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Review

Studies of homogeneous interactions of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water at 298.15 K

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ABSTRACT

The enthalpies of dilution of aqueous solutions of *N*-acetyl-*N*′-methyl-L-glycinamide (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$_3$) (BACHEM) and *N*-acetyl-*N*′-methyl-L-α-serinamide (AcSerNHCH$_3$), *N*-acetyl-*N*′-methyl-L-α-threoninamide (AcThrNHCH$_3$), in water at 298.15 K have been measured. These data were used to calculate the homogeneous enthalpic pair interaction coefficients based on McMillan–Mayer's theory.

Contents

1. Introduction

The interactions of amino acid side substituents in polypeptide chains between themselves as well as with surrounding water molecules are responsible for the formation of tertiary and quaternary protein spatial structures. The interpretation of experimental results determining the interactions between large fragments of protein chains is very difficult due to their size and diversity of specific interactions. Therefore model compounds with analogous functional groups as those in polypeptide chains are studied to describe particular types of interactions. The role of model compounds can be played by amino acid molecules [1–5] and other such as compounds (small peptides and their derivatives) [6–10]. However, the obtained research results present difficulties in separating the energetic effects ascribed to particular functional groups. This especially concerns the contributions made by strong polar amine and carboxyl groups that are components of a zwitterion group.

Thus, it seems to be of interest to study the derivatives of natural amino acids with the general formula: CH$_3$–CO–NH–C$^\alpha$HR–CO–N′H–CH$_3$, in which the amino acid segment composed of C$^\alpha$ combined with a proton and side substituent –R is surrounded by functional groups, analogous to those of peptide bonds, substituted with non-polar methyl groups. The examination of these compounds allow one to determine the energetic effects contributed by amino acid side substituents –R to the global effects, in which polypeptide chains take part.

The molecules of the discussed amide derivatives of amino acids, as opposed to natural amino acids, have no deprotonated carboxyl group (–COO$^-$) and protonated amine group (–NH$_3^+$), thus the significant contribution of Coulomb's forces to the examined interaction mechanisms is eliminated.

In order to determine the interactions between homogeneous molecules of N-acetyl-N′-methyl-L-α-amino acid amides in water, calorimetric measurements of the dilution enthalpies of several selected small peptides solutions were carried out. The obtained values of dilution enthalpy were used to calculate the enthalpic pair interaction coefficients, h_{xx}, derived from McMillan–Mayer's [11] theory modified by Friedman [12] and Franks [13]. The enthalpic homogeneous pair interaction coefficients are a measure

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of the energetic effects of interactions between two homogeneous molecules taking place with the competitive participation of water molecules [1,14]. Based on the determined interactions of N-acetyl-N′-methyl-L-α-amino acid amides between themselves and with water molecules one can conclude about the hydrophilic–hydrophobic character of side amino acid chains, –R.

2. Experimental

The amides used for the investigations: *N*-acetyl-*N*′-methyl-L-glycinamide (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$_3$) (BACHEM) and *N*-acetyl-*N*′-methyl-L-α-serinamide (AcSerNHCH$_3$), *N*-acetyl-*N*′-methyl-L-α-threoninamide (AcThrNHCH$_3$) were synthesized at the Department of Organic Chemistry (University of Łódź). Melting points were determined with a Boetius hot stage apparatus and are uncorrected. The infrared spectra were recorded with a Nexus FT-IR spectrometer (Thermo Nicolet). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 BB (200 MHz) instrument. The synthesis of *N*-methylamides was carried out with the use of L-serine and L-threonine according to the reaction sequence: estrification [15], acetylation [16] and amination [17] described for amino acids. The characteristics of *N*-methylamides are given below. N-Acetyl-L-serin-methylamid, mp 121–122 °C (ethyl acetate) (123–124 °C [18]); IR (KBr) $\nu = 1652\,\mathrm{cm^{-1}}$ (CO); ^{1}HNMR (DMSO-d_6) $\delta = 8.00$–7.65 (2H, m, NH), 4.86 (1H, t, $J = 5.6$ Hz, OH), 3.52 (2H, t, $J = 5.6$ Hz, CH$_2$), 2.57 (3H, d, $J = 4.6$ Hz, NMe), 1.87 (3H, s, COMe); ^{13}C NMR (DMSO-d_6) $\delta = 170.7$, 169.5, 61.9, 55.4, 25.9, 22.9.

N-Acetyl-L-threonin-methylamid, m.p. 166–167 °C (methanol) (164–166 °C [19]); IR (KBr) $\nu = 1644\,\mathrm{cm^{-1}}$ (CO); ^{1}H NMR (DMSO-d_6) $\delta = 7.80$–7.50 (2H, two overlapping br. s, NH), 4.77 (1H, d, $J = 5.2$ Hz, OH), 4.15–3.85 (2H, m, CH), 2.58 (3H, d, $J = 5.1$ Hz, NMe), 1.90 (3H, s, Me), 1.00 (3H, d, $J = 6.3$ Hz); ^{13}C NMR (DMSO-d_6) $\delta = 170.9$, 169.8, 66.5, 58.6, 25.8, 22.9, 20.3.

All the substances were crystallized from water–methanol mixture and dried under reduced pressure at room temperature. The purity of the substances were checked by measuring the temperature of melting point with a differential scanning

Table 1
Enthalpies of dilution ΔH_{dil} aqueous solutions of *N*-acetyl-*N*′-methyl-L-α-amino acid in water at 298.15 K.

m_i (mol kg^{-1})	m_f (mol kg^{-1})	ΔH_{dil} (J mol^{-1})	m_i (mol kg^{-1})	m_f (mol kg^{-1})	ΔH_{dil} (J mol^{-1})
AcGlyNHCH$_3$					
0.3963	0.0159	−227.0	0.5871	0.0297	−365.1
0.3963	0.1399	−148.7	0.5871	0.0386	−340.7
0.3963	0.1473	−146.1	0.7804	0.5277	−140.8
0.4933	0.0259	−268.1	1.5617	0.1530	−740.3
0.4933	0.0316	−274.5	1.5617	0.9011	−348.1
0.4933	0.0480	−256.5	1.5617	1.1380	−219.1
0.4933	0.0574	−266.1			
AcAlaNHCH$_3$					
0.4671	0.0456	−473.2	0.9175	0.4365	−517.60
0.4671	0.1159	−416.9	0.9570	0.1816	−907.20
0.4671	0.2250	−260.1	0.9703	0.0763	−1014.4
0.4671	0.3114	−167.5	0.9703	0.4311	−577.50
0.8049	0.1494	−786.5	0.9703	0.4586	−590.90
0.8049	0.3782	−457.8			
AcValNHCH$_3$					
0.1026	0.00484	−230.3	0.1030	0.00417	−232.2
0.1026	0.01570	−200.4	0.1030	0.00818	−221.9
0.1030	0.00417	−231.8	0.1030	0.01575	−201.0
0.1030	0.00818	−221.9	0.1030	0.01815	−194.9
0.1030	0.01204	−210.2	0.1311	0.00701	−282.3
0.1030	0.01575	−200.8	0.1311	0.01366	−265.2
0.1030	0.01815	−195.1	0.1311	0.01998	−249.0
AcLeuNHCH$_3$					
0.34412	0.0470	−984.7	0.46981	0.0896	−1293.7
0.34412	0.0653	−922.8	0.53346	0.0343	−1837.5
0.34412	0.0667	−973.4	0.53346	0.0899	−1295.4
0.34412	0.0684	−925.1	0.53346	0.1275	−1415.9
0.34412	0.0871	−745.0	0.53346	0.1963	−1097.8
0.34412	0.0967	−817.8	0.53346	0.2338	−950.5
0.46981	0.0579	−1359.3			
AcSerNHCH$_3$					
0.2506	0.1026	−115.6	0.6254	0.2403	−301.7
0.2949	0.1279	−121.3	0.6254	0.2816	−264.6
0.3415	0.0822	−180.0	0.7007	0.4537	−189.2
0.3415	0.1005	−185.6	0.9523	0.2529	−547.6
0.3415	0.1585	−152.9	0.9523	0.2701	−445.8
0.5722	0.3701	−147.9	0.9523	0.3580	−395.9
0.6254	0.1440	−383.0			
AcThrNHCH$_3$					
0.1279	0.0649	−78.50	0.3103	0.1357	−211.0
0.1907	0.0779	−132.8	0.3433	0.1136	−262.8
0.2254	0.0729	−176.1	0.3585	0.2027	−166.9
0.2254	0.1123	−131.9	0.3585	0.1427	−240.5
0.2329	0.0980	−153.9	0.3684	0.2193	−154.1
0.2814	0.0978	−224.9	0.3959	0.2171	−211.0
0.2814	0.1366	−168.0			

calorimeter SETARAM DSC 111. The water used in the experiments was deionized, distilled and degassed. The enthalpies of dilution were measured at 298.15 K with an isothermal calorimeter SETARAM MS-80D 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 solutions and the calorimeter measuring cells were weighed by means of a Mettler AE 240 balance.

3. Results and discussion

The obtained experimental values of dilution enthalpies, ΔH_{dil}, of *N*-acetyl-*N*′-methyl-L-α-amino amides as well as the initial (m_i) and final (m_f) concentrations of the solutions under investigation are given in Table 1.

The values of dilution enthalpies, ΔH_{dil}, were used to calculate enthalpic homogeneous pair interaction coefficients, h_{xx} in water on the basis of modified by Desnoyers [20] McMillana–Mayera's equation [11], in the following form:

$$\Delta H_{\text{dil}} = h_{xx}(m_\text{f} - m_\text{i}) + h_{xxx}(m_\text{f}^2 - m_\text{i}^2) + \cdots$$

where h_{xx} and h_{xxx}—enthalpic interactions coefficients of homogeneous pairs and triples of the examined molecules, respectively. In the present paper, only the interaction coefficients of the second order will be interpreted. Interaction coefficients of a higher order (third or fourth) besides the interaction of three or four molecules include the contributions made by combinations of interactions of lower orders and therefore they will not be considered in this paper. The calculated values of enthalpic pair interaction coefficients, h_{xx} with literature data are listed in Table 2.

The calculated values of coefficients h_{xx} are a measure of the energetic effects of interactions taking place between the examined molecules with the competitive participation of water molecules. The global effect is a sum of superimposing processes: (a) exothermic interactions between polar groups of the examined molecules, (b) endoenergetic effects of partial dehydrations of hydration sheaths of the interacting polar groups. Moreover, water molecules in the direct vicinity of non-polar groups of both amino acid side substituents and methyl groups combined with peptide bonds, reinforce the hydrogen bonds between water molecules in their direct vicinity as a result of hydrophobic hydration [21–23]. The effect of reinforced interactions, as a result of hydrogen bonds cooperation, is transformed onto water molecules hydrating the peptide bonds of the examined small peptides and the polar groups of amino acid side substituents. This brings about the reinforcement of interactions between water molecules and polar groups. Thereby the removal of some water molecules from hydration layers constituting a hindrance to the direct approach of interacting groups requires an increased input of energy. As a result of this process, the total effect of interactions between the molecules of the examined amides becomes more endothermic.

Table 2
Enthalpic homogeneous pair interaction coefficients for *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water at 298.15 K.

N-Acetyl-*N*′-methyl-L-α-amino acid amide	h_{xx}[a] (J kg mol^{-2})	h_{xx}[b] (J kg mol^{-2})	Number of CH$_2$ groups
AcGlyNHCH$_3$	619 ± 15	584.5	0.5
AcAlaNHCH$_3$	1150 ± 120	1180.8	1.5
AcValNHCH$_3$	2600 ± 300		3.5
AcLeuNHCH$_3$	3373 ± 470	3420.1	4.5
AcSerNHCH$_3$	796 ± 38		
AcThrNHCH$_3$	1275 ± 53		

[a] This work.
[b] Blackburn et al. [24].

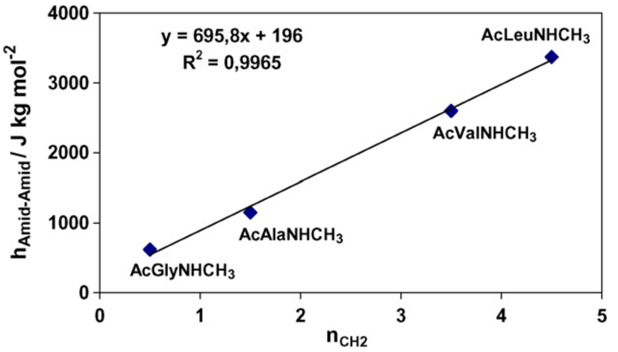

Fig. 1. Dependence of the homogeneous enthaplic pair interaction coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water on the number of CH$_2$ groups in the side chain of amino acid.

The determined values of enthalpic homogeneous pair interaction coefficients, h_{xx}, of the examined amino acid derivatives are positive, which means that the total interaction effect of these homogeneous molecules between themselves is endoenergetic (Table 2).

In the case of amides with alkyl side chains, the values of enthalpic coefficients, $h_{\text{Amid–Amid}}$, increase with the increase in the length of alkyl side chain –R in the following sequence: AcGlyNHCH$_3$ < AcAlaNHCH$_3$ < AcValNHCH$_3$ < AcLeuNHCH$_3$ (Table 2). This is due to the effects of hydrophobic hydration caused by the growing alkyl substituents in the discussed amino acid amide molecules. The illustrated (Fig. 1) dependence of enthalpic homogeneous pair interaction coefficients of small peptides with alkyl side chains on the number of CH$_2$ groups in the side substituents (as proposed by Savage and Wood [25] it is accepted that the CH$_3$ group corresponds to 1.5 CH$_2$ while CH corresponds to 0.5 CH$_2$), is described by the equation: $h_{\text{Amid–Amid}} = 196 + 695.8 n_{\text{CH}_2}$ ($R^2 = 0.9965$). The constant term of the equation describes the interactions between small peptides devoid of side substituent –R (the core of amides). The slope of this function determines the contributions of statistic CH$_2$ groups of amino acid side segments to the total interaction value described by the enthalpic homogeneous pair interaction coefficients $h_{\text{Amid–Amid}}$.

The enthalpic coefficients $h_{\text{Amid–Amid}}$ of L-α-serine derivative are positive, but their value is lower than that of alanine derivative. The replacement of hydrogen atom in the methyl group of alanine derivative side chain (AcAlaNHCH$_3$) by a hydroxyl group (AcSerNHCH$_3$) brings about a decrease in the value of enthalpic pair interaction coefficient. This probably results from the exothermic

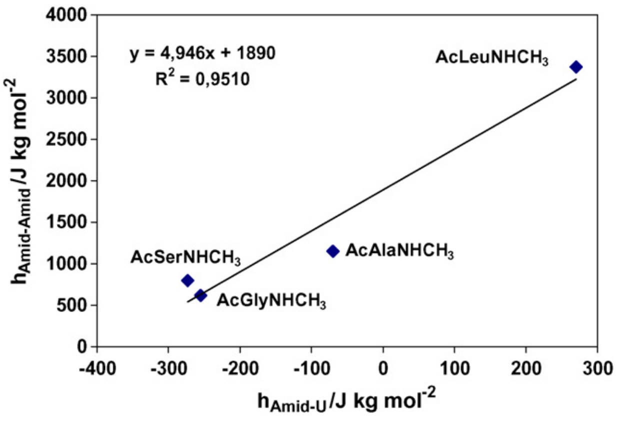

Fig. 2. Relationship between the enthalpic pair interaction coefficients h_{AA} of amino acids in water [1] and the enthalpic pair interaction coefficients $h_{\text{Amid–Amid}}$ of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water.

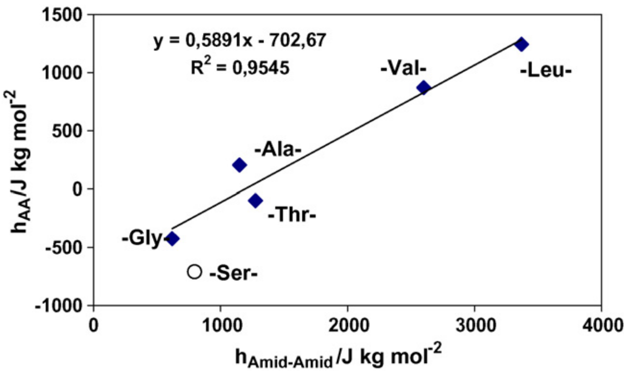

Fig. 3. Relationship between the enthalpic pair interaction coefficients h_{AA} of amino acids in water [1] and the enthalpic pair interaction coefficients $h_{\text{Amid–Amid}}$ of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water.

effects of the interactions between a statistic polar hydroxyl group and other polar groups that are added to the global effect described by the enthalpic coefficient $h_{\text{AcSerNHCH}_3-\text{AcSerNHCH}_3}$ (Table 2).

The addition of both hydroxyl group and $=CH_2$ group (AcThrNHCH$_3$) to the side chain of alanine derivative (AcAlaNHCH$_3$) causes the value of enthalpic coefficient to be comparable with the value of $h_{\text{Amid–Amid}}$ for the initial AcAlaNHCH$_3$. The compensation of exothermic and endothermic effects results from the fact that besides polar group –OH that intensifies the exoenergetic interactions, there is added hydrophobic group $=CH_2$, making an endothermic contribution to the global value of energetic effects associated with the interaction of homogeneous molecule pair $h_{\text{Amid–Amid}}$ (Table 2).

The obtained values of enthalpic homogeneous pair interaction coefficients of *N*-acetyl-*N*′-methyl-L-α-amino acid amides well correlate with the values of enthalpic heterogeneous pair interaction coefficients between the molecules of *N*-acetyl-*N*′-methyl-L-α-amino acid amides and the urea molecule [6] (Fig. 2). The linear relationship suggests that the contributions of amino acid side chains (–R) to the overall interactions between examined homogeneous pair *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water are similar to those of these radicals (–R) made to the interactions between of *N*-acetyl-*N*′-methyl-L-α-amino acid amides and urea in water.

The obtained values of enthalpic homogeneous pair interaction coefficients $h_{\text{Amid–Amid}}$ of *N*-acetyl-*N*′-methyl-L-α-amino acid amides in water well correlate too with the values of the enthalpic pair interaction coefficients h_{AA} of L-α-amino acids in water [1] Fig. 3. The linear relationship indicates similar contributions made by the amino acids side chains to summary effect of interactions taking place in aqueous solutions of the systems under discussion. It is striking however that the endothermic values of enthalpic homogeneous pair interaction coefficients of serine derivatives $h_{(\text{Amid–Amid})}$ are quite high (Figs. 2 and 3). This probably testifies to too small contribution made by hydroxyl groups to the total effect described by the enthalpic coefficient $h_{(\text{Amid–Amid})}$. Such a behavior may be explained in terms of the engagement of the hydroxyl group of serine molecules in intramolecular combination with the adjacent carbonyl group of peptide bond [26,27]. Thereby the hydroxyl group of serine amide does not interact completely as donor and proton acceptor with a homogeneous molecules as it occurs in the case of serine molecules (Ser) [1].

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