Study of the interaction between ethanol and natural amino acids containing ionic side groups in water at $T = 298.15$ K

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Abstract The enthalpies of solution of L-α-aspartic acid, L-α-glutamic acid, L-α-arginine, L-α-lysine, and L-α-histidine have been measured in aqueous ethanol solutions at 298.15 K. From the obtained experimental results, the standard enthalpies of solution of amino acids in water–ethanol solutions have been determined. These data were used to calculate the heterogeneous enthalpic pair interaction coefficients based on McMillan–Mayer's formalism. These values were interpreted in the terms of the ionic or no polar effect of the side chains of L-α-amino acids on their interactions with a molecule of ethanol in water.

Keywords L-α-Amino acids · Ethanol · Aqueous solution · Enthalpies of solution · Enthalpic pair interaction coefficients

List of symbols

$h_{A,EtOH}$	The heterogeneous enthalpic pair interaction coefficient between zwitterions of amino acids and ethanol molecule
$h_{A,EtOH,EtOH}$	The enthalpic triplet interaction coefficient
$\Delta_{sol}H_m^\infty(W)$	Standard enthalpies of solution of L-α-amino acids in water (W)
$\Delta_{sol}H_m^\infty(W + EtOH)$	Standard solution enthalpies of the L-α-amino acids (A) in ethanol (EtOH) and water (W) mixtures
m_{EtOH}	Molal concentration of ethanol in water (mol kg^{-1})

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Introduction

Many research centers carry out studies on factors that exert influence on the stability of the native structure of proteins, processes of their folding and interactions with various components of body fluids. The complex structure of biomacromolecules poses difficulties in the interpretation of the processes, in which they participate. Therefore, simple model systems such as L-α-amino acids [1–3] or small peptides [4–6] are often used for such investigations. Moreover, every living organism contains a constant stock of free natural amino acids, which participate in life processes, interact with the components of intracellular and extracellular fluids.

The aim of our study was to examine the interactions between L-α-amino acids and ethanol proceeding in aqueous solutions. Ethanol occurs in the organisms of yeast and many bacteria as a product of glucose pathway. It is supplied to animal and human organisms as a component of numerous foods, pharmaceutical and cosmetic products. Moreover, ethanol is of interest as a compound possessing two different functional groups with respect to the affinity to water: polar hydroxyl group and non-polar ethyl group.

Based on the values of the solution enthalpy of selected amino acids in aqueous ethanol solutions obtained by the calorimetric method, enthalpic interaction coefficients derived from modified [7, 8] McMillan–Mayer's formalism [9] were determined. These coefficients describe the energetic effects of the interactions between the molecules under investigation proceeding with the competitive co-contribution of water molecules.

Experimental section

Materials

L-α-Aspartic acid (Asp), L-α-glutamic acid (Glu), L-α-arginine (Arg), L-α-lysine (Lys), and L-α-histidine (His) (all mass fraction purity >99%, Fluka) were dried vacuum at 320 K; ethanol (99.8% POCh Poland) was purified by standard methods [10]. The water used in the experiments was deionized, distilled twice, and degassed.

Calorimetric measurements

The enthalpies of solution were measured in water and in aqueous solutions of ethanol (EtOH) using an isoperibol calorimeter [11]. The temperature sensitivity was about 0.00004 K and the temperature stability of the thermostat was better than 0.002 K. The uncertainties in the measured enthalpies did not exceed ±0.5% of the measured value. The examined aqueous solutions containing from 0 to 5 mol of ethanol in 1 kg of water and the aqueous solutions of amino acids (A) (0.001 to 0.005) mol (A) kg^{-1} (solvent) were prepared by weight using Mettler AE 240 balance within the precision ±0.00001 g (buoyancy corrections to the weights were (±0.00002 g). The standard enthalpies of solution of L-α-amino acids (A) were determined by the linear extrapolation to zero amino acids concentration of the results obtained for eight to ten independent measurements within the investigated range of the amino acids concentration.

Results and discussion

The determined standard solution enthalpies of the L-α-amino acids (A) in ethanol (EtOH) and water (W) mixtures $\{\Delta_{sol}H_m^\infty(W + EtOH)\}$, together with their standard deviations, are presented in Table 1.

The values of the standard enthalpies of solution of all the amino acids tested increase with ethanol concentration in water (Table 1). Considering the changes in thermal effects accompanying the dissolution of amino acids in aqueous solution of ethanol, one should take into account the properties of this mixed solvent. Within the concentration range investigated, the addition of ethanol to water brings about the structure reinforcement of the mixture formed [12, 13]. The addition of amino acid to a solvent with a more developed structure such as water/ethanol mixture requires more energy to overcome the reinforced interactions existing in this mixture. Consequently, the dissolution process of amino acids becomes more endothermic.

Information about the interactions proceeding in aqueous solutions between the zwitterions of natural amino acids under investigation and ethanol molecule can be provided by the analysis of the enthalpic interaction coefficients of ethanol molecule–amino acid zwitterion pairs.

The values of standard dissolution enthalpies were used also to obtain the enthalpic heterogeneous pair interaction coefficients by the equation proposed by Desnoyers [14]:

$$\Delta_{sol}H_m^\infty(W + EtOH) - \Delta_{sol}H_m^\infty(W) = 2h_{AEtOH}m_{EtOH} + 3h_{AEtOHEtOH}m_{EtOH}^2 + \cdots \quad (1)$$

where $\Delta_{sol}H_m^\infty(W)$ is the standard enthalpies of solution of L-α-amino acids in water (see Table 1) $h_{A,EtOH}$ is the heterogeneous enthalpic pair interaction coefficient between zwitterions of amino acids and ethanol molecule (see Table 2), $h_{A,EtOH,EtOH}$ is the enthalpic triplet interaction coefficient (see Table 2), and m_{EtOH} denotes the molality concentration of ethanol in water (mol kg^{-1}). The interpretation of the triplet interaction coefficient is obscured by the fact that they also contain some contributions from the pairwise interaction terms and they are not discussed in this article.

The values of the enthalpic pair interaction coefficients between amino acids zwitterions and ethanol molecule in

Table 1 Standard enthalpies of dissolution $\Delta_{sol}H_m^\infty(W + EtOH)$ of L-α-amino acids in aqueous ethanol (EtOH) solutions at 298.15 K

$m_{(EtOH)}$/mol kg^{-1}	$\Delta_{sol}H_m^\infty(W + EtOH)$/kJ mol^{-1}				
	Asp	Glu	Arg	Lys	His
0.0	25.77 ± 0.13	27.11 ± 0.14	6.62 ± 0.09	−15.68 ± 0.08	14.32 ± 0.08
0.5	26.67 ± 0.15	28.05 ± 0.18	7.43 ± 0.09	−14.75 ± 0.08	15.25 ± 0.08
1.0	27.36 ± 0.16	28.78 ± 0.19	8.30 ± 0.09	−13.88 ± 0.08	16.03 ± 0.09
1.5	27.96 ± 0.18	29.45 ± 0.19	8.90 ± 0.09	−13.13 ± 0.07	16.77 ± 0.09
2.0	28.60 ± 0.13	29.94 ± 0.19	9.50 ± 0.09	−12.35 ± 0.09	17.54 ± 0.09
2.5	29.08 ± 0.18	30.52 ± 0.19	9.98 ± 0.09	−11.73 ± 0.07	17.96 ± 0.09
3.0	29.74 ± 0.18	31.07 ± 0.20	10.57 ± 0.10	−11.12 ± 0.10	18.42 ± 0.09
4.0	30.21 ± 0.18	31.64 ± 0.19	11.20 ± 0.09	−10.09 ± 0.05	19.00 ± 0.10
5.0	30.56 ± 0.18	31.98 ± 0.19	11.63 ± 0.09	−9.03 ± 0.07	19.26 ± 0.09

Table 2 Heterogeneous enthalpic pair and triplet interaction coefficients for L-α-amino acids (A) with ethanol (EtOH) in water

Amino acids	$h_{(A,EtOH)}$/J kg mol^{-2}	$h_{(A,EtOH,EtOH)}$/J kg^2 mol^{-3}
L-α-Aspartic acid	871 ± 30	-52.1 ± 3.8
L-α-Glutamic acid	894 ± 22	-54.4 ± 2.8
L-α-Arginine	872 ± 19	-49.6 ± 2.5
L-α-Lysine	926 ± 22	-35.8 ± 2.8
L-α-Histidine	972 ± 18	-63.9 ± 2.3

water (Table 2) describe the energetic effects of the interactions between the molecules investigated proceeding with the competitive co-contribution of water molecules. The enthalpic pair interaction coefficients include the energetic co-contribution of both the water molecules creating hydration layers around polar or ionic groups and the water molecules constituting the hydrophobic sheaths around non-polar groups.

The direct interactions between the hydroxyl group of ethanol molecule and the zwitterion head of $NH_3^+CHCOO^-$ as well as polar or ionic side substituents of amino acids (exothermic process) are possible if some water molecules are removed from their hydration layer (endothermic process). This endothermic process of dehydration is intensified by the hydrophobic effects induced by the ethyl group of ethanol as well as the non-polar groups of amino acid side chain. Water molecules surrounding non-polar groups reinforce the interactions between themselves owing to the cooperativeness of hydrogen bonds. This effect is transferred onto the water molecules of hydration layers of polar or ionic groups. Thereby the removal of some water molecules from hydration sheaths requires an increased energy input (increase in the endothermic process).

The enthalpic pair interaction coefficients, $h_{(A,EtOH)}$ (Table 2), of ethanol and L-α-amino acids (L-α-aspartic acid, L-α-glutamic acid, L-α-arginine, L-α-lysine, and L-α-histidine) possessing ionic substituents are more positive than the value of the enthalpic coefficient for glycine, $h_{(Gly,EtOH)}$ [15]. The high positive values of these coefficients point to the endothermic summary effect of the interaction between the zwitterions investigated and ethanol molecule in water. The positive values of enthalpic coefficients, $h_{(A,EtOH)}$, testify to the predominating effects of partial dehydration of ionic groups in amino acid side chains over the direct interaction between polar groups in the molecules are under discussion.

The calculated enthalpic pair interaction coefficients, $h_{(A,EtOH)}$, describing the interactions of ethanol with L-α-aspartic acid, L-α-glutamic acid, L-α-arginine, L-α-lysine, and L-α-histidine are surprisingly high in comparison with the values of the enthalpic coefficients of urea interaction with the zwitterions of the amino acids under discussion,

$h_{(A,U)}$ [2] (see Table 2). These differences seem to be due to the specific structure of water–ethanol mixtures. The structure of ethanol makes it possible for it to be very well accommodated in the elastic three-dimensional network of hydrogen bonds of water [12, 13] bringing about the reinforcement of water structure [16]. The zwitterion heads of $NH_3^+CHCOO^-$ and polar or ionic side substituents incorporated into the water–ethanol mixtures interact stronger with the surrounding water molecules than with the less accessible hydroxyl group of ethanol. Thereby the direct interactions between polar or ionic groups of L-α-amino acids and the hydroxyl group of ethanol make a smaller than expected exothermic contribution to the global effect of interactions that describe the enthalpic pair interaction coefficients. A crucial contribution to the values of enthalpic coefficients, $h_{(A,EtOH)}$, is made by the endothermic processes of dehydration of zwitterion head, polar or ionic groups in amino acid side chains, and the hydroxyl group of ethanol molecule. Therefore, the total effect described with the enthalpic pair interaction coefficients, $h_{(A,EtOH)}$, is so strongly endothermic.

The substitution of hydrogen atom in the side chain of L-α-alanine ($h_{(Ala,EtOH)} = 658$ J kg mol^{-2}) [15] or L-α-aminobutyric acid ($h_{(Aba,EtOH)} = 820$ J kg mol^{-2}) [17] with carboxylic group leads to the increase of the values of the enthalpic pair interaction coefficients, $h_{(A,EtOH)}$ (Table 2).

The dependence observed is the reverse of that shown in the system with urea, for which the enthalpic interaction coefficients of amino acid–urea pairs, $h_{(A,U)}$, in the case of L-α-alanine ($h_{(A,U)} = -238.2$ J kg mol^{-2}) [2] and L-α-aminobutyric acid ($h_{(A,U)} = -185$ J kg mol^{-2}) [18], have more positive values than those determined for L-α-aspartic acid or L-α-glutamic acid [2] (Table 2).

Comparing the values of enthalpic coefficients, $h_{(A,EtOH)}$, of L-α-aspartic acid and L-α-glutamic acid, one can observe the expected endothermic contribution induced by the presence of non-polar CH_2 group: $h_{(Asp,EtOH)} < h_{(Glu,EtOH)}$

The values of the enthalpic coefficients of the heterogeneous amino acid–ethanol interactions, $h_{(A,EtOH)}$, were correlated with the values of the enthalpic coefficients of

Table 3 Heterogeneous enthalpic pair interaction coefficients for L-α-amino acids (A) with urea molecule (U) in water ($h_{A,U}$) [2] and the free energy transfer of amino acids (ΔG_R) [19]

Amino acids	$h_{(A,U)}$/J kg mol^{-2}	ΔG_R/J mol^{-1}
L-α-Aspartic acid	-831	-5020
L-α-Glutamic acid	-820.8	-3180
L-α-Arginine	-892.8	-8790
L-α-Lysine	-721.5	-2390
L-α-Histidine	-518.3	2680
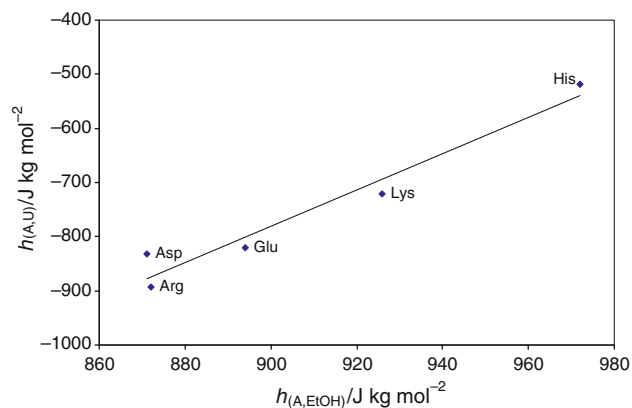
Fig. 1 Relationship between the enthalpic pair interaction coefficients $h_{(A,U)}$ [2] of amino acids zwitterions–urea molecule in water and the enthalpic pair interaction coefficients $h_{(A,EtOH)}$ of amino acids zwitterions–ethanol molecule in water
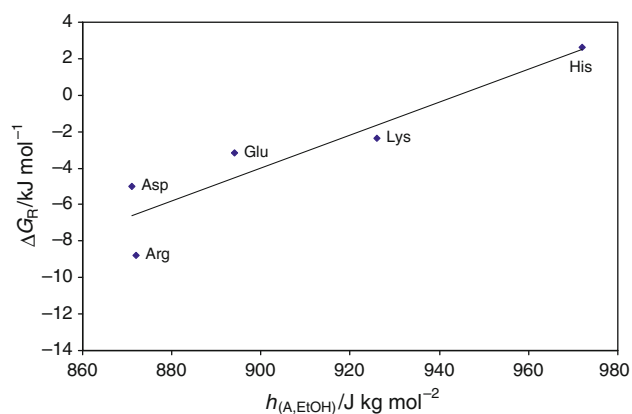
Fig. 2 Relationship between the hydrophobicity parameters of amino acids ΔG_R [19] and the enthalpic pair interaction coefficients $h_{(A,EtOH)}$ of amino acids zwitterions–ethanol molecule in water

amino acid–urea interactions, $h_{(A,U)}$ [2] (see Table 3). This correlation has approximately a linear course ($R^2 = 0.952$, see Fig. 1). This testifies to similar contributions made by the partial effects of the dehydration of solvation layers in polar and ionic groups of the zwitterions of L-α-amino acids and direct interaction between discussed molecules to the total effects of interactions proceeding in examined systems it is: natural amino acids with ethanol ($h_{(A,EtOH)}$) and natural amino acids with urea molecule ($h_{(A,U)}$) in aqueous solution.

The values of the enthalpic pairs coefficients $h_{(A,EtOH)}$ were also compared with lipophilicity scale of amino acids calculated by Eisenberg and McLachlan [19] (see Table 3). The obtained graph (Fig. 2) displays dependence ($R^2 = 0.855$) between the lipophilicity parameters ΔG_R and enthalpic pair interaction coefficients of amino acids and ethanol molecule $h_{(A,EtOH)}$. The observed dependences suggest, as well as above correlation, 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.

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