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ORIGINAL CONTRIBUTION

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Temperature dependence of volume changes on glycine-PEG and L-alanine-PEG in aqueous solution

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Abstract The volume changes on mixing aqueous solutions of glycine-PEG and L-alanine-PEG were respectively measured with a vibration densimeter as functions of concentration and temperature. The volume changes ($\Delta_m V$) for the glycine-PEG-H_2O system were positive, and these $\Delta_m V$ values of different total molality almost linearly decreased with an increase in temperature and converged around 110 °C by extrapolation. On the other hand, those for the L-alanine-PEG-H_2O system were almost zero and independent of temperature. It is considered that the influence of hydration cospheres on glycine-PEG interaction for the glycine-PEG-H_2O system disappears around 110 °C.

Key words PEG – amino acid – volume – hydrophilic hydration – hydrophobic hydration

Introduction

Poly(ethylene glycol) (PEG) is a nonionic linear polymer. The monomer unit of polymer is $-CH_2CH_2O-$, in which hydrophilic oxygens are separated by hydrophobic ethylene units. PEG is widely used for the induction of cell fusion [1], as a fractional precipitating agent for the purification of proteins [2], and for the crystal formation of proteins and nucleic acids [3, 4]. These phenomena have been chiefly explained by the volume exclusion principle, but the influence of PEG on protein structure has not been quantitatively analyzed.

It is widely accepted that the hydration properties of hydrophobic and charged groups play an important role in the conformational stability of biopolymers such as folding–unfolding transitions, ligand interactions, etc. [5–7]. Amino acids are the most convenient substances for analysis of the hydration contribution of such atomic groups because the importance of the hydration of amino acids is generally recognized in connection with the structure and function of proteins.

In previous papers [8, 9], we reported the solubilities of amino acid in aqueous PEG solutions and the volume and compressibility changes on mixing for the ternary amino acid-PEG-H_2O system at 25 °C. We have discussed the exclusion and overlap effects of hydration cospheres of PEG and amino acid on the solubility, volume and adiabatic compressibility changes. We considered that the exclusion effect is due to the structural incompatibility of the hydration cospheres between PEG and charged groups of the amino acid, and the hydrophobic interaction between the ethylene group of PEG and the amino acid side chain becomes stronger with increasing size of the amino acid side chain.

Furthermore, hydration effects in solution are known to be strongly sensitive to temperature [10–11]. Therefore, the study of the temperature dependence of the volume and compressibility changes is of use in order to obtain further information on solute hydration. To gain a better understanding of the hydration of charged and uncharged groups, we report the results regarding the temperature dependence of the volume changes on mixing for the glycine-PEG-H_2O and L-alanine-PEG-H_2O systems.

Experimental

Material

Glycine, L-alanine, and PEG of the highest available purities were purchased from E. Merck Science (FRG) and were used without further purification. The mean molecular weight of PEG was 4000. All amino acids and PEG were dried before use in a vacuum over for 24 h in the presence of silica gel. Distilled and deionized water was used.

Method

The solution densities, d, were measured using an oscillating-tube densimeter (Antor Paar DMA60/602). The temperature around the density meter cell was maintained by circulating water from a constant-temperature bath. Thermal stability of the bath was better than $\pm 1 \times 10^{-2}$ °C. The density meter was calibrated with water and dry air every day. The densities could thus be determined within $\pm 3 \times 10^{-6}$ g cm^{-3}.

Result

As we described in a previous paper [9], the mean apparent molal volume of the mixed solutes in ternary solution, $\phi_v(x, m)$, is defined as follows:

$$\phi_v(x, m) = (1/m)\{(1000 + m_1 M_1 + m_2 M_2)/d - 1000/d_0\}\ , \quad (1)$$

where d and d_0 are the densities of the solution and pure water, respectively, and $m_1(m_2)$ and $M_1(M_2)$ are the molalities and molecular weights of the two solutes. In this paper, 1 denotes amino acid and 2 denotes PEG, and m_2 and M_2 mean the molality and the molecular weight of the PEG monomer unit (–CH_2CH_2O–). The total molality m and mole fraction x are given as

$$m = m_1 + m_2, \quad \text{and} \quad x = m_1/m\ . \quad (2)$$

The volume change on mixing $\Delta_m V$ is given in the following form [12]:

$$\Delta_m V = (m_1 + m_2)\phi_v(x, m) - m_1 \phi_v(1, m) - m_2 \phi_v(0, m)\ , \quad (3)$$

where $\phi_v(1, m)$ and $\phi_v(0, m)$ apply to binary solutions of the pure solute. Using Eq. (3), the volume change on mixing $\Delta_m V$ is obtained directly.

The relations between $\phi_v(x, m)$ and m were obtained from the density data in the temperature ranges from 5° to 45 °C. The results for the glycine-PEG-H_2O and the L-alanine-PEG-H_2O systems are shown in Tables 1 and 2, respectively. All the $\phi_v(x, m)$ values are linearly related to the total molality m as observed in a previous paper [9]. The values of apparent molal volume at infinite dilution were in good agreement with the literature values [13–15].

Table 1 Apparent molal volumes of glycine-PEG in aqueous solution at several temperatures

	$\phi_v(x, m)$ (cm^3 mol^{-1})				
m	$x = 0$	m	$x = 0.5$	m	$x = 1.0$
			$t = 5$ °C		
0.500	36.15	1.000	39.20	0.500	41.96
1.000	36.17	2.000	39.44	1.000	42.40
1.500	36.21	3.000	39.74	1.500	42.94
2.000	36.22	4.000	40.00	2.000	43.38
			$t = 10$ °C		
0.500	36.38	1.000	39.54	0.500	42.48
1.000	36.40	2.000	39.79	1.000	42.90
1.500	36.44	3.000	40.06	1.500	43.42
2.000	36.42	4.000	40.31	2.000	43.85
			$t = 15$ °C		
0.500	36.58	1.000	39.87	0.500	43.00
1.000	36.61	2.000	40.10	1.000	43.37
1.500	36.64	3.000	40.37	1.500	43.85
2.000	36.62	4.000	40.60	2.000	44.22
			$t = 20$ °C		
0.500	36.78	1.000	40.17	0.500	43.38
1.000	36.82	2.000	40.39	1.000	43.74
1.500	36.84	3.000	40.62	1.500	44.19
2.000	36.82	4.000	40.83	2.000	44.54
			$t = 25$ °C		
0.500	36.97	1.000	40.44	0.500	43.74
1.000	37.02	2.000	40.62	1.000	44.06
1.500	37.02	3.000	40.83	1.500	44.48
2.000	37.00	4.000	41.02	2.000	44.80
			$t = 30$ °C		
0.500	37.14	1.000	40.66	0.500	44.04
1.000	37.18	2.000	40.82	1.000	44.33
1.500	37.20	3.000	41.04	1.500	44.70
2.000	37.17	4.000	41.22	2.000	45.01
			$t = 35$ °C		
0.500	37.34	1.000	40.82	0.500	44.30
1.000	37.32	2.000	41.02	1.000	44.58
1.500	37.37	3.000	41.22	1.500	44.91
2.000	37.35	4.000	41.40	2.000	45.23
			$t = 40$ °C		
0.500	37.50	1.000	40.98	0.500	44.50
1.000	37.50	2.000	41.20	1.000	44.76
1.500	37.53	3.000	41.40	1.500	45.10
2.000	37.48	4.000	41.58	2.000	45.38
			$t = 45$ °C		
0.500	37.64	1.000	41.16	0.500	44.64
1.000	37.68	2.000	41.34	1.000	44.98
1.500	37.69	3.000	41.53	1.500	45.26
2.000	37.67	4.000	41.70	2.000	45.52

Colloid & Polymer Science, Vol. 273, No. 8 (1995)

Table 2 Apparent molal volumes of L-alanine-PEG in aqueous solution at several temperatures

		$\phi_v(x,m)$ ($cm^3\ mol^{-1}$)			
m	$x = 0$	m	$x = 0.5$	m	$x = 1.0$
			$t = 5\,°C$		
0.250	36.17	0.500	47.68	0.250	59.20
0.500	36.15	1.000	47.76	0.500	59.37
0.750	36.16	1.500	47.88	0.750	59.55
1.000	36.17	2.000	47.96	1.000	59.74
			$t = 10\,°C$		
0.250	36.40	0.500	48.01	0.250	59.65
0.500	36.38	1.000	48.10	0.500	59.82
0.750	36.38	1.500	48.19	0.750	59.98
1.000	36.40	2.000	48.26	1.000	60.13
			$t = 15\,°C$		
0.250	36.60	0.500	48.29	0.250	60.00
0.500	36.58	1.000	48.40	0.500	60.16
0.750	36.60	1.500	48.48	0.750	60.35
1.000	36.61	2.000	48.56	1.000	60.51
			$t = 20\,°C$		
0.250	36.78	0.500	48.55	0.250	60.32
0.500	36.78	1.000	48.64	0.500	60.49
0.750	36.83	1.500	48.72	0.750	60.63
1.000	36.82	2.000	48.78	1.000	60.79
			$t = 25\,°C$		
0.250	37.00	0.500	48.77	0.250	60.56
0.500	36.97	1.000	48.86	0.500	60.74
0.750	37.97	1.500	48.93	0.750	60.88
1.000	37.02	2.000	49.01	1.000	61.05
			$t = 30\,°C$		
0.250	37.15	0.500	48.98	0.250	60.80
0.500	37.14	1.000	49.07	0.500	60.98
0.750	37.19	1.500	49.14	0.750	61.12
1.000	37.18	2.000	49.21	1.000	61.29
			$t = 35\,°C$		
0.250	37.31	0.500	49.22	0.250	61.08
0.500	37.34	1.000	49.30	0.500	61.24
0.750	37.30	1.500	49.35	0.750	61.36
1.000	37.32	2.000	49.41	1.000	61.50
			$t = 40\,°C$		
0.250	37.47	0.500	49.41	0.250	61.27
0.500	37.50	1.000	49.49	0.500	61.42
0.750	37.52	1.500	49.51	0.750	61.55
1.000	37.50	2.000	49.59	1.000	61.69
			$t = 45\,°C$		
0.250	37.63	0.500	49.58	0.250	61.41
0.500	37.64	1.000	49.65	0.500	61.57
0.750	37.63	1.500	49.70	0.750	61.70
1.000	37.68	2.000	49.75	1.000	61.84

The $\Delta_m V$ values obtained by Eq. (3) for both the glycine-PEG-H_2O and L-alanine-PEG-H_2O systems are summarized in Table 3 and plotted at constant total molality against temperature in Fig. 1. As seen in Fig. 1, the $\Delta_m V$ values for the glycine-PEG-H_2O system are positive and decrease with an increase in temperature; they seem to converge around 110 °C by linear extrapolation. Those for the L-alanine-PEG-H_2O system are almost zero and independent of temperature.

Table 3 Temperature dependence of volume change

	$\Delta_m V$($cm^3\ kg^{-1}$)			
(°C)	$m = 1.0$	$m = 2.0$	$m = 3.0$	$m = 4.0$
Gly-PEG-H_2O system				
5	0.13	0.29	0.48	0.76
10	0.11	0.25	0.45	0.64
15	0.08	0.22	0.41	0.66
20	0.09	0.22	0.39	0.62
25	0.07	0.17	0.29	0.44
30	0.05	0.13	0.26	0.42
35	0.02	0.09	0.23	0.44
40	0.02	0.10	0.23	0.48
45	− 0.01	0.06	0.17	0.40
	$m = 0.5$	$m = 1.0$	$m = 1.5$	$m = 2.0$
Ala-PEG-H_2O system				
5	0.00	− 0.01	0.00	0.00
10	0.00	− 0.01	− 0.01	− 0.01
15	0.00	0.00	0.00	− 0.01
20	0.00	− 0.01	− 0.02	− 0.03
25	0.00	− 0.01	− 0.02	− 0.03
30	0.00	− 0.02	− 0.02	− 0.04
35	0.01	0.01	0.00	− 0.02
40	0.02	0.02	0.00	− 0.03
45	0.03	0.04	0.02	0.00

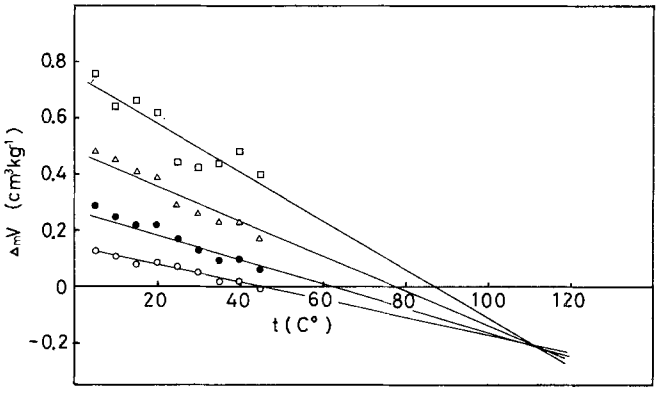

Fig. 1 Temperature dependence of volume changes for the glycine-PEG-H_2O system ○: m = 1.0(mol/kg) ●: m = 2.0(mol/kg) △: m = 3.0(mol/kg) □: m = 4.0(mol/kg)

Discussion

In the PEG/water system, PEG and water are thought to be structurally compatible. Blandamer et al. [16] pointed

out that the distance between alternate oxygen atoms in the PEG chain and the next-nearest-neighbor distance between oxygen atoms in water are similar. Kjellander and Flourin [17] developed this idea and suggested that PEG can adopt conformations where the ethylene groups are encaged by a dynamic network of water molecules at the same time as hydrogen bonds are formed to the ether oxygen. Troyanik [18] showed by the Monte Carlo method that a helical structure of water molecules (water bridges) is formed around the PEG chain by the hydrogen bonds between three water molecules and two ether oxygens or four water molecules and one ether oxygen. The PEG chain thus promotes structuring of the water network by forming hydrogen bonds between the ether oxygen and water molecules. Kaatze et al. [19, 20] measured the complex dielectric constants of aqueous solutions of nonionic linear polymers. According to his results, the hydration number n_h per monomer unit of PEG was $5 \sim 6$, and the values of τ_c^h/τ_c^0 is 2.1 at 25 °C, where τ_c^h and τ_c^0 are the reorientation time of water molecules in the hydration sphere and in pure water, respectively. They considered that the hydration water may be in the hydrophobic hydration state.

As seen in Fig. 1, the most remarkable feature of the $\Delta_m V$ values for the glycine-PEG-H_2O system is that the differences among the positive $\Delta_m V$ values of different total molality m decrease with an increase in temperature and seem to converge around 110 °C by extrapolation. Namely, at this temperature all the $\Delta_m V$ values for the glycine-PEG-H_2O system reach one value ($-0.22\ cm^3/kg^{-1}$). However, this tendency is not seen for the L-alanine-PEG-H_2O system.

It is interesting to attempt to interpret these volume changes in terms of the hydration behavior of amino acid that depends on the hydrophobic and hydrophilic groups. At neutral pH, glycine and L-alanine are zwitterionic molecules. Glycine does not possess a side chain, but L-alanine has a hydrophobic methyl group as a side chain. Consequently, the hydration sphere of glycine is controlled predominantly by electrostatic interactions between water molecules and the oppositely charged amino and carboxyl atomic groups.

As described in the previous paper [9], the interaction between amino acid and PEG can be explained by the cosphere concept of Gurney. According to Desnoyers and his colleagues [21], the sharing of hydrophilic hydration and hydrophobic hydration cospheres results in an overall repulsive force. The repulsive force due to the structural incompatibility of the hydration cospheres of glycine and PEG will cause a positive volume increase on mixing because the electrostriction of water in the hydration cospheres of glycine and PEG may be reduced. Consequently, the results in Fig. 1 for the glycine-PEG-H_2O system may show that the volume effect of electrostriction of water around the charged groups of glycine is reflected in the value of $\Delta_m V$. A decrease in electrostriction means a decrease in the absolute value of $\Delta_m V$. That is to say, an increase in temperature breaks down the structure of bulk water, and water in the bulk medium structurally becomes more like the electrostricted water around the charged groups of glycine at elevated temperatures. This effect would result in a decrease and even a disappearance around 110 °C of the structural difference between the bulk water and the electrostricted water around the charged groups of glycine. Therefore, the positive $\Delta_m V$ values decrease and converge with increasing temperature.

On the other hand, $\Delta_m V$ values for the L-alanine-PEG-H_2O system manifest different behavior than those for the glycine-PEG-H_2O system due to the presence of the hydrophobic $-CH_3$ group. Thus, for L-alanine the hydrophobic hydration as well as the electrostatic one should influence its hydration sphere. As Chalikian et al. [22] pointed out, the hydrophobic hydration of the $-CH_3$ group in L-alanine should not be considered as independently hydrated, because the hydration cospheres of the $-CH_3$ group and the charged groups probably interact via overlapping hydration spheres. For the L-alanine-PEG-H_2O system, the repulsive force due to the structural difference in the hydration cospheres and the hydrophobic interaction between the methyl group of L-alanine and the ethylene group of PEG may compensate each other over the temperature ranges studied.

Chalikian et al. [22] have determined the apparent molar characteristics of glycine and L-alanine over the pressure and temperature ranges of 1 to 1000 bar and $18 \sim 45$ °C and observed a reduction in hydration with increasing pressure. They interpret these observations as suggesting that an increase in pressure makes water in the bulk solvent more like the electrostricted water in the hydration spheres surrounding the charged terminal groups common to both glycine and L-alanine.

Privalov [23] showed that the differences between specific enthalpy values of the unfolding of some globular proteins decrease with an increase in temperature and even disappear around 110 °C, and considered that 110 °C is a characteristic temperature for compact globular proteins which have some common features in their structural organization responsible for their thermodynamic behavior.

In aqueous solutions, the hydrophobic interaction [24] has long been thought to be the most important driving force for many biochemical process. Recently, however, hydrophilic interactions, which are highly dependent on the properties of the solvent, seem to be as important as

hydrophobic interactions in highly specific processes such as protein folding and molecular recognition [25–28]. As observed in the glycine-PEG-H_2O system, 110 °C may be a characteristic temperature for the hydration of a charged group, and a more systematic investigation is required to characterize the hydration properties of such charged and hydrophobic groups as a function of temperature.

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References

1. Ahkong QF, Howell JI, Lucy JA, Safwant F, Davey MR, Cocking EC (1975) Nature 255:66
2. Atha DH, Ingham KC (1981) J Biol Chem 256:12108
3. Lee JC, Lee LLY (1981) J Biol Chem 256:625
4. Mcpherson AJr (1976) J Biol Chem 251:6300
5. Namethy G, Peer WJ, Sheraga H (1981) Annu Rev Biophys Bioeng 10:459
6. Hvidt A (1983) Annu Rev Biophys Bioeng 12:1
7. Westhof E (1988) Annu Rev Biophys Bioeng 17:125
8. Sasahara K, Uedaira H (1993) Colloid and Polym Sci 271:1035
9. Sasahara K, Uedaira H (1994) Colloid and Polym Sci 272:385
10. Kharakoz DP (1989) Biophys Chem 34:115
11. Kharakoz DP (1991) J Phys Chem 95:5634
12. Wen WY, Nara K (1967) J Phys Chem 71:3907
13. Millero FJ, Surdo AL, Shin C (1978) J Phys Chem 82:784
14. Leyendekkers JV (1986) J Phys Chem 90:5449
15. Harada S (1978) J Solu Chem 7:463
16. Blandamer MJ, Fox MF, Powell E, Stafford JW (1967) Makromol Chem 124:222
17. Kjellander R, Flourin E (1981) J Chem Soc Faraday Trans I 77:2053
18. Troyanik AI (1984) Zh Strukt Khim 25:49
19. Kaatze U (1978) Ber Bunsenges Phys Chem 82:690
20. Kaatze U, Gottmann O, Podbielski R, Pottel R, Terveer U (1978) J Phys Chem 82:112
21. Desnoyers JE, Arel M, Perron E, Jolicoeur C (1969) J Phys Chem 73:3346
22. Chalikian TV, Sarvazyan AP, Funck T, Chain CA, Breslauer KJ (1994) J Phys Chem 98:321
23. Privalov PL (1979) Adv Protein Chem 33:167
24. Kozak JJ, Knight WS, Kouzmann W (1968) J Chem Phys 48:675
25. Ben-Naim A (1989) J Chem Phys 90:7412
26. Ben-Naim A (1990) J Phys Chem 94:6893
27. Mezei M, Ben-Naim A (1990) J Chem Phys 92:1359
28. Ben-Naim A (1990) Biopolymer 29:567