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Thermodynamics of Interaction of L-α-Phenylalanine with Urea and Dimethylformamide in Aqueous Solution

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Abstract — The thermal effects of solution of L-phenylalanine in aqueous solutions of urea and dimethylformamide (DMF) at 25°C were determined. The solubility of L-phenylalanine in water and aqueous DMF solutions was measured. The standard enthalpies, free energies, and entropies of solution of the amino acid in aqueous solutions of amides were calculated. The parameters of pair and ternary amino acid–amide interactions were determined within the framework of the McMillan–Mayer theory. The amino acid–amide pair interaction is accompanied by a decrease in the Gibbs free energy, controlled by the entropy term with DMF and by the enthalpy term with urea. The interaction of L-phenylalanine with two amide molecules is repulsive, which in the case of DMF leads to an increase in the standard free energies of solution of the amino acid at the amide mole fraction $X_2 > 0.05$.
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Understanding the mechanism of amino acid–amide interactions in aqueous solutions is important not only for solution chemistry, but also directly for molecular biology, because amide molecules contain the same fragments as protein molecules. Many amides in aqueous solution promote the transition from the native form of globular proteins to the denatured form [1–3]. However, apparently, the mechanism of the action of amides on proteins should be different, because the properties of amides in aqueous solutions strongly depend on the degree of substitution. Indeed, DMF is a strong proton acceptor, whereas urea exhibits, along with proton acceptor properties, also pronounced proton-donor power [4, 5]. DMF is a predominantly hydrophobic substance because of the presence of two methyl groups, and for DMF in water the pressure derivative of the second virial coefficient is negative [3]. For typically hydrophilic urea, this derivative is positive [3]. Hence, the behavior of amino acids as elementary units of a protein molecule in urea and DMF solutions should be different, and the differences should be particularly strong for such hydrophobic amino acids as phenylalanine or leucine. Here we report on a comparative study of the interaction of L-phenylalanine with urea and DMF in water.

The experimental data on the solubility of L-phenylalanine in the water–DMF system are given in Table 1. The dependences of the solubility of L-phenylalanine (X_3) on the composition of water–DMF and water–urea mixtures [1] are compared in Fig. 1. It can be seen that small additions of amides lead to an increase in X_3, especially in aqueous solutions of urea. This is quite natural, because additions of urea and DMF to water considerably increase the solubility of benzene [8, 9]. At the same time, it is well known that L-alanine is salted-out from urea solutions [1]. Therefore, the observed increase in the solubility of L-phenylalanine in the examined range of compositions of water–urea mixtures is exclusively due to the presence of the nonpolar benzene fragment in the amino acid

Table 1. Solubility (*s*, g amino acid/100 g solvent) of L-phenylalanine in water–DMF mixtures at 25°C

X_2	*s*
0	2.79 ± 0.02,[a] 2.80 [1]
0.00883	2.85 ± 0.01
0.01771	2.90 ± 0.04
0.0355	2.88 ± 0.02
0.04997	2.76 ± 0.03
0.08407	2.39 ± 0.03
0.1127	2.08 ± 0.01
0.1468	1.75 ± 0.02

[a] The errors are given as doubled standard deviations of the mean value for a series of 5–9 measurements.

molecule. At the DMF mole fraction $X_2 > 0.05$, the solubility of L-phenylalanine starts to decrease. Apparently, at small additions of DMF to water, the salting-in of the benzene moiety prevails over salting-out of the alanyl residue. With a further increase in the amide concentration, the salting-out effect starts to prevail, which determines a decrease in the solubility of L-phenylalanine. Such a behavior of amino acids in aqueous solutions is not unusual. For example, on adding urea to water, the molal concentration of saturated solutions of leucine, histidine, and glutamine also first increases and then decreases [1].

We showed previously [6, 10] that, in a relatively wide concentration range, m 0.001–0.03 mol kg^{-1}, the experimental enthalpies of solution of L-phenylalanine are independent of the concentration of the amino acid in the aqueous solution. Therefore, as standard enthalpies of solution of L-phenylalanine, $\Delta_s H^0$, in mixtures of water with DMF and urea we took the integral enthalpies $\Delta_s H^m$ obtained in the range of amino acid concentrations 0.003–0.01 mol kg^{-1}. These quantities are listed in Table 2. In Fig. 2 we compare the results of this study with published [11] enthalpies of solution of L-phenylalanine in mixtures of water with urea. It is seen that the two data sets are in good agreement.

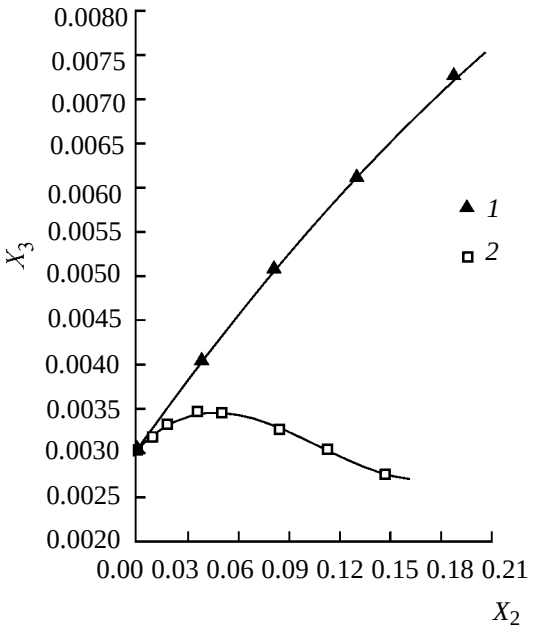

Fig. 1. Solubility (X_3, mole fraction) of L-phenylalanine in (*1*) water–urea and (*2*) water–DMF systems at 25°C. (X_2) Mole fraction of amide; (lines) theoretical description.

It is known [1, 12] that the standard free energy of solution $\Delta_s G^0$ of poorly soluble substances can be determined by the solubility method:

$$\Delta_s G^0 \approx RT\ln(1/X_3), \quad (1)$$

where X_3 is the solute mole fraction in the saturated solution. Then, using data of Tables 1 and 2, it is easy to calculate all the thermodynamic functions of solution of the amino acid in aqueous solutions of the amides. Figures 2 and 3 show the enthalpy ($\Delta_s H^0$) and entropy ($-T\Delta_s S^0$) terms of the free energies of solution of L-phenylalanine in mixtures of water with DMF and urea. In a solution of hydrophilic urea, the enthalpy term decreases and the entropy term increases with an increase in the amide concentration, whereas in a solution of hydrophobic DMF the pattern is opposite: $\Delta_s H^0$ increases, and $-T\Delta_s S^0$ decreases. The dependences $\Delta_s H^0 = f(X_2)$ and $-T\Delta_s S^0 = f(X_2)$ in urea solution intersect at the amide mole fraction X_2 ~0.033, where, apparently, $\Delta_s G^0 = 2\Delta_s H^0 = -2T\Delta_s S^0$. With DMF, in the region of amide mole fractions X_2 ~0.13, the entropy term is virtually zero, so that $\Delta_s G^0 = \Delta_s H^0$.

At small amounts of amides added to water, interactions between the solute and amide are mainly pair (L-phenylalanine–amide) and ternary (amide–L-phenylalanine–amide), and their contributions to thermodynamic properties of solutions can be calculated using the formally rigorous McMillan–Mayer solution theory [3]. The enthalpy parameters of amino acid–amide interactions were calculated as in our previous papers [13–15]. The paameters of pair and ternary

Table 2. Standard enthalpies of solution ($\Delta_s H^0$, kJ mol^{-1}) of L-phenylalanine in aqueous solutions of urea and DMF at 25°C

Water–urea		Water–DMF	
X_2	$\Delta_s H^0$	X_2	$\Delta_s H^0$
0	8.23 ± 0.07[a] [6]	0.02008	9.80
0.006873	7.85	0.02359	10.22
0.02404	7.15	0.03439	10.73
0.03229	6.89	0.03703	11.07
0.04048	6.75	0.04507	11.45
0.04945	6.38	0.06018	12.29
0.06833	5.99	0.07226	12.73
0.07956	5.69	0.07261	12.79
0.1020	4.99	0.09808	13.73
0.1202	4.74	0.1002	13.73
		0.1149	14.02

[a] The error for the enthalpy of solution in water is given as the doubled standard deviation of the mean value.

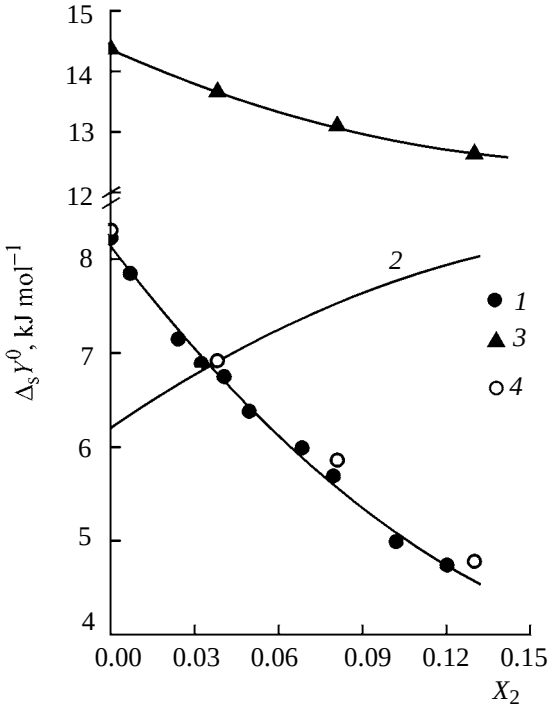

Fig. 2. (*1*) Enthalpy ($\Delta_s H^0$) and (*2*) entropy ($-T\Delta_s S^0$) terms, and (*3*) Gibbs free energy ($\Delta_s G^0$) of solution of L-phenylalanine in water–urea mixtures; (*4*) data of [11].

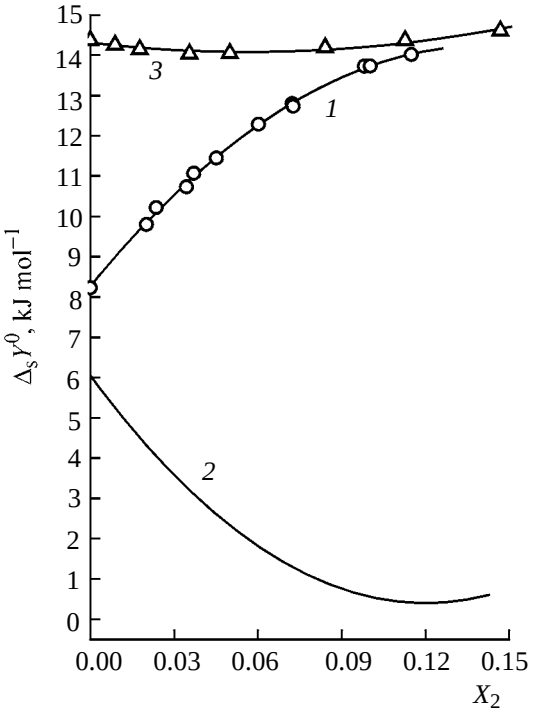

Fig. 3. (*1*) Enthalpy ($\Delta_s H^0$) and (*2*) entropy ($-T\Delta_s S^0$) terms, and (*3*) Gibbs free energy ($\Delta_s G^0$) of solution of L-phenylalanine in water–DMF mixtures.

interactions for the free energy (g_{23}, g_{223}) and entropy ($-Ts_{23}$, $-Ts_{223}$) were calculated from Eqs. (2)–(6):

$$RT\ln\frac{X_3^{\text{water}}}{X_3^{\text{soln}}} = a_1X_2 + a_2X_2^2\,, \qquad (2)$$

$$g_{23}\ \ a_1M_{H_2O}/2, \qquad (3)$$

$$g_{223} = (a_2 - a_1)M_{H_2O}^2/3, \qquad (4)$$

$$-Ts_{23} = g_{23} - h_{23}, \qquad (5)$$

$$-Ts_{223} = g_{223} - h_{223}. \qquad (6)$$

The parameters of pair and ternary interactions of benzene, L-alanine, and L-phenylalanine with urea and DMF in water are compared in Table 3. It is seen that the pair interaction of the solutes with DMF is energetically unfavorable (repulsive from the viewpoint of enthalpy), especially in the case of hydrophobic L-phenylalanine. Interaction of both amino acids with hydrophilic urea is favorable from the viewpoint of enthalpy, whereas the benzene–urea interaction is unexpectedly repulsive.

The free energy parameters show that the pair interaction of L-phenylalanine and benzene with hydrophilic urea is attractive ($g_{23} < 0$), whereas that of L-alanine is weakly repulsive ($g_{23} \geq 0$). The pair interaction of L-phenylalanine, as well as that of nonpolar benzene, with DMF is attractive. The results obtained suggest that just the presence of a nonpolar benzene moiety in the L-phenylalanine molecule is responsible for the interaction being attractive, irrespective of whether the amide is hydrophobic (DMF) or hydrophilic (urea). However, this apparent insensitivity of g_{23} to the nature of the amide is actually a result of compensation of the enthalpy and entropy terms. Table 3 clealry shows that the attraction of L-phenylalanine to hydrophilic urea is enthalpy-controlled (the entropy term prevents the interaction), whereas the attraction of L-phenylalanine to DMF is, on the contrary, entropy-controlled, which is typical of predominantly hydrophobic substances [3].

In all the cases the ternary interaction parameters are smaller than the pair interaction parameters and have a different sign, which indicates that the McMillan–Mayer series rapidly converge and the theory is suitable for practical use [3]. The interaction of L-phenylalanine with two DMF or urea molecules is accompanied by the overall repulsion ($g_{223} > 0$), more noticeable in the case of DMF. Specifically the

Table 3. Enthalpy (h_{23}, J kg mol^{-2}; h_{223}, J kg^2 mol^{-3}) and entropy ($-Ts_{23}$, J kg mol^{-2}; $-Ts_{223}$, J kg^2 mol^{-3}) terms of the Gibbs parameters of pair (g_{23}) and ternary (g_{223}) interactions of amino acids and benzene with DMF (upper row) and urea (lower row) in water at 25°C

Ratio	h_{23}	h_{223}	$-Ts_{23}$	$-Ts_{223}$	g_{23}	g_{223}
L-Phenylalanine	760(21) [a]	–41(2)	–902(23)	66(3)	–142(9)	25(2)
	–351(24), –333(36) [b]	14(3)	187(27)	–8(3)	–164(10) [c]	6(1) [c]
L-Alanine	409(21) [d]	–	–	–	–	–
	–202(28) [b]	9(3) [b]	209(28)	–10(3)	7(4) [c]	–1(0.3) [c]
Benzene	580(36) [e]	–22(5) [e]	<0	–	<0	–
	513(153) [f]	–35(20) [f]	–656(153)	40(20)	–143(5) [f]	5(1) [f]

[a] The errors of the parameters are given as standard deviations from the calculated mean values. [b] Our calculation based on data from [11]. [c] Our calculations using data on solubility of L-phenylalanine and Gibbs free energies of L-alanine in the water–urea mixture at 25°C [1]. [d] Data for D,L-alanine. [e] Our calculation based on data from [14]. [f] Calculated in this study from the activity coefficients of benzene in the water–urea mixture at 0–50°C [8].

contribution from ternary interactions leads to an increase in the standard free energy of solution of the amino acid at DMF mole fractions $X_2 > 0.05$ (Fig. 3).

Table 1 allows one more important conclusion. Apparently, two structural fragments, nonpolar benzene (or toluene) moiety and polar alanyl (zwitterionic) group, can be distinguished in the L-phenylalanine molecule. Then we have a right to expect that, if the Savage–Wood principle of additivity of group contributions [17] is applicable to parameters of pair interaction in the systems under consideration, then y_{23} can be estimated with a good accuracy by summation of the aromatic hydrocarbon–amide and L-alanine (or glycine)–amide interaction parameters. As seen from Table 3, for the enthalpy parameters of L-phenylalanine–DMF pair interactions, the calculated and experimental data are in good agreement. A similar pattern is observed with the parameters g_{23} for L-phenylalanine–urea interactions, but here it results merely from the compensation of the enthalpy and entropy terms. Indeed, by summing up the corresponding parameters of interaction of benzene and L-alanine with urea, we obtain the values that not only considerably differ from the experimental values of h_{23} and $-Ts_{23}$ for L-phenylalanine, but even have a different sign. Summing up the parameters of interaction of toluene and glycine with urea leads to a similar result. Naturally, a question arises: Why is the group additivity method well applicable to solutions of a hydrophobic amide but does not adequately predict even the sign of interaction parameters in solutions of hydrophilic urea? It is intuitively clear that the contributions to y_{23} are made by all the possible orientations of pairs of interacting particles and by all the distances between them where the medium-strength interaction potential is nonzero. Apparently, the dependence of the energy of interaction of different groups in the interacting particles on the distance should not be the same and can be altered upon introduction of a new group into the molecule, especially if this modification gives rise to preferential orientations [3]. Specifically to the preferential orientations arising between *N*-acetyl-L-phenylalaninamide and formamide molecules in aqueous solutions Nelander et al. [18] attributed a substantial discrepancy between the experimental value of h_{23} (47 J kg mol^{-2}) and that calculated using the Savage–Wood additivity scheme (–487 J kg mol^{-2}). Similar phenomenon can be observed in the case of interaction of L-phenylalanine with urea, because these molecules contain the same groups as the substances studied in [18]. It is reasonable to assume that the observed effects arise from the presence of a zwitterionic group in the L-phenylalanine molecule, because the parameters h_{23} for various amino acids are negative and are of the same order of magnitude. Indeed, the enthalpy parameter of glycine–urea interaction, as we calculated from data of [11], is –346(20) J kg mol^{-2}, which is identical to data for L-phenylalanine (Table 3). As the urea and DMF molecules have the same H-acceptor groups and, as shown above, the group additivity principle is observed in DMF solutions, it can be tentatively assumed that the preferential orientations arising between L-phenylalanine and urea in aqueous solutions are caused by interaction of NH_2 groups of urea with the zwitterionic group of the amino acid in aqueous solution.

Thus, it is evident that the hydrophobicity of the amide molecule strongly affects the enthalpy and entropy parameters of pair interaction but affects g_{23} only slightly. The pair interaction of L-phenylalanine

with both hydrophobic DMF and hydrophilic urea is attractive ($g_{23} < 0$). However, the nature of the attraction is quite different: In the case of DMF it is essentially hydrophobic ($Ts_{23} > h_{23} >> 0$), whereas with urea it is hydrophilic ($h_{23} < 0$, $Ts_{23} < 0$). In DMF solutions, the enthalpy and entropy terms of the pair interaction largely compensate each other, which is typical of hydrophobic substances. The interaction of L-phenylalanine with hydrophilic urea is apparently accompanied by appearance of preferential orientations between the polar groups of the interacting substances.

EXPERIMENTAL

The thermal effects of solution were measured on a variable-temperature calorimeter with an isothermal shell and automatic data acquisition and processing [6].

L-α-Phenylalanine [produced in Germany, packed by Labtekh company, TU (Technical Specification) 6-09-4322–78] was used without additional purification after drying in a vacuum at 70°C for several days to constant weight. Water was double-distilled. Dimethylformamide (pure grade) was dried over molecular sieves (3 Å) and double-distilled in a vacuum at 30°C. The residual water content was determined by Fischer titration to be 0.008 wt %. Urea (>99.5 wt %) was used without additional purification. According to Fischer titration, the water content of urea was 0.15 wt %, and it was taken into account when preparing the solutions.

The solubility of the amino acid in water was measured by the isothermal saturation method. A 50-ml cell temperature-controlled with an accuracy of ±0.02 K was charged with the amino acid and water, taken in appropriate proportion. The cell was tightly closed and stirred with a magnetic stirrer for 24 h, which was sufficient to attain the equilibrium. The amount of the amino acid in the saturated solution was determined by the method of dry residue (direct gravimetry) [1, 7]. For this purpose, a solution sample was quickly taken into a temperature-controlled syringe with a stainless steel needle fixed in the cell lid. The sample was filtered through glass frits into special tightly sealed capsules. The filters were kept at a constant temperature (±0.2 K) during the time of sampling and filtration. The filtrate was weighed on an Ohaus balance with an accuracy of $\pm 2 \times 10^{-4}$ g. Then the solvent was evaporated by heating in a stream of hot air at ~60°C. Then the dry residue was vacuum-dried to constant weight.

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