Colloid & Polymer Science Colloid Polym Sci 272: 385–392 (1994)

Volume and compressibility changes on mixing aqueous solutions of the amino acid and poly(ethylene glycol)

K. Sasahara and H. Uedaira[1])

Research Laboratory Fujinomiya, Fuji Photo Film Co., Ltd. Shizuoka, Japan
[1]) College of Engineering, Hosei University, Kajino, Koganei-shi, Tokyo, Japan

Abstract: The volume and compressibility changes on mixing aqueous solutions of the amino acid and poly(ethylene glycol) were measured with a vibration densimeter and a sing-around velocimeter at 298.15 K. For the system of alanine-PEG-H_2O, the additivity rule for the mean apparent molal volume and compressibility at infinite dilution held, and the excess volume and compressibility changes on mixing were obtained. For the system of glycine-PEG-H_2O, the additivity rule for the mean apparent molal compressibility at infinite dilution did not hold. While the mean apparent molal volume and compressibility changes were negative and positive for the systems of another amino acid-PEG-H_2O, respectively, where amino acids were valine, isoleucine, leucine, phenylalanine, and tryptophan. These results suggest that glycine and alanine are excluded from the hydration layer around PEG chain and the amino acids with a larger side chain than alanine are bound to the PEG chain due to the hydrophobic interaction. The hydration number per monomer around PEG chain was estimated to be 3.9.

Key words: PEG – amino acid – excess volume – excess compressibility – hydrophobic interaction

Introduction

In a previous paper [1], we reported that the solubilities of amino acid in aqueous poly(ethylene glycol) (PEG) solutions increased with increasing the size of the hydrophobic side chain of amino acid. This result was interpreted in terms of the exclusion effect due to the structural incompatibility of the hydration cospheres of amino acid and PEG, and the hydrophobic interaction between the side chain of amino acid and the methylene groups of PEG.

Kjellander and Florin [2] suggested that PEG can adopt conformations where the methylene groups are encaged by a dynamic network of water molecules at the same time as hydrogen bonds are formed to the ether oxygen. Troyanik showed by the Monte Carlo method that the helix structure of water molecules 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 [3]. The average hydration number per monomer unit of PEG is 3.5 by his model. Kaatze et al. [4] measured the complex dielectric constants of aqueous PEG solution and considered that the hydration water around PEG chain may be in the hydrophobic hydration state.

Recently, Ishimura and Uedaira [5] measured the ^{17}O relaxation rates of water in aqueous apolar amino acid solutions. They showed that the dynamic hydration number (n_{DHN}) [6], which expresses the hydration characteristics of amino acid, increases with increasing the size of hydrophobic group of the amino acid. The hydration of glycine is hydrophilic and tryptophan has the most hydrophobic character.

Volumetric and compressibility behavior of solutes in aqueous solution can provide useful information about solute-solvent and solute-solute interaction. Wen and Nara [7] measured the volume changes on mixing for the ternary system of KBr-Bu_4NBr-H_2O and obtained the large excess volume changes of mixing with positive signs. The increase of the solution volume by mixing was attributed mainly to the interaction of hydrophobic cation through the change of water structure. Høiland et al. [8] measured the partial molar volumes and isentropic partial molar compressibilities of some nucleobases and nucleosides in aqueous solution. They showed that the self-association (stacking) process of nucleobases leads to a volume contraction and positive compressibility change and considered that this process of stacking is the approach of two hydrophobic solutes from infinity to close contact. It is expected that when some ordered close structure is formed in solution, the volume and the compressibility of the solution will generally change. From these standpoints, it is interesting to investigate the volume and compressibility changes on mixing aqueous solutions of amino acid and PEG to elucidate further the effect of overlap of hydration spheres. In this paper, we report the volume and compressibility changes on mixing of the ternary system of amino acid-PEG-H_2O. The results obtained are discussed in terms of the structural difference of hydration spheres and the hydrophobic interaction between amino acid and PEG.

Experimental

Material

The amino acids used in this study were glycine (Gly), L-alanine (Ala), L-valine (Val), L-isoleucine (Ileu), L-leucine (Leu), L-phenylalanine (Phe) and L-tryptophan (Trp). All the amino acids were special reagent grade products from Protein Chemistry Laboratory Co., Ltd. and they were used without further purification since their purities were ascertained to be excellent from the solubility data in water. PEG (analytical grade, Merck) of a mean molecular weight 4000 was used without further purification. All amino acids and PEG were dried before use in a vacuum oven 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 (Anton Paar DMA60). The solutions were prepared by successive additions of concentrated solution to a known quantity of solvent. The addition was carried out by weight in a mixing chamber connected to the measuring cell with a teflon tube and a flow pump. 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 $\pm 2\times10^{-3}$ K. The density meter was calibrated with water and dry air every day. The densities could thus be determined within $\pm 2\times10^{-6}\,\mathrm{g\,cm^{-3}}$. The ultrasonic velocity measurements in solutions were made with the sing-around velocimeter which is capable of an accuracy of 1 $\mathrm{cm\text{-}s^{-1}}$. The details of the apparatus and procedure have been reported elsewhere [9]. All measurements were carried out at 298.15 K.

Treatment of the data

The apparent molal volume ϕ_v of a solute in binary solution is defined in the molality scale:

$$\phi_v = (V - 55.51 V_0)/m \, , \tag{1}$$

where V_0 is the molal volume of pure water, V the volume of solution, and m the molality of a solute. If we consider a solution in which two solutes are dissolved, Eq. (1) can be modified into the following form by using the density and rewriting ϕ_v as $\phi_v(x, m)$

$$\phi_v(x, m) = (1/m)\{(1000 + m_1M_1 + m_2M_2)/d - 1000/d_0\} \, , \tag{2}$$

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 two solutes. In this paper, 1 denotes amino acid and 2 denotes PEG, and m_2 and M_2 mean the molality and molecular weight of PEG monomer unit. $\phi_v(x, m)$ is the mean apparent molal volume of the mixed solutes at total molality m and mole fraction x, given as

$$m = m_1 + m_2, \quad \text{and} \quad x = m_1/m \, . \tag{3}$$

The excess apparent molal volume, $\phi_v^{ex}(x, m)$, is defined as the deviation from $\phi_v(x, 0)$, the mean apparent molal volume at infinite dilution:

$$\phi_v^{ex}(x, m) = \phi_v(x, m) - \phi_v(x, 0) . \qquad (4)$$

The excess volume of the ternary solution may be defined as the product of the total molality and the excess apparent molal volume:

$$V^{ex}(x, m) = m\phi_v^{ex}(x, m) . \qquad (5)$$

According to Wen and Nara [7], the excess volume change of mixing $\Delta_m V^{ex}$ is given as the deviation from the linear relationship between $V^{ex}(x, m)$ and x at constant total molality:

$$\Delta_m V^{ex} = V^{ex}(x, m) - xV^{ex}(1, m) - (1—x) \times V^{ex}(0, m) . \qquad (6)$$

On substituting Eq. (6) for Eq. (5), we obtain

$$\Delta_m V^{ex} = (m_1 + m_2)\phi_v^{ex}(x, m) - m_1\phi_v^{ex}(1, m) - m_2\phi_v^{ex}(0, m) , \qquad (7)$$

where $\phi_v^{ex}(1, m)$ and $\phi_v^{ex}(0, m)$ apply to binary solutions of the pure solute. From Eq. (7), the excess volume of mixing $\Delta_m V^{ex}$ is obtained directly.

The mean apparent molal adiabatic compressibility $\phi_k(x, m)$ may be written in the following form as the case of volume:

$$\phi_k(x, m) = \{10^3(\beta - \beta_0)/(m_1 + m_2)d_0\} + \beta\phi_v(x, m) . \qquad (8)$$

The coefficient of the adiabatic compressibility, β, is calculated from $1/\beta = dv^2$, where v is the sound velocity of solution. The subscript zero refers to pure water. The excess apparent molal adiabatic compressibility, $\phi_k^{ex}(x, m)$, is defined as the deviation from $\phi_k^{ex}(x, 0)$, the mean apparent molal adiabatic compressibility at infinite dilution:

$$\phi_k^{ex}(x, m) = \phi_k(x, m) - \phi_k(x, 0) . \qquad (9)$$

The excess adiabatic compressibility changes of mixing $\Delta_m K^{ex}$ are defined as follows

$$\Delta_m K^{ex} = (m_1 + m_2)\phi_k^{ex}(x, m) - m_1\phi_k^{ex}(1, m) - m_2\phi_k^{ex}(0, m) , \qquad (10)$$

where $\phi_k^{ex}(1, m)$ and $\phi_k^{ex}(0, m)$ apply to binary solutions of the pure solute.

Results

For the various x, the relations between $\phi_V(x, m)$ and m are obtained from the density data, and are shown in Fig. 1 for the system of Gly-PEG-H_2O and in Fig. 2 for the system of Ala-PEG-H_2O. In both figures, the ordinate represents the excess apparent molal volume $\phi_v^{ex}(x, m)$, defined as the deviation from $\phi_v(x, 0)$. The used $\phi_v(x, 0)$ values were calculated as follows

$$\phi_v(x, 0) = x\phi_v(1, 0) + (1 - x)\phi_v(0, 0) , \qquad (11)$$

where $\phi_v(1, 0)$ and $\phi_v(0, 0)$ are the apparent molal volume at infinite dilution for the solute 1 and 2, respectively. The values of apparent molal volume at infinite dilution of Gly, Ala and PEG in binary solution were 43.20 cm^3mol^{-1}, 60.46 cm^3mol^{-1} and 36.96 cm^3mol^{-1} at 298.15 K, respectively, and they were in good agreement with the literature values [10–12]. As seen in Figs. 1 and 2, the curves of $\phi_v^{ex}(x, m)$ are linear against total molality m and the additivity rule holds for $\phi_v(x, 0)$ as the $\phi_v^{ex}(x, m)$ values converge zero at infinite dilution. The Eq. (11) means that the mixing of two

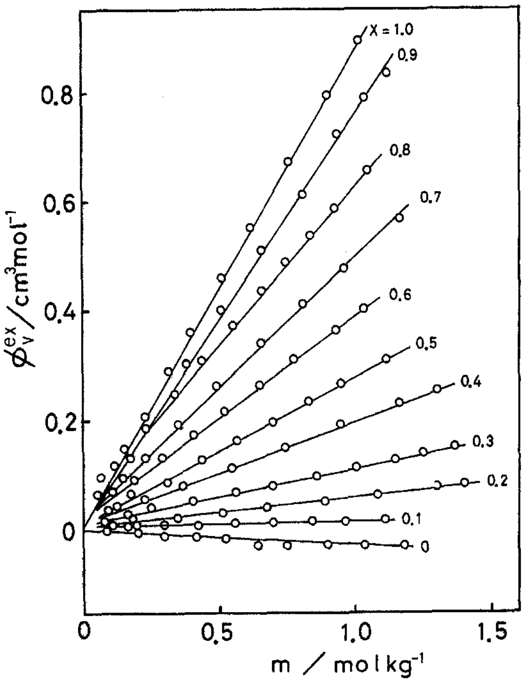

Fig. 1. Relation between excess apparent molal volume and total molality at various mole fractions of Gly for the system of Gly-PEG-H_2O at 298.15 K.

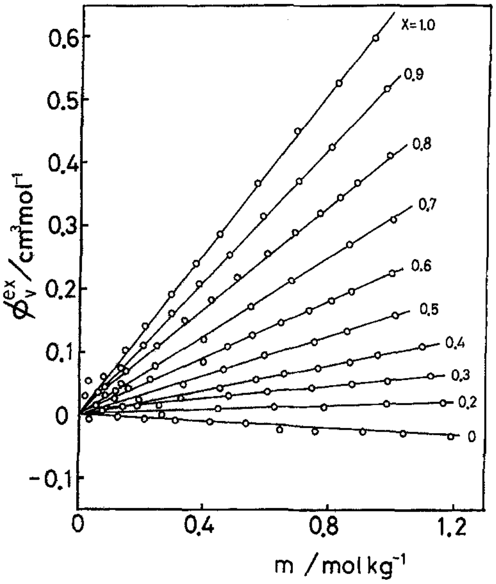

Fig. 2. Relation between excess apparent molal volume and total molality at various mole fractions of Ala for the system of Ala-PEG-H_2O at 298.15 K.

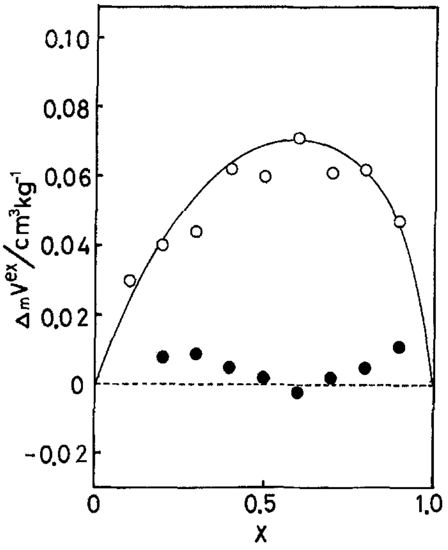

Fig. 3. Excess volume changes of mixing at constant total molality (1.0 mol/kg) as a function of mole fraction of amino acids at 298.15 K. ○: Gly, ●: Ala

binary solutions at infinite dilution produces a ternary solution whose volume is the sum of the volumes of the binary solutions. The $\Delta_m V^{ex}$ values obtained for both systems of Gly-PEG-H_2O and Ala-PEG-H_2O are plotted in Fig. 3 at constant total molality against x, the mole fraction of amino acid. As seen in Fig. 3, the $\Delta_m V^{ex}$ values plotted against x are positive for the system of Gly-PEG-H_2O and almost zero for the system of Ala-PEG-H_2O.

Table 1 shows $\phi_k(x, m)$ values for the systems of Gly-PEG-H_2O and Ala-PEG-H_2O, respectively. The $\phi_k(x, m)$ is linearly related to the total molality m, as observed in the case of $\phi_V^{ex}(x, m)$ in Figs. 1 and 2. The apparent molal compressibilities at infinite dilution for Gly, Ala and PEG in binary solution (respectively, $10^4\phi_k = -26.92$ cm³ mol⁻¹ bar⁻¹, -25.60 cm³ mol⁻¹ bar⁻¹, -0.85 cm³ mol⁻¹ bar⁻¹ at 298.15 K) are in good agreement with the values reported in the literature [10–12]. Table 2 shows both $\phi_k(x, 0)$ values extrapolated ($m \to 0$) and calculated by Eq. (12):

$$\phi_k(x, 0) = x\phi_k(1, 0) + (1 - x)\phi_k(0, 0) , \qquad (12)$$

where $\phi_k(1, 0)$ and $\phi_k(0, 0)$ are the apparent molal compressibility at infinite dilution for the solute 1 and 2, respectively. As seen in Table 2, the $\phi_k(x, 0)$ values obtained by extrapolation are almost equal to those calculated for the system of Ala-PEG-H_2O, but the $\phi_k(x, 0)$ values obtained by extrapolation are smaller than those calculated for the system of Gly-PEG-H_2O. Consequently, the additivity rule seems to hold for $\phi_k(x, 0)$ in the system of Ala-PEG-H_2O, but does not hold in the system of Gly-PEG-H_2O. In the system of Ala-PEG-H_2O, the $\Delta_m K^{ex}$ values obtained by Eq. (10) are shown against x in Fig. 4 at constant total molality and almost zero.

Figure 5 shows the relations between mean apparent molal volume and total molality at various mole fraction of valine for the system of Val-PEG-H_2O. The slopes of the mean apparent molal volumes are negative, and additivity rule at infinite dilution does not hold for this system. Figure 6 shows the relations between mean apparent molal volume and total molality for the systems of another amino acid-PEG-H_2O, where amino acids are Ileu, Leu, Phe and Trp. As observed in the case of Val-PEG-H_2O, the curves are not linear, and the slopes negative. Figure 7 shows the relations between mean apparent adiabatic compressibility and total molality for the systems of amino acid-PEG-H_2O, where amino acids are Leu, Ileu and Trp. We can see the positive compressibility changes in Fig. 7.

Table 1. Mean apparent molal compressibilities of glycine-PEG and alanine-PEG in aqueous solution at 298.15 K.

m	$10^4\ \phi_k(x, m)$ ($cm^3\ mol^{-1}\ bar^{-1}$)					
($mol\ kg^{-1}$)	$x = 0$	$x = 0.2$	$x = 0.4$	$x = 0.6$	$x = 0.8$	$x = 1$
				Gly-PEG-H_2O		
0.1	− 0.85	− 6.46	− 11.43	− 16.80	− 21.73	− 26.45
0.2	− 0.69	− 6.05	− 11.20	− 16.47	− 21.10	− 25.96
0.3	− 0.75	− 6.01	− 10.98	− 16.11	− 20.83	− 25.45
0.4	− 0.75	− 5.90	− 10.86	− 15.82	− 20.38	− 25.05
0.5	− 0.71	− 5.83	− 10.76	− 15.51	− 20.15	− 24.55
0.6	− 0.68	− 5.74	− 10.57	− 15.27	− 19.83	− 24.25
				Ala-PEG-H_2O		
0.1	− 0.85	− 5.82	− 10.51	− 15.71	− 20.50	− 24.96
0.2	− 0.69	− 5.56	− 10.61	− 15.52	− 19.97	− 24.92
0.3	− 0.75	− 5.57	− 10.53	− 15.11	− 19.68	− 24.12
0.4	− 0.75	− 5.57	− 10.32	− 14.93	− 19.33	− 23.64
0.5	− 0.71	− 5.43	− 10.16	− 14.72	− 19.10	− 23.13
0.6	− 0.68	− 5.34	− 10.10	− 14.51	− 18.89	− 22.65

Table 2. Mean apparent molal compressibilities at infinite dilution of glycine-PEG and alanine-PEG in aqueous solution at 298.15 K.

X	$10^4\ \phi_k(x, 0)$ ($cm^3mol^{-1}bar^{-1}$)	
	(extrapo)	(calcu)
	Gly-PEG-H_2O	
0	− 0.85	
0.2	− 6.30	− 6.06
0.4	− 11.55	− 11.27
0.6	− 17.00	− 16.48
0.8	− 22.00	− 21.69
1	− 26.90	
	Ala-PEG-H_2O	
0	− 0.85	
0.2	− 5.80	− 5.80
0.4	− 10.81	− 10.75
0.6	− 15.75	− 15.70
0.8	− 20.55	− 20.65
1	− 25.60	

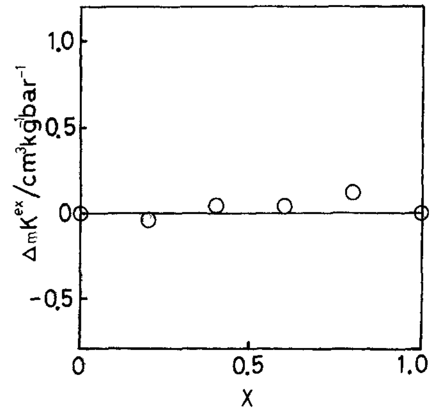

Fig. 4. Excess adiabatic compressibility changes of mixing at constant total molality (0.6 mol/kg) as a function of mole fraction of Ala at 298.15 K.

Discussion

As described in the previous paper [1], the interaction between amino acid and PEG can be explained by the cosphere concept of Gurney. The amino acids having the hydrophilic hydration structure by polar groups are excluded from the hydration layer around PEG chain in terms of the structural incompatibility of hydration cospheres. With increasing the size of hydrophobic group of the amino acid, the hydrophobic interaction between the methylene groups of PEG and the side chain of amino acid becomes stronger than the exclusion effect by polar groups. Therefore, Phe and Trp with the larger hydrophobic side chain have a larger solubility in aqueous PEG solutions than in water.

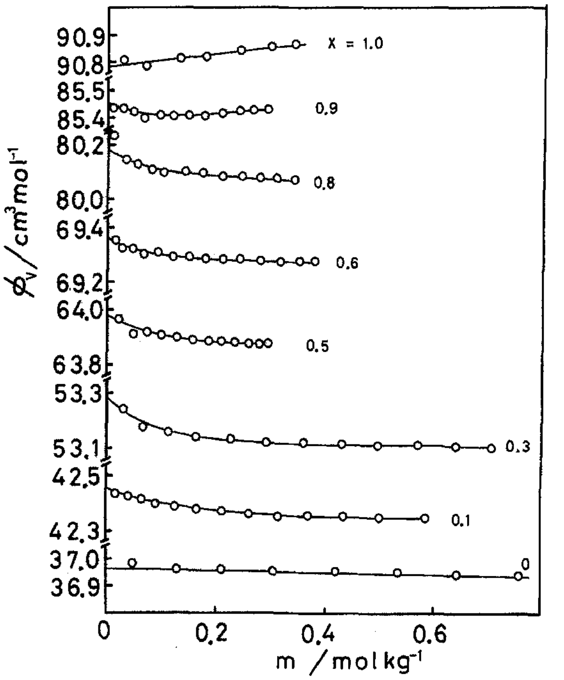

Fig. 5. Relation between mean apparent molal volume and total molality at various mole fractions of Val for the system of Val-PEG-H_2O at 298.15 K.

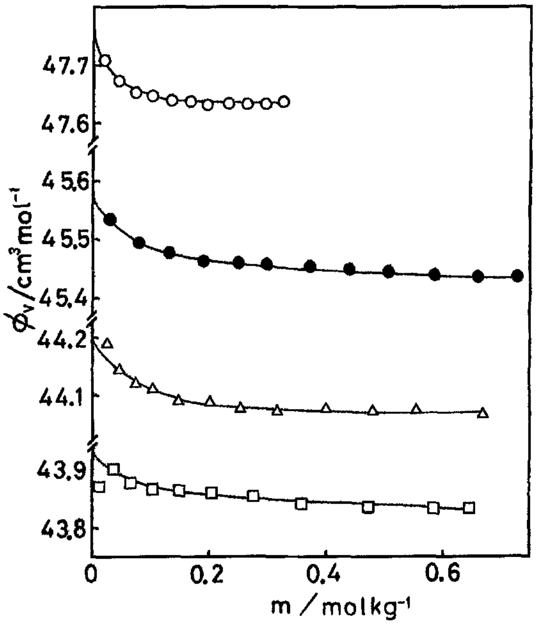

Fig. 6. Relation between mean apparent molal volume and total molality at 0.1 mole fraction of amino acid for the system of amino acid-PEG-H_2O at 298.15 K. □: Ileu, △: Leu, ●: Phe, ○: Trp

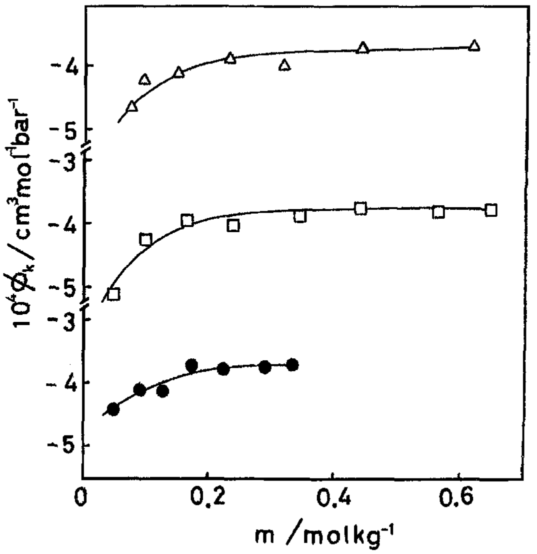

Fig. 7. Relation between mean apparent molal adiabatic compressibility and total molality at 0.1 mole fraction of amino acid for the system of amino acid-PEG-H_2O at 298.15 K. □: Ileu, △: Leu, ●: Trp

For the system of Gly-PEG-H_2O, the $\Delta_m V^{ex}$ values are found to be positive in Figs. 3. Uedaira [13] reported that in the ternary system which contained two structure makers, mutual salting-out was observed, while in the system with a structure maker and a structure breaker, mutual salting-in was found. According to Desnoyers and his colleagues [14], the sharing of hydrophilic hydration and hydrophobic hydration cosphere results in overall repulsive force. The repulsive force due to the structural incompatibility of the hydration cospheres of Gly and PEG will cause this positive volume increase on mixing since the electrostriction of water in the hydration cospheres of Gly and PEG may be reduced. This repulsive force will also explain the smaller $\phi_k(x, 0)$ values obtained by extrapolation ($m \rightarrow 0$) than those calculated by Eq. (12). For the system of Ala-PEG-H_2O, the $\Delta_m V^{ex}$ and $\Delta_m K^{ex}$ values are found to be almost zero in Figs. 3 and 4. It is considered that the repulsive force by the structural difference of the hydration cospheres and hydrophobic interaction between the methyl group of Ala and the methylene groups of PEG are equilibrated apparently and the interaction between Ala and PEG cannot be detected, and the additivity rule for volumes and compressibilities at infinite dilution holds in this system.

As seen in Figs. 5, 6, and 7, the hydrophobic interaction between the methylene groups of PEG and the side chain of amino acid leads to a volume contraction and a compressibility increase. These results suggest that this process by the hydrophobic interaction is the approach of two hydrophobic solutes from infinity to close contact. Such a process must involve removal of only part of the hydration spheres of both solutes and may well be connected with a volume decrease. These curves of $\phi_v(x, m)$ in Figs. 5, 6 are expressed in the form of Eq. (13):

$$\phi_v(x, m) = \phi_v(x, 0) + \sum a_i m^{i/2} \; (i = 1.2\text{---}) \,, \quad (13)$$

and each a_i coefficient is obtained by a least squares fit with a computer program. These coefficients represent the interaction between amino acids and PEG which is contributing to the solution volume. They are not useful, however, for interpreting the volume change in terms of the hydrophobic interaction, except for calculating $\phi_v(x, m)$ at the desired molality. Normally, the solutes are considered incompressible, i.e., the intrinsic compressibility is zero. Therefore, the observed compressibility changes in Fig. 7 may be ascribed to the compressibility of the hydration cospheres as observed in the case of stacking of some nucleobases and nucleoside in water [8]. This will explain the positive compressibility changes observed for the hydrophobic interaction between amino acid and PEG.

Properties and structures of nonionic surfactants having oxyethylene chain as a hydrophilic group have been studied extensively by various experimental methods [15–18]. Based on these experimental observations, most authors conclude that 2 ~ 5 water molecules are needed for complete hydration of one oxyethylene unit. One of the current interests is the hydration of the oxyethylene chain, since the hydration structure is closely related to the size and shape of the micelle and the phase behavior in surfactant and nonionic polymer chemistry [19–20]. We have, therefore, calculated the hydration number per monomer unit around PEG chain due to the two assumptions as follows.

i) Water in aqueous PEG solution consists of two state water: free water and hydrated water.

ii) Ala in aqueous PEG solution dissolves in free water only.

The latter assumption is based on the data that excess volume and compressibility changes on mixing ($\Delta_m V^{ex}$ and $\Delta_m K^{ex}$) are not observed for the system of Ala-PEG-H_2O in Figs. 3 and 4. Thus, using the solubilities of Ala in aqueous PEG solutions at 298.15 K in the previous paper [1] and these two assumptions, it is possible to calculate the hydration number around CH_2CH_2O unit. Table 3 shows the values of the solubilities of Ala in aqueous PEG solutions and the calculated hydration number around PEG monomer unit. The average hydration number obtained for CH_2CH_2O unit is 3.9 at 298.15 K. The obtained result means that 3.9 water molecules take part in the hydration of each CH_2CH_2O group. This result is in agreement with those of Troyanik and Kaatze et al. [3–4].

Table 3. Solubilities of Ala in aqueous PEG solution and hydration number around PEG monomer unit at 298.15 K

Solvent	Solubility[a]) (g/100 g solvent)	Hydration number
Water	16.56	
PEG 3 wt%	15.22	4.1
PEG 5 wt%	14.40	3.9
PEG 7 wt%	13.54	3.9

[a]) reference [1]

References

1. Sasahara K, Uedaira H Colloid Polym Sci (to be published)
2. Kjellander R, Flourin E (1981) J Chem Soc Faraday Trans I 77:2053
3. Troyanik AI (1984) Zh Strukt Khim 25:49
4. Kaatze U (1978) Ber Bunsenges Phys Chem 82:690
5. Ishimura M, Uedaira H (1990) Bull Chem Soc Jap 63:1
6. Uedaira H, Ikura M, Uedaira H (1989) Bull Chem Soc Jap 62:1
7. Wen WY, Nara K (1967) J Phys Chem 71:3907
8. Høiland H, Skauge A, Stokkeland I (1984) J Phys Chem 88:6350
9. Nakajima T, Komatsu T, Nakagawa T (1975) Bull Chem Soc Jap 48:788
10. Millero FJ, Surdo AL, Shin C (1978) J Phys Chem 82:784
11. Leyendekkers JV (1986) J Phys Chem 90:5449
12. Harada S (1978) J Solu Chem 7:463
13. Uedaira H (1977) Bull Chem Soc Jap 50:1298

14. Desnoyers JE, Arel M, Perron E, Jolicoeur C (1969) J Phys Chem 73:3346
15. Liu KJ, Parson JL (1969) Macromolecules 2:529
16. Maxfield J, Shepherd IW (1975) Polymer 16:505
17. Hager SL, Macrury TB (1980) J Applied Polym Sci 25:1559
18. de Vringer T, Joosten JGH, Junginger HE (1986) Colloid Polym Sci 264:623
19. Zulauf M, Rsenbusch JP (1983) J Phys Chem 87:856
20. Mitchell DJ, Tiddy GJT, Waring L, Bostock T, NcDonald MP (1983) J Chem Soc Faraday Trans I 79:975

March 27, 1993;
accepted August 3, 1993

Authors' address:

Kenji Sasahara
Research Laboratory Fujinomiya
Fuji Photo Film Co. Ltd.
200 Ohnakazato
Fujinomiya, Shizuoka
418 Japan