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# Amino Acid Behavior in Aqueous Denaturant Solutions: Temperature Dependence of the L-Histidine−Amide Interaction

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We have studied the thermodynamics of the pair interaction between aromatic amino acid−L-histidine and nonelectrolyte denaturing globular proteins−hydrophilic urea (U) and presumably hydrophobic dimethylformamide (DMF) in the temperature range of 288−328 K. Our study does indicate for the first time the anomalous temperature dependence of the enthalpies and entropies of the L-histidine−U and L-histidine−DMF interaction in water, which is consistent with the previously reported results for water−urea (U) and water−U−L-phenylalanine systems. This phenomenon is found to be closely related to the behavior of water, since in all cases, the extrema observed arise in the temperature range of 300−308 K, where the temperature dependence of the heat capacity of pure water passes through the minimum. The amino acid−urea interaction is shown to be accompanied in a wide temperature range by a large negative enthalpy change, which reveals a strong tendency of urea binding with polar and charged groups of proteins.

## Introduction

Amino acids play a central role both as building blocks of proteins and intermediates in metabolism. The chemical properties of amino acids in proteins determine their biological activity in living systems, the major component of which is water. This explains the existence of a huge number of experimental and theoretical studies directed toward the investigation of the behavior of amino acids, peptides, and their various derivatives in aqueous media (see refs 1−11 and reference therein). The central point of thermodynamic studies over several decades is to obtain experimental information on the energetics of the interaction which occurs between solute species in aqueous medium,[6−11] since it is the energetics that generally determines what is currently called the molecular recognition process.[6] Notwithstanding the fundamental complexity of such systems, a certain progress in predicting their behavior has been reached in terms of the additivity-of-groups approach[6−11] first proposed by Savage and Wood[12] and later used by various scientific groups.[6−11] However, most of these efforts[6−12] have been performed only at 298 K, and at the moment, the information about temperature dependence of the energetics of the solute−solute interaction is fairly fragmentary.[6] In particular, it is not quite clear whether the results obtained at 298 K for amino acids or peptides may be used for explaining and predicting the peculiarities of solute−solute interactions at 310 K.

During the past several years, we have been involved in an extensive and continuous thermodynamic study on the behavior of amino acids in water and nonelectrolyte solutions.[13−18] Our efforts have been mainly directed toward obtaining experimental information to illuminate some of the features that contribute to the protein−solvent and protein−denaturant interaction in aqueous media. In particular, we have found[18] that temperature dependence of the enthalpic and entropic parameters of the L-phenylalanine−U interaction passes through the extrema near the temperature minimum of the heat capacity of pure water, whereas enthalpies of L-alanine interaction with U and DMF have revealed a linear temperature dependence.[16] Thus, to give a deeper insight into this phenomenon, we study here the interaction of U and DMF with another essential aromatic amino acid—L-histidine—and compare the results obtained with those reported earlier.

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## Experimental Section

Distilled water was treated with basic potassium permanganate and then distilled twice in a quartz still to reach the electric conductivity of $1 \times 10^{-5}$ S·m$^{-1}$. Urea (Harnstoff, >99.5%) was dried under reduced pressure at 343 K for several days and used without further purification. DMF (Fluka, >98%) was dried with 4 Å molecular sieves and then distilled under reduced pressure at 303 K. The residual amount of water determined according to Karl Fisher titration was smaller than 0.02 mass %. L-Histidine (free base, MP Biomedicals Inc., >99%) was dried in vacuum at 343 K for several days and used without further purification. Imidazole (Aldrich, >99%) was sublimed under reduced pressure at 373 K and then dried at 333 K for several days.

U and DMF aqueous solutions were prepared by weight from freshly distilled water and pure nonelectrolytes with accuracy of 0.0001 mol fraction. Calorimetric measurements were carried out with automated ampule calorimeters described in detail previously.[19,20]

## Results

The standard enthalpies of solution of L-histidine in water given in Table 1 represent the result of four or more measurements in the range of solute molalities of 0.003−0.06 mol/kg, where the $\Delta H^{m}$ (sol) values do not depend on a solute concentration.[13] The $\Delta H^{m}$ (sol) $\cong \Delta H^{0}$ (sol) values in the mixed solvents listed in Tables 1 and 2 reflect the result of mainly one experiment at the solute molality of 0.005−0.02 mol/kg. The experimental enthalpies of imidazole solution (see Table

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**TABLE 1: Standard Enthalpies of Solution $\Delta H^0$ (sol) in kJ/mol for L-Histidine in Water−U Mixtures at 288.15−318.15 K**

| $X_A$[a] | $\Delta H^0$ (sol) | $X_A$ | $\Delta H^0$ (sol) | $X_A$ | $\Delta H^0$ (sol) |
|---|---|---|---|---|---|
| 288.15 K | | 293.15 K | | 298.15 K[17] | |
| 0[13] | 13.76 ± 0.05[b] | 0 | 13.95 ± 0.04 | 0 | 14.22 ± 0.06 |
| | | | | | 14.32,[11] 13.96[21] |
| 0.019 36 | 12.35 | 0.019 94 | 12.57 | 0.016 04 | 13.26 |
| 0.040 43 | 10.61 | 0.039 95 | 11.39 | 0.018 61 | 13.10 |
| 0.063 33 | 9.34 | 0.060 11 | 10.51 | 0.030 59 | 12.42 |
| 0.080 74 | 8.70 | 0.080 02 | 9.44 | 0.052 10 | 11.40 |
| 0.101 89 | 7.93 | 0.100 1 | 9.11 | 0.061 69 | 10.95 |
| 0.128 3 | 7.57 | | | 0.103 3 | 9.26 |
| | | | | 0.124 1 | 9.07 |
| 306.15 K | | 313.15 K | | 318.15 K | |
| 0 | 14.74 ± 0.05 | 0 | 15.34 ± 0.07 | 0 | 15.76 ± 0.04 |
| 0.008 531 | 14.30 | 0.008 180 | 15.00 | 0.019 72 | 14.61 |
| 0.025 25 | 13.69 | 0.011 09 | 14.70 | 0.039 90 | 13.69 |
| 0.033 98 | 13.18 | 0.014 19 | 14.49 | 0.060 09 | 12.91 |
| 0.050 22 | 12.30 | 0.034 15 | 13.57 | 0.079 57 | 12.51 |
| 0.060 43 | 11.92 | 0.051 16 | 12.97 | 0.099 91 | 11.97 |
| 0.079 53 | 11.33 | 0.076 14 | 12.04 | | |
| 0.100 8 | 10.58 | 0.102 1 | 11.24 | | |
| 0.121 6 | 9.84 | | | | |
| | | | | 328.15 K | |
| | | | | 0 | 16.59 ± 0.07 |
| | | | | 0.009 945 | 15.84 ± 0.05 |

[a] The amide mol fraction. [b] Uncertainties of the $\Delta H^0$ (sol) values from here on represent twice the standard deviation of the mean.

**TABLE 2: Standard Enthalpies of Solution $\Delta H^0$ (sol) in kJ/mol for L-Histidine in Water−DMF Mixtures at 288.15−318.15 K**

| $X_A$ | $\Delta H^0$ (sol) | $X_A$ | $\Delta H^0$ (sol) | $X_A$ | $\Delta H^0$ (sol) |
|---|---|---|---|---|---|
| 288.15 K | | 293.15 K | | 298.15 K[17] | |
| 0.008 950 | 14.00 | 0.010 06 | 14.25 | 0.013 04 | 14.62 |
| 0.028 37 | 14.52 | 0.019 79 | 14.53 | 0.022 85 | 14.85 |
| 0.047 89 | 14.87 | 0.028 92 | 14.74 | 0.034 06 | 15.21 |
| 0.081 52 | 15.37 | 0.041 87 | 15.04 | 0.047 97 | 15.53 |
| 0.094 29 | 15.47 | 0.070 55 | 15.50 | 0.073 66 | 15.89 |
| 0.131 3 | 15.67 | 0.094 65 | 15.78 | 0.118 7 | 16.18 |
| | | 0.115 3 | 15.88 | | |
| 306.15 K | | 313.15 K | | 318.15 K | |
| 0.006 251 | 14.92 | 0.011 09 | 15.92 | 0.020 10 | 16.10 |
| 0.014 15 | 15.06 | 0.024 55 | 16.19 | 0.039 69 | 16.41 |
| 0.029 44 | 15.44 | 0.040 95 | 16.55 | 0.060 01 | 16.69 |
| 0.038 01 | 15.72 | 0.053 81 | 16.75 | 0.079 98 | 16.88 |
| 0.053 50 | 16.07 | 0.072 71 | 17.16 | 0.092 11 | 16.97 |
| 0.074 58 | 16.43 | 0.098 68 | 17.55 | 0.120 1 | 17.19 |
| 0.090 89 | 16.61 | 0.121 3 | 17.77 | | |
| 0.117 1 | 16.86 | | | | |
| | | | | 328.15 K | |
| | | | | 0.018 23 | 16.63 ± 0.06 |

**TABLE 3: Standard Enthalpies of Imidazole Solution $\Delta H^0$ (sol) in kJ/mol in Water−DMF and Water−U Mixtures at 298.15 K**

| $X_A$ | $\Delta H^0$ (sol) | $X_A$ | $\Delta H^0$ (sol) |
|---|---|---|---|
| Water−DMF | | Water−U | |
| 0 | | 12.83 ± 0.06, 12.8[22] | |
| 0.011 14 | 12.95 | 0.009 055 | 12.74 |
| 0.028 66 | 13.17 | 0.027 26 | 12.63 |
| 0.035 47 | 13.25 | 0.039 77 | 12.57 |
| 0.047 62 | 13.35 | 0.061 11 | 12.45 |
| 0.067 07 | 13.46 | 0.081 28 | 12.37 |
| 0.085 70 | 13.50 | 0.090 15 | 12.34 |
| 0.101 6 | 13.52 | 0.100 0 | 12.31 |

**TABLE 4: Enthalpic Parameters of Pair Interaction, $h_{AB}$, in J kg/mol$^2$ of L-Histidine with U and DMF in Water[a]**

| $T$, K | DMF | U |
|---|---|---|
| 288.15 | 254 (7) | −818 (31) |
| 293.15 | 277 (5) | −671 (49) |
| 298.15 | 304 (12)[17] | −579 (10)[17], −518[11] |
| 306.15 | 272 (13) | −460 (27) |
| 313.15 | 218 (11) | −491 (25) |
| 318.15 | 165 (6) | −543 (25) |

[a] Values given in parentless represent standard deviation of the mean.

3) have been measured in the same range of molalities and, thus, are considered to be equal to the standard values. Tables 1−3 show very good agreement between our and the $\Delta H^0$ (sol) values available in the literature.[11,21,22]

## Discussion

**A. Pair Interaction at 298.15 K.** The formally exact theory of solutions developed by McMillan and Mayer[23] and then adapted by Kauzmann[24] and Friedman[25] relates thermodynamic properties of multicomponent system to certain integrals of the mean force potential associated with the interaction between pairs, triplets, and a high number of solute molecules in a highly diluted solution.

The enthalpic parameters of the amino acid−nonelectrolyte pair interaction given in Table 4 have been extracted from the experimental data with the procedure described earlier.[18,19] The L-histidine−U enthalpic parameter is seen to be negative at the standard temperature, which means that such interaction contributes in an attractive sense to the total interaction that is monitored by the free energy. Therefore, the increase in the amino acid solubility in aqueous urea solutions[26] primarily results from the favorable L-histidine−denaturant interaction. The identical behavior reveals other aromatic solutes: L-phenylalanine and L-tryptophan.[18,26] In contrast, the L-histidine interaction with hydrophobic DMF is energetically unfavorable, which results in decreasing amino acid solubility in water−DMF mixtures.[17] Thus, for L-histidine, the enthalpic term dominates in both U and DMF solutions. However, for the water−DMF−L-phenylalanine system, the favorable solute−amide interaction is found to arise from the large and positive entropy.[15] It indicates that in this case, hydrophobic interaction between the phenyl radical of the amino acid and the DMF methyl groups gives a major contribution to the overall free energy change.

It is interesting to note that at the standard temperature, enthalpies of interaction between various amino acids and U are always negative,[11,15−18] and in general, the interaction becomes more favorable when the side-chain hydrophilicity is increased. However, for L-phenylalanine, it is not the case. In fact, the $h_{AB}$ parameters for glycine and L-phenylalanine are found to be almost identical,[18] although the benzene−U pair interaction is accompanied by a large and positive enthalpy change.[18] The application of the additivity-of-group approach to the water−U−L-phenylalanine system gives the $h_{AB}$ and $s_{AB}$ values that differ in sign from the experimental ones.[15,18] We have attributed this effect to the appearance of preferential orientations between the zwitterionic group of the amino acid and the $NH_2$ fragments of a urea molecule.[15,18] If it is true, we could expect that the approach above would predict the L-histidine behavior better, since its side chain contains several hydrophilic centers, which should randomize the solute−urea interaction. The $h_{AB}$ values for imidazole extracted from the data given in Table 3 are equal to −67 (5) and 133 (5) J kg/mol$^2$ for U and DMF, respectively. Using the $h_{AB}$ parameters for L-alanine[16] (see also eqs 3 and 4 given below), we have

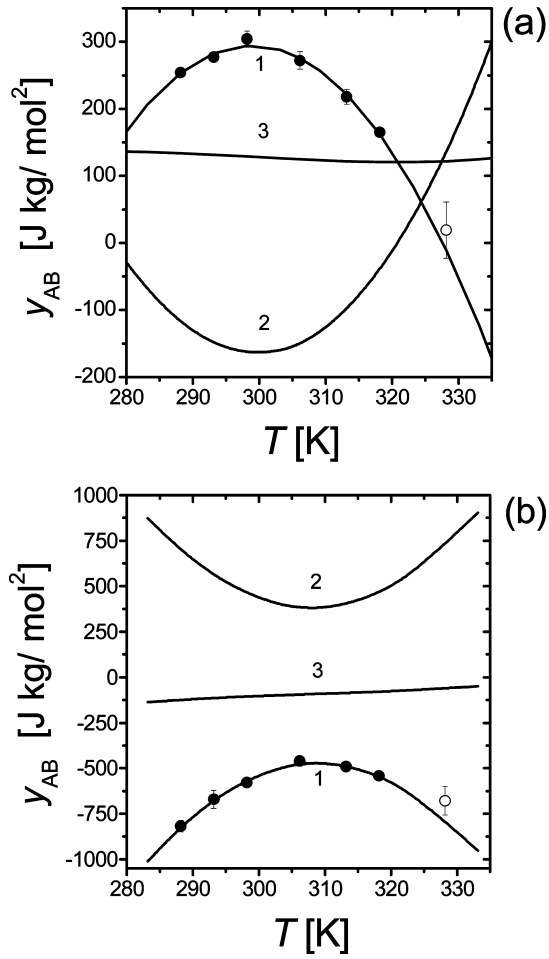


**Figure 1.** Temperature dependence of enthalpic (1), entropic (2), and free energy (3) parameters for the L-histidine−DMF (a) and L-histidine−U (b) interaction in water. Points, experimental $h_{AB}$ values; lines represent the calculation according to eqs 1, 2, and 6; ○, estimated $h_{AB}$ values at 328 K (see text).

obtained −378 and 567 J kg/mol$^2$ for the L-histidine interaction with U and DMF, respectively. These values are only in a semiquantitative agreement with the experimental ones listed in Table 4. Nevertheless, the difference observed is much smaller than for the L-phenylalanine−U interaction.[18] Therefore, for more hydrophilic L-histidine, preferential orientations between U and the zwitterionic group of the amino acid in water are less pronounced.

**B. Temperature Dependence of the Amino Acid−Amide Interaction.** The temperature dependence of the $h_{AB}$ values listed in Table 4 is plotted in Figure 1a, b. In both cases, the curves $h_{AB}$ vs ($T$) are found to fit quite well to the following second-order polynomials:

$$h_{AB}(\text{L-histidine−DMF}, T) = 294\ (5) + 0.50\ (0.46)\cdot (T - 298.15) - 0.5\cdot 0.711\ (0.07)\cdot[(T - 298.15)^2],\quad s_f = 7\ \text{J kg/mol}^2 \quad (1)$$

$$h_{AB}(\text{L-histidine−U}, T) = -564\ (8) + 17.55\ (0.8)\cdot (T - 298.15) - 0.5\cdot 1.65\ (0.12)\cdot[(T - 298.15)^2],\quad s_f = 13\ \text{J kg/mol}^2 \quad (2)$$

where from here on, the first term is the enthalpic parameter of the pair interaction at the reference temperature of 298.15 K; the second term is the heat capacity parameter ($c_{pAB}$, J kg/(mol$^2$ K)), and the third term is its temperature derivative. Values in parentheses represent the standard deviation of the parameters obtained, and $s_f$ is the standard deviation of the fit.

Figure 1a, b shows that both curves pass through maxima. For urea solutions, the maximum appears at 306 K, whereas for the water−DMF system, it is shifted to lower temperatures. Equation 1 predicts that in hot water, the enthalpy of L-histidine−DMF interaction becomes negative. To check this prediction, we have measured the enthalpies of L-histidine solution in pure water and a highly aqueous water−DMF mixture ($X_A = 0.018\ 23$) at 328.15 K (see Tables 1, 2). It allows us to estimate the $h_{AB}$ parameter as follows:[18] $h_{AB} \approx (16.63 - 16.59)\ 0.009/0.01823 = 20$ J kg/mol$^2$. This value shown in Figure 1a as an open circle is seen to be in good agreement with eq 1.

Figure 1b shows that a nearly identical situation occurs in urea solutions. Thus, we may state that in both cases, the $c_{p\ AB}$ values are positive at lower temperatures but become negative in hot water. It indicates that the amino acid transfer from water to highly aqueous amide solutions is accompanied by a positive heat capacity change in cold water, which becomes negative with an increase in the temperature.

It is of particular importance to compare the L-histidine behavior with that for amino acids containing hydrophobic groups in their side chains. The temperature dependence of the enthalpies of the L-alanine and L-phenylalanine interaction with amide molecules is found to be well reproduced with the following equations:[16,18]

$$h_{AB}(\text{L-alanine−DMF}, T) = 434\ (4) - 4.88\ (0.4)\cdot 298.15\cdot (T/298.15\text{-}1),\quad s_f = 8\ \text{J kg/mol}^2 \quad (3)$$

$$h_{AB}(\text{L-alanine−U}, T) = -311\ (8),\quad s_f = 16\ \text{J kg/mol}^2 \quad (4)$$

$$h_{AB}(\text{L-phenylalanine−U}, T) = -349\ (6) - 5.87\ (0.60)\cdot (T - 298.15) + 0.5\cdot 0.95\ (0.09)\cdot[(T - 298.15)^2],\quad s_f = 9\ \text{J kg/mol}^2 \quad (5)$$

Figure 2a shows that the enthalpy of L-alanine−DMF interaction decreases linearly with an increase in the temperature, revealing the same behavior as for interaction between well-established hydrophobic solutes−tetraalkylammonium bromides, and hexamethyl phosphoric triamide.[19] The energetics of the L-alanine−U interaction appears to be constant in the physiological temperature range, which is rather unusual and requires extension of the temperature range of measurements. In contrast, in the water−U−L-phenylalanine system, the minimum on the curve $h_{AB}$ vs ($T$) is reached (see Figure 2b).

Standard thermodynamic manipulations allow us to compute temperature changes of the free energy parameters from the available $h_{AB}$ values:

$$\frac{g_{AB}(T)}{T} - \frac{g_{AB}(298.15)}{298.15} = -\int_{298.15}^{T} \frac{h_{AB}}{T^2}\,dT \quad (6)$$

Then using the previously reported $g_{AB}$ values at 298 K[15,17] and eqs 1−6, we are able to compute entropies and free energies of pair interaction.

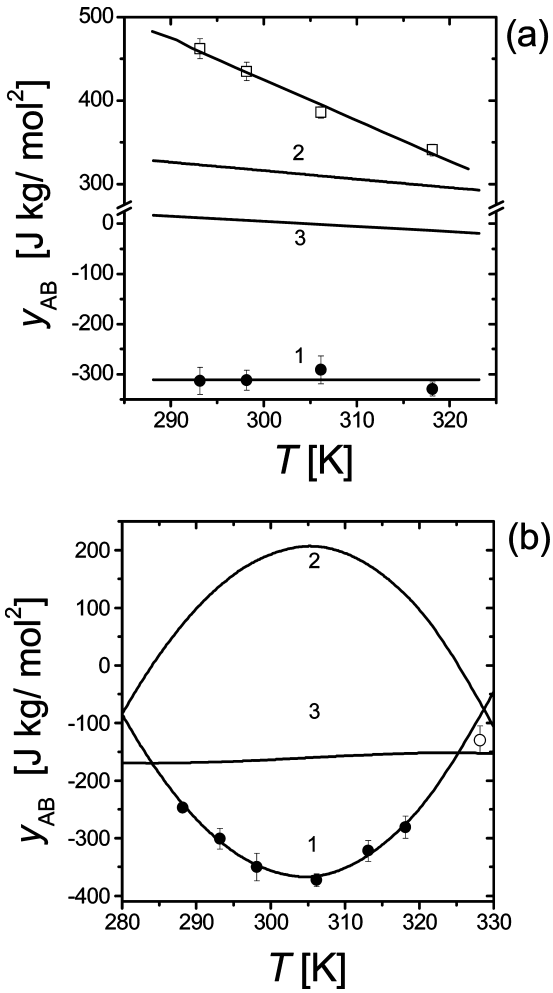


**Figure 2.** Temperature dependence of enthalpic (1), entropic (2), and free energy (3) parameters for the L-alanine−U[16] and (a) and L-phenylalanine−U[18] (b) interaction in water. Points, experimental $h_{AB}$ values; lines represent the calculation according to eqs 3−6; □, enthalpic parameters for the L-alanine−DMF interaction;[16] ○, estimated $h_{AB}$ value at 328 K (see text).

Figures 1 and 2 illustrate their temperature dependences for the systems studied. The results obtained illuminate at least three interesting features that are worth noting. First, the significant enthalpy−entropy compensation exists for all systems so that free energy parameters are small and rather insensitive to the chemical structure of the amino acids and temperature changes. Second, for all the temperatures studied, the amino acid−U interaction remains enthalpically favorable. Finally and generally, both the enthalpic and entropic characteristics in L-histidine solutions reveal extremal temperature dependence that results in rather close $h_{AB}$ and $Ts_{AB}$ values at 298 and 310 K. Therefore, the aromatic amino acid−denaturant interaction appears to be sensitive to the behavior of pure water, that is, to the existence of the temperature minimum of its heat capacity at 308 K.[27]

Figures 1 and 2 show that the position and depth of the extrema observed depend strongly on the hydrophobicity/hydrophilicity of the interacting species. In fact, in the water−U−L-phenylalanine system, the curve $h_{AB}$ vs $T$ passes through the slight minimum, whereas for L-histidine, the pronounced maximum is reached. For the L-histidine−DMF interaction, the curve $h_{AB}$ vs $T$ passes through the maximum at ∼300 K, but for significantly more hydrophobic L-alanine and L-phenylalanine, the enthalpic parameters decrease linearly with an increase in the temperature.[16,28] This comparative analysis allows us to draw a tentative conclusion that the anomalous behavior of enthalpic and entropic parameters is closely related to hydrophilic hydration and interaction between polar and charged groups in the solute molecules.

## Conclusions

Thus, our study does indicate for the first time the anomalous temperature dependence of the L-histidine−urea and L-histidine−dimethylformamide pair interaction in water, which is consistent with previously reported enthalpic characteristics for water−urea[20] and water−urea−L-phenylalanine[18] systems. Since all extrema occur in the temperature range of 300−308 K, where the $c_{pAB}$ values change their signs, it results in rather close enthalpic and entropic parameters at the standard and physiological temperatures. It may serve as a certain justification for using the results obtained at 298 K to explain processes occurring at the physiological temperature. However, it is not a valid approximation for the interaction between presumably hydrophobic solutes, where the $c_{pAB}$ values are found to be constant in the wide temperature range.[16,19,28]

Also remarkable are large negative values for the enthalpic amino acid−urea parameters in the wide temperature range. This observation supports the idea[29] that urea acts rather directly by binding to polar and charged groups of a protein, weakening internal H-bonds and causing denaturation without any significant changes in the properties of the H-bond network of liquid water near apolar groups of macromolecules.

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