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Amino Acid Solvation in Aqueous Kosmotrope Solutions: Temperature Dependence of the L-Histidine–Glycerol Interaction

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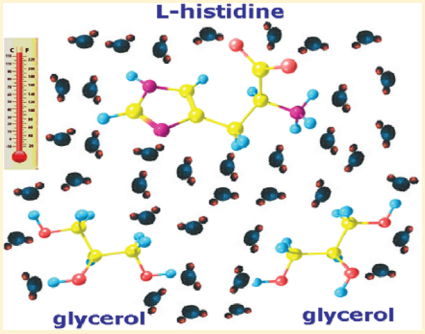
ABSTRACT: We have studied thermodynamics of interaction between the aromatic amino acid L-histidine and glycerol, which is one of the most important stabilizing agents for proteins in water. The pair and triplet interaction parameters have been extracted from enthalpy and solubility data using standard thermodynamic manipulations in a wide temperature range. Our results indicate for the first time that the L-histidine–glycerol pair and triplet interactions are characterized by rather small enthalpy and entropy changes, which do not depend on temperature in either cold or hot water. These temperature-independent enthalpies and entropies of interaction lead to zero heat capacity changes during the amino acid transfer from water to both dilute and rather concentrated aqueous glycerol solutions. We attribute this behavior to a delicate balance between contributions from hydrophobic and hydrophilic fragments in the solute molecules. This unique feature appears to be the major reason that thermodynamics of pair and triplet interactions are nearly identical at standard and physiological temperatures.



INTRODUCTION

The study of the interactions between simple biological solutes and nonelectrolytes in aqueous solutions appears to be of fundamental importance to understand the factors that determine the stability of biopolymers in living systems.[1−4] The main goal of most of the thermodynamic studies over the several decades[5−12] was to obtain the detailed experimental information on the energetics of the interaction between amino acids, amides or their derivatives with nonelectrolyte molecules. These interactions and the corresponding energies generally determine what is currently called the molecular recognition process. However, most of these efforts deal with the behavior of highly soluble amino acids or their derivatives in aqueous solutions at the standard temperature only. Therefore, it is not quite clear whether the results obtained at 298 K for a rather restricted set of solutes can reflect correctly the behavior of biosystems at 310 K.

Recently[13,14] we have shown that enthalpic and entropic parameters of pair interaction of urea with two aromatic amino acids, L-phenylalanine and L-histidine, in water reveal anomalous temperature changes. Both parameters pass through maxima in the temperature range of 300−308 K, indicating that the heat capacity of the solute-denaturant pair interaction changes in sign. This solute behavior is found to be closely related to properties of pure water because the extrema observed arise in the region, where the temperature dependence of the heat capacity of pure water passes through a minimum. It has been also emphasized that for slightly hydrophobic species enthalpic and entropic parameters of solute−solute pair interactions appear to be nearly identical at 298 and 310 K. However, for the interaction between more hydrophobic L-phenylalanine and L-alanine with dimethylformamide (DMF) they differ strongly.

In the present Article, we give a deeper insight into the temperature dependence of the amino acid−nonelectrolyte interaction in water in the case of L-histidine and glycerol solutions over the physiological temperature range. Glycerol is known to be one of the most important cryoprotectants for different organisms and reveals high activity to maintain the structure of biological macromolecules.[1,2,15] It has been shown that both glycerol and sugars indirectly stabilize the native structure of globular protein due to preferential hydration of the protein domain even at high nonelectrolyte concentrations.[1−3] This thermodynamically unfavorable protein−solvent interaction induces the minimization of the surface contact between protein and nonelectrolyte molecules, which stabilizes more folded conformations. Here our efforts are mainly directed toward obtaining thermodynamic information on the L-histidine interactions with one or two glycerol molecules in a dilute aqueous solution using a virial expansion technique to elucidate some of the features that contribute to the protein−nonelectrolyte interactions in water−glycerol mixtures.

EXPERIMENTAL SECTION

Distilled water was treated with basic potassium permanganate and then distilled twice in a quartz still to reach the electric conductivity of 1×10^{-5} S·m^{-1}. Glycerol (Aldrich, > 99.5%) was stored with freshly dried 4 Å molecular sieves and then used without further purification. L-Histidine (free base, MP Biomedicals, >99%) and L-phenylalanine (Carl Roth, >99.5%) were dried in vacuum at 343 K for several days and used

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Table 1. Standard Enthalpies of Solution ΔH^0 (sol) in kilojoules per mole for L-Histidine in Water−Glycerol Mixtures at 288.15−328.15 K

X_{Gl}[a]	ΔH^0 (sol)	X_{Gl}	ΔH^0 (sol)	X_{Gl}	ΔH^0 (sol)
288.15 K		298.15 K		308.15 K	
0	13.76	0	14.15	0	14.90
			14.22 ± 0.06[16]		
	13.76 ± 0.05[16]		14.32,[12] 13.96[6]		
0.01011	13.97	0.00774	14.34	0.01035	15.12
0.02014	14.12	0.02059	14.52	0.01887	15.24
0.03675	14.31	0.04729	14.75	0.02782	15.36
0.04652	14.38	0.04921	14.76	0.04368	15.48
0.06805	14.47	0.07806	14.81	0.05381	15.53
0.08072	14.49	0.08801	14.82	0.06605	15.59
0.1003	14.49	0.1069	14.84	0.08186	15.60
		0.1201	14.84	0.09318	15.60
318.15 K		328.15 K		298.15 (L-phenylalanine)	
0	15.79	0	16.60	0	8.30 ± 0.03
	15.76 ± 0.04[16]		16.59 ± 0.07[17]		8.23,[13] 8.28[7]
0.00461	15.90	0.00513	16.71	0.01806	9.21
0.009900	16.00	0.01269	16.85	0.03306	9.79
0.01691	16.12	0.02413	17.01	0.04996	10.52
0.02363	16.21	0.03664	17.14	0.06123	10.95
0.03874	16.37	0.05498	17.23	0.07825	11.60
0.0497	16.44	0.06649	17.26	0.08914	11.90
0.08113	16.52	0.08810	17.28		
0.1014	16.52	0.1179	17.29		

[a]From here on it denotes the glycerol mol fraction and errors represent the twice standard deviation. The coefficients of eq 2 for L-histidine are: A_0 = 13.76(0.004), A_1 = 21.95 (0.37), A_2 = −219.4(9), A_3 = 727.0(59), s_f = 0.004 kJ/mol (288 K); A_0 = 14.16(0.01), A_1 = 22.14 (0.98), A_2 = −249.4 (20), A_3 = 937.3 (111), s_f = 0.01 kJ/mol (298 K); A_0 = 14.90(0.01), A_1 = 22.01 (1), A_2 = −236.4 (25), A_3 = 864.3(178), s_f = 0.01 kJ/mol (308 K); A_0 = 15.80(0.003), A_1 = 22.93 (0.36), A_2 = −242.1 (9), A_3 = 852.2(52), s_f = 0.005 kJ/mol (318 K); and A_0 = 16.60(0.004), A_1 = 22.42 (0.34), A_2 = −248.3 (8), A_3 = 913.6(40), s_f = 0.005 kJ/mol (328 K). Values in brackets represent the standard deviation.

without further purification. Glycerol aqueous solutions were prepared by weight from freshly distilled water and the pure nonelectrolyte with accuracy of 0.0001 mol fraction.

Calorimetric measurements were carried out with automated ampule calorimeters previously described in detail.[13,14] Solubility measurements were performed with the method of isothermal saturation. Weighed amounts of the amino acid and the solvent were placed in the 50 mL glass hermetic cell and stirred with a magnetic stirrer usually for 24 h. The temperature inside the cell was maintained equal to 298 ± 0.02 K with a HAAKE DC 10 thermostat. Three or four milliliters of the liquid content was quickly taken up with a thermostatted syringe, filtered at 298 ± 0.2 K on a glass filter for 2 min, weighed in a hermetic vessel with analytical balances, and highly diluted by an appropriate solvent for a spectrophotometric control of the solute content in a saturated solution. The absorption band at 211 nm was chosen for all water−glycerol mol fractions studied. Spectrophotometric measurements were repeated with several probes, and the mean value of the absorbance coefficient was selected to compute solubility values.

RESULTS

The standard enthalpies of solution of L-histidine in water listed in Table 1 represent the mean value from two independent measurements in the range of solute molalities of 0.005 to 0.02 mol/kg, where the ΔH^m (sol) values do not depend on the solute concentration.[8−10,16,17] These values are found to be in a very good agreement with the results previously reported.[16] The ΔH^m (sol) ≅ ΔH^0 (sol) values in the mixed solvents listed in Table 1 reflect the result of mainly one experiment at the solute molality of 0.007 to 0.02 mol/kg. On the basis of results of our previous studies (see refs 13, 14, and 16), the overall uncertainty of the ΔH^0 (sol) values is estimated to be <1%. Solubility values given in Table 2 reflect the mean value from at least four measurements. The solubility of L-histidine in water is seen to be in a good agreement with the dry weight technique reported by Nozaki and Tanford.[18,19]

Table 2. Solubility for L-Histidine in Water−Glycerol Mixtures at 298.15[a]

X_{Gl}	S g/100 g solvent
0	4.28 ± 0.02
	4.33,[18] 4.30[19]
0.01132	4.01 ± 0.06
0.02588	3.70 ± 0.04
0.05050	3.35 ± 0.07
0.07011	3.10 ± 0.04
0.09142	2.93 ± 0.06
0.1161	2.82 ± 0.04

[a]Coefficients for eq 4 are: B_1 = 4.72 (0.25), B_2 = −35.58 (2), and s_f = 0.01 kJ/mol.

DISCUSSION

A. Enthalpies of Transfer at the Standard Temperature. Figure 1 illustrates enthalpies of the solute transfer for different amino acids from water to water−glycerol mixtures. This transfer is accompanied by a positive enthalpy change that

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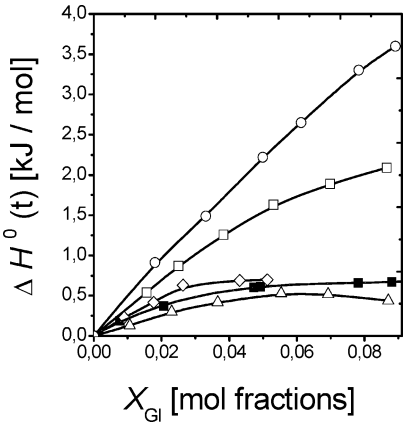

Figure 1. Enthalpies of transfer of L-phenylalanine (○), L-alanine (□),[10] glycine (◇),[8] L-histidine (■), and L-serine (△)[9] from water to water–glycerol mixtures at 298 K. Points: experimental data, lines represent the polynomial description.

depends on the amino acid side-chain nature. For hydrophobic L-alanine and, especially, L-phenylalanine, the enthalpic cost is significantly larger than that for hydrophilic glycine or L-serine. L-Histidine containing the heterocycle side-chain also reveals small enthalpy changes. The comparative analysis clearly shows that in both aqueous glycerol and urea solutions[13,14] hydrophobic groups in the amino acid molecules give positive and hydrophilic ones give negative contributions to the enthalpies of transfer.

Figure 1 shows one interesting feature of L-histidine solvation in an aqueous glycerol solution. Whereas the enthalpy of L-phenylalanine transfer increases monotonously with the glycerol content in the mixture, the ΔH^0 (sol) values for another aromatic amino acid, L-histidine, increases only up to $X_{GI} \approx$ 0.06 mol fractions (3.5 mol of the alcohol per 1 kg of water). For higher glycerol concentrations, the enthalpy remains nearly constant. The same behavior is found for other temperatures. (See Figure 2 below.) One possible explanation of this concentration

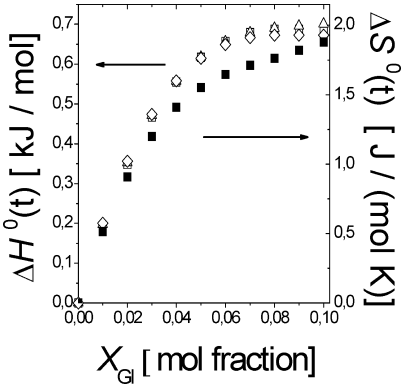

Figure 2. Enthalpies (light symbols) and entropies (dark symbols) of transfer of L-histidine from water to water–glycerol mixtures at 288 (□), 308 (△), and 328 K (◇).

dependence may be the formation of labile complexes between L-histidine and glycerol molecules in the mixed solvent. Because the enthalpy of transfer shown in Figure 1 is positive, the formation of such aggregates should be driven by a favorable entropy change. However, this is unlikely. In fact, molecular dynamics simulations[2] show that alcohol molecules can bind to protein O and N atoms in 30% glycerol aqueous solutions favoring formation of multiple hydrogen bonds. This favorable energetic effect should be accompanied by negative enthalpy changes. However, in our case, enthalpies of transfer are positive and do not depend on the temperature at least in the temperature range of 288–328 K. (See Figure 2 below.) Therefore, our experimental results do not suggest direct L-histidine–glycerol binding. Because L-phenylalanine does not contain hydrophilic groups in its side chain and the enthalpy of L-phenylalanine transfer increases monotonously with the alcohol content, the L-histidine behavior in the binary solvent is determined by hydrophilic nitrogen atoms in the heterocycle side-chain.

B. Temperature Dependence of Thermodynamic Functions. In Figure 2, the enthalpies of L-histidine transfer for 288, 308, and 328 K are plotted versus the glycerol mol fraction. The experiments performed reveal a very interesting and unexpected result: enthalpies of solute transfer are identical in both cold and hot aqueous glycerol solutions. Even for the largest glycerol concentrations studied these quantities differ by only ~60 J/mol, which is within the experimental error. This remarkable experimental fact leads to the following important conclusions. First, heat capacities of transfer are equal to zero both for low and sufficiently large glycerol concentrations in the binary mixture. Therefore, the entropy of transfer should be constant in the temperature range studied, and the free energy should reveal a linear temperature dependence. Second, the standard heat capacity of solution of L-histidine in water has been found to increase linearly in a wide temperature range.[16,17] Because heat capacities of transfer approach zero even at high alcohol concentrations, it is clear that heat capacities of amino acid solvation in water–glycerol mixtures are identical to those observed in pure water in the temperature range studied. Figure 2 also shows that the difference between the enthalpies of L-histidine solvation in pure water and the most concentrated glycerol solution studied is very small, being 0.7 kJ/mol. Therefore, glycerol molecules perturb only slightly the hydration shell of L-histidine, as long as sufficient bulk water is present in the mixed solvent.[15]

For calculating free energies of the solute transfer from water to the mixed solvent, we have applied solubility data given in Table 2 via the following equation[18,19]

$$\Delta G^0(t) = RT \ln \frac{X^w}{X^m} + RT \ln \frac{\gamma^w}{\gamma^m} \approx RT \ln \frac{X^w}{X^m} \qquad (1)$$

where X^w and X^m are solubility values for L-histidine in water and the mixture, respectively, expressed in a mol fraction scale; γ^w and γ^m are the activity coefficients for a saturated solution in water and a mixture, respectively. Because the latter values are unavailable, we assume that the second term in eq 1 approaches zero. This approximation is found to be valid for slightly soluble biological solutes in water–organic mixtures.[18,19] Then, using experimental enthalpic and solubility data as well as the Gibbs–Helmholtz equation, entropies and free energies of transfer can be computed. The entropies of transfer shown in Figure 2 are positive and constant for the temperature range studied. Figure 2 shows that enthalpic and entropic contributions to the free energy of transfer are rather small and almost cancel each other so that only a small free-energy change occurs. This quantity is found to be positive and decreases with the temperature.

C. L-Histidine–Glycerol Interaction. To extract more detailed information about the solute–alcohol interaction from the experimental data, we have applied a thermodynamic formalism similar to our previous studies,[20,21] which relates

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thermodynamic properties of a multicomponent system to certain integrals of the mean force potential associated with the interaction between a fixed set of solute molecules in a highly diluted solution.

The ΔH^0 (sol)$-f(X_{Gl})$ curves have been fitted to third-order polynomials for the whole alcohol concentration range studied

$$\Delta H^0(\text{sol}) = A_0 + A_1 X_{Gl} + A_2 X_{Gl}^2 + A_3 X_{Gl}^3 \quad (2)$$

The corresponding coefficients are given in the footnote of Table 1. The A_1 and A_2 coefficients, obtained in a least-squares fitting routine, are related to the enthalpic parameters of the L-histidine−glycerol pair (h_{GlH}) and triplet interactions (h_{GlGlH}) in the following manner[22]

$$h_{GlH} = A_1 M_{H_2O}/2;\ h_{GlGlH} = (A_2 - A_1) M_{H_2O}^2/3 \quad (3)$$

where M_{H_2O} is molar mass of water (kg/mol). Free-energy parameters have been computed at 298 K with the same procedure from solubility values

$$RT \ln \frac{X^w}{X^m} = B_1 X_{Gl} + B_2 X_{Gl}^2 \quad (4)$$

$$g_{GlH} = B_1 M_{H_2O}/2;\ g_{GlGlH} = (B_2 - B_1) M_{H_2O}^2/3 \quad (5)$$

In this case, however, the two-order polynomial is found to be enough to describe experimental data. The coefficients of eq 4 are listed in the footnote of Table 2. Entropic $-Ts_{GlH}$ and $-Ts_{GlGlH}$ parameters have been computed from enthalpic and free-energy values using a standard thermodynamic manipulation. Thermodynamics of the amino acid−glycerol pair and triplet interactions at different temperatures are presented in Figure 3. The L-histidine interaction with one alcohol molecule in water is accompanied by modest enthalpy and entropy changes, which almost cancel each other. Triplet interaction parameters are found to be several times less than the y_{GlH} values, indicating a rather rapid convergence of a virial expansion. The solute−alcohol pair interaction is slightly repulsive, and the triplet one is slightly attractive in the temperature range studied; in both cases, the free-energy sign is defined by the enthalpic contribution. These quantities are expectably independent of the temperature (see enthalpies of transfer given in Figure 1), providing zero heat capacity changes for both sets of interacting species. Therefore, in both cold and hot water the L-histidine−glycerol interactions are accompanied by identical enthalpy and entropy changes. Free-energy parameters scale linearly with temperature, and above the boiling point of pure water both quantities should change their signs. Because the free energy of pair interaction becomes negative, it is clear that L-histidine solubility in the mixture will be larger than that in pure water. Therefore, the amino acid should be better solvated in a glycerol solution at higher temperatures, but for the physiological temperature range, the opposite situation occurs.

For the interpretation of the temperature-independent enthalpies and entropies of pair and triplet interaction, it is important to understand how hydrophobicity or hydrophilicity may cause such a behavior. It is worth remembering that solute molecules contain both apolar and polar groups that may contribute in different ways to the total y_{GlH} and y_{GlGlH} values. In principle, the addivity-of-groups approach first proposed by Savage and Wood[23] provides useful information about the group behavior at least for the enthalpies of pair interaction. However, much more experimental data are required to apply

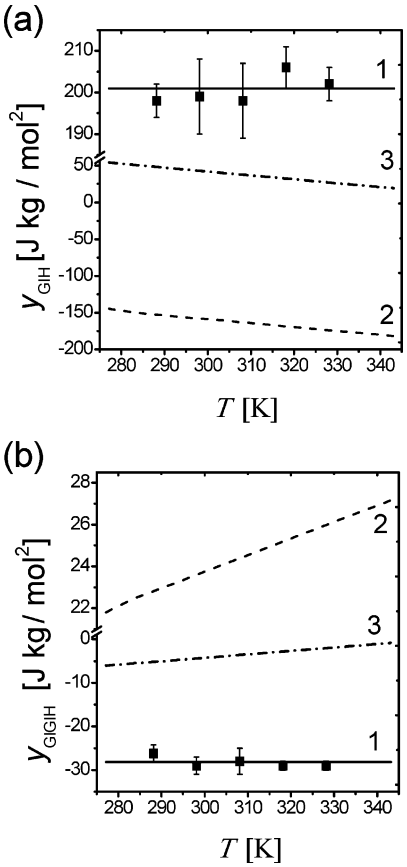

Figure 3. Temperature dependence of enthalpic h (1), entropic $-Ts$ (2), and free energy g (3) parameters of L-histidine−glycerol pair (a) and triplet (b) interactions. Points: experimental h_{GlH} and h_{GlGlH} values, broken lines represent the calculation according to Gibbs−Helmholtz equation, error bars represent the standard deviation.

this concept quantitatively. Moreover, we are not sure that this simple additive concept is able to reproduce the interaction of such complex heterofunctional molecules. The comparative analysis of the L-histidine interaction with different nonelectrolytes in water shows that the attraction between the amino acid and the hydrophilic denaturant urea results from the favorable enthalpy change, whereas the repulsion between L-histidine and DMF arises from the unfavorable enthalpic contribution.[14] In both cases, however, temperature dependences of enthalpic and entropic parameters pass through extrema at 300−308 K. The same behavior reveals L-phenylalanine in aqueous urea solutions but not L-alanine, for which the enthalpy and entropy of pair interaction with urea are almost independent of the temperature.[14] It is clear that the latter two solutes differ strongly from the structural point of view from those studied here, but striking similarity in the behavior of the L-histidine−glycerol and L-alanine−urea pairs allows one to draw a tentative conclusion that both hydrophobic and hydrophilic interactions are responsible for the temperature-independent enthalpies and entropies of interaction shown in Figure 3. In fact, the heat capacity change for the interaction between two apolar groups is positive and that for two polar groups is usually negative.[24] Although the exact information on the cross interaction parameters for L-histidine and glycerol is unavailable, it is reasonable to assume that contributions from apolar and polar groups in the amino acid and alcohol molecules reveal opposite temperature

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dependence, which results in the temperature independent the h and s values.

CONCLUSIONS

Therefore, in conclusion, we can state the following as the result of the present study using compounds, which have got some importance for protein chemistry. First, our results indicate for the first time that the L-histidine−glycerol pair and triplet interactions are accompanied by modest enthalpy and entropy changes, which do not depend on the temperature in both cold and hot water. This induces zero heat capacity change for the L-histidine transfer from water to rather concentrated glycerol solutions. Second, a significant enthalpy−entropy compensation exists for the system studied so that free-energy parameters are small and decrease linearly with the temperature. Slightly above the boiling point of water, the L-histidine−glycerol pair interaction should become attractive, which denotes that solubility of the amino acid in a mixture will be larger than that in pure water. Finally, because both enthalpic and entropic characteristics in aqueous kosmotrope solutions are the temperature-independent, it is clear that thermodynamic parameters at 298 and 310 K are identical. This may serve as some justification of using the experimental data obtained at 298 K to explain processes occurring at the physiological temperature. It is of particular importance to test the temperature dependence of the solute−glycerol interactions for other amino acids and, especially, for L-phenylalanine, which would allow us to compare the contributions from polar and apolar aromatic side-chains. However, this extension of the present research is left for future work.

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Notes

The authors declare no competing financial interest.

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