

## The enthalpies of interactions of some L- $\alpha$ -amino acids with urea molecule in aqueous solutions at 298.15 K

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**Summary.** Dissolution enthalpies of L- $\alpha$ -aminobutyric acid, L- $\alpha$ -isoleucine, L- $\alpha$ -phenylalanine, L- $\alpha$ -methionine, L- $\alpha$ -serine, L- $\alpha$ -threonine, L- $\alpha$ -cysteine, L- $\alpha$ -asparagine and L- $\alpha$ -glutamine in aqueous solutions of urea have been measured by calorimetry at 298.15 K. The obtained results were used to calculate the enthalpic interaction coefficients between the zwitterions of the L- $\alpha$ -amino acids and a molecule of urea in water. These values were interpreted in terms of the hydrophobic or hydrophilic effects of the side chains of amino acids on their interactions with a polar molecule of urea in water.

**Keywords:** L- $\alpha$ -amino acids – Enthalpies of solution – Aqueous solutions of urea

### Introduction

Natural amino acids, occurring in aqueous solutions in the form of zwitterions ( $R-C^{\alpha}HCO_2^{-}NH_3^{+}$ ) as well as the amino acid segments that form peptides and proteins, differ in side chains,  $-R$ , from themselves. It is the amino acid side substituents as the functional groups of polypeptide chains of proteins are responsible for specific interactions and the spatial architecture of these biomolecules. Side chains differentiate also the interactions of amino acids and their derivatives with surrounding molecules of water (that constitutes >90% of body fluids) and with other molecules or biomolecules occurring in an aqueous medium. Therefore, it is interesting to elucidate the mechanisms and energetics of interactions occurring in aqueous solutions between L- $\alpha$ -amino acids and the substances existing in living organisms or organic compounds with analogous functional groups to those in biomolecules. A role of such a model organic molecule is played by urea, a primary biomolecule occurring in living organisms as one of the components of the metabolic urea

cycle, and, in addition, possessing analogous functional groups to those in proteins.

To find the parameters that could differentiate the interactions of amino acid side chains with a molecule of urea in aqueous solutions, the enthalpies of solution of several L- $\alpha$ -amino acids in water and aqueous solutions were measured by calorimetry. The results obtained were used to calculate the enthalpic pair interaction coefficients between zwitterions of amino acids and a molecule of urea in water. The enthalpic heterogeneous pair interaction coefficients derived from the modified theory of McMillan Mayer (McMillan and Mayer, 1945; Franks et al., 1976; Friedman and Krishnan, 1973) well describe the summary energetic effects of interactions between the urea molecule and the molecules of the examined amino acids occurring with the competitive participation of water molecules.

### Experimental

Urea (U) (99.5% from Fluka), L- $\alpha$ -aminobutyric acid (Aba), L- $\alpha$ -isoleucine (Ile), L- $\alpha$ -phenylalanine (Phe), L- $\alpha$ -methionine (Met), L- $\alpha$ -serine (Ser), L- $\alpha$ -threonine (Thr), L- $\alpha$ -cysteine (Cys), L- $\alpha$ -asparagine (Asn) and L- $\alpha$ -glutamine (Gln) (all 99% from Aldrich). The water used in the experiments was deionized, distilled and degassed.

The enthalpies of solution ( $\Delta H_S$ ) were measured at 298.15 K with an isoperibol calorimeter (Pałecz, 1995). The temperature sensitivity was about  $4 \times 10^{-5}$  K, and temperature stability of the water thermostat was better than  $10^{-3}$  K. The ampoules containing the examined L- $\alpha$ -amino acids were filled in a dry box and weighed with a Sartorius RC 210D balance.

### Results and discussion

The dissolution enthalpies of the L- $\alpha$ -amino acids in aqueous solutions of urea containing from 0.5 to

**Table 1.** Standard enthalpies of solution of L- $\alpha$ -amino acids in aqueous urea solutions at 298.15 K

m mol(U)kg <sup>-1</sup> H <sub>2</sub> O	ΔH <sup>0</sup> <sub>S(W+U)</sub> kJ mol <sup>-1</sup>	Ser	Thr	Cys	Asn	Gln	Aba	Ile	Met	Phe
0.0	11.49 ± 0.03	10.33 ± 0.03	11.15 ± 0.03	21.64 ± 0.05	22.50 ± 0.06	0.795 ± 0.01	3.06 ± 0.01	11.30 ± 0.03	8.28 ± 0.03	
0.5	10.97 ± 0.04	10.02 ± 0.05	10.84 ± 0.04	20.90 ± 0.07	21.75 ± 0.09	0.41 ± 0.01	2.85 ± 0.02	11.12 ± 0.04	8.18 ± 0.04	
1.0	10.51 ± 0.05	9.68 ± 0.04	10.46 ± 0.05	20.30 ± 0.08	21.26 ± 0.10			10.91 ± 0.05	8.10 ± 0.04	
1.5	10.14 ± 0.04	9.33 ± 0.04	10.22 ± 0.04	19.30 ± 0.09	20.65 ± 0.09	0.16 ± 0.01	2.74 ± 0.01	10.78 ± 0.04	8.04 ± 0.05	
2.0	9.74 ± 0.04	9.04 ± 0.04	9.93 ± 0.04	18.69 ± 0.09	20.15 ± 0.09			10.64 ± 0.05	7.96 ± 0.04	
2.5	9.36 ± 0.04	8.71 ± 0.03	9.72 ± 0.04	18.09 ± 0.08	19.40 ± 0.8	-0.28 ± 0.01	2.57 ± 0.01	10.38 ± 0.04	7.92 ± 0.04	
3.0	9.04 ± 0.03	8.51 ± 0.04	9.51 ± 0.04	17.70 ± 0.08	19.20 ± 0.09	-0.50 ± 0.02	2.44 ± 0.01	10.26 ± 0.05	7.86 ± 0.04	
4.0										

3.0 mol(U) kg<sup>-1</sup> (water). The standard enthalpies of solution of L- $\alpha$ -amino acids (A) in water ( $\Delta H^0_{S(W)}$ ) and aqueous solutions of urea ( $\Delta H^0_{S(W+U)}$ ) were calculated as the mean of the results of 8 to 12 independent measurements within the concentration range 0.001 to 0.010 mol(A) kg<sup>-1</sup> (solvent). The determined standard enthalpies of solution of the L- $\alpha$ -amino acids in aqueous solutions of urea, together with standard deviations, are presented in Table 1.

The increase in the concentrations of urea in aqueous solutions brings a decrease in the values of standard dissolution enthalpies of the L- $\alpha$ -amino acids (Table 1).

The standard solution enthalpies of amino acids in water and in aqueous solution of urea were used to obtain the enthalpic heterogeneous pair interaction coefficients  $h_{AU}$  for the interaction between amino acids zwitterion and the urea molecule in water (Desnoyers et al., 1976). The standard solution enthalpies of the L- $\alpha$ -amino acids in aqueous solutions of urea are presented as a polynomial function:

$$\Delta H^0_{S(W+U)} = \Delta H^0_{S(W)} + 2h_{AU}m_U + 3h_{AUU}m_U^2 + \dots \quad (1)$$

where  $m_U$  is molal concentration of urea (mol kg<sup>-1</sup>),  $h_{AU}$  is the enthalpic pair interaction coefficient and  $h_{AUU}$  denotes the enthalpic triplet interaction coefficient. The enthalpic pair and triplet interaction coefficients determined in this work are listed in Table 2. The interpretation of the triplet interaction coefficient is obscured by the fact that it also contain pairwise interaction terms.

The values of enthalpic interaction coefficients of heterogeneous pairs describe the global effect that is a sum of exothermic processes of direct interactions between the

**Table 2.** Heterogeneous enthalpic pair interaction coefficients for L- $\alpha$ -amino acids with urea in water at 298.15 K

L- $\alpha$ -amino acids	CH <sub>2</sub>	$h_{AU}/J\text{ kg mol}^{-2}$	$h_{AUU}/J\text{ kg}^2\text{ mol}^{-3}$
Glycine (Gly)	0.5	-390.2*	
Alanine (Ala)	1.5	-238.2*	
Aminobutyric acid (Aba)	2.5	-185	3.4
Valine (Val)	3.5	-116*	
Leucine (Leu)	4.5	-98*	
Isoleucine (Ile)	4.5	-95	2.9
Phenylalanine (Phe)		-97	6.2
Methionine (Met)		-180	1.1
Serine (Ser)		-511	23
Threonine (Thr)		-350	9.2
Cysteine (Cys)		-358	18.8
Asparagine (Asn)		-819	33
Glutamine (Gln)		-690	28.2

\* Palecz, 1998

statistic zwitterions of the examined amino acids and a statistic molecule of urea in aqueous solutions, occurring with the participation of water molecules. To provide conditions for direct interactions between two polar molecules or ions, some amount of water molecules of hydration layers must be removed as they are a spheric hindrance to their direct approach. Water molecules pushed out from the hydration sheaths deep into the solution assume an order typical for bulk water. Thus, the enthalpic interaction coefficients of amino acid zwitterions with a molecule of urea are a sum of:

- the direct interaction between the zwitterionic "head" ( $-\text{CHCO}_2^--\text{NH}_3^+$ ) of the examined amino acid and the polar molecule of urea (exothermic process),
- partial dehydrations of hydration sheaths of the zwitterionic "head" of amino acid and the polar molecule of urea (endothermic processes).

In the case of amino acids with polar groups in side chains, the global effects described by the enthalpic pair interaction coefficients, become supplemented with exothermic processes connected with the interaction of these polar groups with the urea molecule as well as with endothermic processes of the partial hydration of these polar groups.

For amino acids possessing nonpolar side substituents,  $-\text{R}$ , the global effect of their interaction with the molecule of urea comprises also additional processes generated by the phenomenon of hydrophobic hydration. Water molecules in the direct vicinity of hydrophobic groups of side chains reinforce hydrogen bonds between themselves (Ide et al., 1997; Palecz et al., 2000). As a result of the cooperativeness of hydrogen bonds, the effect of the reinforced interactions is transferred to water molecules surrounding polar or ionic groups in the amino acid molecule under investigation. This brings about the reinforcement of interactions between water molecules of the hydration layer and polar or ionic groups. Therefore, the removal of some water molecules from the hydration sheath, which are a hindrance to the direct approach of the interacting groups, requires an increased input of energy. Thus, the exothermic effect of direct interactions between the polar groups of the examined molecules is weakened and sometimes dominated by the endothermic effects of partial dehydrations of the solvation layers of interacting molecules (the values of enthalpic pair interaction coefficients become then positive).

The enthalpic interaction coefficient of urea molecule with glycine molecule (Gly) determined in aqueous solutions, in which  $-\text{H}$  is a side substituent at  $\text{C}^\alpha$ , possess a

negative value (Palecz, 1998) indicating a dominating exothermic effect of direct interactions of glycine zwitterions with the urea molecule over the endothermic dehydration effects. The replacement of hydrogen at  $\text{C}^\alpha$  in the amino acid molecule with an alkyl substituent in the case of L- $\alpha$ -aminobutyric acid (Aba) or L- $\alpha$ -isoleucine (Ile), similarly as in the case of L- $\alpha$ -alanine (Ala), L- $\alpha$ -valine (Val) or L- $\alpha$ -leucine (Leu) (Palecz, 1998), brings about an increase in the values of enthalpic pair interaction coefficients ( $h_{\text{AU}}$ ) between the urea molecule and amino acid zwitterions (Table 2). This testifies to an increased endothermic contribution to the summary effect described by enthalpic pair interaction coefficients, connected with the effects of partial dehydration, intensified by the hydrophobic hydration of nonpolar alkyl groups. The values of enthalpic interaction coefficients,  $h_{\text{AU}}$ , of L- $\alpha$ -amino acids containing alkyl side chains in their molecules increase in the following sequence: Gly < Ala < Aba < Val < Leu < Ile (Table 2, Fig. 1). The increase in the length of alkyl side substituent linked to the zwitterion head causes the summary effect of urea-amino acid interaction in water to become more endothermic. The increase in the values of enthalpic interaction coefficients,  $h_{\text{AU}}$ , of the amino acid under consideration becomes smaller and smaller as the side chain is extended with additional  $\text{CH}_2$  groups (according to Wood and Hiltzik, 1980, it is accepted that the  $\text{CH}_3$  group corresponds to 1.5  $\text{CH}_2$ , while  $\text{CH}$  corresponds to 0.5  $\text{CH}_2$ ). Probably the phenomena of hydrophobic hydration that reinforce the interactions between water molecules surrounding the more remote alkyl groups, are transferred to a lesser extent onto water molecules that hydrate the zwitterion "head" of amino acid. Thus, the endothermic effect of partial dehydration of the solvation sheath in the polar portion of amino acid does

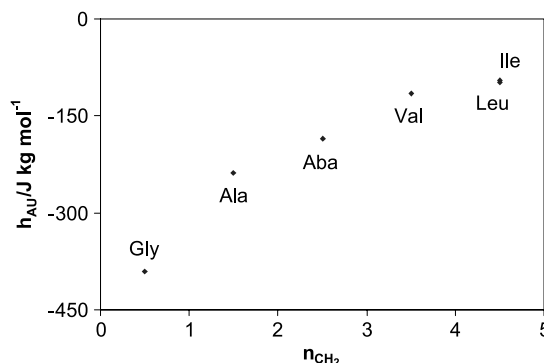


Fig. 1. Dependence of the enthalpic pair interaction coefficients of L- $\alpha$ -amino acids and urea in water on the number of  $\text{CH}_2$  groups in the side chain of amino acid molecule

not increase proportionally to the number of carbon atoms in the side chain, similarly as in aqueous solutions of amino acids and electrolytes (Palcz, 2000).

In the case of other examined natural amino acids containing nonpolar side chains: L- $\alpha$ -methionine (Met), L- $\alpha$ -phenylalanine (Phe), the endothermic dehydrating effect also grows and contributes to the summary effect that describes the interactions between zwitterions of these amino acids and urea molecules, in comparison with glycine zwitterions (Table 2).

The extension of L- $\alpha$ -alanine side chain with a polar thiol group, -SH, brings about a decrease in the enthalpic heterogeneous pair interaction coefficient of urea molecule and cysteine zwitterion ( $h_{\text{Ala-U}} > h_{\text{Cys-U}}$ ), see Table 2. This results from the exothermic contribution made by the interactions between the polar molecule of urea and -SH to the global effect of interaction between urea and zwitterions of amino acid. The exothermic effect connected with direct interactions between the statistic urea molecule and cysteine zwitterions is greater than the endothermic effects of partial dehydrations of these interacting molecules (Table 2).

Similarly, the replacement of hydrogen atom in the alkyl side chain of L- $\alpha$ -alanine (Ala) with the strongly polar group -OH (L- $\alpha$ -serine) brings about a drop in the value of enthalpic interaction coefficient of the urea molecule with serine molecule ( $h_{\text{Ser-U}}$ ) in comparison with the molecule of alanine ( $h_{\text{Ala-U}}$ ) (Table 2). The addition of a nonpolar group -CH<sub>2</sub>- to the side chain of L- $\alpha$ -serine, resulting to the formation of L- $\alpha$ -threonine (Thr), brings about an increase in the enthalpic pair interaction coefficient of urea-threonine ( $h_{\text{Thr-U}}$ ) in comparison with serine ( $h_{\text{Ser-U}}$ ) (Table 2). These changes are caused by the presence of the hydrophobic group -CH<sub>2</sub>- in the side chain of threonine that through hydrophobic hydration intensifies the increase in the endothermic contribution connected with the dehydration effects of interacting polar groups.

Analyzing the values of enthalpic pair interaction coefficients of urea and zwitterions of L- $\alpha$ -alanine, L- $\alpha$ -cysteine and L- $\alpha$ -serine, one can observe a decrease in the value of enthalpic coefficient  $h_{\text{AU}}$  with the substitution of the alanine side chain with polar groups such as -SH and -OH (Table 2). The values of enthalpic heterogeneous pair interaction coefficients of alanine and its derivatives change in the following sequence:  $h_{\text{Ala-U}} > h_{\text{Cys-U}} > h_{\text{Ser-U}}$ . The replacement of -SH with -OH brings about an increase in direct exothermic interactions between the urea molecule and amino acid zwitterions, which seems to be caused by the greater ability of the -OH

group of serine to form donor-acceptor bonds in comparison with the -SH group of cysteine (Pillai et al., 1979).

The replacement of the hydrogen atom by the polar amide group in alanine (Ala) or in the amino butyric acid (Aba) leads to rise of asparagines (Asn) and glutamine (Gln). The fall in values of the enthalpic pair interaction coefficients between describing amino acids and the urea molecule (Table 2), reflects the exothermic contribution what brings in global effect of the interactions, amide groups in side chains of these amino acids with polar molecule of urea.

The determined enthalpic pair interaction coefficients between the urea molecule and amino acid zwitterions ( $h_{\text{AU}}$ ) (Table 2) describing the summary process of interactions between the examined statistical molecules in solution that takes place with the competitive participation of water molecules well correlate with the hydrophobicity parameter of amino acid side chains found on the basis of the enthalpic homogeneous pair interaction coefficients of natural amino acids (Palcz, 2002) (Fig. 2). The above relationships shown in Fig. 2 are described by the equation of straight line, for which  $R^2 = 0.967$ . The linear relationship indicates similar contributions made by the effects of partial dehydration of hydration layers of interacting zwitterion "heads" as well as by the polar side chains of amino acids and the processes connected with the hydrophobic effects of nonpolar side chains to the summary effect of interactions taking place in aqueous solutions of the system under discussion.

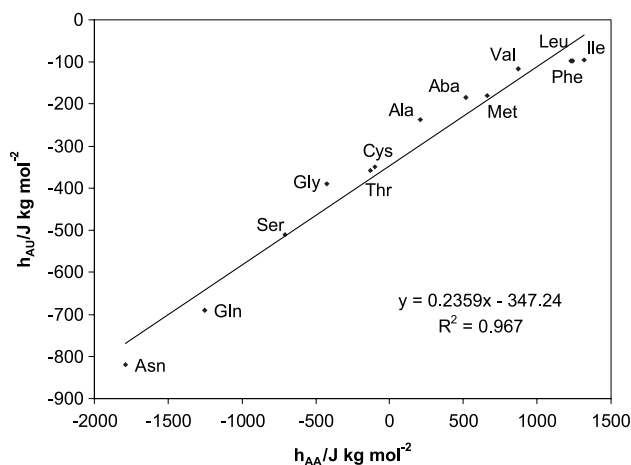
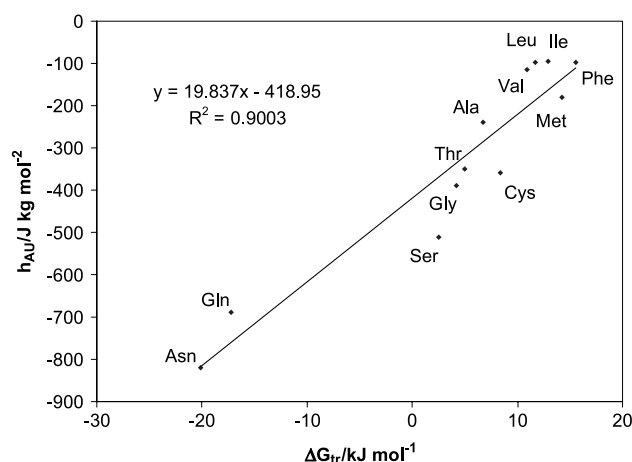


Fig. 2. Relationship between the enthalpic homogeneous pair interaction coefficients  $h_{\text{AA}}$  of amino acids in water and the enthalpic pair interaction coefficients  $h_{\text{AU}}$  amino acids zwitterions – urea molecule in water



**Fig. 3.** Relationship between the free energy transfer of amino acids (Engelman et al., 1986) and the enthalpic pair interaction coefficients amino acids zwitterions – urea molecule in water

The maintained values of the enthalpic pair interaction coefficients of studied amino acids with the molecule of urea we compared with calculated by Engelman (Engelman et al., 1986) of free energy transfer of the helices (in amino acids sequences) built from given individual the amino acid from interior of membrane protein to water. These values according to Stryer (1995) differentiate well the hydrophobicity of side amino acids chains, permitting to identify the helices of transmembranous proteins. The received graph (Fig. 3) displays the dependence, evidencing the possibility of the utilization of enthalpic pair interaction coefficients between zwitterions of amino acids and the urea molecule as parameters characterizing the affinity of side chains of amino acids to water.

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