed solvents are larger than the corresponding values in water, particularly in $2m$ alkali-chloride solutions. However, the B values of Thr in $1m$ LiCl and $1m$ NaCl are almost the same as those in water and are rather small in $1m$ KCl. The reason for this is not clear at present. Concerning the effects of the salts on the B-coefficient of each amino acid, there seems to exist no regularity. The D-coefficients in the mixed solvents have almost the same value as the corresponding values in water.

For a dilute solution of unsolvated spherical colloidal suspensions, Einstein has derived this relation:

$$\eta_r = 1 + 2.5\phi, \qquad (11)$$

where ϕ is the volume fraction of the solute.[9] If this equation is valid for the amino acids, Eq. 11 becomes:

$$\eta_r = 1 + 0.0025\, V_h\, c, \qquad (12)$$

where V_h is the hydrodynamic volume in $cm^3\,mol^{-1}$. Since the Dc^2 term in Eq. 7 can be assumed to be negligible in a dilute solution, the following relation holds:

$$B = 0.0025\, V_h. \qquad (13)$$

As has been noted by Desnoyers and Perron, V_h may be assumed to be the partial molal volume of the unsolvated solute particle in a continuum solvent.[5] Thus, if the amino-acid molecule has no interaction with the solvent molecules, the larger B value in the mixed solvent might mean a larger hydrodynamic volume in the mixed solvent. However, as has been mentioned above, judging from both the volume and the compressibility data, it must be concluded that, in the mixed solvents, the amino-acid molecules are less hydrated than in water. One possible explanation may be as follows: the hydrated amino-acid molecules interact with the solvent molecules, reducing the structure of water and thus bringing about a dimunution of the B value; this effect must be larger in water than in the mixed solvents, for in the mixed solvents the structure of water is already broken to some extent by the cosolvent alkali chloride. Another explanation may be the ion-dipolar pair formation between the amino-acid molecule and the ions. However, since there is no definite relationship among the effects of ions upon the B values of the amino acids, it is not possible at present to conclude the reason why the B coefficients in the mixed solvents are larger than in water, notwithstanding the less hydration.

Alkali-metal-cation Effects. As may be seen from Tables 1 and 2, for the alkali-metal ions the order of decreasing dehydration effect is:

$$Na^+ > K^+ > Li^+.$$

This sequence is not that of either the increasing or decreasing crystal ionic radii of the alkali metals. The main factor determining the order may be the difference in the interaction of the alkali cations with the amino-acid molecules. Having a rather large dipole moment, the amino-acid molecules should have a rather large interaction with the ions. According to Frank and Wen, a small ion in aqueous solutions is surrounded by three concentric regions.[10] The innermost (Region A) is immobilized; the second (Region B) one is a less ice-like water structure, and the third (Region C) contains normal water. If some hydrated water molecules are lost by the interaction, the electrostriction effect should decrease, and the volume and also the compressibility of the amino acid should increase, in the mixed solvent. Li^+ should have water molecules firmly bounded in Region A because of its small crystal radius. On the other hand, Na^+ and K^+ have larger radii; thus, they should have the water molecules held less firmly than Li^+. If only Na^+ and K^+ ions lose their water molecules in Region A when these three alkali cations interact strongly with the amino acid, this order of increasing effective radius: $Na^+ > K^+ > Li^+$ may result. This accounts for this order of decreasing dehydration effect $Na^+ > K^+ > Li^+$. However, for a further discussion the difference in the hydration number of the alkali cations must be taken into consideration.

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