ISSN 1070-3632, Russian Journal of General Chemistry, 2008, Vol. 78, No. 4, pp. 597–601. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © I.N. Mezhevoi, V.G. Badelin, 2008, published in Zhurnal Obshchei Khimii, 2008, Vol. 78, No. 4, pp. 616–620.

Enthalpy Characteristics of Solution of *L*-Cysteine and *L*-Asparagine in Water–Alcohol Mixtures at 298.15 K

I. N. Mezhevoi and V. G. Badelin

Institute of Solution Chemistry, Russian Academy of Sciences,
ul. Akademicheskaya 1, Ivanovo, 153045 Russia
e-mail: inm@isc-ras.ru

Received June 28, 2007

Abstract—The integral enthalpies of solution $\Delta_{sol}H^m$ of *L*-cysteine and *L*-asparagine in mixtures of water with ethanol, *n*-propanol, and isopropanol at a mole fraction of alcohol of up to 0.32 were determined by calorimetry of solution. The standard enthalpies of solution ($\Delta_{sol}H^0$) of *L*-serine and of its transfer ($\Delta_{tr}H^0$) from water to a mixed solvent were calculated. The dependences of $\Delta_{sol}H^0$ and $\Delta_{tr}H^0$ on the composition of water–alcohol mixtures pass through a maximum. The calculated enthalpy coefficients of pair interaction of amino acids with alcohol molecules are positive and increase in the order ethanol, *n*-propanol, isopropanol. The data obtained were interpreted from the viewpoint of various types of interaction in solution and effect of the amino acid residue on the thermochemical characteristics of solution.

DOI: 10.1134/S1070363208040142

The selectivity of interaction of side substituents in amino acids with water and their hydrophobic–hydrophilic character determine the properties and steric features of the tertiary and quaternary structure of proteins. The solvation, being the initial step of biochemical processes, determines the biological activity of macromolecules of complex structure. Aqueous-alcoholic solvents are also of interest because of different nature of interactions between solution components depending on the cosolvent concentration.

The features of thermochemical behavior of various amino acids in aqueous-alcoholic solutions in a wide range of cosolvent concentrations practically were not studied. The usually examined composition range is restricted to the mole fraction of organic component of 0.09 [1], whereas the shape of the dependences of the thermochemical characteristics of solution and transfer changes at higher concentrations of the cosolvent. In this study we measured the enthalpies of solution of two amino acids, *L*-cysteine (*L*-Cys) and *L*-asparagine (*L*-Asn), in the water–ethanol (EtOH), water–*n*-propanol (PrOH), and water–isopropanol (*i*-PrOH) systems. The results were compared with the data that we obtained previously for *L*-serine (Ser).

The examined compounds are derivatives of *L*-alanine [H_2N–$CH(CH_2R)$–COOH]. The substituent R is a hydrogen atom in *L*-alanine, an –SH group in *L*-cysteine, an O=C–NH_2 group in *L*-asparagine, and an –OH group in *L*-serine. Our goal was to elucidate the role of different side substituents in amino acids in the solvation in water–alcohol solvents. The experimental data on the standard enthalpies of solution $\Delta_{sol}H^0$ of amino acids in mixed aqueous-alcoholic solvents are given in Table 1. The dependences of the enthalpies of transfer $\Delta_{tr}H^0$ of Cys and Asn from water to aqueous-alcoholic solvents on the mole fraction of the cosolvent (X_2) are plotted in Figs. 1 and 2, respectively.

As seen from Figs. 1 and 2, $\Delta_{tr}H^0$ changes sign with variation of the mixed solvent composition at X_2 ~0.2 for the system with PrOH and X_2 ~0.23 for the system with *o*-PrOH. In Asn solutions, the enthalpy of transfer changes sign in all the three solvent systems (at X_2 ~0.18 for EtOH, ~0.15 for PrOH, and ~0.17 for *i*-PrOH). This means that the enthalpies of transfer are determined by different contributions with opposite signs from various kinds of interactions and solvation processes competing in the solution. To interpret the dependences observed, we used a model approach based on solute–cosolvent interactions, suggested in [2]. In amino acid–alcohol–water ternary systems, the

Table 1. Standard enthalpies of solution $\Delta_{sol}H^0$ (kJ mol^{-1}) of amino acids in mixed water–alcohol solvents at 298.15 K

EtOH–H_2O		PrOH–H_2O		*i*-PrOH–H_2O	
m_y[a]	$\Delta_{sol}H^0$	m_y[a]	$\Delta_{sol}H^0$	m_y[a]	$\Delta_{sol}H^0$
L-Cys					
0	10.81±0.06	0	10.81±0.06	0	10.81±0.06
0.7375	11.64±0.06	0.8325	12.23±0.06	0.8555	12.40±0.07
1.7961	12.74±0.07	1.5225	13.06±0.06	1.4774	13.50±0.06
2.9320	14.13±0.05	2.1511	13.90±0.08	2.1518	14.70±0.06
4.1841	15.14±0.07	3.1648	14.71±0.07	3.1645	15.72±0.07
5.6550	15.72±0.07	4.0872	15.24±0.07	4.1405	16.41±0.08
7.3546	16.30±0.07	5.3967	15.61±0.07	5.3965	16.94±0.08
9.2937	16.48±0.09	7.1200	15.77±0.05	7.1212	17.40±0.09
11.5012	16.69±0.10	8.7601	15.34±0.08	8.7615	17.11±0.08
14.1490	16.54±0.08	11.0810	13.99±0.06	11.0813	16.08±0.09
17.2780	15.92±0.08	12.9057	12.25±0.07	12.9050	14.27±0.07
21.4003	15.04±0.08	15.9980	9.37±0.06	15.9988	11.31±0.06
25.5690	14.14±0.07	19.0600	6.54±0.04	19.0607	8.48±0.06
L-Asn					
0	22.57±0.10	0	22.57±0.10	0	22.57±0.10
0.8544	23.58±0.11	0.7301	23.57±0.12	0.7202	23.67±0.11
1.8137	24.23±0.11	1.2831	24.21±0.12	1.2849	24.51±0.11
3.0326	24.95±0.12	2.3001	24.85±0.13	2.3011	25.44±0.14
4.1540	25.55±0.14	3.1514	25.35±0.11	3.1510	26.29±0.12
5.7465	25.73±0.13	4.2910	25.66±0.12	4.2919	26.76±0.12
7.2560	25.67±0.12	5.4343	25.75±0.14	5.4362	27.05±0.13
9.3915	24.52±0.12	7.1665	24.99±0.14	7.1669	26.76±0.14
11.3595	23.17±0.13	8.5624	23.80±0.13	8.5635	25.55±0.13
14.4417	19.74±0.11	10.5980	20.90±0.12	10.5981	23.24±0.11
17.0610	15.73±0.12	12.7121	17.53±0.11	12.7121	20.31±0.12

[a] (m_y) Molal concentration of alcohol, mol kg^{-1}.

hydrated solute molecules occur at a sufficiently short distance for their hydration shells to overlap. The obtained values of $\Delta_{tr}H^0$ for amino acids will be determined by a sum of terms of different signs:

$$\Delta_{tr}H^0 = -\Delta H_1 - \Delta H_2 + \Delta H_3 + \Delta H_4, \quad (1)$$

where ΔH_1 refers to ion–bipolar interactions between the zwitterionic centers of the amino acids and the OH group of alcohols; ΔH_2, to hydrophilic–hydrophilic interactions between the –OH, –SH, and NH_2 groups of amino acids and the –OH group of alcohol by the hydrogen bonding mechanism; ΔH_3, to hydrophobic–hydrophilic interactions between the nonpolar moieties of amino acids or alcohols and side groups of amino acids (or OH group of alcohol) or zwitterionic centers of amino acids; and ΔH_4, to hydrophobic–hydrophobic interactions between the nonpolar moieties of amino acids and nonpolar moieties of alcohols.

According to this model, the first and second types of interactions make negative contributions, and the third and fourth types, positive contributions to $\Delta_{tr}H^0$. The positive values of $\Delta_{tr}H^0$ in solutions of Cys in water–PrOH and water–*i*-PrOH solvents and in solutions of Asn with all the examined aqueous-alcoholic solvents at the concentrations indicated above (Figs. 1, 2) suggest the prevalence of the third

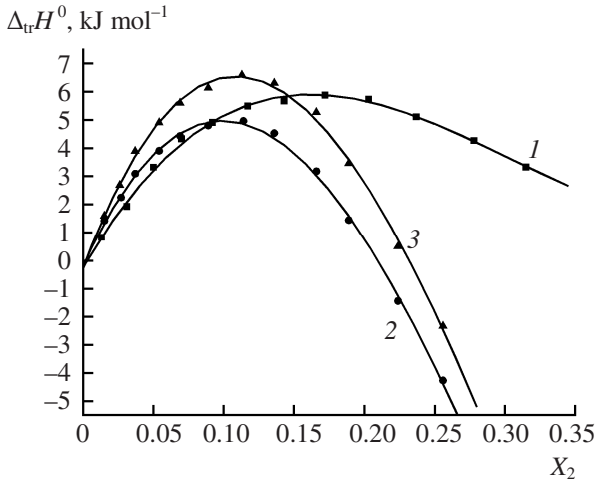

Fig. 1. Enthalpy of transfer ($\Delta_{tr}H^0$) of *L*-cysteine from water into its mixtures with (*1*) EtOH, (*2*) PrOH, and (*3*) *i*-PrOH as a function of the mole fraction of alcohol X_2 at 298.15 K.

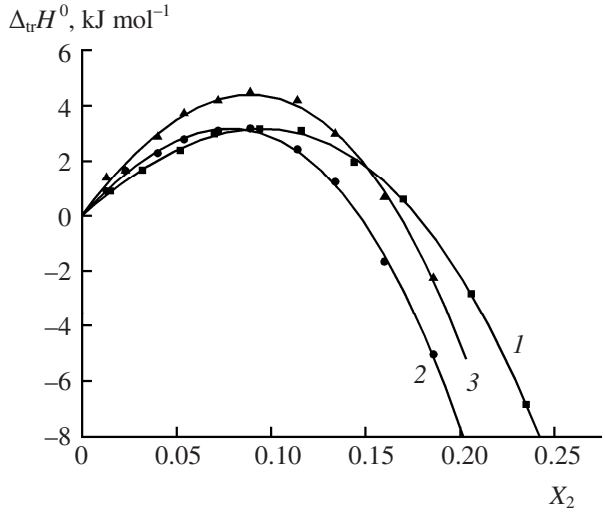

Fig. 2. Enthalpy of transfer ($\Delta_{tr}H^0$) of *L*-asparagine from water into its mixtures with (*1*) EtOH, (*2*) PrOH, and (*3*) *i*-PrOH as a function of the mole fraction of alcohol X_2 at 298.15 K.

(ΔH_3) and fourth (ΔH_4) types of interaction over the first and second types. After passing through a maximum, the $\Delta_{tr}H^0$ values start to decrease and then become negative, suggesting the prevalence of ion–bipolar and hydrophilic–hydrophilic interactions. Apparently, with an increase in the cosolvent concentration, the interactions between the OH groups of alcohols and side substituents in amino acids become more intense, which leads to an increase in the share of exo effects in the ternary solution. In addition, an increase in the concentration of alcohols in aqueous solution leads to a decrease in the effect of hydrophobic hydration, characterized by stronger hydrogen bonding of water around nonpolar groups, e.g., in *i*-PrOH [3]. In interaction with amino acids, the effects of alcohol dehydration become more energy-consuming, which makes the dissolution of biomolecules more endothermic.

The amino acids with nonpolar substituents (e.g., *DL*-α-alanine [4]) behave similarly. The effect of their dissolution is more endothermic compared to amino acids with side polar groups. The maxima at various mole fractions of alcohols show that the contributions from the hydrophobic–hydrophilic and hydrophobic–hydrophobic group interactions are maximal at the corresponding cosolvent concentration. The flatter shape of the maximum for ethanol and its shift toward higher alcohol concentrations compared to PrOH and *i*-PrOH are determined by strong association of EtOH.

The interparticle interactions in ternary aqueous systems were characterized within the framework of the McMillan–Mayer theory [5] by calculating the enthalpy coefficients of pair interactions (h_{xy}) of amino acids with alcohol molecules. For their calculation, the concentration dependence $\Delta_{sol}H^0 = f(m_y)$ for amino acids in aqueous-alcoholic solutions was approximated by a third-degree polynomial:

$$\Delta_{sol}H^0 = a_0 + a_1 m_y + a_2 m_y^2 + a_3 m_y^3, \quad (2)$$

where m_y is the molal concentration of alcohol; the coefficients a_0, a_1, and a_2 are calculated by the least-squares method.

As a result, we obtained Eqs. (3)–(5) for Cys and (6)–(8) for Asn in water–ethanol, water–*n*-propanol, and water–isopropanol mixtures. The absolute terms in Eqs. (3)–(5) correspond to the standard enthalpies of solution of *L*-cysteine in pure water and are well consistent with the data we obtained (Table 1) and published data, $\Delta_{sol}H^0$(Cys) = 11.15 ± 0.03 [6]. The published data for *L*-asparagine [Eqs. (6)–(8)] are essentially different: $\Delta_{sol}H^0$(Asn) = 20.95 ± 0.14 [7], 24.06 [8], and 31.51 kJ mol^{-1} [9]. The value we obtained for the enthalpy of solution of *L*-Asn in water (Table 1) is well consistent with the data of [7] and [8] and can be considered to be reliable.

$$\Delta_{sol}H^0 = (10.92\pm0.18) + (1.24\pm0.07)m_y\text{–}\ldots,$$
$$R\ 0.989,\ SD\ 0.197,\ N\ 12\ , \quad (3)$$

$$\Delta_{sol}H^0 = (10.89\pm0.10) + (1.71\pm0.05)m_y\text{–}\ldots,$$
$$R\ 0.999,\ SD\ 0.094,\ N\ 12, \quad (4)$$

$$\Delta_{sol}H^0 = (10.84\pm0.16) + (2.09\pm0.09)m_y\text{–}\ldots,$$
$$R\ 0.998,\ SD\ 0.155,\ N\ 12, \quad (5)$$

$$\Delta_{sol}H^0 = (22.66\pm0.12) + (1.08\pm0.06)m_y\text{–}\ldots,$$
$$R\ 0.999,\ SD\ 0.095,\ N\ 10, \quad (6)$$

$$\Delta_{sol}H^0 = (22.63\pm0.18) + (1.36\pm0.12)m_y\text{–}\ldots,$$
$$R\ 0.998,\ SD\ 0.140,\ N\ 10, \quad (7)$$

$$\Delta_{sol}H^0 = (22.45\pm0.18) + (1.79\pm0.12)m_y\text{–}\ldots,$$
$$R\ 0.997,\ SD\ 0.141,\ N\ 10. \quad (8)$$

To calculate the enthalpy coefficient of pair interactions h_{xy}, we used the coefficient a_1 related to h_{xy} by $h_{xy} = a_1/2$ [10]. The values we obtained for Ser [11] in alcohol solutions and the published data for *L*-alanine (Ala) [12] in mixtures with ethanol are given in Table 2.

As seen from the table, all the coefficients are positive in all the alcohols and decrease for EtOH solutions in the order h_{xy}(Ala) > h_{xy}(Cys) > h_{xy}(Ser) > h_{xy} (Asn). This means that endothermic processes associated with structural rearrangement of the ternary solution and release of water molecules from the hydration shells of amino acids and alcohols prevail over direct interactions of solvated polar groups of the interacting molecules. The coefficient for Ala containing a nonpolar $–CH_3$ group is higher than for the amino acids with polar substituents, which is associated with the effects of hydrophobic hydration. Replacement of the –SH group by –OH causes enhancement of the exothermic interactions between the molecules of alcohols and zwitterions of the amino acid, which is due to stronger capability of the –OH group of serine to form donor–acceptor bonds compared to the –SH group [13]. The more negative coefficient for Asn is determined by the presence in it of an additional center of specific solvation, $O=C–NH_2$, making the dissolution more exothermic. We observed previously a similar increase in the exothermicity of dissolution in going from glycine to diglycine as a result of appearance of an additional peptide group O=C–N–H [14].

Table 2. Enthalpy coefficients of pair interactions h_{xy} (J kg mol^{-2}) of amino acids with alcohols in aqueous solutions at 298.15 K

Substance	EtOH	PrOH	*i*-PrOH
L-Asn	540±30	682±61	895±61
L-Ser[a]	568±27	825±27	1023±63
L-Cys	623±35	853±24	1043±40
L-Ala[b]	658±26	–	–

[a] Data of [11]. [b] Data of [12].

Thus, the enthalpy characteristics of solution of the examined amino acids are determined by the nature of the side substituents (polar or nonpolar groups, donor–acceptor properties), effects of structural rearrangement of a ternary solution (dehydration of the interacting substances and hydrophobic effects), and also concentration and structural features of alcohol molecules.

EXPERIMENTAL

The integral enthalpies of solution were measured in a hermetically sealed four-ampule variable-temperature calorimeter with an isothermal shell, allowing successive measurements of a series of thermal effects in dissolution of several weighed portions of a substance in the same volume of the solvent without recharging the calorimetric cell [15]. The reaction part of the calorimeter and all the internal parts contacting the solution were made of VT-1 titanium alloy. The capacity of the calorimetric beaker was ~110 ml. The stability of the temperature-control system in the course of calorimetric measurements was maintained with an accuracy of 10^{-3} K. The thermometric and energetic sensitivities of the calorimeter were 2×10^{-4} K and 1×10^{-3} J per millimeter of the recorder scale. The thermal effect was compensated by electric current. To assess the accuracy and reliability of the operation of the calorimetric unit, we measured the thermal effects of solution of KCl in water at 298.15 K. From ten independent measurements of the enthalpies of solution of KCl in H_2O and enthalpies of dilution given in [16], we obtained the standard enthalpy of solution $\Delta_{sol}H^0 = 17.23 \pm 0.06$ kJ mol^{-1}, well consistent with the commonly accepted value of 17.22 ± 0.33 kJ mol^{-1}.

The concentration of amino acids was varied in the range 0.005–0.008 mol kg^{-1}. The enthalpies of solution $\Delta_{sol}H^0$ of amino acids in the examined concentration range (up to 0.008 mol kg^{-1}) are independent of the concentration of the biomolecules; therefore, as standard values of $\Delta_{sol}H^0$ we took the arithmetic mean values of the thermal effects of solution $\Delta_{sol}H^m$ from 5–7 measurements. Before experiments, *L*-cysteine

and *L*-asparagine (Aldrich, 99%) were recrystallized from the water–ethanol mixture and vacuum-dried at 343 K for 48 h. Water after deionization was double-distilled (specific conductivity 10^{-5} Ω^{-1} cm^{-1}). Alcohols were purified by standard procedures [17]. The water content was monitored by Fischer titration [18]. It did not exceed 0.05 wt % in ethanol, 0.03 wt % in n-propanol, and 0.04 wt % in isopropanol. The mixtures were prepared gravimetrically.

ACKNOWLEDGMENTS

The study was financially supported by the Grant Council at the President of the Russian Federation for young Russian scientists and their scientific advisers (MK-4905.2006.3).

REFERENCES

1. Palecz, B., *Fluid Phase Equil*., 2000, vol. 167, no. 2, p. 253.
2. Franks, H.S. and Evans, E.W., *J. Chem. Phys.*, 1945, vol. 13, p. 507.
3. Talukdar, H. and Kundu, K.K., *J. Phys. Chem*., 1992, vol. 96, p. 970.
4. Smirnov, V.I., Mezhevoi, I.N., and Badelin, V.G., *Zh. Fiz. Khim.*, 2004, vol. 78, no. 2, p. 280.
5. McMillan, W.G. and Mayer, J.E., *J. Chem. Phys.,* 1945, vol. 13, p. 276.
6. Palecz, B. and Nadolna, A., *Fluid Phase Equil.*, 2006, vol. 250, no. 1, p. 49.
7. Abu-Hamdlyyah, M. and Shehabuddin, A., *J. Chem. Eng. Data*, 1982, vol. 27, no. 1, p. 74.
8. Zittle, C.A. and Schmidt, C.L.A., *J. Biol. Chem.,* 1935, vol. 108, p. 161.
9. Rodante, F., *Thermochim. Acta*, 1989, vol. 149, nos. 1–2, p. 157.
10. Piekarski, H. and Tkaczyk, M., *J. Chem. Soc., Faraday Trans*., 1991, vol. 87, p. 3661.
11. Mezhevoi, I.N. and Badelin, V.G., *Zh. Fiz. Khim.,* 2008, vol. 82, no. 4, p. 789.
12. Palecz, B., *Thermochim. Acta*, 2005, vol. 435, no. 1, p. 99.
13. Pillai, L., Boss, R.D., and Greenberg, M.S., *J. Solution Chem.*, 1979, vol. 8, no. 7, p. 635.
14. Smirnov, V.I. and Badelin, V.G., *Zh. Fiz. Khim.*, 2005, vol. 79, no. 4, p. 673.
15. Mezhevoi, I.N., *Cand. Sci. (Chem.) Dissertation,* Ivanovo, 2004.
16. Parker, V.B., *NSRDS-NBS 2*, Washington, 1965, p. 66.
17. Gordon, A.J. and Ford, R.A., The Chemist's Companion. *A Handbook of Practical Data, Techniques, and References,* New York: Wiley, 1972.
18. Klimova, V.A., *Osnovnye metody analiza organicheskikh soedinenii* (Main Methods for Analysis of Organic Compounds), Moscow: Khimiya, 1967.