



# Enthalpies of solution of L-threonine in a (water + alcohol) mixture at 298.15 K

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## ABSTRACT

The enthalpies of solution of L-threonine in the (water + methanol), (water + ethanol), (water + *n*-propanol), and (water + *i*-propanol) mixtures, with an alcohol content up to 0.4 mol fractions, have been measured calorimetrically at  $T = 298.15$  K. The standard enthalpies of solution and transfer of L-threonine from water to an aqueous alcohol have been calculated. The effect of the structure properties of the mixed solvent on the specified enthalpy characteristics of L-threonine is discussed. The enthalpy coefficients of pairwise interactions between amino acid and alcohol molecules have been computed. It has been found that these coefficients become increasingly positive in the methanol, ethanol, *n*-propanol, and *i*-propanol consequence. A comparative analysis of the thermodynamic characteristics of dissolution of L-threonine and some other amino acids (glycine, L-alanine and L-valine) in the mixtures studied has been made.

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## 1. Introduction

It is well known that ethanol (EtOH) is a strong denaturant of globular proteins, and the amino acids, being the structural fragments of proteins, participate in all the physiological processes of a living cell. Therefore, the important place in disclosing the mechanism of denaturation of proteins and biopolymers occupy the works devoted to interactions between the amino acids and alcohol (mainly, ethanol) molecules [1–5]. As a rule, the solutions of amino acids in the aqueous–alcoholic mixtures containing no more than 8 mol of co-solvent per 1 kg of water have been studied [1–3] and there are virtually no data on the thermodynamic characteristics of the specified systems with higher alcohol concentrations. Because the (water + ethanol) mixtures with rather high alcohol concentrations are the “real” denaturing media for proteins, would be interesting to study other aqueous–alcoholic solutions of amino acids, as a comparison.

This paper is an extension of our previous works on the study of enthalpic effects of interaction between amino acids (peptides) and alcohols [6–10] as well as amides [11,12] and other organic co-solvents [13–17] in aqueous media. We report here the enthalpies of solution,  $\Delta_{\text{sol}}H^m$ , for L-threonine in aqueous EtOH, methanol (MeOH), *n*-propanol (*n*-PrOH) and *i*-propanol (*i*-PrOH), with a view to gaining information on energy-related changes in the amino acid–alcohol intermolecular interaction under the influence of the co-solvent nature. The alcohol content in mixtures ranged up to

0.4 mol fractions. The choice of L-threonine is caused by that the given amino acid is an irreplaceable one being a part of many proteins. Herewith, unlike glycine, L-alanine and L-valine, L-threonine contains a hydroxyl group.

The results obtained in the present paper were compared with those for the same solutions of glycine [6,18], L-alanine [7] and L-valine [10].

## 2. Experimental

Chromatographically homogeneous amino acids (Reanal Co., Hungary) were recrystallized twice from ( $\text{H}_2\text{O} + \text{EtOH}$ ) mixture, dried in a vacuum chamber at 333 K for 48 h, and kept over  $\text{P}_2\text{O}_5$  under vacuum in desiccators. The molal concentration ( $m$ ) of the L-threonine solutions was varied in the range of  $0.005 < m < 0.015$  mol per 1 kg mixed solvent. The alcohols were purified as recommended earlier [19,20]. The water content determined by the Karl Fisher titration [21] did not exceed 0.03, 0.05, 0.03, and 0.04 wt.% in MeOH, EtOH, *n*-PrOH, and *i*-PrOH, respectively. Water was purified by deionization and double distillation until a specific conductivity of ca.  $1.0 \times 10^{-5} \text{ S m}^{-1}$ . All the mixtures were prepared by weight.

The values of  $\Delta_{\text{sol}}H^m$  for L-threonine were measured at  $(298.15 \pm 0.005) \text{ K}$  using an isoperibol hermetic calorimeter fitted with a 60  $\text{cm}^3$  reaction vessels and electrical calibration. The calorimeter setup and experimental procedure were described in detail previously [22–24]. The relative random error of measurements did not exceed 0.5%. The calorimeter was tested by measuring (10 experiments) the enthalpy of solution of potassium chloride (KCl) in water at 298.15 K according to [22,23,25]. Our values of  $\Delta_{\text{sol}}H^m$  ( $m = 0.111 \text{ mol kg}^{-1}$ ) =  $17.60 \pm 0.04 \text{ kJ mol}^{-1}$  and  $\Delta_{\text{sol}}H^0 = 17.23 \pm 0.07 \text{ kJ mol}^{-1}$  agree with the recommended liter-

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

| $m_2^a$ | MeOH         | $m_2^a$ | EtOH         | $m_2^a$ | <i>n</i> -PrOH | $m_2^a$ | <i>i</i> -PrOH |
|---------|--------------|---------|--------------|---------|----------------|---------|----------------|
| 0.712   | 11.15 ± 0.02 | 0.721   | 11.51 ± 0.02 | 0.733   | 11.68 ± 0.02   | 0.715   | 11.93 ± 0.02   |
| 1.357   | 11.78 ± 0.02 | 1.452   | 12.78 ± 0.02 | 1.215   | 12.25 ± 0.02   | 1.181   | 12.74 ± 0.02   |
| 2.034   | 12.56 ± 0.02 | 2.368   | 13.65 ± 0.02 | 1.982   | 13.57 ± 0.02   | 1.742   | 13.85 ± 0.02   |
| 2.837   | 13.11 ± 0.02 | 3.437   | 14.59 ± 0.02 | 2.726   | 14.71 ± 0.02   | 2.581   | 15.04 ± 0.02   |
| 4.117   | 14.93 ± 0.03 | 4.512   | 15.99 ± 0.03 | 3.375   | 15.54 ± 0.03   | 3.356   | 16.19 ± 0.03   |
| 5.529   | 15.99 ± 0.03 | 5.435   | 17.23 ± 0.03 | 4.028   | 16.02 ± 0.03   | 4.285   | 18.16 ± 0.03   |
| 7.371   | 16.98 ± 0.03 | 7.616   | 18.79 ± 0.03 | 4.815   | 16.96 ± 0.03   | 5.387   | 19.83 ± 0.03   |
| 10.302  | 18.18 ± 0.03 | 9.011   | 19.96 ± 0.03 | 6.021   | 17.89 ± 0.03   | 6.417   | 18.98 ± 0.03   |
| 10.882  | 18.20 ± 0.03 | 12.268  | 20.91 ± 0.03 | 8.878   | 18.82 ± 0.03   | 9.149   | 19.83 ± 0.03   |
| 16.067  | 19.55 ± 0.03 | 17.247  | 21.51 ± 0.03 | 13.417  | 18.89 ± 0.03   | 12.838  | 20.78 ± 0.03   |
| 24.303  | 20.16 ± 0.03 | 24.089  | 20.69 ± 0.03 | 18.749  | 17.81 ± 0.03   | 17.966  | 20.22 ± 0.03   |
| 35.716  | 20.92 ± 0.03 | 34.344  | 19.19 ± 0.03 | 26.796  | 16.56 ± 0.03   | 25.659  | 19.06 ± 0.03   |

<sup>a</sup> The molal concentration of alcohols (mol/kg).

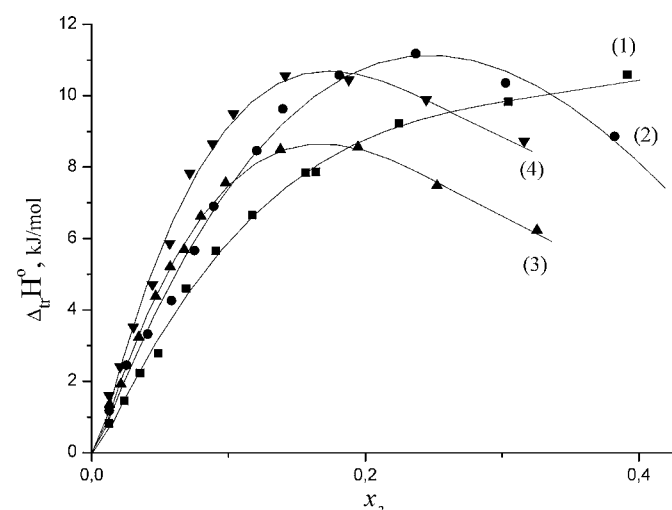
ature values ( $17.56 \pm 0.02 \text{ kJ mol}^{-1}$  [26]/ $17.58 \pm 0.02 \text{ kJ mol}^{-1}$  [27] and  $17.22 \pm 0.04 \text{ kJ mol}^{-1}$  [22,24], respectively).

### 3. Results

The standard enthalpies of solution,  $\Delta_{\text{sol}}H^\circ$  ( $\equiv \Delta_{\text{sol}}H^\infty$ ), were calculated by averaging the results of five independent measurements of  $\Delta_{\text{sol}}H^m$  for each composition of an aqueous alcohol, because no dependences of  $\Delta_{\text{sol}}H^m$  from concentration of L-threonine were observed in the mixed solvents studied. The confidence interval half-width ( $\pm \xi_n$ ) of the  $\Delta_{\text{sol}}H^\circ$  value was determined by the Peters formula [28] for the root-mean-square error,  $\xi_n = t_{0.95}(4/5) \sum_{i=1}^n |x_i - \bar{x}_i| / [n(n-1)]^{1/2}$ , with correction for a Student criterion of  $t_{0.95} = 2.78$ . Here ( $n=5$ ) is the number of runs,  $x_i = \Delta_{\text{sol}}H^m$ , and  $\bar{x}_i = |\Delta_{\text{sol}}H^m|_{\text{av}}$ . The experimental data on  $\Delta_{\text{sol}}H^\circ$  obtained for L-threonine in aqueous alcohols under study are summarized in Table 1. The enthalpies of transfer of L-threonine from water to an aqueous alcohol, ( $\Delta_{\text{tr}}H^\circ$ ), graphically presented in Fig. 1 were calculated from the experimental enthalpies for L-threonine dissolution in the pure water, ( $\Delta_{\text{sol}}H^\circ(w)$ ), and in the alcohol-containing aqueous solution, ( $\Delta_{\text{sol}}H^\circ(w+y)$ )

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

Enthalpy of dissolution of L-threonine in water ( $\Delta_{\text{sol}}H^\circ(w) = 10.33 \pm 0.06 \text{ kJ mol}^{-1}$ ) was taken from [29].



**Fig. 1.** Enthalpies of transfer  $\Delta_{\text{tr}}H^\circ$  of L-threonine from water into the  $\text{H}_2\text{O} + \text{MeOH}$  (1),  $\text{H}_2\text{O} + \text{EtOH}$  (2),  $\text{H}_2\text{O} + n\text{-PrOH}$  (3), and  $\text{H}_2\text{O} + i\text{-PrOH}$  (4) mixed solvent as functions of the alcohol mole fraction ( $x_2$ ) at 298.15 K.

### 4. Discussion

One can see from Fig. 1, that  $\Delta_{\text{tr}}H^\circ(x_2)$  for L-threonine depends appreciably on alcohol structure properties. In all cases, the dissolution of L-threonine in an aqueous alcohol is the endothermic process within the investigated concentration range. Herewith the alcohol capability to association influences the presence or absence of a maximum on the curve of  $\Delta_{\text{tr}}H^\circ$  against  $x_2$ . So, in the case of ( $\text{H}_2\text{O} + \text{MeOH}$ ) mixtures, the maximum on the concentration dependence of  $\Delta_{\text{tr}}H^\circ$  is absent, whereas for aqueous EtOH, the given maximum is weakly pronounced. This phenomenon was observed previously for like solutions of other amino acids [6,7,10]. It is apparent that such a change in  $\Delta_{\text{tr}}H^\circ$  for L-threonine is connected simultaneously to the destruction of alcohol self-associates (in a wide concentration range of the mixture) and their hydration with further dehydration at interacting with the L-threonine molecules.

Dependences of  $\Delta_{\text{tr}}H^\circ$  versus  $x_2$  for ( $\text{H}_2\text{O} + n\text{-PrOH}$ ) and ( $\text{H}_2\text{O} + i\text{-PrOH}$ ) mixtures have obviously pronounced endothermic maximum which is displaced in the range of lower concentrations of alcohol when *n*-PrOH is replaced by *i*-PrOH. Also, noteworthy is that the specified concentration dependences are virtually symmetric relative to each other. It points to the similar character of interactions between *n*-PrOH or *i*-PrOH and L-threonine molecules. The higher endothermicity of L-threonine dissolution in ( $\text{H}_2\text{O} + n\text{-PrOH}$ ) compared to ( $\text{H}_2\text{O} + i\text{-PrOH}$ ) mixtures can be connected with the greater hydrophobicity of the isomeric alcohol. Herewith, although the  $\Delta_{\text{tr}}H^\circ$  value increases monotonically with increasing the alcohol concentration (up to  $x_2 \approx 0.15/0.2$ ), the “slope” of this curve is various, increasing in a series  $\text{MeOH} < \text{EtOH} < n\text{-PrOH} < i\text{-PrOH}$ .

The interparticle interactions in this region can be characterized in terms of the McMillan–Mayer theory [30] adapted by Kauzmann [31], Krishnan and Friedman [32], and Desnoyers et al. [33] for calculating the enthalpy coefficients of pairwise interactions  $h_{xy}$ . For this purpose, the  $\Delta_{\text{sol}}H^\circ$  versus  $m_2$  functions were approximated by the third-order polynomial equation:

$$\Delta_{\text{sol}}H^\circ = a_0 + a_1m_2 + a_2m_2^2 + a_3m_2^3 \quad (2)$$

where  $m_2$  is the molal concentration of the alcohol, and  $a_i$  are coefficients calculated by a least-squares method. The correlation coefficient,  $R$ , and the Student criterion value,  $t_\alpha$ , ranged from 0.993 to 0.997 and from 0.170 to 0.312, respectively. The  $h_{xy}$  value was calculated from the  $a_1$  coefficient related to the coefficient of pairwise interactions as  $h_{xy} = a_1/2$  [34]. Table 2 shows the  $h_{xy}$  quantities for interactions between L-threonine and alcohol molecules obtained in this work and reported previously for glycine [6], alanine [7] and valine [10].

In the binary solvents studied, the L-threonine molecule has a mixed solvation shell, which consists of both alcohol and water molecules, with the latter predominating (at least, at low concen-

**Table 2**

Enthalpic coefficients of pairwise interactions ( $h_{xy}$ , J kg mol<sup>-2</sup>) between L-threonine (glycine, DL-alanine, L-valine) and alcohols in aqueous solutions at 298.15 K.

| Substance      | MeOH     | EtOH      | <i>n</i> -PrOH | <i>i</i> -PrOH |
|----------------|----------|-----------|----------------|----------------|
| L-threonine    | 611 ± 30 | 796 ± 25  | 939 ± 29       | 1157 ± 51      |
| Glycine [6]    | –        | 551 ± 35  | 776 ± 48       | 915 ± 63       |
| DL-Alanine [7] | –        | 571 ± 112 | 923 ± 53       | 1071 ± 80      |
| L-valine [10]  | –        | 1009 ± 73 | 1098 ± 41      | 1370 ± 62      |

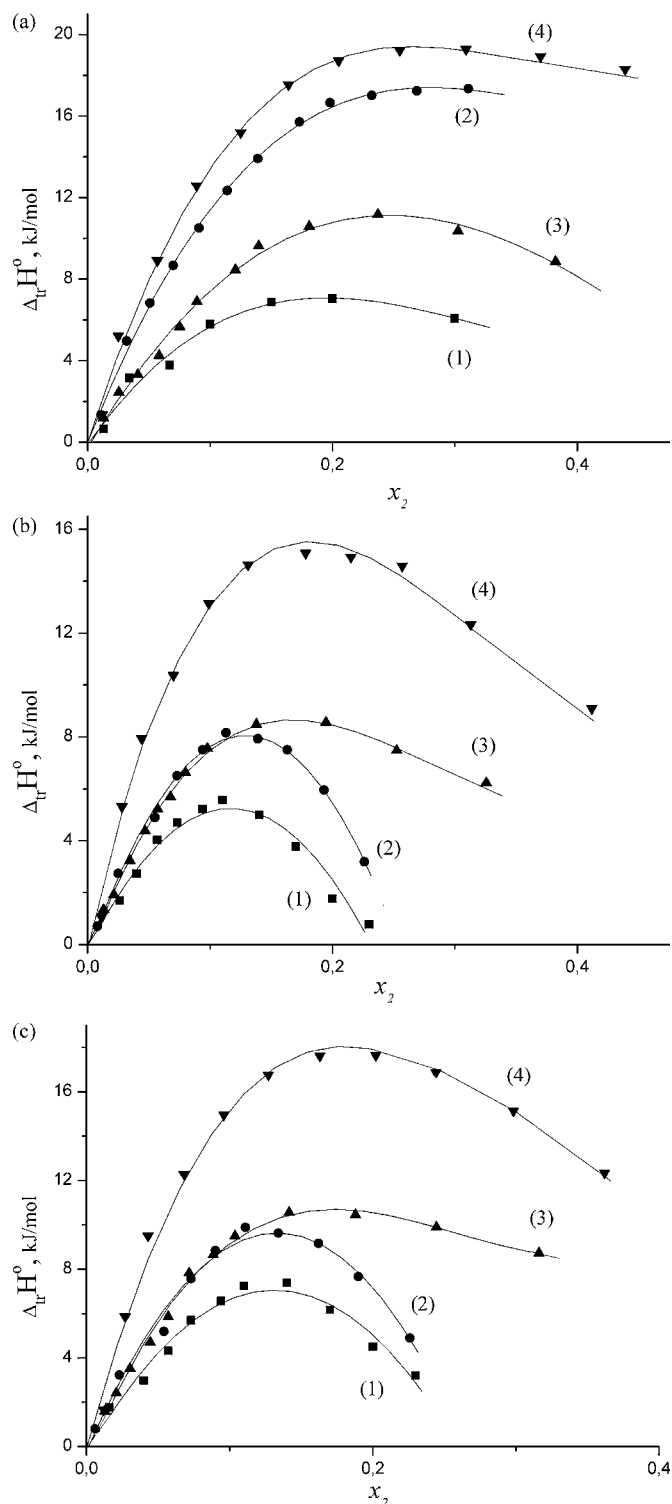
trations of alcohol:  $x_2 < 0.15$ ). The interaction of L-threonine and alcohol molecules is accompanied by overlapping of their hydration shells, resulting in a partial reorganization of these shells and in a change of the L-threonine–alcohol interactions. Therefore, the  $h_{xy}$  values reflect totally the dehydration, solvation and direct L-threonine–alcohol interaction effects. For amino acids (such as L-threonine) having the alkyl side chains, these processes are accompanied additionally by the hydrophobic effect in the vicinity of the non-polar fragments. As a consequence, the sign and magnitude of  $h_{xy}$  are determined by the superposition of the above effects.

It can be seen from data of Table 2 that the pairwise enthalpy coefficients of interaction of L-threonine with an alcohol are positive by sign. This is caused by the high endothermicity of process of partial dehydration of the solvation shells of both the L-threonine and alcohol molecules. This effect predominates over the exothermic effect of a direct interaction between the zwitterion of L-threonine and the alcoholic polar groups. Such a conclusion is in agreement with the results obtained earlier for glycine [6], L-alanine [7] and L-valine [10].

The differences in  $h_{xy}$  for L-threonine depend mainly on the structure properties of alcohol molecules and become increasingly positive in a consequence MeOH < EtOH < *n*-PrOH < *i*-PrOH. According to the inferences [35], it can be connected with strengthening of the alcohol hydrophobicity in the specified order.

Analysis of changes in  $\Delta_{tr}H^\circ$  (Fig. 2a–c) and  $h_{xy}$  (Table 2) for various amino acids allows to notice the following features.

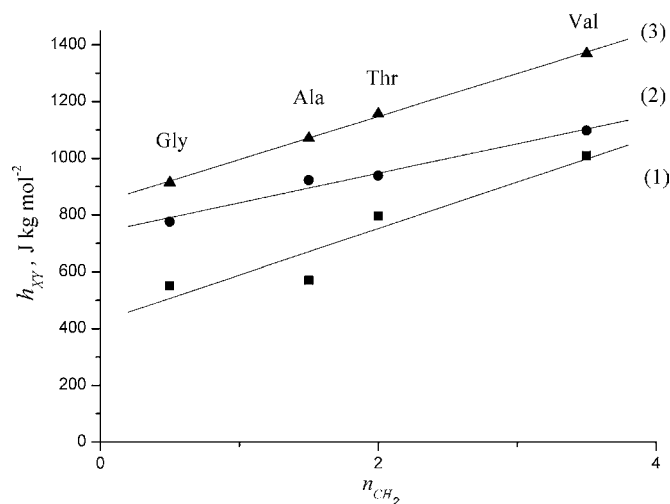
To begin with, the replacement of a hydrogen atom in a glycine molecule by an alkyl group results in an increase of positive (in sign) value of  $h_{xy}$  which is caused by the decrease in exothermic contributions from electrostatic interactions between a zwitterion of DL-alanine and a polar molecule of alcohol under the influence of the hydrophobic hydration of an amino-acid CH<sub>3</sub>-group. Compared to L-alanine, there is one OH-group on the side chain of L-threonine, resulting in the H-bond formation with alcohol molecules. The enthalpy effect of such hydrogen bonding will make up for the dehydration energy. As a result, the  $h_{xy}$  value for the interaction of L-threonine with an alcohol becomes less positive than that for L-alanine with the same alcohol. Here-with, it should be remembered that processes of dehydration of molecules both L-threonine and alcohol, and also hydrophobic interactions between the non-polar groups of the amino acid and an alcohol are remained still to be dominating. The values  $h_{xy}$  of L-threonine and L-valine have the essential distinctions in magnitude. It is possible to explain as follows. Side chain of a molecule of L-valine can be considered as the L-threonine side chain in which OH-group is substituted on CH<sub>3</sub>-group. The additional CH<sub>3</sub>-group in the L-valine molecule even more weakens direct interactions of L-valine with molecules of alcohol as a result of the hydrophobic hydration. Besides, two alkyl groups in the L-valine molecule decreases the interactions between the given amino acid and alcohol due to the steric effect, which complicates a formation of H-bonds. Therefore the intermolecular interactions between L-valine and molecules of alcohol in comparison with L-threonine are weakened. The greatest difference in value  $h_{xy}$  between L-threonine and L-valine is observed in aqueous–alcoholic



**Fig. 2.** Enthalpies of transfer  $\Delta_{tr}H^\circ$  of (1) glycine [6], (2) alanine [7], (3) L-threonine and (4) L-valine [10] from H<sub>2</sub>O into its mixtures with (a) EtOH, (b) *n*-PrOH and (c) *i*-PrOH versus alcohol mole fraction ( $x_2$ ) at 298.15 K.

mixtures in which an alcohol shows the big hydrophobic and steric effects.

Furthermore, the good correlations between  $h_{xy}$  and number of CH<sub>2</sub>-groups ( $n_{CH_2}$ ) in the amino acids molecule were found for glycine, L-alanine, L-threonine and L-valine solutions in various (H<sub>2</sub>O + alcohol) mixtures. These correlations are shown in Fig. 3. It follows from this figure that these functions are linear and they



**Fig. 3.** Dependence of the enthalpic coefficients of pairwise interaction of some amino acids in  $H_2O + EtOH$  (1),  $H_2O + n\text{-}PrOH$  (2) and  $H_2O + i\text{-}PrOH$  (3) mixtures on the number of  $CH_2$ -groups in the amino acids molecules (as proposed by Savage and Wood [36,37], it is accepted that the  $CH_3$  group corresponds to  $1.5CH_2$  while  $CH$  corresponds to  $0.5CH_2$ ).

can be described by the following equations:

$$h_{xy} = (424.8 \pm 83.3) + (163.7 \pm 38.4)n_{CH_2}, \quad R = 0.949, \quad EtOH \quad (3)$$

$$h_{xy} = (738.6 \pm 23.4) + (104.2 \pm 10.8)n_{CH_2}, \quad R = 0.989, \quad n\text{-}PrOH \quad (4)$$

$$h_{xy} = (843.8 \pm 8.3) + (151.7 \pm 3.8)n_{CH_2}, \quad R = 0.999, \quad i\text{-}PrOH \quad (5)$$

The constant term in the above equations for  $h_{xy}$  corresponds to the contribution of the interaction of the amino-acid zwitterion with an alcohol molecule. A positive sign of this contribution testifies that the endothermic effect of dehydration of the interacting polar groups is larger than the opposite (in sign) effect of direct interaction between the zwitterions and alcohol molecules. The latter effect increases in a series  $(H_2O + EtOH) < (H_2O + n\text{-}PrOH) < (H_2O + i\text{-}PrOH)$ . The slope of the  $h_{xy}$  versus  $n_{CH_2}$  function is a measure of the contribution of  $CH_2$  group to the value of the enthalpy coefficient considered. The pairwise interactions between molecules of threonine and alcohol in the  $(EtOH + L\text{-threonine}) < (n\text{-}PrOH + L\text{-threonine}) < (i\text{-}PrOH + L\text{-threonine})$  consequence are weakened. Increasing positive values  $h_{xy}$  points also to decreasing the exothermic contribution from electrostatic interactions between a zwitterion of L-threonine and a molecule of alcohol under the influence of hydrophobic hydration of the alcoholic alkyl groups. The similar changes in such interactions with the alcohol molecules are characteristic for other amino acids, too (see in Fig. 3).

To estimate the influence of hydrophobicity of the amino-acid side chains on the  $h_{xy}$  values for amino acid–alcohol interactions, the semi-empiric scale of hydrophobicity proposed by Nozaki and Tanford for similar systems can be used [38]. The given scale has been successfully used by author [29] for correlating the “homogeneous” coefficients of pairwise interactions ( $h_{xx}$ ) for some L-amino acids with parameter  $\Delta f_t$ , which characterizes the hydrophobicity of amino-acid side chains. From this, it is interesting to establish interrelation between the “heterogeneous” coefficients of pairwise interactions in question ( $h_{xy}$ ) and the hydrophobicity scale ( $\Delta f_t$ ) for the amino-acid side chains. The observed relationship is described by the linear equations (6)–(8):

$$h_{xy} = (558.1 \pm 33.2) + (72.9 \pm 9.7)(\Delta f_t), \quad R = 0.983, \quad EtOH \quad (6)$$

$$h_{xy} = (814.8 \pm 32.3) + (47.5 \pm 9.5)(\Delta f_t), \quad R = 0.963, \quad n\text{-}PrOH \quad (7)$$

$$h_{xy} = (958.5 \pm 52.7) + (67.6 \pm 15.4)(\Delta f_t), \quad R = 0.952, \quad i\text{-}PrOH \quad (8)$$

We believe that positions of both  $h_{xy}$  against  $\Delta f_t$  and  $h_{xy}$  against  $n_{CH_2}$  dependences relative to the  $x$ -coordinate are determined by the hydrophobicity of alcohol (see Fig. 3). If in the range of  $x_2$  from 0 to 0.15, the solvation shell of a L-threonine molecule consists mainly of water molecules, at  $x_2 > 0.15$ , a gradual substitution of water molecules by alcohol molecules takes place. At  $0.15 < x_2 < 0.2$ , the endothermic effects caused by the solvent structural reorganization with dehydrating the L-threonine and alcohol molecules are sequentially compensated by the exothermic effects of direct interactions between polar groups of the interacting components. Here the endothermic maxima on the curves of  $\Delta_{tr}H^\circ$  against  $x_2$  are observed for the majority of amino acids. The maximum height depends on the structure properties of both amino acid and alcohol. The increase in the molecular size of amino acid as well as in the alcohol hydrophobicity shifts this extremum in the water-rich region. If changes in  $\Delta_{tr}H^\circ$  at transfer from one aqueous alcohol to another are found to be analogous,  $(H_2O + EtOH) < (H_2O + n\text{-}PrOH) < (H_2O + i\text{-}PrOH)$ , for all the amino acids considered, the interpretation of such changes cannot be definitive. For example, in the concentration range  $0 < x_2 < 0.15$ , the dependences of  $\Delta_{tr}H^\circ$  from  $x_2$  for L-threonine and alanine have an approximately analogous character (see Fig. 2b and c). However, at  $x_2 > 0.2$ , a conspicuous divergence in dynamics of change of  $\Delta_{tr}H^\circ$  for L-threonine and alanine is observed. Also, one can notice that, at lower concentration of alcohol in mixtures (except for aqueous methanol), the character of solvation of the small (in size) amino acids (glycine and alanine) is reversed.

Thus, the above-presented results make possible to understand, how the change in energy of interaction between the amino acid and alcohol depends on both the structure properties of components and the composition of the aqueous–alcoholic mixture.

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