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PHYSICAL CHEMISTRY OF SOLUTIONS

Energetics of the Molecular Interactions of L-Cysteine, L-Serine, and L-Asparagine in Aqueous Propylene Glycol Solutions at 298.15 K

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Abstract—Integral enthalpies of dissolution $\Delta_{sol}H^m$ of L-cysteine, L-serine, and L-asparagine in aqueous solutions of 1,3-propylene glycol at organic solvent concentrations of up to 0.26 mole fraction are measured via the thermochemistry of dissolution. Standard enthalpies of dissolution ($\Delta_{sol}H^\circ$) and transfer ($\Delta_{tr}H^\circ$) of amino acids from water to a mixed solvent are calculated. It is found that the calculated enthalpy coefficients of pair interactions of the amino acids with polyhydric alcohol molecules have positive values. The effect the arrangement of the hydroxyl group in the structure of polyhydric alcohols has on the enthalpy of interaction of amino acids in aqueous solutions is revealed. The effect of different types of interactions in solutions and the structural features of biomolecules and cosolvents on the enthalpy of dissolution of amino acids is analyzed.

Keywords: molecular interactions, aqueous solutions, calorimetry, enthalpies of dissolution, amino acids, polyhydric alcohols.

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INTRODUCTION

The chemical stability and thermodynamic equilibria of protein systems and enzymes in aqueous solutions is an important problem in the pharmacology and biochemistry of biologically active biopolymers. Complex biomolecules are greatly affected by slight changes in temperature, the chemical affinity of a solvent to the molecular groups on a protein's surface, and changes in osmotic pressure, often leading to the denaturation of proteins [1, 2]. One way of stabilizing polymers is to select stabilizing organic cosolvents such as polyhydric alcohols. Examination of the thermodynamic characteristics of dissolution of model protein compounds (amino acids and oligopeptides) with binary solutions allows us to select the optimum concentration of organic cosolvent for the stabilization of complex biomolecules and to better understand the mechanisms of interaction in ternary systems.

EXPERIMENTAL

Integral enthalpies of dissolution were measured using a sealed variable-temperature four-ampoule calorimeter equipped with an isothermal shell of our own design to make consecutive measurements of a series of thermal effects during the dissolution of several weighed portions of a substance in the same amount as the solvent without refilling the calorimeter cell [3]. The reaction vessel of the calorimeter and all of its internal parts that came in contact with the solution were made of VT-1 titanium alloy. The volume of the calorimetric cell was ~110 mL. The stability of the temperature control system during calorimetric measurements was maintained with an accuracy of 10^{-3} K. The thermometric and energy sensitivities of the calorimeter were 2×10^{-4} K/mm and 1×10^{-3} J/mm, respectively, according to the recording device's scale. The thermal effect was compensated for with an electric current. The thermal effects of the dissolution of KCl in water at 298.15 K were measured to estimate the accuracy and reliability of the calorimetric setup's operation. A standard value of $\Delta_{sol}H^\circ = 17.23 \pm 0.06$ kJ/mol was derived from ten independent measurements of the enthalpies of dissolution of KCl in H_2O and data on the enthalpies of dilution [4]; these were in good agreement with the value of 17.22 ± 0.04 kJ/mol recommended in the literature [5, 6].

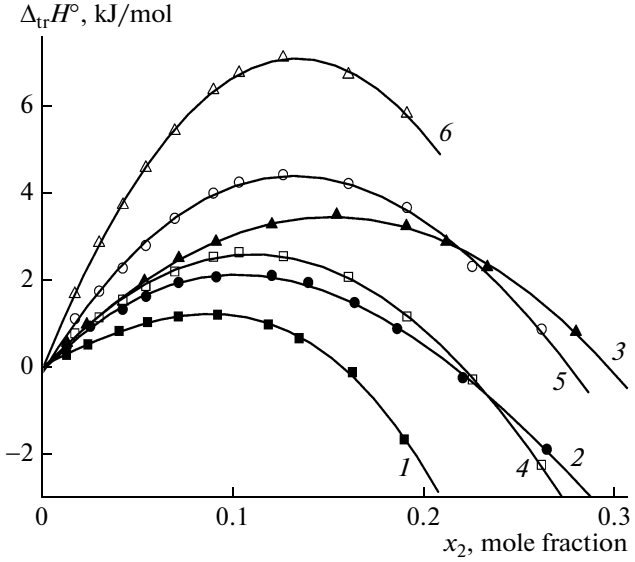
The amino acid concentration was varied in the range of 0.003–0.009 mol/kg. Enthalpies of dissolution $\Delta_{sol}H^m$ of the amino acids in the investigated range of concentrations (up to 0.009 mol/kg) did not depend on the biomolecule concentration, so the arithmetic means of the thermal effects of dissolution $\Delta_{sol}H^m$ taken from the results of two or three measurements were taken as our standard $\Delta_{sol}H^\circ$ values. Prior to each experiment, L-cysteine (Aldrich, 97%), L-serine (Aldrich, 99%), and L-asparagine (Aldrich, 98%) were recrystallized from a water–ethanol mixture and dried in vacuum at 343 K for 48 h. Water was deionized

Table 1. Standard enthalpies of dissolution ($\Delta_{sol}H°$, kJ/mol) of amino acids in a mixed water–1,3-propylene glycol solvent at 298.15 K

m_y	$\Delta_{sol}H°$	m_y	$\Delta_{sol}H°$	m_y	$\Delta_{sol}H°$
L-Cysteine		L-Serine		L-Asparagine	
0.7285	11.36 ± 0.07	0.7259	11.49 ± 0.08	0.7202	22.85 ± 0.10
1.3274	11.78 ± 0.07	1.4274	11.94 ± 0.08	1.3587	23.09 ± 0.10
2.4465	12.31 ± 0.09	2.4396	12.33 ± 0.09	2.3265	23.40 ± 0.10
3.1373	12.78 ± 0.08	3.1925	12.63 ± 0.09	3.2387	23.60 ± 0.11
4.2734	13.31 ± 0.09	4.2665	12.95 ± 0.08	4.2543	23.73 ± 0.10
5.5576	13.68 ± 0.09	5.5678	13.08 ± 0.08	5.6076	23.77 ± 0.11
7.5813	14.08 ± 0.08	7.5834	13.11 ± 0.09	7.4461	23.55 ± 0.10
10.1145	14.30 ± 0.08	8.9812	12.95 ± 0.08	8.6258	23.23 ± 0.10
13.0765	14.04 ± 0.08	10.8534	12.49 ± 0.08	10.7632	22.45 ± 0.09
14.8954	13.68 ± 0.07	12.6745	11.89 ± 0.07	12.9865	20.91 ± 0.10
16.8703	13.10 ± 0.07	15.6783	10.76 ± 0.08		
21.5332	11.61 ± 0.07	19.9238	9.12 ± 0.06		

m_y is the molality of 1,3-propylene glycol, mol kg^{-1}.

and then doubly distilled (specific conductivity, 10^{-5} Ω^{-1} cm^{-1}); 1,3-propylene glycol (Panreac, 98%) was used without further purification. Mixed aqueous solvents were prepared gravimetrically.



Dependences of the enthalpies of transfer ($\Delta_{tr}H°$) of (*1*) L-asparagine, (*2*) L-serine, (*3*) L-cysteine, (*4*) DL-Alanine [23], (*5*) L-alanine [23], and (*6*) DL-valine (6) [23] from water to a water–1,3-propylene glycol mixture on organic cosolvent concentration x_2 at 298.15 K.

RESULTS AND DISCUSSION

Integral enthalpies of dissolution $\Delta_{sol}H°$ of L-cysteine (L-Cys), L-serine (L-Ser), and L-asparagine (L-Asn) in mixed solutions of water with 1,3-propylene glycol (1,3-PrD) are given in Table 1. Dependences of the enthalpies of transfer $\Delta_{tr}H°$ of polar amino acids from water to aqueous solutions of 1,3-propylene glycol on the mole fraction of the cosolvent (x_2) are shown in the figure. For comparison, the figure shows data derived earlier for aliphatic biomolecules from another subclass, i.e., nonpolar hydrophobic amino acids.

It is evident from the figure that for all the amino acids, the dependences of $\Delta_{tr}H°$ on the mole fraction of the cosolvent exhibit extreme points and enthalpies of transfer with opposite signs in the investigated range of concentrations. The enthalpies of transfer were determined from different contributions with opposite signs resulting from interactions of different types and the solvation processes that compete in the solution. The observed dependences are discussed using a common approach based on the analysis of different types of interactions that occur during the dissolution of crystalline substances in a solvent [7–9]. In amino acid–polyhydric alcohol–water ternary systems, the molecules of hydrated solutes are located fairly close to each other. Because of this, the solvation shells of the molecules undergo partial rearrangement, changing the pattern of interparticle interactions in ternary solutions [10]. The resulting enthalpies of transfer for

amino acids are determined by the sum of the contributions with opposite signs. The changes that occur in the thermochemical quantities in the system can be expressed through the equation

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

where ΔH_1 denotes the ion bipolar interactions that occur between the zwitterion centers of the amino acids and the OH groups of the polyhydric alcohol; ΔH_2 represents the hydrophilic–hydrophilic group interactions between the polar groups of the amino acids and the –OH group of the polyhydric alcohols that occur through hydrogen bonding (ΔH_2); ΔH_3 signifies the hydrophobic–hydrophilic interactions between the nonpolar portions of the amino acids or the organic cosolvent and the OH groups of the cosolvent or the zwitterions centers of the amino acids; and ΔH_4 stands for the hydrophobic–hydrophobic interactions between the nonpolar portions of the amino acids and the polyhydric alcohol.

The first two types of interactions make a negative contribution to the enthalpies of transfer of the amino acids from water to the aqueous organic solvent, while the other interactions make a positive contribution. The positive values of the enthalpies of transfer for all the amino acids to the intersection with the axis of respective concentrations (see figure) suggest that the third (ΔH_3) and fourth (ΔH_4) types of interactions with the solvent predominate. After passing through maxima, the enthalpies of transfer gradually decline, indicating an increase in the fraction of ion bipolar interactions. The transition to the negative range of the enthalpies of transfer is most pronounced for strongly polar amino acids (L-asparagine and L-serine) with pendant groups capable of forming hydrogen bonds with the molecules of the solvent. As the polyhydric alcohol concentration increases, the interactions between the polar groups of the cosolvents and the zwitterions and polar centers of the amino acids become stronger, raising the exothermicity of dissolution of biomolecules in the solution. An extended range of concentrations with negative $\Delta_{tr}H^\circ$ values is also observed for DL-alanine (DL-Ala); it can be attributed to the presence of D-isomers of alanine in the racemic mixture, which lowers the degree of hydration of the DL-forms of the amino acid [11]. The extreme points in the dependences of the enthalpies of transfer of the amino acids from water to aqueous solutions of 1,3-propylene glycol suggest that the contributions from the hydrophobic–hydrophilic and hydrophobic–hydrophobic group interactions are strongest at these concentrations.

The interparticle interactions in aqueous solutions were quantitatively estimated via regression analysis using terms of the McMillan–Mayer theory [12] by calculating the enthalpy coefficients of pair interactions (h_{xy}) between amino acids and molecules of 1,3-propylene glycol. Concentration dependences $\Delta_{sol}H^\circ = f(m_y)$ of the amino acids in the polyhydric alcohol were treated with a third-degree polynomial:

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

where m_y is the molal concentration of the polyhydric alcohol and a_0, a_1, a_2, and a_3 are approximation coefficients calculated by the least squares method.

The following regression equations for L-cysteine, L-serine, and L-asparagine in aqueous solutions of 1,3-propylene glycol were derived as a result of this analysis:

$$\Delta_{sol}H^\circ = (10.81 \pm 0.05) + (0.77 \pm 0.22)m_y - \ldots, \quad R = 0.998, \quad SD = 0.050, \quad N = 12, \quad (3)$$

$$\Delta_{sol}H^\circ = (11.01 \pm 0.03) + (0.72 \pm 0.01)m_y - \ldots, \quad R = 0.999, \quad SD = 0.029, \quad N = 12, \quad (4)$$

$$\Delta_{sol}H^\circ = (22.57 \pm 0.05) + (0.44 \pm 0.03)m_y - \ldots, \quad R = 0.999, \quad SD = 0.039, \quad N = 10. \quad (5)$$

The values of the free terms of Eqs. (3)–(5) correspond to the standard enthalpies of dissolution of the amino acids in pure water, which are in good agreement with our values and those in the literature; e.g., $\Delta_{sol}H^\circ$(L-Cys) = 10.81 ± 0.06 [13], $\Delta_{sol}H^\circ$(Cys) = 11.15 ± 0.03 [14], $\Delta_{sol}H^\circ$(L-Ser) = 11.01 ± 0.09 [15], $\Delta_{sol}H^\circ$(L-Ser) = 11.49 ± 0.09 [16], and $\Delta_{sol}H^\circ$(L-Asn) = 22.57 ± 0.10 kJ/mol [13]. The enthalpy coefficients of pair interactions h_{xy} between the amino acids and the alcohols were calculated using the relation $h_{xy} = a_1/2$ [17]. The values calculated in this work and derived earlier for amino acids in polyhydric alcohol solutions are listed in Table 2.

Table 2. Enthalpy coefficients of pair interactions (h_{xy}, J kg mol^{-2}) between amino acids and 1,2-propylene glycol (I) and 1,3-propylene glycol (II) in aqueous solutions at 298.15 K

Amino acid	I	II
L-Asparagine	357 ± 16 [18]	220 ± 17
L-Serine	471 ± 16 [19]	358 ± 7
L-Cysteine	536 ± 13 [20]	387 ± 11
DL-Alanine	531 ± 17 [21]	403 ± 9 [23]
L-Alanine	694 ± 19 [22]	584 ± 16 [23]
DL-Valine	–	944 ± 23 [23]

All of the enthalpy coefficients of pair interactions h_{xy} for the investigated amino acids had positive values, suggesting that the endothermic processes associated with the structural rearrangement of a ternary solution and the release of water molecules from the hydration

shells of amino acids and polyhydric alcohols dominate over direct interactions between the solvated polar groups of the interacting molecules.

Hydrophobic hydration and donor–acceptor interactions play an important role in many protein systems and have been objects of close scrutiny for many researchers [24, 25]. Hydrophobic interactions describe association by nonpolar substances or nonpolar portions of amphiphilic molecules in aqueous solutions. Electrostatic interactions of different nature and hydrogen bonds in aqueous solutions also contribute to hydrophobic interactions. Enthalpy coefficients of pair interaction h_{xy} of the investigated amino acids with pendant hydrocarbon radicals exhibiting lyophilic and lyophobic properties increase in the order L-Asn < L-Ser < L-Cys < DL-Ala < L-Ala < DL-Val. The lowest h_{xy} coefficient for Asn is determined by the presence of an additional center of donor–acceptor interaction O=C–NH_2, which raises the exothermicity of dissolution. The highest h_{xy} coefficient for DL-valine in solutions of 1,2-propylene glycol is attributed to the presence of an additional –CH–CH_3 group in the molecule; this contributes to hydrophobic hydration, as is evident from the strengthening of the hydrogen bonds of water around the CH_3 groups [26]. This enhances the structural rearrangement of the solution during the interaction between the solvated molecules, magnifies the endothermic effects, and raises h_{xy}. Substituting the –OH group for the –SH group accelerates the exothermic interactions between the alcohol molecules and the amino acid zwitterions, due to the better ability of the –OH group of serine to form donor–acceptor bonds, relative to the –SH group of cysteine [27].

The basic condition for the chiral recognition of amino acid molecules in mixed solutions is the presence of bipolar ions and pendant alkyl radicals [28]. It is evident from Table 2 that the enthalpy coefficients of interaction h_{xy} with 1,3-propylene glycol for a racemic mixture of the DL-forms of alanine are lower than for the natural L-form of the amino acid. This means that the presence of D-isomers of alanine in the racemic mixture lowers the degree of hydration of the DL-forms of the amino acid. Because of this, the degree of interaction with the cosolvent and the exothermicity of dissolution during the dissolution of DL-alanine in aqueous solutions of polyhydric alcohols are higher for the racemic mixture than for the L-forms of the amino acid, as can be seen from the lower positive value of h_{xy}.

The position of the OH group in propylene glycol has a fairly large effect on the resulting thermodynamic characteristics. Table 2 shows that the enthalpy coefficients of pair interactions for the amino acids in 1,3-propylene glycol are lower than those in 1,2-propylene glycol. This difference can be attributed to the stronger effects from the hydrophobic hydration of the methyl (CH_3) and methylene (CH_2) groups, which shield the hydroxyl group in 1,2-propylene glycol and hinder direct interaction with amino acids. In addition, the structure and conformation of 1,3-propylene glycol are probably prone to forming stronger intermolecular hydrogen bonds, magnifying the exothermic effects in a ternary solution [29].

CONCLUSIONS

We have shown that the arrangement of pendant groups and the stereoisomerism of the interacting molecules of polar and nonpolar aliphatic amino acids and propylene glycol have a considerable effect on the thermochemical characteristics of the dissolution of biomolecules. Enhancement of the hydrophobic effects and shielding of the OH group of propylene glycol in position 2 by pendant hydrocarbon radicals was observed for all of the investigated amino acids.

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