The limiting partial molar volume and apparent molar volume of glycylglycine in aqueous KCl solution at 298.15 and 308.15 K

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Abstract

Apparent molar volumes V_Φ of glycylglycine in aqueous KCl solutions have been obtained from densities at 298.15 and 308.15 K measured with a vibrating-tube densimeter. These data have been used to deduce partial molar volumes of transfer $\Delta \text{trs} V_\Phi^0$ from water to different KCl–water mixtures. $\Delta \text{trs} V_\Phi^0$ values are positive. This result arises from the interaction of KCl with the charged centers of glycylglycine. The results show that $\Delta \text{trs} V_\Phi^0$ depends less on temperature. Hydration numbers are calculated from V_Φ^0 data and $\Delta \text{trs} V_\Phi^0$ are interpreted in terms of various interactions.
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1. Introduction

The partial molar volumes of proteins are a characteristic parameter that has been used to elucidate several processes such as protein conformation changes, protein aggregation or polymerization [1–6]. Because of the complexities of proteins, it is difficult to investigate thermodynamic properties directly. It is common practice to use amino acids and oligopeptides as model compounds [7–10]. Most previous studies on oligopeptides have been restricted to water [11–14]. However, biological fluids are not pure water, and the properties of proteins such as their structure, solubility, denaturation, activity of enzymes, etc. are greatly influenced by electrolytes [15–18]. Although there are some investigations of this property of glycylglycine in aqueous NaCl solutions [19,20], no systematic studies exist on the volume properties of oligopeptides in electrolyte solutions. This paper presents the densities ρ, apparent volumes V_Φ and infinite dilution apparent molar volume V_Φ^0 in aqueous KCl solutions at 298.15 and 308.15 K.

2. Experimental

2.1. Materials

Glycylglycine (BR mass fraction ≥ 0.98) was procured from China Medicine (Group) Shanghai Chemical Reagent Corporation, dried for 6 h at 60 °C, and stored over silica gel in a vacuum desiccator for 48 h before use. Potassium chloride (AR mass fraction ≥ 0.995) was procured from Mingo Chemical Reagent Factory, purified by two recrystallizations from distilled and deionized water obtained by a quartz subboiling purifier, dried at 110 °C, and stored over silica gel in a vacuum desiccator for 48 h before use.

Solutions were prepared by weight using a Mettler AE 200 analytical balance with a precision of 0.0001 g. All liquids were degassed ultrasonically.

2.2. Density measurements

Densities were determined by a vibrating-tube digital densimeter (DMA55 Anton Paar) thermostated to ± 0.01 K (HAAKE C). The densimeter was calibrated with twice distilled water and dry air. The density of water at 298.15

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Table 1
Densities ρ and apparent molar volumes V_Φ of aqueous solutions of glycylglycine and KCl at 298.15 and 308.15 K

m (mol kg^{-1})	ρ (g cm^{-3}) (298.15 K)	V_Φ (cm^3 mol^{-1}) (298.15 K)	ρ (g cm 3) (308.15 K)	V_Φ (cm^3 mol^{-1}) (308.15 K)
$m_{KCl} = 0.0000$				
0.0949	1.00254	76.58 (0.21)[a]	0.99921	77.29 (0.21)
0.2052	1.00850	76.75 (0.10)	1.00510	77.46 (0.10)
0.2997	1.01349	76.92 (0.07)	1.01003	77.63 (0.07)
0.4062	1.01899	77.12 (0.05)	1.01547	77.82 (0.05)
0.5069	1.02406	77.32 (0.04)	1.02050	77.98 (0.04)
$m_{KCl} = 0.1020$				
0.0944	1.00723	76.93 (0.21)	1.00389	77.65 (0.21)
0.1877	1.01223	77.11 (0.11)	1.00883	77.83 (0.11)
0.2977	1.01799	77.32 (0.07)	1.01453	78.01 (0.07)
0.3837	1.02242	77.43 (0.05)	1.01889	78.17 (0.05)
0.4730	1.02690	77.62 (0.04)	1.02333	78.32 (0.03)
$m_{KCl} = 0.4941$				
0.0910	1.02469	77.94 (0.21)	1.02107	78.67 (0.21)
0.1929	1.03000	78.14 (0.10)	1.02631	78.89 (0.10)
0.2840	1.03466	78.24 (0.07)	1.03091	78.89 (0.07)
0.3859	1.03976	78.39 (0.05)	1.03594	79.15 (0.05)
0.4836	1.04454	78.54 (0.04)	1.04066	79.29 (0.04)
$m_{KCl} = 1.0204$				
0.0983	1.04759	79.38 (0.19)	1.04367	79.69 (0.19)
0.1975	1.05254	79.51 (0.09)	1.04860	79.83 (0.09)
0.2855	1.05685	79.62 (0.07)	1.05289	79.95 (0.07)
0.3942	1.06207	79.73 (0.05)	1.05809	80.05 (0.05)
0.4762	1.06592	79.83 (0.04)	1.06193	80.13 (0.04)
$m_{KCl} = 2.0117$				
0.0835	1.08628	80.67 (0.21)	1.08221	81.43 (0.21)
0.1821	1.09093	80.77 (0.09)	1.08677	81.64 (0.09)
0.2747	1.09518	80.96 (0.06)	1.09096	81.77 (0.06)
0.3687	1.09942	81.06 (0.05)	1.09511	81.92 (0.05)
0.4540	1.10317	81.18 (0.04)	1.09883	81.97 (0.04)
$m_{KCl} = 2.7822$				
0.0875	1.12130	81.71 (0.18)	1.11675	82.08 (0.19)
0.1780	1.12530	81.85 (0.09)	1.12073	82.22 (0.09)
0.2556	1.12863	82.03 (0.06)	1.12406	82.35 (0.06)
0.3460	1.13245	82.16 (0.05)	1.12787	82.46 (0.05)
0.4419	1.13640	82.30 (0.04)	1.13180	82.62 (0.04)

[a] Estimated uncertainties for V_Φ in parentheses.

and 308.15 K was, respectively, taken as 0.99729 and 0.99403 g cm^{-3} [21] and the density of air as 1.1845×10^{-3} and 1.1460×10^{-3} g cm^{-3} [22].

An average of triplicate measurements was the final result.

3. Results

3.1. The partial molar volume at infinite dilution and transfer partial molar volume

Apparent molar volumes, V_Φ, were calculated from the density data with the equation:

$$V_\Phi = M/\rho - 1000(\rho - \rho_0)/m\rho\rho_0, \qquad (1)$$

where M is the molar mass of the solute (glycylglycine) in g mol^{-1}, m the molality of the solute in aqueous KCl solution in mol kg^{-1}, ρ and ρ_0 are the densities of solution (glycyl-

glycine, KCl and water) and aqueous KCl solution, respectively, in g cm^{-3}. Estimated uncertainties in apparent molar volumes, δV_Φ, were calculated using the equation reported by Hedwig [23]

$$\delta V_\Phi = -(M + 1000/m)\delta\rho/\rho^2 \qquad (2)$$

The uncertainty in solution density, $\delta\rho$, was estimated to be 2×10^{-5} g cm^{-3}. In using Eq. (2), the contributions to δV_Φ arising from uncertainties in solution concentrations (0.03–0.15%) are assumed to be negligible compared with those arising from the uncertainties in density. The densities and apparent molar volumes of the glycylglycine and the uncertainties are presented in Table 1.

Partial molar volumes at infinite dilution were obtained by least-squares fitting to the equation:

$$V_\Phi = V_\Phi^0 + S_v m \qquad (3)$$

Table 2
The partial molar volumes at infinite dilutions V_Φ^0 and the transfer partial molar volumes Δ trs V_Φ^0 at 298.15 and 308.15 K

m_{KCl} (mol kg^{-1})	V_Φ^0 (cm^3 mol^{-1}) (298.15 K)	V_Φ^0 (cm^3 mol^{-1}) (308.15 K)	Δ trs V_Φ^0 (cm^3 mol^{-1}) (298.15 K)	Δ trs V_Φ^0 (cm^3 mol^{-1}) (308.15 K)
0.0000	76.39 (0.019)[a,b]	77.12 (0.009)		
0.1020	76.77 (0.015)	77.49 (0.008)	0.38 (0.038)	0.37 (0.017)
0.4941	77.82 (0.021)	78.56 (0.027)	1.43 (0.042)	1.44 (0.036)
1.0204	79.27 (0.014)	79.59 (0.020)	2.88 (0.033)	2.47 (0.029)
2.0117	80.54 (0.025)	81.34 (0.043)	4.15 (0.042)	4.22 (0.052)
2.7822	81.57 (0.023)	81.95 (0.011)	5.18 (0.046)	4.83 (0.020)

[a] Uncertainties for V_Φ^0 in parentheses.
[b] Literature values of V_Φ^0 (cm^3 mol^{-1}) of glycylglycine in water: 76.43 ± 0.04 [20], 76.28.0.02 [19] at 298.15 K; 77.10 ± 0.01[19] at 308.15 K.

where S_v is the experimental slope and m the molality of the solute (glycylglycine) in mixtures. Apparent molar volumes of glycylglycine were found to be a linear function of molality over the concentration range studied. Transfer volumes were calculated by

$$\Delta \text{ trs } V_\Phi^0 \{\text{water to KCl (aq)}\} = V_\Phi^0 \{\text{in KCl (aq)}\}$$
$$- V_\Phi^0 \{\text{in water}\} \quad (4)$$

The partial molar volumes at infinite dilution, the standard deviations of fitting to Eq. (3), and the transfer partial molar volumes are presented in Table 2.

3.2. The hydration number

The partial molar volume at infinite dilution of a nonelectrolyte can be divided into two parts [24]:

$$V_\Phi^0 = V_{int} + V_{elect}, \quad (5)$$

where V_{int} is the intrinsic molar volume of the nonelectrolyte solute and V_{elect} the electrostriction partial molar volume due to the hydration of the nonelectrolyte. Millero et al. [25] reported the intrinsic molar volume could be estimated from the molar volume of nonelectrolyte crystal:

$$V_{int} = (0.7/0.634)V_{cryst}, \quad (6)$$

where 0.7 is the packing density for molecules in organic crystals, 0.634 the packing density for random packing spheres, and V_{cryst} the molar volume of the nonelectrolyte crystal. The V_{cryst} of the glycylglycine is 1.534 g cm^{-3} [26] at 298.15 K, and crystal density of the glycylglycine at 308.15 K was assumed to be the same as at 298.15 K.

Millero et al. [27] reported a relation between the electrostriction volume and the hydration number of nonelectrolyte in solution:

$$V_{elect} = N_h(V_e^0 - V_b^0), \quad (7)$$

where V_e^0 is the molar volume of electrostricted water and V_b^0 the molar volume of bulk water. If 1 mol of water molecules in the bulk moves to the solvating sphere of the nonelectrolyte, the volume change is $(V_e^0 - V_b^0)$. Following the procedure descried by Millero et al. [32], we found that $(V_e^0 - V_b^0)$ was -3.3 cm^3 mol^{-1} at 298.15 K and -4.0 cm^3 mol^{-1} at

Table 3
The hydration numbers of glycylglycine in the aqueous KCl solutions of the different concentrations at 298.15 and 308.15 K

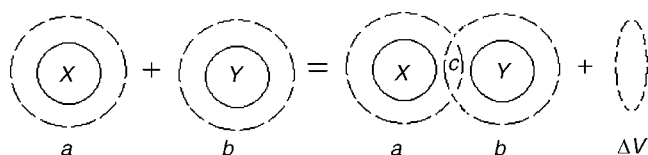| m_{KCl} (mol kg^{-1})	N_h/entries	
	298.15 K	308.15 K
0.0000	5.67	4.49
0.1020	5.55	4.40
0.4941	5.23	4.13
1.0204	4.79	3.88
2.0117	4.41	3.44
2.7822	4.10	3.29

Fig. 1. The structural interaction for two cospheres.

308.15 K. Hydration numbers are presented in Table 3 estimated by using Eqs. (5)–(7).

4. Discussion

4.1. The transfer partial molar volume

The partial molar volumes at infinite dilution V_Φ^0 and the transfer partial molar volume Δ trs V_Φ^0 are both positive, which can be explained by the cosphere overlap model [28,29]. In Fig. 1, if X and Y are hydrophobic, $\Delta V < 0$. If X is hydrophobic and Y is ionic or dipolar, then $\Delta V < 0$. If X and Y are both ionic or dipolar, $\Delta V > 0$. The overlap of cospheres of two ionic species relaxes some solvating water to the bulk so that overall structure is increased, giving rise to a positive volume change. This tendency can also be explained by Eq. (8) [30]:

$$V_\Phi^0 = V_{v,w} + V_{void} - n\sigma_s \quad (8)$$

where $V_{v,w}$ is the van der waal's volume, V_{void} the void or empty volume, σ_s the shrinkage in volume caused by the interaction of a hydrogen-bonding group with water molecules, and n the potential number of hydrogen bonding sites in a

molecule. If we assume that $V_{v.w}$ and V_{void} are the same in water and KCl solutions, the positive volume change might arise from a decrease in σ_s in KCl solution. Because of the presence of KCl, the interaction of the glycylglycine and ions (K^+, Cl^-) becomes stronger, and the number of hydrogen bonds between glycylglycine and water molecules decreases, thus causing a decrease in σ_s.

Though glycylglycine is a nonelectrolyte, interactions with the charged centers COO^- and NH_3^+ are important in aqueous solutions. There are four interactions in mixtures of glycylglycine, KCl and water: (a) ion–ion interaction between K^+ ions with COO^- groups, and Cl^- ions with NH_3^+ groups; (b) ion–apolar group interactions; (c) ion–peptide group interactions; and (d) apolar group–apolar group interactions. According to the cosphere overlap model, (a) and (c) lead to a positive volume contribution, but (b) and (d) lead to a negative volume contribution. The positive signs of V_Φ^0 and Δ trs V_Φ^0 thus indicate that interactions involving the charged centers of glycylglycine dominate the apolar group–apolar group and apolar group–ion interactions.

The partial molar volumes of glycylglycine increase with increasing temperature. Eq. (8) shows that the limiting apparent molar volume V_Φ^0 consists of structural ($V_{v.w}$, V_{void}) and solvation ($n\sigma_s$) contributions. Trends in group contributions of the $-COOH$, $-CH2-$ [11] and $-CHCONH-$ [31] to the volume increase with increasing temperature. On the other hand, increasing temperature reduces the electrostriction and hence n and σ_s decrease. Because Δ trs V_Φ^0 is the difference of V_Φ^0 (in mixtures) and V_Φ^0 (in water), the influence of temperature is minimal.

4.2. The hydration number

In mixtures, decrease of the hydration number of glycylglycine with increasing KCl molality indicates that interactions involving ions (K^+, Cl^-) with the charged centers of the peptide become stronger. That weakens the electrostriction of the charged centers with water molecules and strengthens the hydration competition between ions and the charged centers of the peptide.

In summary, the values of Δ trs V_Φ^0 and V_Φ^0 are positive and increase with increasing ionic cosolute concentrations. The positive Δ trs V_Φ^0 of glycylglycine from water to aqueous KCl solutions show that interactions involving the charged centers of peptides as well as ions are dominating, and the positive Δ trs V_Φ^0 also indicate that globular proteins will be stabilized by salts in the concentrations range studied [20]. These results also provide significant information on the nature of interaction of the ionic cosolutes with the peptide backbone that will help to elucidate the mechanism of interaction of the salts with proteins, which, in turn, determines the stability of the proteins.

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