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Calorimetric studies of interactions of some peptides with electrolytes, urea and ethanol in water at 298.15 K

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Abstract The standard molar enthalpies of solution at infinite dilution $\Delta_{sol}H_m^{\infty}$ of glycylglycine, DL-alanyl-DL-alanine and glycylglycylglycine in aqueous solutions of potassium chloride and ethanol as well as of glycylglycine and glycylglycylglycine in the solutions containing urea and water have been determined by calorimetry at the temperature 298.15 K. Changes of solution enthalpy, expressed in a form so-called heterotactic interaction coefficients, h_{xy} were used for analysis of interactions occurring between the investigated solutes in water. The group contributions illustrating the interactions of KCl, urea and ethanol with selected functional groups in the peptide molecules, namely CH_2, "pep," and "ion" groups, were calculated and discussed.

Keywords Aqueous solutions · Dissolution enthalpy · Enthalpic pair-wise coefficients · Ethanol · Peptides · Potassium chloride · Urea

Introduction

Molecular interactions in aqueous solutions of peptides as well as of model compounds that contain some fragments of natural biomolecules have been frequently examined, using different experimental methods, among them calorimetry, until the present time [1–5]. Such studies have been performed also in our laboratory. Recently, we have reported results of measurements of dissolution enthalpies of some peptides in aqueous solutions of sodium chloride and sodium iodide [6]. We have found that the enthalpic effect of interactions of investigated peptides with a large, polarisable iodide anion is stronger than that with small chloride ion, similarly as it was observed in other systems [7]. The object of this study was to determine and analyse the energetic effect of interactions between simple oligopeptides and potassium chloride, urea and ethanol in diluted solutions in water. Potassium chloride was selected for these investigations due to its particular physiological importance. Urea and ethanol are known as denaturants of globular proteins, but the mechanism of their interactions with the protein is different. Therefore, it seemed interesting to check whether or to which extent the calorimetric measurements can reveal differences of behaviour of the mentioned above reagents. To this goal, the enthalpies of solution of glycylglycine (diglycine) and glycylglycylglycine (triglycine) were measured in water and in aqueous solutions of potassium chloride, urea and ethanol. Additionally, the same measurements for DL-alanyl-DL-alanine in aqueous solutions of potassium chloride and ethanol were made. From the determined standard dissolution enthalpies $\Delta_{sol}H_m^{\infty}$ of peptides in aqueous electrolyte and nonelectrolyte solutions the enthalpic pair interaction parameters h_{xy} in water were calculated and analysed.

Experimental

Samples of glycylglycine, glycylglycylglycine and DL-alanyl-DL-alanine (Aldrich Chemical Co., Ltd., mass fraction: 0.99) were dried under reduced pressure at room temperature. Potassium chloride and urea (Aldrich Chemical Co., Ltd., mass fraction: 0.99) were dried for several days at $T = 333$ K

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also under reduced pressure. All aqueous solutions of potassium chloride, urea as well as ethanol ("analytically pure", POCh S.A. Gliwice, Poland) were prepared by mass. The water was deionised and distilled twice before the preparation of these solutions.

The enthalpies of solution of the peptides were measured in water and in aqueous solutions of potassium chloride, urea and ethanol at 298.15 K, using the "isoperibol" type calorimeter [8]. The examined aqueous solutions contained from 0.25 to 3.00 moles of KCl or urea in 1 kg of water and from about 0.4 (0.75 mol%) to about 8.0 moles (12.5 mol%) of ethanol in 1 kg of water. The enthalpy of solution of triglycine in ethanol solutions was measured in the solutions containing up to 6 mol% of ethanol only due to the relatively low solubility of this peptide in the mixtures containing more alcohol. The peptide concentration range was (4×10^{-3} to 1×10^{-2} mol kg^{-1}). The measured molar enthalpies of solution of peptides were found to be independent of the peptide content within the investigated range of the peptide concentration, hence, the standard enthalpies of solution, $\Delta_{sol}H_m^{\infty}$ at 298.15 K were calculated as the mean of $\Delta_{sol}H_m$ values of all independent calorimetric runs at given mixed solvent composition.

Results and discussion

The molar enthalpies of solution at infinite dilution of peptides in water and aqueous solutions of potassium chloride and urea are collected in Table 1 and they are shown in Fig. 1 as a function of the solution molality. The analogous data for water–ethanol mixtures are given in Table 2, and they are also presented graphically in Fig. 2 as a function of mol% of ethanol. The enthalpies of solution of peptides depend on the kind of peptide molecule as well as on the cosolute (KCl, urea or ethanol) and its content in the system. The $\Delta_{sol}H_m^{\infty}$ values for diglycine and triglycine in aqueous solutions of KCl or urea are positive, and they decrease when the concentration of the cosolute increases. The solution enthalpy of triglycine is more positive than that of diglycine within the whole investigated range of the cosolute content. The dissolution effect of the same peptide (diglycine and triglycine) in the urea solutions is more endothermic than that in aqueous potassium chloride solutions. The values of dissolution enthalpy of DL-alanyl-DL-alanine are negative. They decrease within the molality range 0–2.5 mol kg^{-1}, pass through a small minimum and then increase.

The standard enthalpy of solution for each of examined here peptides in the systems containing ethanol (Fig. 2) grows upon addition of ethanol to water solvent and in the case of diglycine it passes through a maximum near 7.5–10 mol% of alcohol. It is worthy to mention that a similar shape of the dissolution enthalpy curves with the maximum at about 10 mol% of ethanol was observed for almost all simple electrolytes and numerous nonelectrolytes in the water–ethanol mixtures examined thus far. It is also typical for the dissolution enthalpies in the mixtures of water with other alcohols (methanol, propanol, isopropanol and *tert*-butanol) in the range of low alkanol content [9, 10]. It is generally accepted that these maxima are related to a structure-promotion or structure-stabilizing effect of added alkanol on water due to the hydrophobic hydration of the alcohol alkyl group [10]. Therefore, more energy is necessary to extract a solvent molecule in order to form a solute solvation shell and, as a result, the dissolution enthalpy becomes more endothermic or less exothermic. When the alkanol content in the mixed solvent still increases, the mixed solvent structure gets less stable and the energetic effect of the dissolution becomes more favourable (i.e. more exothermic or less endothermic).

Table 1 Standard enthalpies of solution $\Delta_{sol}H_m^{\infty}$ of peptides in aqueous solutions of KCl and urea at 298.15 K

m/mol kg^{-1}	$\Delta_{sol}H_m^{\infty}$*/kJ mol^{-1}	
	KCl	Urea
Glycylglycine		
0.00	11.84 ± 0.02	11.84 ± 0.02
0.25	11.35 ± 0.06	11.49 ± 0.03
0.50	10.91 ± 0.05	11.15 ± 0.02
1.00	10.10 ± 0.03	10.57 ± 0.02
1.50	9.39 ± 0.02	10.00 ± 0.04
2.00	8.80 ± 0.05	9.53 ± 0.03
2.50	8.40 ± 0.07	9.08 ± 0.05
3.00	8.10 ± 0.06	8.79 ± 0.03
Glycylglycylglycine		
0.00	17.69 ± 0.02	17.69 ± 0.02
0.25	16.96 ± 0.04	17.18 ± 0.06
0.50	16.30 ± 0.03	16.69 ± 0.05
1.00	15.07 ± 0.05	15.78 ± 0.03
1.50	13.99 ± 0.02	14.94 ± 0.04
2.00	13.07 ± 0.05	14.24 ± 0.05
2.50	12.25 ± 0.06	13.81 ± 0.03
3.00	11.74 ± 0.04	13.38 ± 0.06
DL-alanyl-DL-alanine		
0.00	−7.59 ± 0.03	
0.25	−7.96 ± 0.04	
0.50	−8.25 ± 0.03	
1.00	−8.69 ± 0.02	
1.50	−8.90 ± 0.05	
2.00	−9.05 ± 0.04	
2.50	−9.11 ± 0.05	
3.00	−8.87 ± 0.05	

* Uncertainties are given as standard deviations

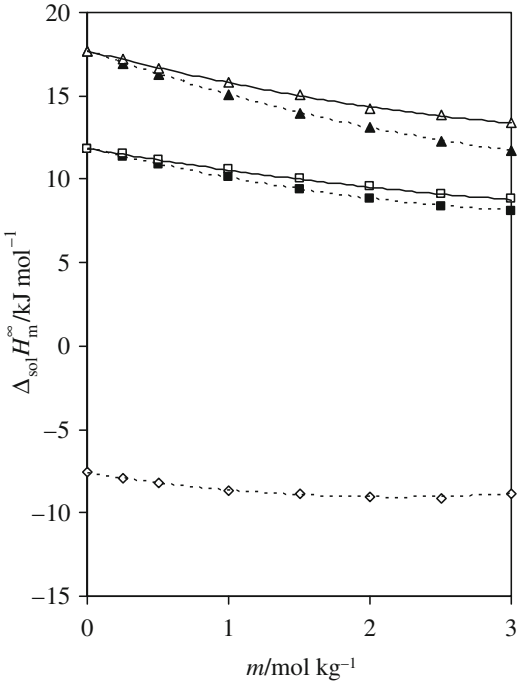

Fig. 1 Standard enthalpies of solution $\Delta_{sol}H_m^{\infty}$ of diglycine (*square*), triglycine (*triangle*) and DL-alanyl-DL-alanine (*diamond*) in aqueous solutions of KCl (*dotted line*) and urea (*line*) as a function of the solution molality m

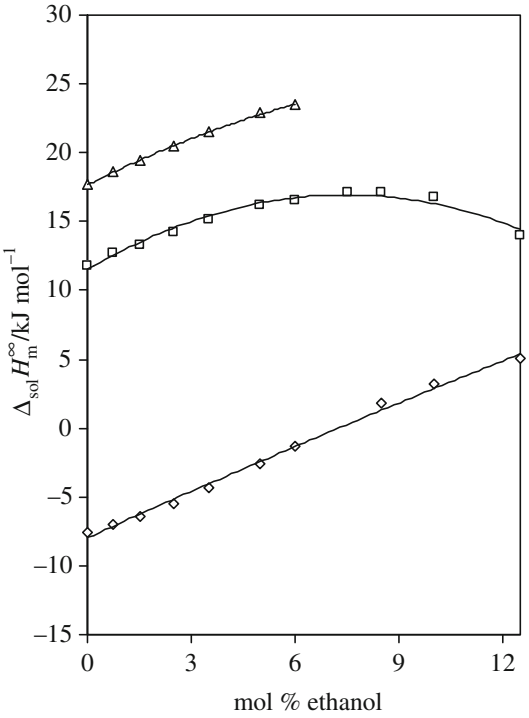

Fig. 2 Standard enthalpies of solution $\Delta_{sol}H_m^{\infty}$ of diglycine (*square*), triglycine (*triangle*) and DL-alanyl-DL-alanine (*diamond*) in aqueous solutions of ethanol as a function of the mol% of ethanol

However, the maximum of $\Delta_{sol}H_m^{\infty}$ is not observed for examined here triglycine and DL-alanyl-DL-alanine. In the case of triglycine, it can be caused by the fact that examined range of the water–ethanol mixture composition was to narrow. Another explanation can be proposed for the latter dipeptide. It is possible that DL-alanyl-DL-alanine which exhibits stronger than diglycine hydrophobic properties additionally stabilizes structure of water even in the mixtures containing more than 10 mol% of ethanol. It means also that DL-alanyl-DL-alanine is preferentially hydrated in the whole examined range of the mixed solvent composition and water molecules in its solvation shell are not replaced by ethanol molecules despite of growing alcohol content. This conclusion can be testified through the analysis of the enthalpic pair interaction coefficients. As it is known the enthalpic pair interaction coefficients, h_{xy} derived from McMillan–Mayer theory [11] are regarded as a measure of the enthalpic effect of interactions

Table 2 Standard enthalpies of solution $\Delta_{sol}H_m^{\infty}$ of peptides in aqueous solutions of ethanol at 298.15 K

Mol% ethanol	$m_{ethanol}$/mol kg^{-1}	$\Delta_{sol}H_m^{\infty}$*/kJ mol^{-1}		
		Glycylglycine	Glycylglycylglycine	DL-alanyl-DL-alanine
0.00	0.0000	11.84 ± 0.02	17.69 ± 0.02	−7.59 ± 0.03
0.75	0.4195	12.69 ± 0.05	18.58 ± 0.06	−7.03 ± 0.04
1.50	0.8453	13.34 ± 0.04	19.41 ± 0.06	−6.40 ± 0.04
2.50	1.4233	14.28 ± 0.06	20.49 ± 0.03	−5.47 ± 0.05
3.50	2.0133	15.12 ± 0.02	21.51 ± 0.03	−4.36 ± 0.06
5.00	2.9215	16.17 ± 0.03	22.88 ± 0.02	−2.58 ± 0.05
6.00	3.5431	16.58 ± 0.03	23.47 ± 0.05	−1.29 ± 0.04
7.50	4.5008	17.18 ± 0.04		
8.50	5.1566	17.18 ± 0.05		1.77 ± 0.04
10.00	6.1677	16.74 ± 0.06		3.19 ± 0.05
12.50	7.9299	14.05 ± 0.05		5.03 ± 0.04

* Uncertainties are given as standard deviations

between two solute particles X and Y in infinitely diluted solution [11, 12]. Consequently, in this way we can analyze the energetic effect of interactions between single molecule of peptide and single molecule of cosolute (urea or ethanol) or, in the case of electrolyte, a sum of the interaction effects between a peptide and the cation and the anion.

The enthalpic pair interaction coefficients h_{xy} for peptide–KCl, peptide–urea and peptide–ethanol pairs in water were determined from the dissolution enthalpies of the peptide X in aqueous solutions of appropriate cosolute Y (KCl, urea, and ethanol) measured in this work. To this aim, the method proposed by Desnoyers et al. [13] was used. The standard dissolution enthalpies of the peptides in the examined solutions were expressed as a function of the cosolute Y molality, m_y in the form:

$$\Delta_{sol}H_m^{\infty} = \Delta_{sol}H_m^{\infty}(H_2O) + 2h_{xy}m_y + 3h_{xyy}m_y^2 \quad (1)$$

where $\Delta_{sol}H_m^{\infty}$ is the standard molar enthalpy of solution of peptide X in aqueous solution of KCl, urea or ethanol respectively and $\Delta_{sol}H_m^{\infty}(H_2O)$ is the standard enthalpy of solution of the same peptide in water. The h_{xyy} coefficient denotes the enthalpic triplet interaction coefficient, which regards to the interactions of one molecule of peptide with two cosolute particles. These coefficients will not be discussed here as they contain also some energy contributions from different type pair interactions and their meaning is obscured. The calculated enthalpic pair interaction coefficients for the examined here systems are presented in Table 3 together with the appropriate literature data concerning similar systems [6, 14–17], which were helpful for analysis of our results.

The enthalpic pair interaction coefficients illustrate a sum of endothermic effects of a partial dehydration of interacting species (molecules or ions) and exothermic effect of a direct X–Y interactions leading to a replacement of the water molecule in the X surrounding by the Y particle. The negative values of h_{xy} for the systems containing peptide and potassium chloride or urea suggest that the interactions between electrolyte as well as urea and peptide molecules dominate the effects of dehydration of the substances present in the solution. The replacement of the hydrogen atoms in hydrocarbon chains of diglycine molecule with a methyl groups causes the same increase (within the error limits) in the value of the enthalpic pair interaction coefficient for the pairs of peptide both with potassium chloride as well as with urea. The pair interaction coefficients for the systems containing glycine and its derivatives (diglycine and triglycine) become more negative when the size of the oligopeptides grows.

The enthalpic pair interaction coefficients for peptide–ethanol pairs are positive which indicates that the hydrophobic properties of peptide as well as of alcohol molecules play a dominant role in the behaviour of these systems. Moreover, in contrast to the systems discussed above the pair interaction coefficients become more positive when the size of the oligopeptides grows.

Some regularity in the h_{xy} values observed in the examined systems suggests that the energetic effect of interactions could be additive with respect to the contributions from functional groups present in the oligopeptide molecule. These contributions were calculated using the Savage and Wood group additivity model [18].

For glycine and oligopeptide molecules, the CH_2 group and the peptide CONH group, denoted "pep" were distinguished as interacting groups. Additionally, the terminal charged groups (NH_3^+ and COO^-) were assumed to be the one defined group and denoted "ion." The examined electrolytes, urea and ethanol were assumed as an interacting species Y.

The h_{xy} coefficient was then presented as a sum:

$$h_{xy} = n_{ion}h_{ion-Y} + n_{pep}h_{pep-Y} + n_{CH_2}h_{CH_2-Y} \quad (2)$$

In the above formula, n_{ion}, n_{pep}, n_{CH_2} denote the number of the terminal charged groups, the peptide groups and the CH_2 groups, respectively, h_{ion-Y}, h_{pep-Y}, and h_{CH_2-Y} are the group coefficients for the interactions of the same groups with the salt, urea and ethanol molecule Y. The results of calculations are presented in Table 4. The obtained data are set up together with the group contributions for the interactions of the same oligopeptides with sodium chloride and sodium iodide [6].

The group coefficients for interactions between the solvated urea molecule or electrolyte and the solvated apolar group in the peptide molecule are positive due to a breaking of the structured water around the methylene group. The other group coefficients that describe the

Table 3 Enthalpic interaction parameters h_{xy} of glycine and some peptides with electrolytes, urea and ethanol in water at 298.15 K

Solute	h_{xy}/J kg mol^{-2}				
	KCl	Urea	Ethanol	NaCl	NaI
Glycine	−470 [14]	−390 [15]	551 [16]	−480 [14]	−940 [14]
Glycylglycine	−1013 ± 22	−715 ± 27	995 ± 61	−828 ± 19 [6]	−1542 ± 24 [6]
Glycylglycylglycine	−1479 ± 39	−1119 ± 51	1122 ± 65	−1278 ± 24 [6]	−2233 ± 34 [6]
DL-alanyl-DL-alanine	−673 ± 56	−354 [17]	674 ± 37	−623 ± 31 [6]	−969 ± 64 [6]

Table 4 Group-additivity coefficients h_{g-y} for the systems investigated

Interacting group/g	h_{g-y}/J kg mol^{-2}				
	KCl	Urea	Ethanol	NaCl [6]	NaI [6]
CH_2	157 ± 18	194 ± 19	−108 ± 75	120 ± 24	301 ± 21
"Pep"	−661 ± 29	−558 ± 29	393 ± 118	−519 ± 40	−947 ± 33
"Ion"	−640 ± 38	−571 ± 39	712 ± 158	−583 ± 54	−1227 ± 45
r^2	0.9983	0.9973	0.9219	0.9952	0.9988

interactions between the same cosolutes and the hydrated peptide group or terminal ionic groups in the oligopeptide molecule are negative, which points to domination of the attraction forces of the ion–dipole or ion–ion type.

The determined values of the group contributions for interactions of NaCl and KCl with the peptide group are very close to those for interactions of the same electrolytes with the peptide group in simple amides reported in literature [7, 19]. On the other hand, the effect of interactions of the examined electrolytes with CH_2 group in simple amides is energetically more unfavourable than that in oligopeptides. One can suppose that due to the presence of highly polar peptide group in vicinity of methylene apolar group in oligopeptide molecule the hydrophobic envelope of CH_2 group is relatively weak and it can be easier destroyed than in the case of simple amides. It is also noteworthy, that the energetic effect of interactions of KCl with peptide group, both in amides and in oligopeptides is more exothermic than the effect of interactions of NaCl with this group. As the enthalpy of hydration of KCl is less exothermic than that of NaCl, it means that the effect of interaction of the peptide group with potassium cation is more favourable than with sodium cation. Taking into account that both discussed salts exert different influences on peptide molecules the observed differences, though not so high, can play an important role in biological processes.

The group coefficients which characterize the interactions with ethanol molecules have the signs opposite to those described above, i.e. negative for CH_2–ethanol and positive for "pep"–ethanol and "ion"–ethanol interactions. The thermochemically unfavourable interactions of polar ethanol molecule with polar peptide or ionic groups seem to be a consequence of strong hydration of the both interacting molecules, i.e. ethanol and oligopeptide ones and relatively weak direct interaction between ethanol molecule and distinguished charged groups in peptide molecule due to rather low dipole moment of the ethanol molecule. As a result, the exothermic effect of the direct interaction does not exceed the endothermic effects of partial dehydration of ethanol molecules and strongly hydrated polar and "ion" groups in peptide molecule.

It is more difficult to explain a favourable energetic effect of interactions between apolar groups in peptide and ethanol molecule. The peptide apolar group is not able to interact with the ethanol hydroxyl group. The van der Waals type interactions are too weak to destroy the hydration shells of the apolar groups both of the peptide and of the ethanol molecules. The enthalpic effect of interactions between hydrophobically hydrated moieties (so called hydrophobic interaction) is endothermic. A possible explanation of the observed negative effect of the group interactions is that hydrated ethanol molecule does not destroy the hydrophobic cage of the oligopeptide apolar group, but the hydrogen bond(s) between the ethanol molecule and the molecule(s) of water of hydration shell of peptide apolar group is formed. These observations confirm our earlier suggestion that the examined peptides interact stronger with solvent water than with alcohol molecules.

The above discussion based on the pair interaction coefficients shows that from a thermochemical point of view the effect of urea on oligopeptides is similar to that of inorganic electrolytes, while the ethanol molecules interact with the peptides in aqueous solutions in other way. These observations, to some extent, seem to illustrate quantitatively a difference in denaturation process of simple peptides under influence of urea and ethanol.

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