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Studies of heterogeneous interactions between *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea molecules in water at 298.15 K

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ABSTRACT

The enthalpies of solution of *N*-acetyl-*N*′-methyl-L-α-threoninamide, *N*-acetyl-*N*′-methyl-L-α-tyrosinamide, *N*-acetyl-*N*′-methyl-L-α-histidinamide, *N*-acetyl-*N*′-methyl-L-α-tryptophanamide have been measured in water and in aqueous urea solutions at 298.15 K. The mixing enthalpies of aqueous urea solution with aqueous *N*-acetyl-*N*′-methyl-L-α-valinamide solution and their respective enthalpies of dilution by water have been determined at 298.15 K. The experimental results were used to obtain the enthalpic coefficients of the interaction between amino acid amides and urea molecules in water based on McMillan–Mayer's model. The values of the interaction parameters were interpreted in terms of the hydrophobic or hydrophilic properties of the amino acid radicals in the examined amide molecules and the influence on their interactions with a urea molecule in water.

1. Introduction

The interpretation of research results concerning the interactions, in which protein polypeptide chains take part, is difficult due to the very complex structure of these macromolecules. For that reason studies have been carried out on low-molecular model compounds containing the same functional groups as those of polypeptide chains. The model compounds include amino acids [1–5], amides of natural amino acids [6–10], dipeptides [11–14] and tripeptides [15,16]. From among the compounds mentioned only the molecules of *N*-acetyl-*N*′-methyl-L-α-amino acid amides posses no terminal ionic groups, which allows one to omit coulombic forces in the analysis of the interactions. Urea is one of the elements of the living organism fluids and can also perform the function of a factor that diversifies the hydrophobic–hydrophilic properties of amino acid side substituents [3]. In the present study, the interactions between several selected *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea molecule in aqueous solutions were examined with the use of calorimetric methods.

Based on the values of the enthalpy of solution of *N*-acetyl-*N*′-methyl-L-α-threoninamide, *N*-acetyl-*N*′-methyl-L-α-tyrosinamide, *N*-acetyl-*N*′-methyl-L-α-histidinamide, *N*-acetyl-*N*′-methyl-L-α-tryptophanamide in water and in aqueous solutions of urea and on the enthalpy of interactions between *N*-acetyl-*N*′-methyl-L-α-valinamide and urea molecule in water, the enthalpic heterogeneous pair interaction coefficients were calculated. These parameters were derived from McMillan–Mayer's theory [17] and modified by Friedman and Krishnan [18], Franks et al. [19] and Desnoyers et al. [20].

2. Experimental

The amino acid amides used for investigations: *N*-acetyl-*N*′-methyl-L-α-threoninamide, *N*-acetyl-*N*′-methyl-L-α-tyrosinamide, *N*-acetyl-*N*′-methyl-L-α-histidinamide, *N*-acetyl-*N*′-methyl-L-α-tryptophanamide were prepared for examinations at the Department of Organic Chemistry at the University of Łódź. The amides were synthesized from commercially available amino acids according to the reaction sequence: esterification [21], acetylation [22] and amination [23]. Melting points of obtained compounds were determined initially with a Boetius hot stage apparatus and next with a differential scanning calorimeter SETARAM TG-DSC 111. The final purity of all the samples was checked by the same differential scanning calorimeter using the peak profile method [24–26] and ranged from 98.5 to 99.6 mol%. Indium (NIST-RM 758, purity 99.99%) was used as reference material.

The infrared spectra were registered with a Nexus FT-IR spectrometer (Thermo Nicolet). The ^{1}H and ^{13}C NMR spectra were

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recorded at 200 MHz and 50 MHz, respectively, using a Varian Gemini 200 instrument. The characterizations of obtained compounds are given below:

N-Acetyl-*N*′-methyl-L-α-tyrosinamide, melting point (m.p.) 198–199 °C (Boetius) and 191–192 °C (DSC 111) (ethyl acetate–methanol, 4:1) (191–192 °C [23]); IR (KBr) ν 1656 and 1615 cm^{-1} (CO); ^1H NMR (DMSO-d_6) δ = 9.17 (1H, s, OH), 8.06 and 8.02 (1H, two s, NH), 7.91–7.80 (1H, m, NH), 6.98 (2H, d, J = 8.5 Hz, 3.5-H), 6.62 (2H, d, J = 8.5 Hz, 2.6-H), 4.40–4.22 (1H, m, α-H), 2.81 (2H, dd, J = 5.0 and 14.0 Hz, β-H), 2.68–2.48 (3H, NCH_3 overlapping with DMSO), 1.75 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ = 171.8, 169.1, 155.8, 130.1, 128.3, 114.9, 54.6, 37.2, 25.7, 22.7.

N-Acetyl-*N*′-methyl-L-α-histidinamide, m.p. 259–262 °C (Boetius) and 258–259 °C (DSC 111) (methanol) (260 °C [27]); IR (KBr) ν 1656 and 1615 cm^{-1} (CO); ^1H NMR (DMSO-d_6) δ = 11.69–11.73 (1H, m, 3-$H_{imidazole\text{-}4\text{-}yl}$), 8.06 and 8.02 (1H, two s, NH), 7.88–7.72 (1H, m, NH), 7.50 (1H, s, 2-$H_{imidazole\text{-}4\text{-}yl}$), 6.72 (1H, s, 5-$H_{imidazole\text{-}4\text{-}yl}$), 4.50–4.30 (1H, m, α-H), 2.60–2.83 (2H, m, β-H), 2.58–2.46 (3H, NCH_3 overlapping with DMSO), 1.75 (3H, s CH_3); ^{13}C NMR (DMSO-d_6) δ = 171.9, 169.4, 134.8, 123.4, 116.9, 53.0, 29.8, 25.8, 22.8.

N-Acetyl-*N*′-methyl-L-α-tryptophanamide, m.p. 188–189 °C (Boetius) and 184–185 °C (DSC 111) (water) (184–185 °C [28]); IR (KBr) ν 1677 and 1637 cm^{-1} (CO); ^1H NMR (DMSO-d_6) δ = 10.77 (1H, s, 1-NH), 8.06 and 8.02 (1H, two s, NH), 7.88–7.10 (1H, m, NH), 7.56 (1H, d, J = 8.0 Hz, 7-H), 7.30 (1H, d, J = 7.5 Hz, 4-H), 7.14–6.88 (3H, m, 2,5,6-H), 4.52–4.34 (1H, m, α-H), 3.06 (1H, dd, J = 5.4 and 14.5 Hz, β-H), 2.86 (1H, dd, J = 8.9 and 14.5 Hz, β-H), 2.58–2.46 (3H, NCH_3 overlapping with DMSO), 1.76 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ = 172.2, 169.2, 136.2, 127.5, 123.6, 121.0, 118.6, 118.4, 111.5, 110.5, 53.7, 28.2, 25.8, 22.8.

The results of analogous analysis for *N*-acetyl-*N*′-methyl-L-α-threoninamide was described previously [10]. The samples of *N*-acetyl-*N*′-methyl-L-α-valinamide (Bachem) and urea (U) (99.5%, Fluka) were recrystallized from water–methanol mixture and were dried under reduced pressure at 298 K for 72 h.

Table 1
Names, abbreviations and structures of *N*-acetyl-*N*′-methyl-L-α-amino acid amides examined and discussed in this work.

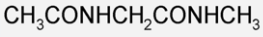
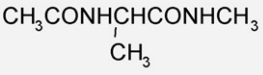
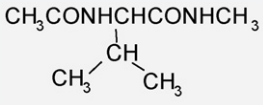
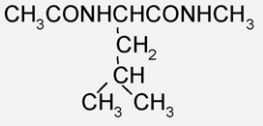
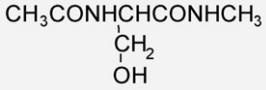
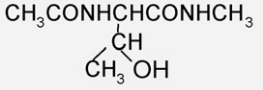
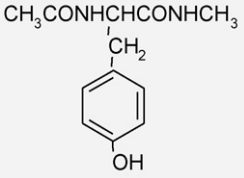
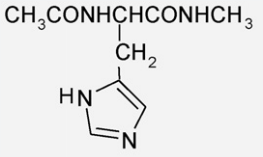
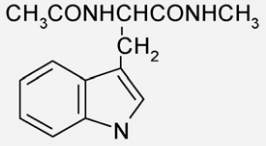
Name	Abbreviation	Structure
N-Acetyl-*N*′-methylglycinamide	$AcGlyNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-alaninamide	$AcAlaNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-valinamide	$AcValNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-leucinamide	$AcLeuNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-serinamide	$AcSerNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-threoninamide	$AcThrNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-tyrosinamide	$AcTyrNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-histidinamide	$AcHisNHCH_3$	
N-Acetyl-*N*′-methyl-L-α-tryptophanamide	$AcTrpNHCH_3$	

Table 2
Standard enthalpies of solution $\Delta_{sol}H_m^{\infty}$ of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in aqueous urea solutions at 298.15 K with the standard deviations of the obtained values.

m_U (mol/kg)	$\Delta_{sol}H_m^{\infty}$ (kJ mol^{-1})			
	AcThrNHCH$_3$	AcTyrNHCH$_3$	AcHisNHCH$_3$	AcTrpNHCH$_3$
0.00	−6.95 ± 0.02	11.64 ± 0.06	12.05 ± 0.03	13.50 ± 0.07
0.25	–	11.50 ± 0.06	–	13.16 ± 0.06
0.50	−7.02 ± 0.03	11.35 ± 0.09	11.56 ± 0.03	12.74 ± 0.06
1.00	−7.10 ± 0.03	10.98 ± 0.05	11.10 ± 0.05	12.10 ± 0.06
1.50	–	10.63 ± 0.09	10.67 ± 0.03	11.68 ± 0.08
2.00	−7.17 ± 0.03	10.30 ± 0.06	10.34 ± 0.05	11.22 ± 0.06
2.50	−7.21 ± 0.04	10.00 ± 0.03	10.27 ± 0.05	11.10 ± 0.07
3.00	−7.24 ± 0.05	9.65 ± 0.03	10.24 ± 0.08	11.00 ± 0.07

The water content of the substances was tested with a differential scanning calorimeter SETARAM TG-DSC 111 and was lower than 0.2% of sample mass. The water used in the experiments was deionised and distilled three times. The structures of the *N*-acetyl-*N*′-methyl-L-α-amino acid amides investigated and analyzed in this work are given in Table 1.

The enthalpies of solution of *N*-acetyl-*N*′-methyl-L-α-threoninamide, *N*-acetyl-*N*′-methyl-L-α-tyrosinamide, *N*-acetyl-*N*′-methyl-L-α-histidinamide and *N*-acetyl-*N*′-methyl-L-α-tryptophanamide were measured in water and in aqueous solutions of urea using an isoperibol calorimeter [14]. The accuracy of enthalpy measurements was $0.005\Delta_{sol}H_m$. The examined aqueous solutions containing from 0.25 to 3.00 mol(U)/kg(water) and the samples of *N*-acetyl-*N*′-methyl-L-α-amino acid amides (Am) (1×10^{-3} to 5×10^{-3}) mol(Am)/kg(solvent) were prepared by weight using Mettler AE240 balance within the precision $\pm 10^{-5}$ g. The standard enthalpies of solution of selected amides were determined by the linear extrapolation to zero concentration of the enthalpies of solution values obtained for six to eight independent measurements.

Because of low solubility of *N*-acetyl-*N*′-methyl-L-α-valinamide in water the enthalpies of dilution (instead of the enthalpies of solution) were determined with an isothermal calorimeter SETARAM MS-80D at 298.15 K. This instrument was equipped with cells made of stainless steel having a diameter of 17 mm. The measurements were carried out in a reversal mixing vessel with a capacity of 10 cm^3 with a small compartment of 0.6 cm^3 or 6.7 cm^3 in the volume. The apparatus and procedure used were the same as those described in earlier papers [10,29].

3. Results and discussion

The obtained standard enthalpies of solution of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water and in aqueous solutions of urea together with standard deviations are listed in Table 2. The enthalpy of solution data was used to obtain the enthalpic heterogeneous pair interaction coefficients h_{AmU} for the interaction between amides and the urea molecule in water. To this aim, the standard solution enthalpies of the amides $\Delta_{sol}H_m^{\infty}(W+U)$ in aqueous solutions of urea were presented as a following polynomial function [20]:

$$\Delta_{sol}H_m^{\infty}(W+U) = \Delta_{sol}H_m^{\infty}(W) + 2m_U h_{Am} + 3m_U^2 h_{AmUU} + \cdots \quad (1)$$

in which $\Delta_{sol}H_m^{\infty}(W)$ is the standard enthalpy of solution of amide in water, m_U is the molal concentration of urea (mol/kg), h_{AmU} is the enthalpic pair interaction coefficient and h_{AmUU} denotes the enthalpic triplet interaction coefficient. The enthalpic interaction parameters determined in this work are listed in Table 3. As the h_{AmUU} coefficients contain some contributions from the pairwise interaction terms [30] they are not discussed in this paper.

Table 3
Heterogeneous enthalpic pair h_{AmU} and triplet h_{AmUU} interaction coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides with urea in aqueous solution at 298.15 K.

N-Acetyl-*N*′-methyl-L-α-amino acid amides	h_{AmU} (J kg mol^{-2})	h_{AmUU} (J kg^2 mol^{-3})
AcGlyNHCH$_3$	−255[a]	–
AcAlaNHCH$_3$	−70[a]	–
AcValNHCH$_3$	177 ± 34	−10 ± 5
AcLeuNHCH$_3$	270[a]	–
AcSerNHCH$_3$	−273[a]	–
AcThrNHCH$_3$	−73 ± 13	6 ± 4
AcTyrNHCH$_3$	−344 ± 24	3 ± 2
AcHisNHCH$_3$	−632 ± 78	71 ± 24
AcTrpNHCH$_3$	−835 ± 140	112 ± 45

[a] Nowicka et al. [8].

The value of the enthalpic coefficient of interaction of *N*-acetyl-*N*′-methyl-L-α-valinamide molecule with urea molecule in aqueous solution h_{AmU} was determined on the basis of measurements of mixing enthalpy of aqueous solutions of these substances and the dilution enthalpy of aqueous solutions of amide in water and the dilution enthalpy of aqueous solutions of urea in water. Using the experimental values of the functions mentioned above the enthalpy of interaction ΔH^{**} of *N*-acetyl-*N*′-methyl-L-α-valinamide molecules and urea can be determined as follows [31]:

$$\Delta H^{**} = \Delta_{mix}H(m_{Am,i}m_{U,i} \rightarrow m_{Am,f}m_{U,f}) - \Delta_{dil}H(m_{Am,i} \rightarrow m_{Am,f}) - \Delta_{dil}H(m_{U,i} \rightarrow m_{U,f}) \quad (2)$$

where $m_{Am,i}$, $m_{Am,f}$, $m_{U,i}$, $m_{U,f}$ designate the initial and final molalities of amide and urea, respectively. The obtained ΔH^{**} values are given in Table 4.

Additionally, the enthalpy of interaction of amide and urea molecules ΔH^{**} was described with the equation proposed by Desnoyers et al. [20,31]:

$$\Delta H^{**} = 2h_{AmU}m_{U,f}(m_{Am,f} - m_{Am,i}) + 3h_{AmAmU}m_{U,f}(m_{Am,f}^2 - m_{Am,i}^2) + 3h_{AmUU}m_{U,f}(m_{Am,f} - m_{Am,i})(m_{U,f} + m_{U,i}) + \cdots \quad (3)$$

in which h_{AmU}, h_{AmAmU}, h_{AmUU} are the pairwise and the triplet enthalpic interaction coefficients. The h_{AmU} values were determined by means of the multi-parameter linear regression and are also listed in Table 3.

The values of the enthalpic heterogeneous pair interaction coefficients h_{AmU} are a measure of interactions proceeding between amide and urea molecules with the competitive co-contribution of water molecules. The global effect is a sum of superimposing processes:

Table 4
The enthalpies of interactions ΔH^{**} of *N*-acetyl-*N*′-methyl-L-α-valinamide molecules and urea in water at 298.15 K.

$m_{Am,i}$ (mol/kg)	$m_{Am,f}$ (mol/kg)	$m_{U,i}$ (mol/kg)	$m_{U,f}$ (mol/kg)	ΔH^{**} (J kg^{-1})
0.0534	0.0088	0.0456	0.0100	4.263
0.0534	0.0103	0.0456	0.0199	4.132
0.0534	0.0133	0.0456	0.0243	4.097
0.0534	0.0352	0.0456	0.0291	4.243
0.0652	0.0121	0.0546	0.0192	4.087
0.0652	0.0213	0.0546	0.0265	4.049
0.0652	0.0348	0.0546	0.0291	4.139
0.0869	0.0368	0.0742	0.0289	3.990
0.0869	0.0398	0.0742	0.0345	3.942
0.0869	0.0429	0.0742	0.0477	3.813
0.0869	0.0460	0.0742	0.0523	3.805
0.0881	0.0139	0.0760	0.0189	3.987
0.0881	0.0334	0.0760	0.0265	3.988
0.0881	0.0475	0.0760	0.0380	3.973

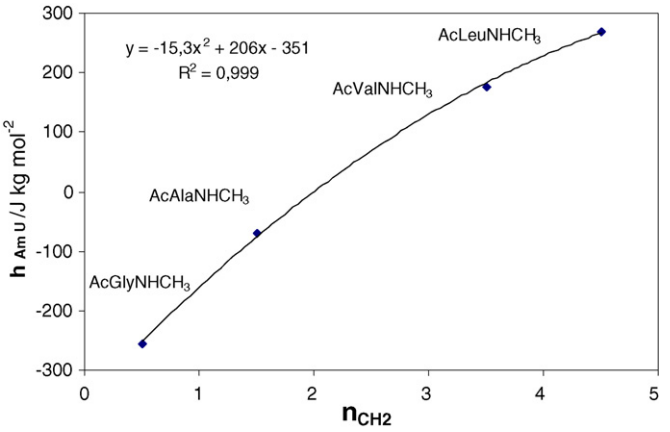


Fig. 1. Dependence of the heterogeneous enthalpic pair interaction coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea in aqueous solution h_{AmU} on the number of methyl groups n_{CH_2} in the amino acid side chain.

(a) exothermic interactions between polar groups of the molecules investigated;
(b) endothermic partial dehydration of the hydration shields of reacting polar groups.

From the analysis of the values of the enthalpic heterogeneous pair interaction coefficients (Table 3) of *N*-acetyl-*N*′-methyl-L-α-amino acid amides with alkyl side substituents and *N*-acetyl-*N*′-methylglycinamide it follows that these coefficients increase with the increasing length of alkyl chain. These changes are brought about by hydrophobic effects that intensify the interactions between water molecules in direct neighbourhood of alkyl groups. The effects of intensified interactions due to the cooperation of hydrogen bonds are transferred onto water molecules that hydrate peptide bonds, polar or ionic groups of the amino acid side substituents of the amide under investigation. Consequently, the dehydration effects of the reacting polar groups require more energy, which results in the increased values of the enthalpic interaction coefficients (domination of endothermic effects). This is shown by the increase in the dehydration contributions, which changes the sign of coefficients for $AcValNHCH_3$–urea and $AcLeuNHCH_3$–urea systems (Table 3), indicating that the dehydration effects contributions dominate over the effects of direct interactions between the molecules of these amides and urea molecules. These changes are well illustrated by the diagram of dependence of the enthalpic heterogeneous pair interaction coefficients h_{AmU} on the number of CH_2 groups in the side substituents of these amides (Fig. 1), using the convention in which the CH_3 group corresponds to 1.5 CH_2 while CH corresponds to 0.5 CH_2 [32]. The enthalpic heterogeneous pair interaction coefficients increase with increasing length of the alkyl side substituent in the amide investigated in the following sequence: $AcGlyNHCH_3 < AcAlaNHCH_3 < AcValNHCH_3 < AcLeuNHCH_3$.

As we have observed earlier [8], the replacement of hydrogen atom in the methyl group in the side substituent of methyl amide of *N*-acetyl-L-α-alanine with the hydroxyl group ($AcSerNHCH_3$) brings about a decrease in the value of the enthalpic pair interaction coefficient h_{AmSerU}. This is due to the exothermic interactions between the statistical polar OH group and urea molecule. On the other hand, the results presented in this work show that the replacement of the same hydrogen atom in *N*-acetyl-*N*′-methyl-L-serinamide with CH_3 group ($AcThrNHCH_3$) causes an increase in the enthalpic pair interaction coefficient h_{AmThrU} (Table 3). These changes seem to be due to hydrophobic effect caused by the present of additional methyl group in the molecule of *N*-acetyl-*N*′-methyl-L-α-threoninamide.

The replacement of hydrogen atom in the methyl group in the side substituent of methyl amide of *N*-acetyl-L-α-alanine with 4-hydroxyphenyl ring ($AcTyrNHCH_3$) brings about a decrease in the value of the enthalpic coefficient of interaction between the molecule of *N*-acetyl-*N*′-methyl-L-α-tyrosinamide and urea molecule. Probably, such negative shift in the h_{AmU} values results from the increase in the exothermic interactions caused by the presence of hydroxyl group. Moreover, the exothermic contribution to these interactions can be made by the aromatic benzene ring, whose delocalized electrons forming π bonds can interact with the urea molecule. A similar effect is observed in the other amides with aromatic substituents, i.e. $AcHisNHCH_3$ and $AcTrpNHCH_3$. Therefore, the enthalpic heterogeneous pair interaction coefficients of the three analyzed above amino acid amides have more negative values than the enthalpic coefficients describing the interactions of *N*-acetyl-*N*′-methylglycinamide with the urea molecule (Table 3).

The values of the enthalpic coefficients of amide–urea interactions h_{AmU} were compared with those of the enthalpic coefficients of amino acid–urea interactions h_{AU} [3], belonging to the scale describing the hydrophobic–hydrophilic properties of the amino acid side chains. The relationship shown in Fig. 2 illustrates the changes that testify to a similar contribution of amino acid side substituents to the total effects described by the enthalpic heterogeneous pair interaction coefficients h_{AmU} and h_{AU}.

In our previous papers [3,8,10,14], we have found the correlations between the enthalpic interaction coefficients for the urea–amino acid derivatives pairs and the various parameters characterizing the properties of amino acid side chains. As is known, it is difficult to find parameter which would allow one to classify of amino acids with nonpolar and polar side substituents. We have selected to analyse of results presented here the Engelman's scale [33], which differentiates the hydrophobic and hydrophilic properties of amino acid side groups. The values of transfer free energy for amino acid side chains in α-helical polypeptides given by Engelman et al. are the sum of hydrophobic and hydrophilic components for each amino acid. Moreover, the authors taken into account the participation of serine and threonine residues in the formation of hydrogen bonds with the backbone carbonyl groups in α-helical segments of protein. Similarly, we have suggested recently [10] the possibility of involvement of hydroxyl groups of the *N*-acetyl-*N*′-methyl-L-α-serinamide and *N*-acetyl-*N*′-methyl-L-α-threoninamide molecules in the intermolecular bond. Therefore, in Fig. 3 we have presented the relationship between the

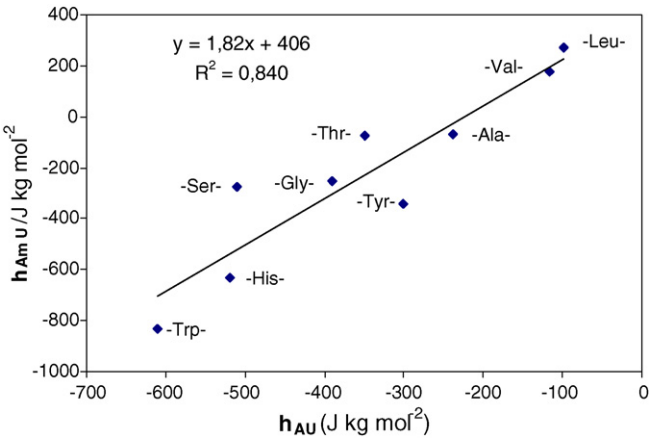


Fig. 2. Relationship between the enthalpic interaction coefficients of pairs *N*-acetyl-*N*′-methyl-L-α-amino acid amide–urea (h_{AmU}) and the enthalpic interactions coefficients of pairs L-α-amino acid–urea (h_{AU}) in water [3].

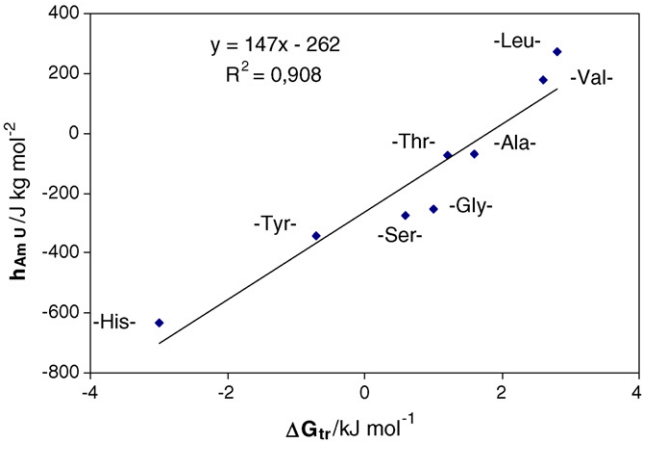


Fig. 3. Relationship between the transfer free energy of amino acids ΔG_{tr} [33] and the enthalpic pair interactions coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea (h_{AmU}) in water.

Engelman's parameters and the enthalpic pair interaction coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea in water. In this statement, L-α-tryptophan and its derivative are omitted since L-α-tryptophan is more hydrophilic than L-α-alanine whereas the Engelman's parameter does not show that. The observed dependences suggest the similar contributions of the amino acid side substituents to the variability of numerical values of the correlated parameters that describe the behavior of the system in aqueous medium.

4. Conclusion

The correlations presented in this work indicate that the enthalpic interaction coefficient of pair *N*-acetyl-*N*′-methyl-L-α-amino acid amides – urea h_{AmU} can play the role of parameter which diversifies the effects of the hydrophobic or hydrophilic amino acid side groups on the interactions of discussed amides with urea molecule in water.

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