Limiting Partial Molar Volumes of Glycine, L-Alanine, and L-Serine in Ethylene Glycol + Water Mixtures at 298.15 K

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Densities of solutions of glycine, L-alanine, and L-serine have been measured respectively with an oscillating-tube densimeter at 298.15 K in ethylene glycol + water mixtures with mass fractions of ethylene glycol ranging from 0 to 0.50. From the experimental data, apparent molar volumes and limiting partial molar volumes of each amino acid in the mixtures have been calculated. The trends of transfer volumes are interpreted by the cosphere overlap model. It is concluded that the presence of ethylene glycol in water decreases the extent of electrostriction caused by the amino acid. Substituent effects are also discussed.

Introduction

Protein hydration is an important factor that is responsible for stabilizing the native structure of globular proteins in aqueous solutions. The specific interactions of water with various functional groups on the proteins as well as other solvent-related effects contribute to the formation of the stable folded structure of proteins in solutions (Hvidt and Westh, 1998). The direct study of these important protein–water interactions is difficult because of the complexity of the interactions in a protein macromolecule. However, one useful approach that assists in our understanding of these interactions is to study small molecule–water interactions in compounds chosen to mimic some aspects of the protein structure. This model compound approach has been widely used in recent years (Lilley, 1993, 1994).

As amino acids are the basic building blocks of proteins, it is not surprising that they have been used extensively as model compounds. A variety of thermodynamic properties, such as enthalpies of dissolution (Palecz and Piekarski, 1997; Lou and Lin, 1998), enthalpies of dilution (Castronuovo et al., 1991; Andini et al., 1996), partial molar heat capacities (Marriott et al., 1998), partial molar volumes (Hakin et al., 1994; Yan et al., 1999), and adiabatic compressibilities (Kikuchi et al., 1995), have been determined for aqueous solutions of amino acids. Recently, investigations on systems containing amino acids in aqueous mixed solvents have aroused much attention (Wadi and Ramasami, 1997; Yan et al., 1998; Kumar, 1999; Castronuovo et al., 1999). The reasons for extending the study of amino acids to systems in mixed solvents are not only that aqueous mixed solvents are extensively used in chemistry and related fields to control factors such as solubility, reactivity, and stability of systems but also that biological fluids are ultimately not pure water.

As a continuation of volumetric and enthalpic investigations on amino acids in various aqueous mixed solvents in our laboratory (Ren et al., 1998, 1999a,b, Li et al., 1999; Lin et al., 2000), the present work is aimed at limiting partial molar volumes of glycine, L-alanine, and L-serine

in ethylene glycol–water mixtures. Although ethylene glycol does not exist in cellular or extracellular fluids of living organisms, it finds wide application in pharmacology and the cosmetics industry. When introduced into a living organism as a vehicle for pharmaceuticals or cosmetics, it affects the components of cellular fluids.

Experimental Section

Biochemical reagent-grade glycine, L-alanine, and L-serine were used after being recrystallized twice from methanol–water mixtures and drying at 100 °C for 12 h in an infrared drier and then in vacuo over P_2O_5 at room temperature for at least 48 h. Analytical reagent-grade ethylene glycol was used without further purification. Water was deionized and distilled using a quartz sub-boiling purifier. Both the ethylene glycol–water mixtures in which the mass fractions of ethylene glycol (w) ranged in 0.10 increments from 0 to 0.50 and the amino acid solutions (amino acid + ethylene glycol + water) were prepared by mass on the molality scale.

The densities of solutions were measured with an Anton Paar vibrating-tube digital densimeter (DMA 55) thermostated to better than ±0.01 °C. The temperature in the measuring cell was monitored with a Anton Paar DT 100-20 digital thermometer. The densimeter was calibrated with twice-distilled water and dry air. The density of pure water at 298.15 K was taken as 0.99705 g·cm^{-3} (Kell, 1975). The accuracy of density values was ±10^{-5} g·cm^{-3}.

Results and Discussion

Densities of solutions (ρ) are shown in Tables 1–3. The apparent molar volumes (V_Φ) of amino acids were calculated from the equation

$$V_\Phi = M_S/\rho - 1000(\rho - \rho_0)/m_S\rho\rho_0 \tag{1}$$

where M_S, m_S, and ρ_0 are the molar mass of the solute, the molality of the solute, and the density of the solvent, respectively. The calculated values of V_Φ are also gathered in Tables 1–3.

Since, for each solute studied, the apparent molar volume was found to be a linear function of the molality over the range studied, the standard-state (infinite dilution) partial

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Table 1. Densities and Apparent Molar Volumes of Glycine in *w* Ethylene Glycol + (1 − *w*) Water Mixtures at 298.15 K

m	ρ	Φ_v	m	ρ	Φ_v
mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}
	$w = 0$			$w = 0.30$	
0.0000	0.99707		0.0000	1.03612	
0.0500	0.99869	42.6225	0.0445	1.03745	44.5625
0.1000	1.00030	42.6843	0.0892	1.03877	44.6770
0.2000	1.00346	42.8896	0.1976	1.04192	44.8640
0.3001	1.00657	43.0383	0.2987	1.04479	45.0374
0.3997	1.00959	43.2439	0.3994	1.04761	45.1532
0.5000	1.01262	43.3338	0.4996	1.05036	45.2805
	$w = 0.10$			$w = 0.40$	
0.0000	1.00938		0.0000	1.04931	
0.0505	1.01008	43.1916	0.0468	1.05065	45.4916
0.1003	1.01254	43.3068	0.0991	1.05213	45.5672
0.2002	1.01562	43.5053	0.1997	1.05496	45.6023
0.2994	1.01864	43.6199	0.2998	1.05771	45.7325
0.3996	1.02158	43.8782	0.3977	1.06036	45.8229
0.4998	1.02452	43.9810	0.4988	1.06306	45.9045
	$w = 0.20$			$w = 0.50$	
0.0000	1.02255		0.0000	1.06200	
0.0496	1.02408	43.8613	0.0539	1.06350	45.9524
0.0975	1.02553	44.0462	0.1043	1.06489	46.0000
0.2011	1.02863	44.2424	0.1981	1.06743	46.1432
0.3010	1.03155	44.4240	0.3069	1.07033	46.2580
0.4001	1.03439	44.5976	0.4039	1.07287	46.3502
0.5000	1.03722	44.7128	0.4997	1.07531	46.4880

Table 2. Densities and Apparent Molar Volumes of L-Alanine in *w* Ethylene Glycol + (1 − *w*) Water Mixtures at 298.15 K

m	ρ	Φ_v	m	ρ	Φ_v
mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}
	$w = 0$			$w = 0.30$	
0.0000	0.99707		0.0000	1.03603	
0.1218	1.00056	60.3294	0.1030	1.03876	61.1545
0.2132	1.00312	60.4514	0.1861	1.04091	61.2762
0.2608	1.00444	60.4937	0.2335	1.04213	61.3041
0.3110	1.00581	60.5648	0.3068	1.04397	61.4174
0.4092	1.00846	60.6699	0.4364	1.04715	61.6008
0.4986	1.01082	60.7861	0.4997	1.04868	61.6634
	$w = 0.10$			$w = 0.40$	
0.0000	1.00961		0.0000	1.04931	
0.0959	1.01231	60.4809	0.1172	1.05227	61.7959
0.1950	1.01505	60.5589	0.1976	1.05427	61.8274
0.2377	1.01621	60.6163	0.2480	1.05550	61.8852
0.2934	1.01772	60.6507	0.3111	1.05702	61.9484
0.4082	1.02077	60.7464	0.3999	1.05912	62.0515
0.5000	1.02317	60.8287	0.4896	1.06121	62.1334
	$w = 0.20$			$w = 0.50$	
0.0000	1.02271		0.0000	1.06201	
0.1170	1.02591	60.7823	0.1046	1.06453	62.3790
0.1879	1.02781	60.8678	0.1773	1.06625	62.4491
0.2323	1.02899	60.9040	0.2420	1.06676	62.4950
0.3129	1.03110	60.9814	0.2568	1.06810	62.5159
0.4172	1.03378	61.0928	0.3832	1.07099	62.5894
0.4938	1.03572	61.1539	0.4730	1.07300	62.6486

Table 3. Densities and Apparent Molar Volumes of L-Serine in *w* Ethylene Glycol + (1 − *w*) Water Mixtures at 298.15 K

m	ρ	Φ_v	m	ρ	Φ_v
mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}	mol·kg^{-1}	g·cm^{-3}	cm·mol^{-1}
	$w = 0$			$w = 0.30$	
0.0000	0.99707		0.0000	1.03611	
0.1147	1.00216	60.4469	0.1002	1.04029	62.3108
0.1921	1.00553	60.5979	0.1992	1.04433	62.4955
0.2629	1.00857	60.7087	0.2498	1.04637	62.5401
0.3008	1.01018	60.7578	0.3000	1.04838	62.5848
0.4065	1.01461	60.9216	0.4018	1.05237	62.7447
0.4991	1.01839	61.1255	0.4999	1.05612	62.9256
	$w = 0.10$			$w = 0.40$	
0.0000	1.00940		0.0000	1.04929	
0.1023	1.01387	60.9571	0.1014	1.05337	63.3572
0.1994	1.01802	61.1517	0.1940	1.05702	63.4949
0.2503	1.02018	61.1951	0.2278	1.05834	63.5586
0.2990	1.02221	61.2895	0.3003	1.06112	63.6591
0.3993	1.02629	61.5617	0.4002	1.06488	63.8190
0.4987	1.03031	61.6819	0.4999	1.06861	63.8937
	$w = 0.20$			$w = 0.50$	
0.0000	1.02262		0.0000	1.06200	
0.1008	1.02690	61.8963	0.1143	1.06645	64.1571
0.1992	1.03100	62.0277	0.1985	1.06968	64.1889
0.2504	1.03311	62.0634	0.2225	1.07059	64.2050
0.2993	1.03511	62.1389	0.3007	1.07353	64.2564
0.3993	1.03911	62.2708	0.4019	1.07728	64.3210
0.4999	1.04304	62.4572	0.5016	1.08091	64.3824

Table 4. Volumetric Parameters of Eq 2 for Glycine in *w* Ethylene Glycol + (1 − *w*) Water Mixtures at 298.15 K

w	V_Φ^0 cm^3·mol^{-1}	v_{AA} cm^3·mol^{-2}·kg	R
0	42.54 (0.02)[a]	1.66 (0.08)	0.9956
	43.20 (0.03)[b]	0.82 (0.04)[b]	
	42.48 (0.02)[c]	4.26 (0.16)[c]	
0.10	43.12 (0.03)	1.77 (0.09)	0.9944
0.20	43.84 (0.04)	1.85 (0.13)	0.9904
0.30	44.53 (0.03)	1.56 (0.09)	0.9940
0.40	45.45 (0.02)	0.91 (0.05)	0.9931
0.50	45.89 (0.01)	1.19 (0.04)	0.9979

[a] Entries in parentheses are the standard errors on each coefficient. [b] Wadi and Ramasami, 1997. [c] Ren et al., 1998.

Table 5. Volumetric Parameters of Eq 2 for L-Alanine in *w* Ethylene Glycol + (1 − *w*) Water Mixtures at 298.15 K

w	V_Φ^0 cm^3·mol^{-1}	v_{AA} cm^3·mol^{-2}·kg	R
0	60.19 (0.01)	1.19 (0.02)	0.9992
	60.62 (0.05)[a]	0.79[a]	
0.10	60.40 (0.01)	0.86 (0.02)	0.9985
0.20	60.67 (0.01)	0.98 (0.02)	0.9989
0.30	61.02 (0.01)	1.30 (0.04)	0.9979
0.40	61.66 (0.02)	0.96 (0.06)	0.9921
0.50	62.31 (0.01)	0.75 (0.05)	0.9923

[a] Hakin et al., 1994.

molar volumes (V_Φ^0) were obtained by least-squares fitting to the equation

$$V_\Phi = V_\Phi^0 + v_{AA}m \qquad (2)$$

in which v_{AA} is the volumetric pairwise interaction coefficient (Hedwig et al., 1991). The evaluated values of V_Φ^0 and v_{AA}, together with their standard errors and correlation coefficients (R) for eq 2, are given in Tables 4−6, in which, for comparison, literature values of V_Φ^0 and v_{AA} for the three amino acids in pure water are also listed. The agreement between them is good.

Transfer volumes of each amino acid, ΔV_Φ^0, from pure water to ethylene glycol−water mixtures were calculated using the equation

$$\Delta V_\Phi^0 = V_\Phi^0 \text{ (in ethylene glycol−water mixture)} -$$
$$V_\Phi^0 \text{ (in pure water)} \quad (3)$$

The results are illustrated in Figure 1.

The value of V_Φ^0 is by definition free from solute−solute interactions and therefore provides information regarding solute−solvent interactions. As can be seen in Figure 1,
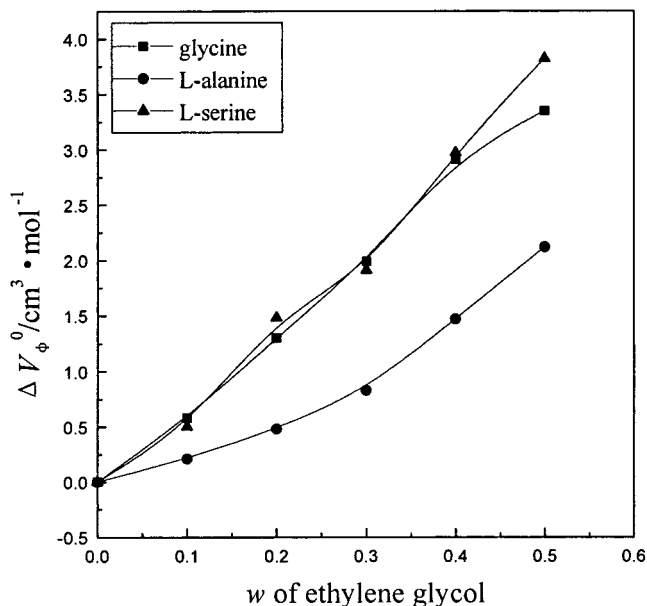
Figure 1. Transfer volumes of amino acids from water to w ethylene glycol + $(1 - w)$ water mixtures as a function of the mass fraction of ethylene glycol in the mixed solvents (w) at 298.15 K.
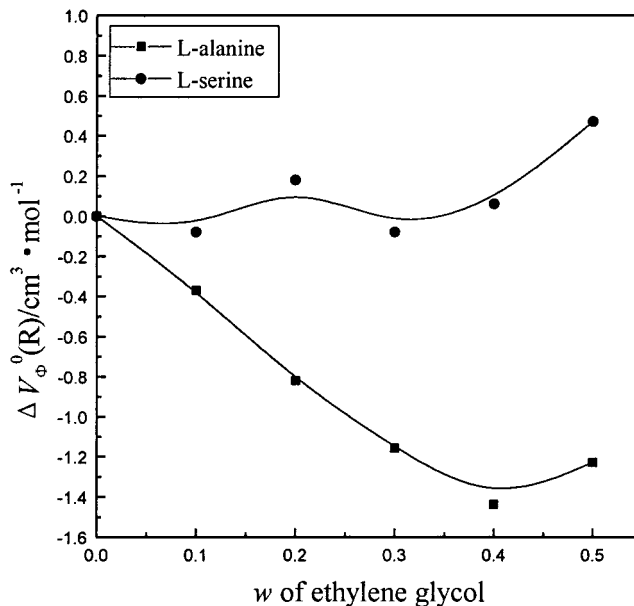

Figure 2. Contributions of R groups to transfer volumes $\Delta V_\Phi^0(R)$ versus mass fraction of ethylene glycol in the mixed solvents (w) at 298.15 K.

Table 6. Volumetric Parameters of Eq 2 for L-Serine in w Ethylene Glycol + $(1 - w)$ Water Mixtures at 298.15 K

| | V_Φ^0 | v_{AA} | |
w	cm$^3\cdot$mol^{-1}	cm$^3\cdot$mol$^{-2}\cdot$kg	R
0	60.26 (0.02)	1.70 (0.06)	0.9976
	60.62 (0.01)[a]	1.27 (0.02)[a]	
0.10	60.76 (0.04)	1.89 (0.12)	0.9925
0.20	61.74 (0.02)	1.38 (0.08)	0.9941
0.30	62.17 (0.02)	1.47 (0.08)	0.9945
0.40	63.23 (0.02)	1.39 (0.08)	0.9938
0.50	64.08 (0.01)	0.58 (0.04)	0.9935

[a] Jolicoeur and Boileau, 1978.

the values of ΔV_Φ^0 for the three amino acids are all positive and increase monotonically with the mass fractions of ethylene glycol in mixed solvents. These results can be explained by the cosphere overlap model, as developed by Friedman and Krishnan (Friedman and Krishnan, 1973), according to which the effect of overlap of hydration cospheres is destructive. The overlap of hydration cospheres of two ionic species results in a net increase in volume, whereas the overlap of hydration cospheres of hydrophobic—hydrophobic groups and ion—hydrophobic groups results in a net decrease in volume. As is well-known, at neutral pH, amino acids exist as zwitterions and upon dissolution in water there is an overall decrease in the volume of the water. This is due to the contraction of the water near the end groups and is termed as electrostriction. The observed increasing positive volumes of transfer suggest that in the ternary solutions (amino acid + ethylene glycol + water), the ion—hydrophilic and hydrophilic—hydrophilic groups interactions are predominant over ion—hydrophobic and hydrophobic—hydrophobic groups interactions. The interactions of two OH groups of ethylene glycol with amino acid zwitterion are localized at the headgroups (COO$^-$ and NH$_3^+$). Due to these interactions, the electrostriction of water caused by the charge centers of the amino acid will be reduced, which results in an increase in volume.

The observed trends of ΔV_Φ^0 can also be explained with the equation (Natarajan et al., 1990)

$$V_\Phi^0 = V_{Vow} + V_v - V_s \qquad (4)$$

in which the limiting partial molar volume of the amino acid may be considered to be made up of the van der Waals volume (V_{Vow}), the volume associated with voids (V_v) or empty space, and the volume due to shrinkage (V_s) that arises from the electrostriction of the solvent caused by the hydrophilic groups present in the amino acid. The presence of ethylene glycol in water decreases the extent of electrostriction caused by the amino acid, which results in a decrease in shrinkage volume. Assuming that V_{Vow} and V_v are not significantly affected by the presence of ethylene glycol, an increase in V_Φ^0 results and consequently a positive value of ΔV_Φ^0 is observed.

It may be noted that ΔV_Φ^0 values of L-alanine are less than those of glycine and L-serine and that ΔV_Φ^0 values of glycine are almost the same as those of L-serine over the whole range of mass fraction of the mixed solvents studied (Figure 1). The introduction of a methyl group in L-alanine provides an additional tendency of hydrophilic—hydrophobic and hydrophobic—hydrophobic groups interactions, and as a result, greater electrostriction of the solvent water is produced, leading to smaller values of ΔV_Φ^0. When one H atom of L-alanine is replaced by one OH group (L-serine), an additional propensity of hydrophilic—hydrophilic groups interactions is provided, and consequently less electrostriction of the solvent water is caused, resulting in comparable values of ΔV_Φ^0 to those of glycine.

It is of interest to examine the contribution to transfer volumes of side groups on the α-carbon of amino acids (R = $-CH_3$, $-CH_2OH$). For this purpose, the ΔV_Φ^0 of glycine is subtracted from that of each of the other two amino acids

$$\Delta V_\Phi^0(R) = \Delta V_\Phi^0 \text{ (substituted amino acid) } -$$
$$\Delta V_\Phi^0 \text{ (glycine)} \qquad (5)$$

where $\Delta V_\Phi^0(R)$ is the contribution of the R group to the transfer volume ΔV_Φ^0. In this calculation, it is being assumed that the volume contribution of the H atom in glycine can be neglected. The results are shown in Figure 2.

The contribution of the $-CH_3$ of L-alanine to the transfer volume is significant and always negative, while that of

the $-CH_2OH$ of L-serine is slight and generally positive. The small negative values of $\Delta V_\Phi^0(R)$ for L-serine at 0.10 and 0.30 mass fractions may be due to the cumulation of errors in the successive calculation. The result also supports the conclusion that the introduction of a methyl group in L-alanine provides an additional tendency of hydrophilic—hydrophobic and hydrophobic—hydrophobic groups interactions, while the replacement of one H atom of L-alanine by one OH group (L-serine) arouse an additional propensity of hydrophilic—hydrophilic groups interactions.

The values of volumetric pairwise interaction coefficients, v_{AA}, which represent the volume changes when two hydrated solute molecules move from infinite distance to proximity, are found to be all positive (Tables 4—6). According to the Friedman—Krishnan model (Friedman and Krishnan, 1973), when nonbonding favorable interactions occur, water molecules are released to the bulk from the hydration cospheres as a consequence of their overlap. Because of the different structural organizations of water molecules in these two domains, the volume change would be negative when water is released from a cosphere that is more structured than the bulk and positive when the bulk is more structured. When the NH_3^+ and COO^- charged centers interact in the pairwise interaction, the overlap of hydration cospheres results in a positive volume change as some electrostricted water returns to the bulk solvent that is more structured.

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