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Research Article

Effect of Hydroxylamine Sulfate on Volumetric Behavior of Glycine, *L*-Alanine, and *L*-Arginine in Aqueous Solution

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The apparent molar volumes of glycine, *L*-alanine, and *L*-arginine in aqueous hydroxylamine sulfate solutions have been determined at $T = 298.15$ K and atmospheric pressure. The standard partial molar volumes, V_2^0, corresponding partial molar volumes of transfer, $\Delta_{tr}V_2^0$, and hydration numbers, N_H, have been calculated for these α-amino acids from the experimental data. The $\Delta_{tr}V_2^0$ values are positive for glycine, *L*-alanine, and *L*-arginine and are all increased with the increase in the concentration of hydroxylamine ions. These parameters obtained from the volumetric data are interpreted in terms of various mixing effects between amino acids and hydroxylamine sulfate in aqueous solutions.

1. Introduction

In recent years, the denaturation and renaturation behavior of biological molecules such as proteins in aqueous solutions has been the subject of extensive investigation [1–3]. However, the complex configurational and conformational factors affecting the structure of protein in aqueous solutions make the direct interpretation from studies on proteins very difficult [4]. Volumetric and transport properties of amino acids such as density and viscosity have been extensively investigated and can provide valuable information for conformational stability and unfolding behavior of globular proteins in aqueous solutions [5–13].

It is well recognized that inorganic and organic salts can produce remarkable effects on the conformation and properties of proteins in aqueous solutions. Hydroxylamine derivatives are always used by biologists for their ability to introduce random mutations by switching different base pairs [14]. Hydroxylamine can also be applied to selectively cleave peptide bonds in peptides and proteins [15]. The highly biological activities of hydroxylamine derivatives make it quite interesting to study the behaviour of proteins in the presence of hydroxylamine salts [16]. Consequently, the knowledge of thermophysical and thermodynamic properties of hydroxylamine with small molecules will be required and useful for obtaining information about various interactions occurring in aqueous hydroxylamine solutions with macromolecules.

In this study, we present experimental densities of glycine, *L*-alanine, and *L*-arginine in aqueous and aqueous hydroxylamine sulfate solutions of (0.10, 0.25, and 0.40) mol kg^{-1} at $T = 298.15$ K and atmospheric pressure. From these data, the standard partial molar volumes (V_2^0), standard partial molar volumes of transfer ($\Delta_{tr}V_2^0$), and molar hydration number (N_H) have been calculated. The results are discussed in terms of various solute-solute interactions and also their nature occurring in the ternary systems (amino acids + hydroxylamine sulfate + water).

2. Experimental

Glycine, *L*-alanine, and *L*-arginine that were obtained from Amresco company have mass fraction purities greater than 0.99. These samples were recrystallized from aqueous ethanol solutions and dried in vacuum oven for 12 h at 333 K before use. Analytical grade hydroxylamine sulfate with purity of minimum mass fraction of 0.995 was obtained from UBE (Japan) and used as such after drying for 72 h in a vacuum desiccator at room temperature. The ultrapure water used for the preparation of the mixture with resistivity greater than

18.2 MΩ·cm was supplied by Nanopure Diamond UV/UF Water Purification System (Thermo Scientific Barnstead, USA). All of the studied solutions were freshly prepared by mass on an electronic balance to an accuracy of $\pm 1 \times 10^{-5}$ g.

The densities of the solutions were measured using an Anton Paar DMA 4500 density meter whose measurement cell temperature was controlled automatically within ±0.01 K of the selected value. The density meter was checked with freshly boiled ultrapure water and with dry air at atmospheric pressure. The uncertainty of the digital vibrating glass tube densimeter is stated as $\pm 5.0 \times 10^{-5}$ g·cm^{-3} in the density range of 0 to 3 g·cm^{-3}.

3. Results and Discussion

The measured densities of glycine, *L*-alanine, and *L*-arginine in aqueous and aqueous hydroxylamine sulfate solutions with different concentrations of (0.10, 0.25, and 0.40) mol·kg^{-1} at $T = 298.15$ K and atmospheric pressure have been shown in Tables 1 and 2, respectively.

The apparent molar volume, V_2, defined as the difference between the volume of the solution and the volume of the pure solvent per mole of solute, is given by the following equation:

$$V_2 = \frac{M}{\rho} - \frac{1000\,(\rho - \rho_0)}{m\rho\rho_0}, \tag{1}$$

where M and m are the molar mass of the amino acid and the molality of the solute and ρ and ρ_0 are the density of the aqueous solution and solvent, respectively. From Tables 1 and 2, we can find that the apparent molar volume increased with the increase in both concentrations of the amino acids and hydroxylamine sulfate.

In diluted conditions, the variation of the apparent molar volume, V_2, for amino acids with the molality of hydroxylamine sulfate can be described by the linear relation

$$V_2 = V_2^0 + B_v m, \tag{2}$$

where B_v is an experimentally determined slope parameter and V_2^0 is the apparent molar volume at infinite dilution also known as the standard partial molar volume. The standard partial molar volumes for the amino acids in aqueous and aqueous solutions of hydroxylamine sulfate are reported in Table 3. The standard partial molar volumes for pure amino acids in aqueous solution agree well with these values from [17, 18]. It is well recognized that the standard partial molar volume is not necessarily the actual volume that was assigned to the amino acid because the water molecule that was near the amino acid does not have the same molar volume as pure water does. The water near the polar molecules or ions is electrostricted compressed by the electric field of solute. On the other hand, the standard partial molar volumes are all increased with the increase of both amino acids and hydroxylamine sulfate concentration in water. This may attribute to the fact that the hydrophilic-hydrophilic and ion-ion interactions between amino acids and hydroxylamine sulfate make their shrinking ability in water decrease and make more water to be released.

Partial molar volumes of transfer, $\Delta_{tr}V_2^0$, at infinite dilution from water to aqueous hydroxylamine sulfate solutions have been calculated from the relation

$$\Delta_{tr}V_2^0 = V_2^0\,(\text{in aqueous salt solution}) - V_2^0\,(\text{in pure water}). \tag{3}$$

The transfer partial molar volume values are also shown in Table 3. The cosphere overlap model is always used to interpret the volume shrinking or expansion effects in terms of solute-solute interactions [19–22]. According to the theory, the existence of hydrophilic-hydrophilic and ion-ion interactions will lead to a positive $\Delta_{tr}V_2^0$ value, while the hydrophobic-hydrophobic interactions lead to a negative value. The $\Delta_{tr}V_2^0$ for amino acids in aqueous hydroxylamine sulfate solution show that the hydrophilic-hydrophilic and ion-ion interactions are in domination. These interactions may be in existence between zwitterionic groups (NH_3^+ and COO^-) in amino acids and ions of hydroxylamine sulfate (NH_3OH^+ and SO_4^{2-}). In these mixtures, noncovalent ion pairs will be formed and thus the electrostriction of neighboring water molecules will be released, which result in the increase of the apparent molar volumes of the amino acids in solution. It is further confirmed that when the H atom in glycine is changed to the more hydrophobic $-CH_3$ group in *L*-alanine, the $\Delta_{tr}V_2^0$ value is further decreased. On the contrary, when the H group is instead of the more hydrophilic or positively charged guanidino group in *L*-arginine, much more positive $\Delta_{tr}V_2^0$ value is observed.

It is interesting to find that the $\Delta_{tr}V_2^0$ values in the presence of hydroxylamine sulfate are smaller to those in the presence of metal salts. For example, the $\Delta_{tr}V_2^0$ values of glycine in 0.1 M $CdCl_2$ [23] and 0.1 M $ZnCl_2$ [24] are about 1.83 and 2.77 at 298.15 K, respectively. Similar trends are observed for *L*-alanine. It reflects that interactions between amino acids and hydroxylamine sulfate may be smaller than those with metal salts. This can be attributed to the fact that these metal ions have more electronic polarizabilities than organic ions, such as hydroxylamine ion. On the other hand, the $\Delta_{tr}V_2^0$ values in hydroxylamine sulfate are larger than those in some aqueous nonionic organic compounds. For example, the $\Delta_{tr}V_2^0$ values for glycine and *L*-alanine in 0.2 M *N*-Methylformamide [25] are observed for 0.05 and −0.02, which are smaller than those in this study. However, it needs more experimental or spectroscopic data to draw a conclusion for the comparison of interaction between amino acids with organic, nonorganic, and metal ions.

The hydration numbers (N_H) that reflect the electrostriction effect of the charged center of the amino acids on neighboring water can been calculated by using the following method [26]:

$$N_H = \frac{V_\phi^0\,(\text{elect})}{V_e^0 - V_b^0}, \tag{4}$$

where V_e^0 and V_b^0 are the molar volumes of electrostricted water and bulk water, respectively. According to Millero et al. [26], for water molecules taken from the bulk phase to the

Table 1: Density, ρ, and apparent molar volumes, V_2, of amino acids in aqueous solutions as a function of amino acids concentration, m, at $T = 298.15$ K.

m mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}	m mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}	m mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}
	Glycine			*L*-Alanine			*L*-Arginine	
0.0000	0.99707		0.0000	0.99707		0.0000	0.99707	
0.0203	0.99772	43.05	0.0211	0.99768	60.23	0.0196	0.99804	124.87
0.0405	0.99837	42.95	0.0412	0.99826	60.23	0.0398	0.99901	125.40
0.0604	0.99900	43.07	0.0588	0.99876	60.34	0.0599	0.99994	126.17
0.1023	1.00033	43.10	0.1043	1.00005	60.43	0.0653	1.00018	126.44
0.1561	1.00201	43.24	0.1465	1.00124	60.47	0.0767	1.00071	126.52
0.2054	1.00355	43.28	0.1981	1.00267	60.58	0.0834	1.00101	126.71
0.2565	1.00514	43.33	0.2445	1.00396	60.59	0.1021	1.00186	126.91

Table 2: Density, ρ, and apparent molar volumes, V_2, of amino acids in aqueous hydroxylamine sulfate solutions as a function of amino acids concentration, m^a, at $T = 298.15$ K.

m^a mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}	m^a mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}	m^a mol·kg^{-1}	$10^3\rho$ kg·m^{-3}	10^6V_2 m^3·mol^{-1}
				Glycine				
	$m_{HA} = 0.1005$ mol·kg^{-1}			$m_{HA} = 0.2512$ mol·kg^{-1}			$m_{HA} = 0.4017$ mol·kg^{-1}	
0.0000	1.00736		0.0000	1.02199		0.0000	1.03588	
0.0205	1.00801	43.32	0.0199	1.02261	43.67	0.0200	1.03648	44.53
0.0417	1.00866	43.72	0.0396	1.02320	44.12	0.0391	1.03704	44.74
0.0614	1.00926	43.94	0.0611	1.02385	44.25	0.0586	1.03758	45.36
0.1043	1.01056	44.14	0.1022	1.02505	44.64	0.1004	1.03878	45.43
0.1581	1.01220	44.14	0.1595	1.02670	44.97	0.1529	1.04026	45.59
0.2166	1.01397	44.16	0.1954	1.02774	45.03	0.2042	1.04173	45.52
0.2588	1.01523	44.21	0.2503	1.02933	45.05	0.2635	1.04340	45.54
				L-Alanine				
	$m_{HA} = 0.1005$ mol·kg^{-1}			$m_{HA} = 0.2512$ mol·kg^{-1}			$m_{HA} = 0.4017$ mol·kg^{-1}	
0.0000	1.00736		0.0000	1.02199		0.0000	1.03588	
0.0204	1.00795	59.97	0.0203	1.02255	60.71	0.0207	1.03643	61.20
0.0406	1.00851	60.47	0.0399	1.02307	61.21	0.0409	1.03693	62.03
0.0619	1.00909	60.80	0.0578	1.02353	61.55	0.0574	1.03735	62.06
0.1163	1.01055	61.21	0.0978	1.02456	61.85	0.0988	1.03839	62.18
0.1514	1.01150	61.23	0.1452	1.02579	61.89	0.1455	1.03952	62.47
0.2097	1.01306	61.31	0.1869	1.02683	62.08	0.1994	1.04085	62.48
0.2465	1.01403	61.36	0.2484	1.02839	62.12	0.2442	1.04192	62.58
				L-Arginine				
	$m_{HA} = 0.1005$ mol·kg^{-1}			$m_{HA} = 0.2512$ mol·kg^{-1}			$m_{HA} = 0.4017$ mol·kg^{-1}	
0.0000	1.00736		0.0000	1.02199		0.0000	1.03588	
0.0198	1.00825	128.48	0.0201	1.02286	128.87	0.0201	1.03672	129.19
0.0407	1.00916	129.13	0.0412	1.02372	130.05	0.0398	1.03749	130.26
0.0624	1.01010	129.33	0.0601	1.02452	129.85	0.0657	1.03853	130.27
0.1008	1.01174	129.55	0.1014	1.02617	130.43	0.0968	1.03973	130.60
0.1524	1.01393	129.59	0.1486	1.02810	130.30	0.1514	1.04185	130.66
0.2019	1.01598	129.75	0.1995	1.03012	130.40	0.1963	1.04350	131.03
0.2518	1.01804	129.76	0.2471	1.03190	130.78	0.2460	1.04536	131.05

[a]Molality of amino acids in the mixture of hydroxylamine sulfate + water as pure solvent.

Table 3: Standard partial molar volumes and transfer partial molar volumes of glycine, *L*-alaine, and *L*-arginine in aqueous solution of hydroxylamine sulfate at $T = 298.15$ K.

	V_2^0 cm^3/mol				$\Delta_{tr}V_2^0$ cm^3/mol		
Amino acid	Water	0.1005[a]	0.2512[a]	0.4017[a]	0.1005[a]	0.2512[a]	0.4017[a]
Glycine	42.97	43.60	43.85	44.81	0.63	0.88	1.84
L-Alanine	60.21	60.27	61.02	61.61	0.06	0.81	1.40
L-Arginine	124.47	128.85	129.42	129.69	4.38	4.95	5.22

[a]Molality of hydroxylamine sulfate in water.

Table 4: hydration number of glycine, *L*-alaine, and *L*-arginine in aqueous and aqueous solution of hydroxylamine sulfate at $T = 298.15$ K.

Amino acid	In water	0.1005[a]	0.2512[a]	0.4017[a]
Glycine	2.70	2.51	2.43	2.14
L-Alanine	3.50	3.48	3.25	3.07
L-Arginine	6.27	4.94	4.77	4.69

[a]Molality of hydroxylamine sulfate in water.

region near the amino acids, the volumes $(V_e^0 - V_b^0)$ are -3.3 cm^3·mol^{-1} at 298.15 K. The electrostriction partial molar volume, V_ϕ^0(elect), can be expressed as

$$V_\phi^0\,(\text{elect}) = V_\phi^0 - V_\phi^0\,(\text{int})\,, \tag{5}$$

where V_ϕ^0(int) represents the intrinsic partial molar volume of the amino acids. Millero et al. [26] calculated the V_ϕ^0(int) from the molar crystal volume for amino acids by using the following relationship:

$$V_\phi^0\,(\text{int}) = \frac{0.7}{0.634} V_\phi^0\,(\text{cryst})\,, \tag{6}$$

where 0.7 is the packing density for the molecule in an organic crystal and 0.634 is the packing density for a random packing sphere. The crystal volume can be calculated from the density of the dry solid amino acid [27].

The hydration numbers of amino acid in aqueous hydroxylamine sulfate solutions at 298.15 K are given in Table 4. The N_H for three amino acids in aqueous hydroxylamine sulfate solutions are positive and according to the order N_H (*L*-arginine) > N_H (*L*-alanine) > N_H (glycine). These data are all smaller than those in pure water and are further decreased with the increasing concentration of hydroxylamine sulfate. On the other hand, as the hydrophilic part side chains of amino acids increase, the decrease becomes progressively larger. In the case of *L*-alanine, the hydration number is only slightly larger than that in pure water. This is reasonable, since the existence of ion-ion and hydrophilic-hydrophilic interaction makes the shrinking water to be more released. It is again demonstrated that the ion-ion and hydrophilic-hydrophilic interactions are dominated between amino acids and hydroxylamine sulfate in aqueous solutions.

4. Conclusions

The apparent molar volumes of glycine, *L*-alanine, and *L*-arginine in aqueous and aqueous hydroxylamine sulfate solutions have been determined at $T = 298.15$ K. The interactions between amino acids and hydroxylamine sulfate have been studied using volumetric properties, including the standard partial molar volumes, V_2^0, corresponding partial molar volumes of transfer, $\Delta_{tr}V_2^0$, and hydration numbers, N_H. The $\Delta_{tr}V_2^0$ values are positive for glycine, *L*-alanine, and *L*-arginine and are increased with the increase in the concentration of hydroxylamine ions. The positive values of $\Delta_{tr}V_2^0$ for all amino acids in aqueous hydroxylamine sulfate solution suggest that the hydrophilic-hydrophilic and ion-ion interactions predominate. The $\Delta_{tr}V_2^0$ values show that the $-CH_3$ group in *L*-alanine weakens the interaction between its hydrophilic or charged group with hydroxylamine sulfate, and the guanidino group in *L*-arginine enhances the hydrophobic-hydrophilic interactions. The N_H for three amino acids are positive and according to the order *L*-arginine > *L*-alanine > glycine.

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