Enthalpies of solution of DL-α-alanyl-DL-α-asparagine in aqueous alcohols at 298.15 K

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ABSTRACT

The enthalpies of solution of DL-α-alanyl-DL-α-asparagine (AlaAsn) were measured in aqueous methanol, ethanol, 1-propanol and 2-propanol with an alcohol mole-fraction content x_2 (from 0 to 0.4) at 298.15 K. The experimental results were used to calculate the enthalpies of transfer of AlaAsn from water to these mixtures as well as the enthalpy coefficients of pair-wise interactions (h_{xy}) between AlaAsn and alcohol molecules in water, according to the McMillan–Mayer's model. The h_{xy} values were found to be positive and increasing in a series methanol < ethanol < 1-propanol < 2-propanol.

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1. Introduction

It is known that alcohols are widely used in the processes of protein denaturation whereas amino acids, small peptides and their derivatives are the basic building blocks of proteins. Hence many works were devoted to the study of interactions of amino acids and peptides with components of a (water + alcohol) mixture in order to understand clearly the mechanism of protein denaturation [1–6]. Earlier [7–9], we have studied the interactions between molecules of DL-α-alanyl-DL-α-glycine (AlaGly), DL-α-alanyl-DL-α-alanine (AlaAla), L-α-alanyl-L-α-alanine (LAlaAla) or DL-α-alanyl-DL-α-valine (AlaVal) and an alkanol. In the given report, we extend a range of DL-alanine derivatives to determine the similar interaction-related characteristics, depending on the structure of an amino acid residue. As an object of this study, the DL-α-alanyl-DL-α-asparagine (AlaAsn) was chosen. The mixtures of water with methanol (MeOH), ethanol (EtOH), 1-propanol (1-PrOH) and 2-propanol (2-PrOH) were used as the dissolving media.

2. Experimental

Calorimetric measurements of enthalpies of solution of AlaAsn were carried out using an isoperibol hermetic calorimeter [10] at $T = (298.15 \pm 0.005)$ K and the mole-fraction content of an alkanol ranging from $x_2 = 0$ to 0.4. The experimental procedure was detailed

earlier [11]. The relative random error of the measurements did not exceed 0.5%.

Chromatographically homogeneous AlaAsn (Reanal Co., Hungary, assay: ≥98%) was used as such. Before experiments, the substance was recrystallized twice from (H_2O + EtOH) mixture, dried in a vacuum chamber at 333 K for 48 h and then kept over P_2O_5 in a desiccator. For AlaAsn, the concentration range was 0.005–0.015 mol kg^{-1} at all the (water + alkanol) compositions studied. The alcohols (Sigma–Aldrich, purum, assay: ≥99%) were purified as recommended previously [12,13]. The water content of these liquids determined with a Karl Fisher titration [14] did not exceed 0.04, 0.05, 0.03, and 0.04 wt% in MeOH, EtOH, 1-PrOH, and 2-PrOH, respectively. The water was purified by deionization and double distillation up to a specific conductivity of *ca.* 1.0×10^{-6} S cm^{-1}. Mixtures were prepared by weight.

3. Results

The standard enthalpies of solution, $\Delta_{sol}H^\circ$ ($\equiv \Delta_{sol}H^\infty$), were calculated by averaging the results of five independent measurements of $\Delta_{sol}H^m$ for each composition of an aqueous alkanol because no dependences of $\Delta_{sol}H^m$ vs. m for AlaAsn were observed in the mixtures studied. The changes of $\Delta_{sol}H^\circ$ for the AlaAsn in aqueous alkanols, together with standard deviations, are listed in Table 1. The enthalpies of transfer, $\Delta_{tr}H^\circ(w \to w + y)$, of AlaAsn from water ($w$) to aqueous alcohol ($w + y$) were defined as:

$$\Delta_{tr}H^\circ(w \to w + y) = \Delta_{sol}H^\circ(w + y) - \Delta_{sol}H^\circ(w) \tag{1}$$

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Table 1
Standard enthalpies (kJ mol^{-1}) of dissolution ($\Delta_{sol}H^\circ$) of AlaAsn in aqueous solution of alcohols at 298.15 K.

m_2[a]	MeOH	m_2[a]	EtOH	m_2[a]	1-PrOH	m_2[a]	2-PrOH
0.712	16.84 ± 0.02	0.721	17.17 ± 0.02	0.733	17.08 ± 0.02	0.715	17.17 ± 0.02
1.357	17.92 ± 0.02	1.452	18.58 ± 0.02	1.215	18.67 ± 0.02	1.181	18.92 ± 0.02
2.034	19.19 ± 0.02	2.368	20.06 ± 0.02	1.982	20.25 ± 0.03	1.742	20.45 ± 0.02
2.837	20.65 ± 0.02	3.437	20.89 ± 0.02	2.726	21.42 ± 0.02	2.581	21.49 ± 0.02
4.117	21.91 ± 0.03	4.512	22.13 ± 0.03	3.375	22.11 ± 0.03	3.356	22.65 ± 0.03
5.529	22.96 ± 0.03	5.435	23.12 ± 0.02	4.028	22.69 ± 0.02	4.285	23.43 ± 0.03
7.371	23.68 ± 0.03	7.616	24.37 ± 0.02	4.815	23.14 ± 0.02	5.387	24.21 ± 0.02
10.302	24.59 ± 0.02	9.011	25.46 ± 0.03	6.021	23.76 ± 0.03	6.417	25.16 ± 0.02
10.882	24.96 ± 0.02	12.268	26.84 ± 0.03	8.878	24.39 ± 0.02	9.149	26.51 ± 0.02
16.067	25.17 ± 0.02	17.247	27.63 ± 0.02	13.417	23.82 ± 0.03	12.838	26.24 ± 0.03
24.303	25.57 ± 0.03	24.089	28.27 ± 0.03	18.749	22.77 ± 0.03	17.966	24.24 ± 0.03
35.716	25.95 ± 0.03	34.344	28.45 ± 0.03	26.796	21.64 ± 0.03	25.659	18.29 ± 0.03

[a] The molal concentration of alcohols, mol kg^{-1}.

Enthalpy of solution of AlaAsn in pure water was found to be 16.55 ± 0.02 kJ mol^{-1}, which agrees perfectly with the reliable literature value [15] being 16.56 kJ mol^{-1}. The changes in $\Delta_{tr}H^\circ$ for AlaAsn against the alkanol mole-fraction content are shown in Fig. 1.

4. Discussion

One can see from Fig. 1 that transfer enthalpies for AlaAsn are positive for all the (water + alkanol) mixtures studied. Herewith $\Delta_{tr}H^\circ$ values depend on both the structure peculiarities of a co-solvent and the mixture composition, increasing monotonically as the alkanol content rises up to $x_2 \approx 0.1$. At the same time the slope of this dependence is found to be increasing in a series MeOH < EtOH < n-PrOH < i-PrOH. The main difference between systems {AlaAsn + H$_2$O + MeOH (EtOH)} and {AlaAsn + H$_2$O + 1-PrOH (2-PrOH)} consists in the presence or lack of an endothermic maximum on the curve $\Delta_{tr}H^\circ$ vs. x_2. The fact of absence of the maximum in the case of the aqueous ethanol or methanol is most likely connected with the formation of a stable hydration shell around its molecules. Herewith the endothermic enthalpy contributions from the simultaneous dehydration of alkanol and AlaAsn molecules probably prevail over exothermic contributions from the peptide−alcohol interactions in all the studied concentration range. For aqueous 1-PrOH and 2-PrOH in concentration range $0.15 < x_2 < 0.25$, the maximum is rather pronounced. The more

endothermicity of AlaAsn dissolution in (H$_2$O + 2-PrOH) compared to (H$_2$O + 1-PrOH) is caused primarily by the greater hydrophobicity of the isomeric alcohol. At $x_2 > 0.2$, the exothermic contribution from AlaAsn–propanol interactions to the total enthalpy interaction effect is beginning to be predominant and, as a consequence, the $\Delta_{tr}H^\circ$ vs. x_2 curve becomes opposite in direction.

From Fig. 2a–c, it can be seen that the introduction of the more hydrophobic residue into a DL-alanine molecule causes generally the increase in endothermicity of transfer of the peptide formed. Herewith the changes in $\Delta_{tr}H^\circ$ for AlaAsn are more exothermic than that for other derivatives presented (in the case of aqueous EtOH, it takes place at $x_2 < 0.2$). For cases of transfer of AlaAla and AlaVal, the difference from $\Delta_{tr}H^\circ$ (AlaAsn) is found to be the most pronounced. Unlike AlaAla and AlaVal molecules, the AlaAsn molecule has the NH$_2$-group and O-atom on the side chain that results in hydrogen-bonding with alkanol molecules. The heat effect of such hydrogen-bonding will make up for the loss energy at dehydrating. As a result, the $\Delta_{tr}H^\circ$ value for the AlaAsn–alkanol interaction becomes less positive than that for AlaAla (AlaVal)–alcohol one, in the case of the same co-solvent (see Fig. 2a–c). The distinction in $\Delta_{tr}H^\circ$ between AlaAla and AlaVal can be explained by the presence of two end alkyl groups in the latter molecule. It creates the additional steric hindrances for the AlaVal−alkanol interaction via hydrogen-bonding.

In the ternary aqueous system containing the small amount of co-solvent, the interparticle interactions can be characterized in terms of McMillan–Mayer's theory [16]. Based on the formalism of this theory [17–19] adapted for estimating the enthalpy coefficients of pair-wise interactions, h_{xy}, the $\Delta_{sol}H^\circ$ vs. m_2 functions were approximated by a polynomial of the form

$$\Delta_{sol}H^\circ = a_0 + a_1 m_2 + a_2 m_2^2 + a_3 m_2^3, \qquad (2)$$

where m_2 is the molal concentration of the co-solvent, and a_i is the regression coefficients calculated by using the least squares method. The correlation coefficient (R) and the Student criterion (t_α) were ranged from 0.961 to 0.997 and from 0.266 to 0.596, respectively. The h_{xy} value was calculated from the a_1 coefficient

Fig. 1. Enthalpies of transfer, $\Delta_{tr}H^\circ$, of AlaAsn from water to (H$_2$O + MeOH) (1), (H$_2$O + EtOH) (2), (H$_2$O + 1-PrOH) (3) and (H$_2$O + 2-PrOH) (4) as a function of the alkanol mole-fraction, x_2, at 298.15 K.

Table 2
Enthalpy coefficients of pair-wise interactions (h_{xy}, J kg mol^{-2}) between AlaAsn and alcohols in aqueous solutions and enthalpy coefficients of pair-wise interactions (h_{A+W}, J kg mol^{-2}) of the alcohols and water at 298.15 K.

Substance	MeOH	EtOH	1-PrOH	2-PrOH
AlaAsn	700 ± 30	740 ± 35	990 ± 65	1065 ± 70
H$_2$O[a]	−35.6 ± 0.7	−45.7 ± 2.5	−58.2 ± 4	−79 ± 3

[a] Enthalpy coefficients of pair-wise interactions (h_{xy}) between H$_2$O and alcohols were calculated according to Eq. (2) using literature data on enthalpies of water + alcohol mixing [22–24].
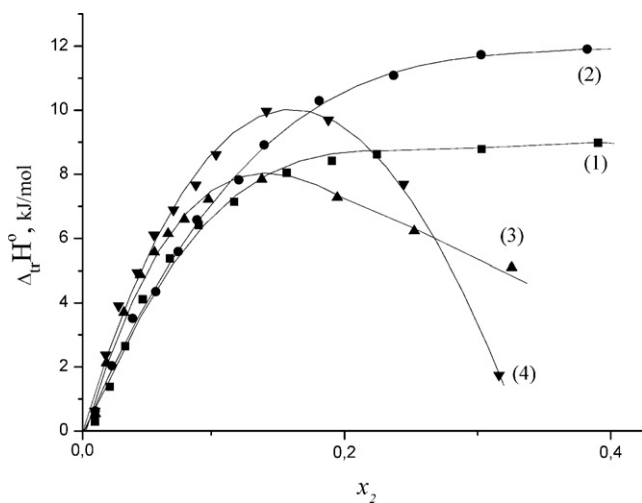
Fig. 3. Correlation between the enthalpy coefficients of pair-wise interactions, h_{xy}, of AlaAsn with alcohols and the enthalpy coefficients of pair-wise interactions, h_{A+W}, of alcohols with water for: AlaAsn (1), AlaGly (2) [7], AlaAla (3) [7], AlaVal (4) [9].

which are reflected in the sign and magnitude of h_{xy}. One may suggest that the contribution in h_{xy} from a direct interaction between AlaAsn and alkanol molecules is less pronounced than that from a dehydration of these molecules in the mixture. Herewith the interaction effect expressed in terms of h_{xy} becomes logically increasing in magnitude in a consequence MeOH < EtOH < 1-PrOH < 2-PrOH.

As was shown in our earlier reports [7–11] as well as in the literature [21,22], the strong interactions between water and organic co-solvent molecules should result in the endothermic contribution to h_{xy}. The same situation is observed in Fig. 3, too. It can be see in the figure that the dependence of h_{xy} for AlaAsn–alkanol interactions (1) against h_{A+W} for alcohol–water those is linear. This linearity is also characteristic for similar dependences relating to AlaGly (2), AlaAla (3) and AlaVal (4).

The difference in the behavior of both peptides and amino acids are due to the difference in properties of their R-side chains. Earlier [3], an off-beat "averaged" scale of hydrophobicity of amino acids or their side-chain substitutes ($P_{hydr-pho}$) has been offered. Since the molecules of dipeptides studied have side chains like those in the molecules of amino acids from the work [3], it would be interesting to verify the presence or absence of correlation dependence between h_{xy} for the alanyl-dipeptide–alkanol interactions and $P_{hydr-pho}$ parameters in an aqueous solution. It can be seen from Fig. 4 that such dependence is close to linear. Herewith the increasing size (strengthening of hydrophobicity) of the side chain in a dipeptide molecule increases the endothermic contribution to h_{xy} in a series AlaAsn < AlaGly < AlaAla < AlaVal.

The contribution of the side chain (R) to h_{xy} for the R-alanyl-dipeptide can be estimated from the difference

$$\Delta h_{xy}(R) = h_{xy}(\text{Ala-R}) - h_{xy}(\text{AlaGly}) \qquad (3)$$

where AlaGly is the amino acid with a R $\equiv$ –CH side chain.

Table 3
Quantitative contribution ($\Delta h_{xy}(R)$, J kg mol^{-2}) to h_{xy} of R-alanyl-dipeptide with alcohols in water solution for R = –CH$_3$, –CH(CH$_3$)$_2$, –CH$_2$CONH$_2$.

R	EtOH	1-PrOH	2-PrOH
–CH$_3$	121	306	276
–CH(CH$_3$)$_2$	397	390	659
–CH$_2$CONH$_2$	−305	−332	−514

Fig. 2. Enthalpies of transfer, $\Delta_{tr}H°$, of DL-α-alanine (1), AlaGly (2), AlaAla (3) [7], AlaVal (4) [9] and AlaAsn (5) from H$_2$O to its mixtures with EtOH (a), 1-PrOH (b) and 2-PrOH (c) as a function of alkanol mole-fraction (x_2) at 298.15 K.

related to the coefficient of pair-wise interactions as $h_{xy} = a_1/a_2$ [20]. The obtained h_{xy} values are listed in Table 2.

The h_{xy} values for AlaAsn–alkanol interactions are positive. It means that the interaction between AlaAsn and alkanol molecules in aqueous solutions is accompanied by overlapping their hydration shells. The given effect results in both a partial reorganization of these shells and a change of the above-marked interactions,
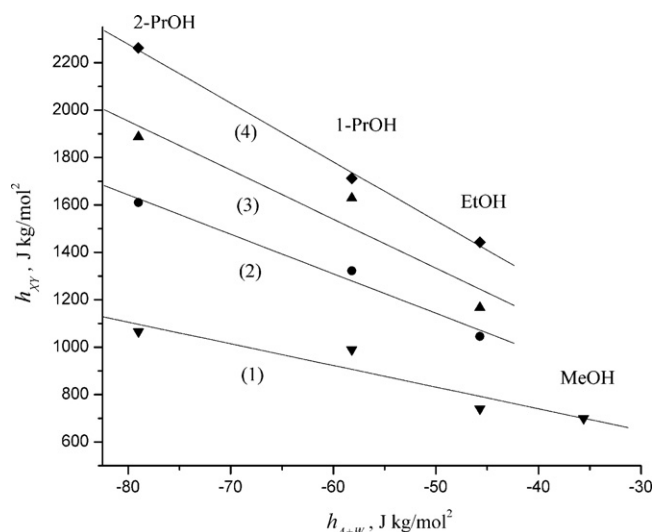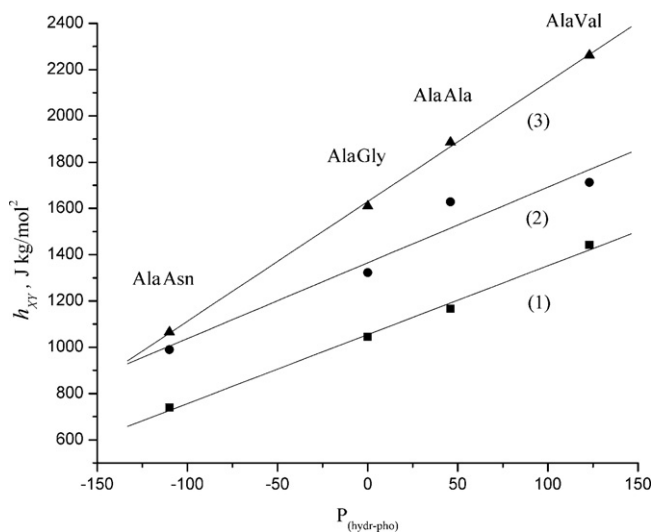
Fig. 4. Correlation between the enthalpy coefficients of pair-wise interactions, h_{xy}, of AlaAsn, AlaGly [7], AlaAla [7] and AlaVal [9] with EtOH (1), 1-PrOH (2) and 2-PrOH (3) in aqueous solution and the hydrophobicity parameters $P_{hydr-pho}$ [3].

Hence the $\Delta h_{xy}(R)$ value (Table 3) can be regarded quantitatively as a contribution of the R-chain to h_{xy} for the R-alanyl-dipeptide where $R \equiv -CH$, $-CH_3$, $-CH(CH_3)_2$ or $-CH_2CONH_2$. The positive values of $\Delta h_{xy}(R)$ for AlaAla and AlaVal confirm the earlier made findings that a R-alanyl-dipeptide molecule containing hydrophobic CH_3-groups weakens considerably its interaction with the alkanol molecules in an aqueous medium. On the contrary, the negative $\Delta h_{xy}(R)$ values for AlaAsn testify to the strengthening of interactions between $-CH_2CONH_2$-containing dipeptide and alcohol molecules in each of (water + alkanol) mixtures studied.

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