Thermal properties and interpartical interactions of L-proline, glycine, and L-alanine in aqueous urea solutions at 288–318 K

V. P. Korolev · O. A. Antonova · N. L. Smirnova

Abstract The enthalpies of solution of L-proline have been measured in aqueous urea solutions at 0–6 mol urea kg^{-1} water at 288.15, 298.15, 308.15, and 318.15 K by the calorimetric method. The two-parameter relation connecting the values of solution enthalpies of proline with urea concentration and temperature has been obtained. The enthalpy and heat capacity parameters of pair interaction of L-proline with urea in water have been computed. Using the thermodynamic relations, the temperature changes of reduced enthalpy, and also the change of entropy and reduced Gibbs energy of solution of L-proline in aqueous solutions of urea at the temperature rise from 288 to 318 K have been determined. Their comparison with the data for glycine and L-alanine has been carried out. It has been shown that the entropy–enthalpy compensation (Barclay–Butler rule) takes place for dissolution and transfer processes.

Keywords L-Alanine · Aqueous solutions · Enthalpy and heat capacity parameters of pair interaction · Enthalpy of solution · Entropy–enthalpy compensation · Gibbs energy · Glycine · L-Proline · Urea

Introduction

The imino acid proline plays a decisive role in the protection against cell injuries caused by freezing and dehydration in diatoms and several higher plants (see [1, 2] and references therein). This also applies to overwintering poikilotherms, especially insects [2]. Proline accumulation can provide a quick mechanism for maintaining osmoticum of cells and tissues in response to stress (see [1–4] and references therein). Under water stress conditions it was demonstrated that free proline is accumulated in the plant cells, probably in the cytoplasm. After dewatering, the enhanced level of proline decreases very rapidly. The origin of proline is not due to a hydrolysis of a proline-rich protein, but to a new synthesis from reserved substances and from exogenously added nitrogen sources via the glutamic acid pathway [1, 2].

Proline is a contradictory and enigmatic substance. It has the greatest parameter of hydrophobicity π [5] in series, namely: glycine, L-alanine, and L-proline. However, the solubility of proline in water is much higher than even glycine and its enthalpy of solution in water is exothermic unlike other amino acids. It was shown that in terms of heat capacity of solution of amino acid in water [6] and the value of parameter of pair interaction of amino acid with urea in aqueous solution [7] which can serve as a criteria of hydrophobicity, proline is located between glycine and alanine.

The interest to the investigation of thermodynamics of dissolution and solvation of amino acids in aqueous solutions of organic substances destabilizing (denaturing) and stabilizing proteins native structure is caused by the desire to understand the biochemical systems better. Such works with aqueous solutions of urea which is usually used as a denaturing agent were made by various methods, including calorimetry, only at standard temperature [7–11], at the fixed urea concentration [10], and in a narrow interval of concentration [11].

It is necessary to note that according to the classification of Castronuovo et al. [12], urea can be identified as hydrophilic structure breaker solute. The investigation of a

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binary water–urea mixture and urea's action on hydrophobic interaction continues to attract attention of researchers [13–17]. It is interesting to remark that urea reduces the temperature of protein denaturation [18].

It is important to investigate thermodynamic properties (including thermal properties) at different temperatures and in a wide concentration intervals. Unfortunately, such works are rare. There are data on the enthalpies of solution in aqueous urea solutions only for glycine [19] and alanine [20] in the literature.

This study reports the results of calorimetric measurements of solution enthalpies of L-proline in aqueous urea solutions at different temperatures and various urea concentrations. The aim of this article is to (i) report the enthalpies of solution of L-proline in water–urea binary system at 288–318 K, (ii) obtain the two-parameter relation connecting the values of solution enthalpies of proline with urea concentration and temperature, (iii) compute the enthalpy and heat capacity parameters of pair interaction of L-proline with urea in water, (iv) calculate the changes of reduced enthalpy of solution, entropy of solution and the reduced Gibbs energy at the temperature rise from 288 to 318 K, and (v) to discuss the obtained characteristics using the results for glycine [19] and L-alanine [20].

Experimental

Materials

L-Proline (Fluka (Analytical) > 99.5%) and urea ("pur.p.a." (Reag. ACS, Riedel de Haën)) have been used without additional purification. The water content in urea defined by Fisher's method has been 0.15 mass.% and taken into account while preparing the solutions, which have been prepared by weight on the molality concentration scale using bidistilled water. We have used freshly prepared aqueous urea solutions.

Solution calorimetry

The enthalpies of solution of L-proline in aqueous urea ($\Delta_{sol}H^m_{(W+U)}$) have been measured with calorimeter provided with a 60 sm^3 titanium vessel. The construction and test of calorimeter were reported earlier [21–23]. The temperature sensitivity of the apparatus has been about 4×10^{-5} K and the temperature stability of the thermostat has been better than 10^{-3} K. The accuracy has been ±1%. The measurement of solution enthalpies of proline had been carried out within the range from 0.01 to 0.024 mol proline kg^{-1} aqueous urea solvent.

The standard enthalpies of solution of L-proline in aqueous urea solutions have been equal to the experimental enthalpies of solution, according to the results [5, 6], where it was shown that the integral enthalpies of solution were independent on the concentration within the proline concentration range under investigation.

Results

The standard enthalpies of solution of L-proline in aqueous urea solutions at different temperatures are presented in Table 1. The data of the standard enthalpies of solution of L-proline in water have been taken from Korolev et al. [6]. The standard enthalpies of solution of L-proline in aqueous urea solutions (Table 1) show the strengthening of an exothermic effect with the increase urea concentration and the weakening of the one with the temperature rise.

The enthalpies of solution of L-proline in aqueous urea solutions measured by the authors at 298.15 K and data

Table 1 Standard enthalpies of solution ($\Delta_{sol}H^0_{(W+U)}$/kJ mol^{-1}) of L-proline in aqueous urea solutions

m_U/mol kg^{-1}	m_P/mol kg^{-1}	q/J mol^{-1}	$\Delta_{sol}H^0_{(W+U)}$
288.15 K			
0.541	0.019	−5.46	−3.69
1.436	0.019	−5.77	−4.16
2.922	0.019	−6.09	−4.75
4.540	0.010	−3.59	−5.30
6.285	0.014	−5.38	−5.79
298.15 K			
0.511	0.015	−4.57	−3.46
1.434	0.015	−4.20	−3.89
2.943	0.014	−4.86	−4.46
4.529	0.014	−4.73	−4.97
6.202	0.024	−8.64	−5.46
308.15 K			
0.521	0.021	−5.12	−3.16
1.423	0.021	−5.00	−3.53
2.951	0.017	−4.73	−4.07
4.529	0.018	−4.83	−4.55
6.209	0.016	−5.40	−5.07
318.15 K			
0.512	0.018	−3.58	−2.87
1.429	0.014	−3.43	−3.19
2.945	0.023	−4.64	−3.69
4.546	0.016	−4.32	−4.14
6.297	0.020	−7.30	−4.68

q The heat effect of dissolution of an appropriate number of mole of proline, the data of the standard enthalpies of solution of L-proline in water are given in [6] and equal −3.41, −3.22, −2.94 kJ mol^{-1} at 288.15, 298.15, 308.15 K, respectively; the value of $\Delta_{sol}H^0$ of L-proline in water at 318.15 K have been calculated by Eq. 7 (see text) and equals −2.66 kJ mol^{-1}
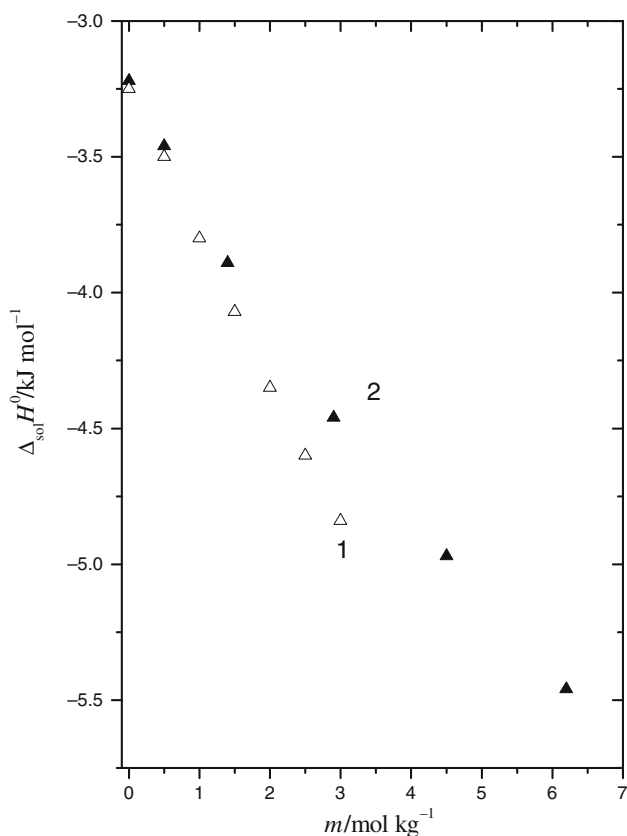
Fig. 1 The standard solution enthalpies of L-proline in aqueous urea solutions at 298.15 K: 1: [11], 2: this study

[11] are shown in Fig. 1 for the comparison. The values are in reasonable agreement in the range of molalities $m_U = 0–1.5$ mol kg^{-1}. Our value of enthalpy of solution equals -4.5 kJ mol^{-1} at $m_U = 3$ mol kg^{-1} and differs from the value -4.8 kJ mol^{-1} [11] on 6%.

Discussion

The obtained standard solution enthalpies of L-proline in aqueous urea solutions (Table 1) have been used to determine enthalpic interaction parameters (derived from McMillan–Mayer theory [24]) between L-proline and urea according to the equation [25]:

$$\Delta_{sol}H^0_{(W+U)} = \Delta_{sol}H^0_{(W)} + 2h_{PU}m_U + 3h_{PUU}m_U^2, \quad (1)$$

where h_{PU} and h_{PUU} denote the enthalpic pair and triplet interaction parameters, respectively. The values obtained at different temperatures are listed in Table 2. The h_{PU} value determined in this study at 298.15 K equals -239 ± 4 J kg mol^{-2} and differs from -280 J kg mol^{-2} [11] on $\sim 7\%$.

For the purpose of comparison the enthalpic parameters of pair and triplet interaction between glycine or L-alanine and urea calculated from coefficients of second-order polynomials [19, 20] are presented in Table 3. For water–urea–amino acid systems being considered the enthalpic pair interaction coefficients are negative at temperatures investigated. It testifies to the predominance of the effect of direct interaction of amino acid with urea over the endothermic processes of partial dehydration of urea and amino acid. The temperature influences the energy of interaction of amino acids with urea in different ways. So, with the temperature rise the values of parameter of pair interaction of proline (Table 2) or glycine (Table 3) with urea become less negative, and the attraction weakens. Enthalpy of interaction of alanine with hydrophilic urea probably does not depend on temperature.

The temperature dependences of enthalpic interaction parameters of amino acids from Tables 2 and 3 have been described by Eqs. 2, 3 for L-proline and Eqs. 4, 5 for glycine:

$$h_{PU} = (-237.8 \pm 0.8) + (3.027 \pm 0.068)(T - 298.15),$$
$$R = 0.9995, \text{ sd } = 1.5 \text{ J kg mol}^{-2} \quad (2)$$

$$h_{PUU} = (6.254 \pm 0.040) - (0.2123 \pm 0.0033)(T - 298.15),$$
$$R = 0.99976, \text{ sd } = 0.07 \text{ J kg}^2\text{mol}^{-3} \quad (3)$$

$$h_{GU} = -349 + 7.133(T - 298.15) \quad (4)$$

$$h_{GUU} = 9 - 0.267(T - 298.15) \quad (5)$$

From data of Table 3 for L-alanine one can judge that there are no dependences of h_{AU} and h_{AUU} on temperature. Therefore, we have accepted that the enthalpic pair and triple interaction parameters of alanine have been calculated as the mean of the results from Table 3 for alanine:

Table 2 Parameters of Eq. 1 for water–urea–L-proline system

T/K	288.15	298.15	308.15	318.15
$\Delta_{sol}H^0_{(W)}$/kJ mol^{-1}	-3.41 [6]	-3.22 [6]	-2.94 [6]	-2.66^a
h_{PU}/J kg mol^{-2}	-267.0 ± 4.7	-238.7 ± 4.1	-208.7 ± 4.5	-176.1 ± 3.9
h_{PUU}/J kg^2 mol^{-3}	8.33 ± 0.59	6.34 ± 0.51	4.10 ± 0.57	2.00 ± 0.48
sd/kJ mol^{-1}	0.021	0.017	0.019	0.014
R	0.99980	0.99984	0.99978	0.99987

^{a} Calculated by Eq. 7 (see text)

Table 3 Enthalpic pair and triplet interaction parameters of glycine and L-alanine with urea in water

Glycine	h_{GU}/J kg mol^{-2}	h_{GUU}/J kg^2 mol^{-3}
298.15 K	-349 ± 15	9.4 ± 0.9
313.15 K	-242 ± 19	4.8 ± 0.7
L-Alanine	h_{AU}/J kg mol^{-2}	h_{AUU}/J kg^2 mol^{-3}
293.15 K	-318 ± 16	16.5 ± 2.0
298.15 K	-297 ± 12	16.0 ± 1.5
306.15 K	-274 ± 13	12.3 ± 1.7
318.15 K	-316 ± 11	19.5 ± 1.4

Parameters are calculated using the coefficients of second-order polynomials for glycine [19] and L-alanine [20]

$$h_{AU} = -301 \pm 13 \text{ J kg mol}^{-2}, h_{AUU} = 16.1 \pm 1.7 \text{ J kg}^2\text{mol}^{-3} \tag{6}$$

The heat capacity parameters of interaction ($c_p = \partial h / \partial T$) of amino acids with urea are equal 7.1, 3.0, and 0 J kg mol^{-2} K^{-1} for glycine, L-proline, and L-alanine, respectively.

The dependence of standard enthalpies of solution of L-proline in water [6] at 283–313 K versus temperature has been described by the equation:

$$\Delta_{sol}H^0_{(W)} = (-3.17 \pm 0.01) + (0.0254 \pm 0.0011) \times (T - 298.15),$$
$$R = 0.995, \text{ sd } = 0.03 \text{ kJ mol}^{-1} \tag{7}$$

Substitution Eqs. 2, 3, 7 in Eq. 1 result in the equation for solution enthalpy of L-proline in aqueous urea solution at various temperatures as follows:

$$\Delta_{sol}H^0_{(W+U)}(\text{Pro}) = -3.17 - 0.476m_U + 0.01876m_U^2 + (0.0254 + 0.00605m_U - 0.000637m_U^2)(T - 298.15) \tag{8}$$

Analogous equations for glycine and L-alanine have been obtained. The $\Delta_{sol}H^0$ values for glycine at 293–313 K [19, 26, 27] and L-alanine at 288–318 K [20, 27] in water have been described as a function of temperature by Eqs. 9 and 10:

$$\Delta_{sol}H^0_{(W)}(\text{Gly}) = (14.17 \pm 0.02) - (0.0469 \pm 0.0022) \times (T - 298.15),$$
$$R = 0.997, \text{ sd } = 0.03 \text{ kJ mol}^{-1} \tag{9}$$

$$\Delta_{sol}H^0_{(W)}(\text{Ala}) = (7.80 \pm 0.03) + (0.0490 \pm 0.0023) \times (T - 298.15),$$
$$R = 0.995, \text{ sd } = 0.06 \text{ kJ mol}^{-1} \tag{10}$$

Equations 4–6, 9, 10 and Eq. 1 have been used to obtain the equations of solution enthalpy of glycine and L-alanine in aqueous urea solutions at various temperatures:

$$\Delta_{sol}H^0_{(W+U)}(\text{Gly}) = 14.17 - 0.698m_U + 0.027m_U^2 + (-0.0469 + 0.0143m_U - 0.0008m_U^2) (T - 298.15) \tag{11}$$

$$\Delta_{sol}H^0_{(W+U)}(\text{Ala}) = 7.8 - 0.602m_U + 0.0483m_U^2 + 0.049(T - 298.15) \tag{12}$$

Equations 8, 11, 12 allow to determine the changes of reduced enthalpy, and also those of entropy and reduced Gibbs energy of solution of amino acids at the temperature changes from T_1 to T_2 using known thermodynamic relations:

$$\Delta(\Delta_{sol}H^0/T) = \Delta_{sol}H^0_2/T_2 - \Delta_{sol}H^0_1/T_1 \tag{13}$$

$$\Delta\Delta_{sol}S^0 = \Delta_{sol}S^0_2 - \Delta_{sol}S^0_1 = \int_{T1}^{T2} \frac{\Delta C^0_p}{T}dT \tag{14}$$

$$\Delta(\Delta_{sol}G^0/T) = \Delta(\Delta_{sol}H^0/T) + (-\Delta\Delta_{sol}S^0) \tag{15}$$

The values of changes of $\Delta_{sol}H^0/T$, $\Delta_{sol}S^0$ and $\Delta_{sol}G^0/T$ at the temperature rise from $T_1 = 288.15$ to $T_2 = 318.15$ K for glycine, L-proline, and L-alanine in aqueous urea solutions are presented in Table 4. From the data of Table 4 it is seen that in water the $\Delta(\Delta_{sol}G^0/T)$ values for glycine and alanine are negative unlike those of proline. These values are defined by the enthalpic contribution in case of glycine and proline and by the entropic one in case of alanine.

The $\Delta(\Delta_{sol}G^0/T)$ values become less negative for glycine and alanine and more positive for proline in 6 m aqueous urea solutions. Both entropic and enthalpic components are negative for glycine in 6 m aqueous urea solution. In aqueous urea solutions of alanine the negative value of $\Delta(\Delta_{sol}G^0/T)$ is determined by an entropic component as in

Table 4 Changes of thermodynamic characteristics (J mol^{-1} K^{-1}) of dissolution of amino acids in water and 6 m aqueous urea and those of transfer from water to aqueous urea solution at the temperature rise from 288 to 318 K

Amino acid	Glycine	L-Proline	L-Alanine
Water			
$\Delta(\Delta_{sol}H^0/T)$	-7.8	3.5	2.1
$-\Delta\Delta_{sol}S^0$	3.9	-2.5	-4.8
$\Delta(\Delta_{sol}G^0/T)$	-3.9	1.0	-2.7
6 m_U			
$\Delta(\Delta_{sol}H^0/T)$	-2.6	5.7	2.7
$-\Delta\Delta_{sol}S^0$	-0.5	-4.0	-4.8
$\Delta(\Delta_{sol}G^0/T)$	-3.1	1.7	-2.1
Water → 6 m_U			
$\Delta(\Delta_{tr}H^0/T)$	5.2	2.2	0.6
$-\Delta\Delta_{tr}S^0$	-4.4	-1.5	0
$\Delta(\Delta_{tr}G^0/T)$	0.8	0.7	0.6
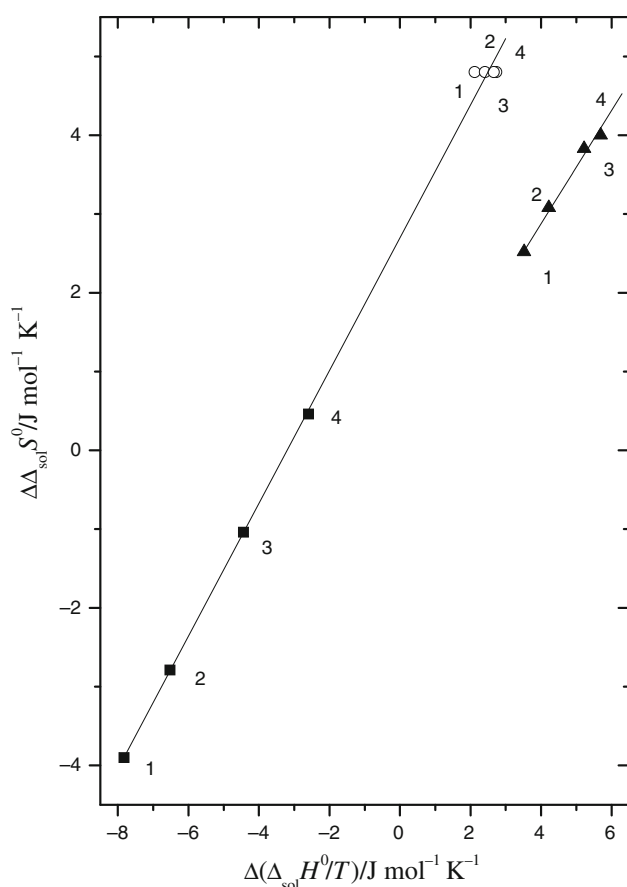
Fig. 2 The $\Delta\Delta_{sol}S^0$ values of amino acids in aqueous urea solutions versus the $\Delta(\Delta_{sol}H^0/T)$ values at the temperature rise from 288.15 to 318.15 K at $m_U = 0$ (1), $m_U = 1$ (2), $m_U = 3$ (3), $m_U = 6$ mol kg^{-1} (4). Glycine (*filled square*), L-alanine (*open circle*), L-proline (*filled triangle*). *Lines*—Eqs. 17, 18

Fig. 3 The $\Delta\Delta_{tr}S^0$ values of transfer of amino acids from water to aqueous urea solutions versus the $\Delta(\Delta_{tr}H^0/T)$ values at the temperature rise from 288.15 to 318.15 K at $m_U = 0$ (1), $m_U = 1$ (2), $m_U = 3$ (3), $m_U = 6$ mol kg^{-1} (4). Glycine (*filled square*), L-alanine (*open circle*), L-proline (*filled triangle*). *Line*—Eq. 19

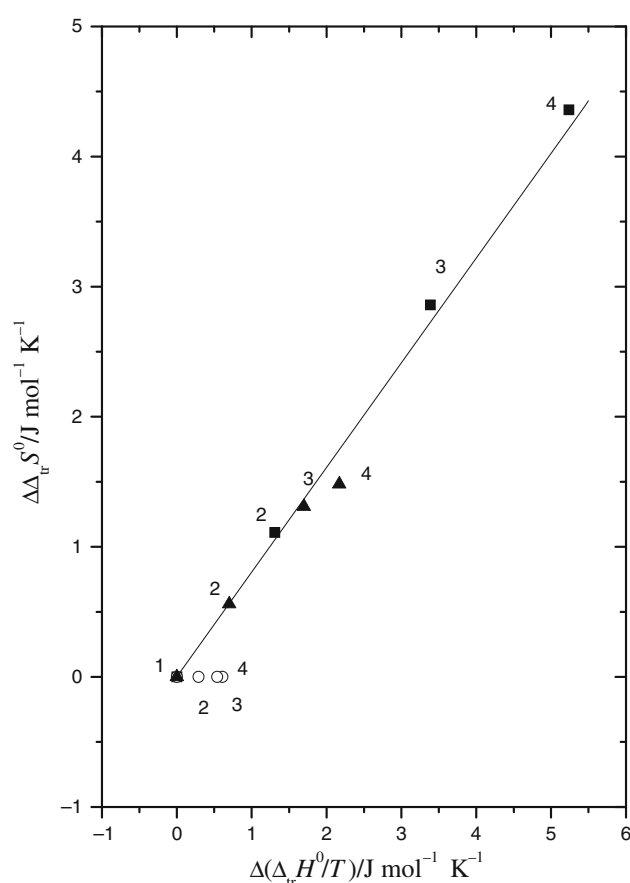water, while for proline-by the enthalpic one as in water as well.

The entropic component for proline is negative in water, as well as for alanine and becomes more negative in urea solution. In this sense proline is hydrophobic, as well as alanine. But on the basis of the $\Delta\Delta_{sol}S^0$ values proline is less hydrophobic, than alanine. The $\Delta\Delta_{sol}S^0$ values are increasing in the following sequence: glycine, proline, and alanine. The $\Delta(\Delta_{sol}G^0/T)$ values are negative for glycine and alanine, and are positive for proline, and are growing in series: glycine, alanine, proline.

In thermodynamic analysis of ternary systems the functions of transfer are often used and expressed as:

$$\Delta_{tr}Y^0_{(W\rightarrow W+U)} = \Delta_{sol}Y^0_{(W+U)} - \Delta_{sol}Y^0_{(W)}, \quad (16)$$

where $Y = G/T$, H/T or S in our case.

The sign of the $\Delta(\Delta_{tr}G^0/T)$ value for amino acids in question is determined by the enthalpic contribution (Table 4). The values of both components, namely, $\Delta(\Delta_{tr}H^0/T)$ and $\Delta\Delta_{tr}S^0$, are decreasing in series: glycine, proline, alanine.

It has been interesting to check up, whether the correlation between entropic and enthalpic components, known as a Barclay–Butler rule [28] is carried out. It has turned out that such relation takes place.

The $\Delta(\Delta_{sol}H^0/T)$ and $\Delta\Delta_{sol}S^0$ values of amino acids being studied have been calculated by Eqs. 8, 11–14 at $m_U = 0, 1, 3, 6$ mol kg^{-1}. In Fig. 2, the $\Delta\Delta_{sol}S^0 = f(\Delta(\Delta_{sol}H^0/T))$ dependences of amino acids in aqueous urea solutions of several concentrations are shown. These thermodynamic quantities for glycine and alanine obey the dependence:

$$\Delta\Delta_{sol}S^0 = (2.70 \pm 0.06) + (0.843 \pm 0.014)\Delta(\Delta_{sol}H^0/T),$$
$$R = 0.999, \text{ sd } = 0.17 \text{ Jmol}^{-1}\text{K}^{-1} \quad (17)$$

The corresponding values for proline belong to direct proportional dependence:

$$\Delta\Delta_{sol}S^0 = (0.719 \pm 0.008)\Delta(\Delta_{sol}H^0/T),$$
$$R = 0.994, \text{ sd } = 0.07 \text{ J mol}^{-1}\text{K}^{-1} \quad (18)$$

At a complete compensation a slope of the $\Delta\Delta_{sol}S^0 = f(\Delta(\Delta_{sol}H^0/T))$ dependence should be equal to 1. In reality

in both cases the changes of an enthalpic component slightly exceed the changes of an entropic one: the value of slope is less than 1, the $\Delta(\Delta_{sol}G^0/T)$ value has the same sign as the enthalpic component value. The greater slope of the dependence (17), than (18) one, means the more complete entropy–enthalpy compensation in case of glycine and alanine unlike proline.

We have also analyzed the thermodynamic characteristics of transfer of amino acids from water to aqueous urea solutions. In Fig. 3, the $\Delta\Delta_{tr}S^0 = f(\Delta(\Delta_{tr}H^0/T))$ dependence is presented. These values for amino acids under investigation belong to the common direct proportional dependence:

$$\Delta\Delta_{tr}S^0 = (0.805 \pm 0.033)\Delta(\Delta_{tr}H^0/T),$$
$$R = 0.99, \; sd = 0.23 \; J \; mol^{-1}K^{-1} \quad (19)$$

One can conclude about an identical degree of entropy–enthalpy compensation for glycine, alanine, and proline, while considering the transfer process.

Conclusions

The enthalpies of solution of L-proline in the mixtures of urea with water at $m_U = 0–6 \; mol \; kg^{-1}$ have been measured at 288.15, 298.15, 308.15, and 318.15 K by the calorimetric method for the first time. The two-parameter relation connecting the values of solution enthalpies of proline with urea concentration and temperature have been obtained.

For water–urea–L-proline the enthalpic pair interaction parameters are negative at temperatures investigated. It testifies to the predominance of the effect of direct interaction of L-proline with urea over the endothermic processes of partial dehydration of urea and amino acid. The enthalpic parameters of pair interaction become less negative with the temperature rise.

The temperature changes of reduced enthalpy, and also the change of entropy and reduced Gibbs energy of solution of L-proline in aqueous solutions of urea at the temperature rise from 288 to 318 K have been determined. Their comparison with the data for glycine and L-alanine has been carried out. It has been shown that the entropy–enthalpy compensation (Barclay–Butler rule) takes place for dissolution and transfer processes.

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