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# Effect of chain length on interactions of aliphatic alcohols with suspended human serum albumin

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**Abstract**

Enthalpy changes on the immersion of human serum albumin (HSA) into *n*-butanol, *n*-propanol, ethanol and methanol containing different amounts of water have been measured calorimetrically at 25°C. Water sorption isotherms on HSA were also determined in water–*n*-butanol and water–ethanol mixtures. From comparison of the calorimetric and sorption data, it was concluded that there is a significant enthalpy change on the HSA immersion into methanol and ethanol even under conditions where there is no change in the quantity of adsorbed water. No similar contribution was found in the *n*-butanol based suspensions. Water monolayer capacity evaluated from the Langmuir model decreases also significantly when going from ethanol to *n*-butanol. Considering this non water sorption contribution, values of the monolayer capacity and the shape of the experimental dependences, it was inferred that a relatively small change of the solvent molecule structure (from *n*-propanol to ethanol) affects strongly the interactions of the protein with the solvent. © 1997 Elsevier Science B.V.

*Keywords:* Human serum albumin; Aliphatic alcohols; Interactions; Enthalpy of immersion; Water sorption; Langmuir model

## 1. Introduction

An examination of the solid protein–organic component interactions would be appropriate and enlightening for understanding both the solid state protein chemistry and biotechnological implications as the enzymatic catalysis in nonaqueous media. So, it was demonstrated that the direct organic solvent–enzyme interactions suppress the transesterification reaction catalyzed by subtilisin Carlsberg suspended in toluene [1]. On the other hand, denaturating cosolvents are able to activate enzymes suspended in nonaqueous media which was explained with easing the flexibility constraints imposed by protein–protein contacts [2]. Enantioselectivity of suspended enzymes was found to be greatly effected by the anhydrous solvents [3].

As such, solvent–protein interactions require further fundamental study [4], and there is a need in the thermodynamic data describing the effect of such kind of interactions on the protein suspension properties. To our knowledge, the typical approach for examination of the direct solvent–solid protein interactions contributing to the thermodynamic properties of suspensions involves the comparison of the water sorption isotherms in suspensions and in the gas phase [5–7]. It was concluded that when the water

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sorption is plotted as a function of the water activity, sorption isotherms obtained for proteins in suspensions are similar to the water vapor isotherms. Some suppression of the water sorption relative to sorption of water vapor was noted for protein suspensions in alcohols [5,7]. In particular, such a suppression was mentioned for bovine serum albumin [5]. On the other hand, it was suggested that when the water activities are evaluated more accurately with the UNIFAC and NRTL models, the same sorption isotherms do not depend significantly on the properties of solvents or the nature of proteins [6]. In contrast, tremendous suppression of water sorption relative to the water vapor sorption was observed also for subtilisin Carlsberg and $\alpha$-chymotrypsin suspended in hexane with high water activity [8]. This suppression was interpreted in terms of competition between hexane and water for hydrophobic regions of the enzyme surface.

Based on calorimetric and sorption measurements [9], we demonstrated the different mechanisms of hydration of human serum albumin (HSA) in different organic solvents. It was shown [9–11] that depending on the organic solvent, the immersion of HSA into the water–organic mixtures may involve both the water sorption/desorption on the solid HSA preparation and a second process non related to the water sorption. This additional process is followed with the significant heat effect and the increase in the surface area accessible for sorption of water. It was considered to include the rupture of the protein–protein contacts in the solid phase induced by protein–organic component or/and protein–water interactions [9,11].

Water binding by albumins was determined in many studies [12–15]. However, less work has been done concerning the water interactions with solid albumins suspended in organic solvents. To our knowledge, there was the only study in which sorption of water on bovine serum albumin was determined in some organic solvents at 40°C [16]. This data set was compared with the water vapor sorption on horse serum albumin in the above cited Refs. [5,6].

Considering the combination of calorimetric and sorption measurements as an informative tool to test for the protein–solvent interactions, in this paper we reported new data on the enthalpy changes on immersing HSA into the solutions of water in homologous alcohols and the corresponding water sorption isotherms. We want to show the significant difference in the solid protein–solvent interactions occurring in the homologous series of solvents in which the solvent properties are changed relatively smoothly.

## 2. Experimental part

Human serum albumin was purchased from Sigma (Product No. A1887). Water contents of the protein preparation was $10.0 \pm 0.5\%$ (weight to weight of dry protein) as found by the Karl Fischer method. Alcohols were distilled and purified according to the standard methods [17]. Methanol, ethanol and *n*-propanol are completely miscible with water. Maximal water contents (7 M) in the water–*n*-butanol mixtures under consideration was lower than solubility of water in *n*-butanol (20.5% (w) [18] or 9.6 M).

Heat effects on immersing the hydrated HSA powder into solutions of water in alcohols were measured at 298 K with a Setaram BT-215 microcalorimeter according to the described procedure [19]. Typically, the sample of 4–8 mg of HSA contacted with 4.0 ml of a solvent in the calorimetric cell. Microcalorimeter was calibrated using the Joule effect and tested with dissolving sodium chloride in water according to the recommendations [20]. In most cases enthalpy changes were observed as one heat evolution peak completed for 30–40 min. Only in the ethanol based suspensions with water contents less than 3.5 M of water, a complicated calorimetric profile demonstrated the strong superposition of an exothermic effect on an endothermic effect. This profile was similar to the observed previously for HSA in water–dimethyl sulfoxide mixtures [11]. Resolution of this profile could not be done with confidence. In this case, the heat evolution was completed for 2 h, and the total change of the enthalpy was determined.

The obtained enthalpy changes $\Delta H$ corresponding to the immersion of the hydrated HSA powder (10% water) into mixtures of a solvent and water are in J to g of dry HSA. After the calorimetric experiment had been finished, the water contents $C_w$ of the liquid phase containing less than 3 M of water was

measured using Karl Fisher method. Equilibrium water contents $C_w$ of more concentrated mixtures (more than 3 M of water) was assumed equal to the initial value prepared.

At low water concentrations, the equilibrium $C_w$ was different than the initial water concentration because of the water desorption/sorption by the protein powder. However, this difference was relatively small. As an example, release of water from 4 mg of the hydrated protein (10% of water) immersed into 4 ml of *n*-butanol with the minimal initial water concentration produced approximately 0.005 M of water. This concentration increase was less than 6% of the minimal equilibrium water concentration in *n*-butanol (0.08 M). The similar effect of the water release from HSA to ethanol or methanol was even less because the minimal equilibrium water concentrations were bigger (0.21 M and 0.10 M, respectively). According to data from Ref. [21], the partial solution enthalpies for water are constant, within the experimental error, over the water concentrations 0.07 ÷ 0.24 M in methanol, 0.02 ÷ 0.16 M in ethanol, 0.06 ÷ 0.51 M in *n*-propanol and 0.05 ÷ 0.32 M in *n*-butanol (concentrations were calculated from the mol fraction compositions). Hence, the contribution of the water concentration change induced by the protein to the total measured heat at low water contents was considered to be negligible.

Evidently, for higher concentrations of water this relative change in the $C_w$ values resulted from the immersion of the protein powder in a solvent should be much less. As such, we concluded that the heat effect of the solvent composition change caused by the protein powder was insignificant.

Water sorption isotherms were determined also in separate experiments using the Karl Fisher method. Technique for determination of the water amount bound to HSA was described in detail in Ref. [22]. Typically, 4–10 mg of solid HSA contacted in the sorption cell with 4.0 ml of a solvent. The amount of water on HSA was determined after maintaining the suspensions for 2–3 h in closed vials at 298 K. This time period exceeded the time corresponding to the completion of the heat effect in all calorimetric experiments. Maximal water contents in water–alcohol mixtures correspond to the upper limit of the water concentrations for which the water sorption on HSA may be determined confidently by the described technique [22]. The effect of the exposure time of HSA in water–alcohol mixtures was tested also in the mixtures containing 0.09, 0.65 and 1.2 M of water in *n*-butanol and 0.25, 1.6, 2.2 and 4.8 M of water in ethanol. No noticeable variation of the amount of bound water on HSA was observed after maintaining the suspensions during 24 h. Water sorption ($A$) obtained corresponds to g of water to g of dry HSA, in %, w/w.

Insolubility of HSA in water–*n*-butanol mixtures containing less than 7 M of water and in water–methanol mixtures containing less than 25 M of water was tested by measurements on a Specord M-40 Spectrophotometer at 280 nm. No variation in the absorbance of the liquid phase was observed after exposing the HSA preparation for 6 h to these water–alcohol mixtures.

For purposes of comparison, water activities referred to the pure liquid standard state were calculated in several water–organic mixtures using literature data on the vapor–liquid equilibrium. For most systems such vapor–liquid equilibrium data were found in the compilation [23] which collected original data for water mixtures with methanol, *n*-propanol, *n*-butanol [24], ethanol [25], pyridine [26] and 1,4-dioxane [27]. Data for the mixtures of water with acetonitrile and dimethyl sulfoxide were taken from Refs. [28] and [29], respectively. Based on the vapor–liquid equilibrium data, water activities in mixtures under study were interpolated by a polynomial of the minimal degree which was necessary in order to describe adequately the initial data set. As such, the polynomial of degree 4 was used to represent the water activities in most mixtures. Water activities in *n*-butanol were interpolated by the polynomial of degree 2.

Literature data on vapor–liquid phase equilibria for most systems correspond to 298 K. However, data for water mixtures with ethanol and pyridine found in the compilation [23] are at 303 K. This temperature difference was neglected because the change of the water partial enthalpy on mixing is relatively small in the water mixtures with ethanol and pyridine. As an example, these partial enthalpy changes at infinite dilution of water are $-1.78$ and $-1.92$ kJ mol$^{-1}$, in ethanol [21] and pyridine [30], respectively. These enthalpy values at infinite dilution of a solute produce usually a maximal tempera-

ture effect on the solute activity coefficient in the mixture. They would involve only the 6% change of the activity coefficient when the temperature is changed from 303 K to 298 K. In addition, we tested the temperature effect on the water activities using the UNIFAC model [31]. The calculated effect was insignificant for the water mixtures with ethanol and pyridine, especially in the most important middle and high water activity regions.

## 3. Results and discussion

The enthalpy changes $\Delta H$ on immersing HSA into water solutions in *n*-butanol, *n*-propanol, ethanol and methanol are presented in Figs. 1 and 2 as the function of the water concentration $C_w$ in the solvents. The amounts ($A$) of water bound to the HSA preparation are shown also in Figs. 1 and 2. One can see that there is the similarity in the data pattern for suspensions in *n*-butanol and *n*-propanol, and in ethanol and methanol. The enthalpy changes obtained in water mixtures with two highest alcohols decrease on increasing the water contents in the solvents (Fig. 1). This decrease reflects the water

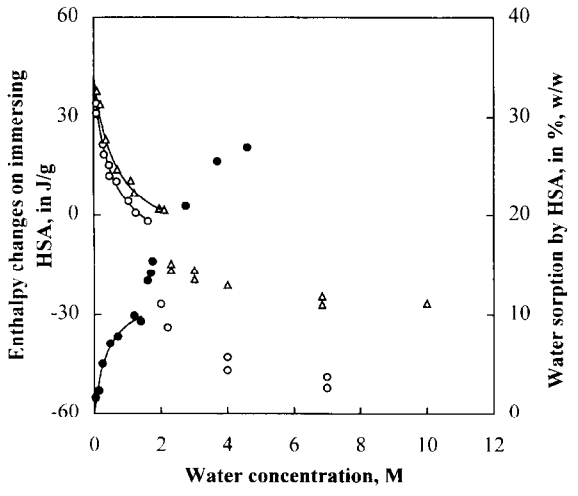


Fig. 1. Enthalpy changes $\Delta H$ on immersing HSA into *n*-propanol–water mixtures (△), *n*-butanol–water mixtures (○) and the amounts of water ($A$) on HSA in *n*-butanol–water mixtures (●) plotted against the equilibrium water concentration $C_w$ in mixtures. Water concentration ranges: 0.08 ÷ 10.0 M (△); 0.08 ÷ 7.0 M (○); 0.05 ÷ 4.6 M (●).

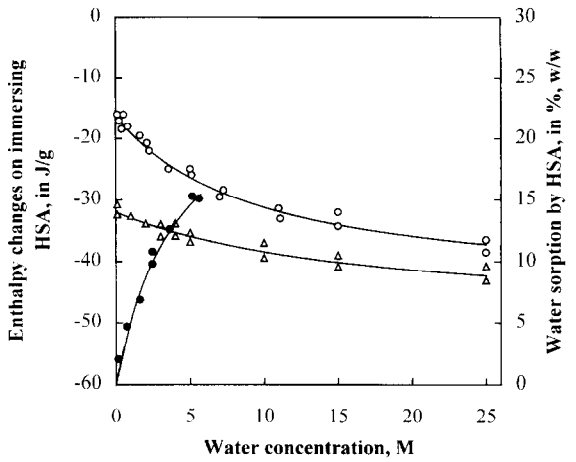


Fig. 2. Enthalpy changes $\Delta H$ on immersing HSA into methanol–water mixtures (△), ethanol–water mixtures (○) and the amounts of water ($A$) on HSA in ethanol–water mixtures (●) plotted against the equilibrium water concentration $C_w$ in mixtures. Water concentration ranges: 0.08 ÷ 25.0 M (△); 0.21 ÷ 25.0 M (○); 0.16 ÷ 5.4 M (●).

sorption on HSA as it can be seen from the water sorption isotherm in *n*-butanol. At a some water content the enthalpy changes fall down significantly, and then, they become nearly constant. Water sorption by HSA in *n*-butanol also demonstrates an additional increase in the water uptake in a relatively close range of the water concentration in solution.

As distinct from the *n*-butanol and *n*-propanol based suspensions, calorimetric data for HSA in water mixtures with ethanol and methanol show the smooth dependence on the water concentration (Fig. 2). Nevertheless, the enthalpy changes at high water concentrations tend apparently to a some saturation level in all studied water–alcohol mixtures. The saturation region corresponds to the water activities exceeding 0.70. These saturation values are approximately −42, −39, −29 and −49 J $g^{-1}$ in the mixtures containing methanol, ethanol, *n*-propanol and *n*-butanol, respectively. These $\Delta H$ limits are in a good agreement with the analogous saturation values found previously [9,32] for other water–organic mixtures (−39, −38 and −40 J $g^{-1}$ for the mixtures containing pyridine, 1,4-dioxane and acetonitrile, respectively). Of all suspensions, only the *n*-propanol based mixtures stand out as demonstrating a more positive saturation value. In addition,

these enthalpy changes on the immersion of HSA in organic solvents with high water contents are also in keeping with the solution enthalpy of HSA in water which is $-43.5 \pm 3.8$ J g$^{-1}$ at a protein concentration of 1 g l$^{-1}$ [19]. Therefore, as one would expect, at high water concentration the hydrated state of the solid HSA is nearly the same in all four alcohol–water mixtures and approaches the state of the protein dissolved in water.

Considering this similar final status of the hydrated HSA in various water–organic mixtures and the smooth calorimetric dependences obtained in methanol and ethanol (Fig. 2), one should admit that (a) interactions resulting in the sharp changes of the $\Delta H$ values and the water sorption on HSA in *n*-butanol and *n*-propanol (Fig. 1) had to occur in pure methanol and ethanol or (b) these interactions are 'smeared out' over the whole concentration range of the water–methanol and water–ethanol mixtures.

We want to demonstrate that as distinct from two highest alcohols the solid HSA–solvent interactions do occur in methanol and ethanol.

To show this, we fitted calorimetric and sorption data in Figs. 1 and 2 with the Langmuir isotherm in the following form (Eqs. (1) and (2)):

$$\Delta H = A_0 \Delta h \left[ \frac{K_c \cdot C_w}{1 + K_c \cdot C_w} - \Theta_0 \right] + \text{const} \qquad (1)$$

$$A - 10.0 = A_0 \cdot 18 \cdot 100 \cdot \left[ \frac{K_c \cdot C_w}{1 + K_c \cdot C_w} - \theta_0 \right] \qquad (2)$$

where $K_c$ and $\Delta h$ are the equilibrium sorption constant ($M^{-1}$), and the sorption enthalpy (J mol$^{-1}$), respectively. $A_0$ is the sorption capacity of the Langmuir monolayer (in mol per g of dry protein); $\Theta_0$ is the fraction of sorption sites occupied in the initial preparation of HSA. const describes the non water sorption contribution to the measured $\Delta H$ values; it corresponds to the enthalpy change on immersing the HSA preparation into a solvent at a fixed initial amount of the bound water. Different const values in various media prove different solvent–protein interactions. 10.0 is the water amount on the initial HSA preparation, in %, w/w. 18 is the molar weight of water, and $A_0 \cdot 18 \cdot 100$ reflects the water percentage in the filled monolayer.

All experimental data obtained in methanol and ethanol (Fig. 2) were included in the fitting procedure. Since data in the *n*-propanol and *n*-butanol based suspensions do not show evidently the Langmuir type of sorption on the all composition range, only the points measured before the sharp changes of properties in Fig. 1 may be included in the fitting. Calculated parameters of Eqs. (1) and (2) are presented in Table 1. Sorption enthalpies $\Delta h$ obtained by dividing $A_0 \Delta h$ on $A_0$ are also included in Table 1.

The fitted curves are shown in Figs. 1 and 2 by the solid lines. One can see that such a simple model is enough for describing the enthalpy changes and water sorption both at low and big concentrations of water in the solvent. This effectiveness of the Langmuir model in describing the calorimetric and water sorption data for solid HSA in organic solvents was demonstrated by us also earlier [9–11,19,22,32]. The $K_c$ values in the water–*n*-butanol mixtures obtained by calorimetry and from the sorption measurements are in a well agreement (Table 1). Considering the low water affinity to sorption on HSA from the ethanol mixtures, the correspondence between the sorption constant values determined by two methods seems to be also quite enough.

The product $A_0 \cdot 18 \cdot 100 \cdot \Theta_0$ calculated in the *n*-butanol and ethanol based mixtures corresponds to the amount of water on the initial HSA preparation (10.0, 9.4 and 10.0%, w/w, respectively). This means that there is no the tightly bound water which does not take part in the sorption equilibrium in the studied water concentration range.

The const values were evaluated from the parameters of Eqs. (1) and (2) (Table 1). This const value in mixtures containing *n*-butanol is nearly zero. This indicates that there is no non water sorption process when the HSA preparation is placed in *n*-butanol at low water contents. However, the const value in the water–ethanol mixtures is $-26.3 \pm 8.5$ J g$^{-1}$. This parameter clearly shows that the HSA–ethanol interactions influence on the thermodynamic quantities of a suspension. In addition, one can see that the enthalpy changes $\Delta H$ in water–methanol mixtures become more negative when the water content is increased (Fig. 2). This means that the water sorption enthalpy is a negative value (see also the enthalpy $-A_0 \Delta h$ of the water monolayer formation in Table 1). Corresponding to the sorption data in *n*-butanol and ethanol (Figs. 1 and 2), one should expect that at

Table 1
Parameters of Eqs. (1) and (2)[a]

| Alcohol | Equation | $K_c$ ($M^{-1}$) | $-A_0\Delta h$ (J $g^{-1}$) | $A_0 \cdot 18 \cdot 100$ (%, w/w) | $A_0 \cdot 18 \cdot 100 \cdot \Theta_0$ (%, w/w) | $-A_0\Delta h \cdot \Theta_0 + \text{const}$ (J $g^{-1}$) | $\sigma$[b] (J $g^{-1}$) | $\Delta h$ (kJ $mol^{-1}$) | const (J $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| *n*-butanol[c] | Eq. (1) | $2.5 \pm 0.8$ | $54.9 \pm 1.8$ | – | – | $41.6 \pm 1.6$ | 1.2 | $-7.8 \pm 0.8$ | $-1.8 \pm 0.5$ |
| *n*-butanol | Eq. (2) | $2.5 \pm 0.8$ | – | $12.7 \pm 0.8$ | $10.0 \pm 1.5$ | – | 0.35 | – | |
| *n*-propanol | Eq. (1) | $1.5 \pm 0.3$ | $54.0 \pm 2.3$ | – | – | $42.1 \pm 1.7$ | 1.0 | – | – |
| Ethanol | Eq. (1) | $0.10 \pm 0.03$ | $28.7 \pm 2.9$ | – | – | $-16.8 \pm 0.7$ | 1.4 | $-1.8 \pm 0.4$ | $-26.3 \pm 8.5$ |
| Ethanol | Eq. (2) | $0.19 \pm 0.06$ | – | $28.4 \pm 3.7$ | $9.4 \pm 2.9$ | – | 0.46 | – | – |
| Methanol | Eq. (1) | $0.05 \pm 0.02$ | $17.2 \pm 3.6$ | – | – | $-32.2 \pm 0.6$ | 0.9 | – | – |

[a] $\pm$ corresponds to the standard errors.
[b] Residual standard deviation of the fitting procedure.
[c] Calorimetric data on the $\Delta H$ values for HSA in *n*-butanol–water mixtures were measured by us also earlier [32]. However, to exclude a possible effect of the preparation quality, we determined a new calorimetric data set presented in this paper. Nevertheless, differences in the evaluated parameters of the Langmuir model based on two sets of calorimetric data are within of the estimation errors. For comparison, parameters from Ref. [32] are as follows: $K_c = 4.2 \pm 1.0$ $M^{-1}$; $-A_0\Delta h = 52.9 \pm 2.5$ J $g^{-1}$; $-A_0\Delta h \cdot \Theta_0 + \text{const} = 36.2 \pm 3.6$ J $g^{-1}$.

low water content methanol also strips water molecules from the HSA preparation which makes this desorption heat contribution a positive value. Total measured enthalpy changes on the immersion of HSA into mixtures at low water contents are negative. Therefore, the non water sorption contribution const to the $\Delta H$ values in the water–methanol mixtures has to be significant and even more negative than the total enthalpy changes measured at low water contents (Fig. 2).

Monolayer capacity is also changed when going from *n*-butanol to ethanol. As it can be seen from the $A_0$ values, surface accessible for water sorption on HSA in ethanol is significantly more than in water–*n*-butanol mixtures. The percentage of water in the filled monolayer $A_0 \cdot 18 \cdot 100$ calculated for HSA in *n*-butanol is more close to the values estimated from the vapor water sorption on solid proteins [12]. On the other hand, this capacity obtained in the ethanol surrounding is in a good agreement with data found in good solvating media as acetonitrile and dimethyl sulfoxide (28.6%, w/w [10] and 23.4%, w/w [11], respectively).

It appears that depending on the solvent, one can expect the different availability of the protein surface for the water sorption. As distinct from the possible weak competition effect of the good solvating solvents on the water sorption isotherm [5,7], one can expect even an opposite increasing effect of the direct protein–solvent interactions on the surface area available for water.

To illustrate the absence of the artifact caused by use of the Langmuir model, we compared directly the calorimetric and sorption data. For this purpose, using parameters of Eq. (2), the sorbed amounts of water on HSA in the *n*-butanol and ethanol based mixtures were calculated exactly for the water concentrations corresponding to the measured $\Delta H$ values. The comparison of measured calorimetric data with interpolated sorption data at the same water concentration is given in Fig. 3. One can see from Fig. 3 that there are good linear dependences between the $\Delta H$ values and the change $\Delta A$ of the water amount bound to HSA on its suspending. The slope of such a linear dependence is the differential enthalpy of water desorption. The differential enthalpies of water sorption calculated from the slopes of the lines ($-7.4 \pm 0.4$ and $-1.5 \pm 0.4$ kJ mol$^{-1}$

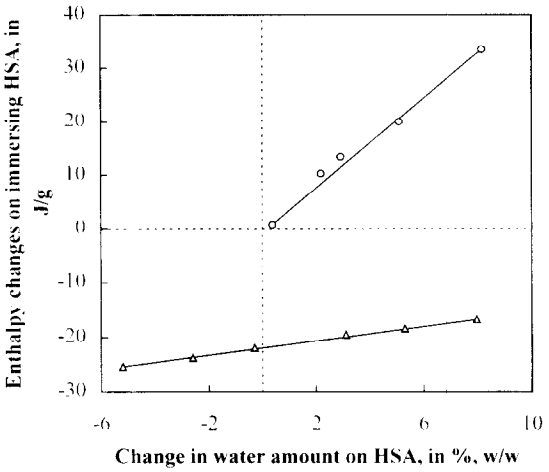


Fig. 3. Enthalpy changes $\Delta H$ plotted against the change in the water amount on HSA $\Delta A$ in the ethanol–water (△) and *n*-butanol–water (○) mixtures. The change $\Delta A$ of amount of water bound to HSA on its suspending was calculated as the difference $10.0 - A$. Ethanol–water mixtures: $\Delta H = (-21.8 \pm 0.8) + (0.8 \pm 0.2) \cdot \Delta A$, $r = 0.987$, $\sigma = 0.5$. *n*-butanol–water mixtures: $\Delta H = (0.4 \pm 0.9) + (4.1 \pm 0.2) \cdot \Delta A$, $r = 0.997$, $\sigma = 1.1$.

in the *n*-butanol and ethanol based mixtures, respectively) are in a good agreement with the sorption enthalpies estimated from the data fitting with the Langmuir model (Table 1). Intercepts of the lines correspond to the enthalpy change on the immersion of the HSA preparation at the fixed initial amount of the bound water. Hence, these intercepts are, in essence, the non water sorption contributions const in Eq. (1). Their values ($0.4 \pm 0.9$ and $-21.8 \pm 0.8$ J g$^{-1}$ in the *n*-butanol and ethanol based mixtures, respectively) correspond also to the const values found from the fitting procedure (Table 1).

An important point is that this kind of analysis does not depend on the current interpretation of the parameters of the fitted Langmuir model. In this comparison the Langmuir model should be considered above all else as the model approximating the sorption data. Thus, such a comparison of calorimetric and sorption data shows clearly that a change in the water amount on HSA immersed in water–alcohol mixtures involves a proportional contribution to the enthalpy change $\Delta H$ in the broad range of the water concentrations. Depending on the alcohol nature, additional enthalpic term may occur even when

there is no change in the quantity of water sorbed by HSA.

Water sorption on bovine serum albumin suspended in ethanol was determined also at 40°C in Ref. [16]. Water sorption measured in Ref. [16] was less than sorption obtained in the present study. There is some difficulty in explanation of discrepancy between two sets of data obtained at different temperatures with different techniques. Water sorption shown in Fig. 2 was measured using technique for the direct determination of the total amount of water on the solid protein [22]. Data from Ref. [16] were determined from the difference of the water concentration in the solvent induced by the water sorption. However, it is important to pay attention that according to data [16] the point in which the water amount on HSA corresponds to its initial value (10%) would be achieved at higher water concentrations than in the present study. From calorimetric data on Fig. 2 it follows that the corresponding enthalpy change $\Delta H$ would be even more negative. Therefore, enthalpy change on the HSA immersion into ethanol under conditions where there is no change in the quantity of adsorbed water would be even more significant.

Earlier, referring to the literature on the sorption by the polymer glasses, the dual-mode sorption model based on a linear combination of the Langmuir sorption and Henry's law expressions was suggested for describing the water sorption by solid proteins in organic solvents [7]. In accordance with this model, the Langmuir term describes the water binding to the surface ionizable residues. The Henry's law term may correspond both to the linear portion of other Langmuir contributions and to the physical partitioning similar to the solute nonspecific distribution between two immiscible liquids.

Hence, the total calorimetric heat evolved on the water sorption by the protein should be also considered as the sum of two contributions. First is the product of the Langmuir sorption term on its enthalpy of sorption. Second contribution is the product of the Henry's law sorption term on the differential enthalpy change corresponding to this kind of sorption. Considering the Langmuir sorption and Henry's law sorption as two independent contributions to the total water uptake by the protein, it would be reasonably to say that the differential sorption enthalpies corresponding to these contributions have to be also different. Then, the water sorption by the protein and the corresponding total calorimetric heat effect cannot be connected with a linear dependence.

Therefore, from the linear dependences in Fig. 3 it follows that the Henry's law term in this dual-mode model seems to be excessive for the HSA suspensions under consideration. As such, the calorimetric data (and their combination with the sorption data) are evidently more critical for evaluating the sorption model than the sorption isotherms alone.

Following the interpretation outlined recently in Ref. [9], we consider the obtained const values for HSA in methanol and ethanol and the 'jump' stage in the *n*-butanol and *n*-propanol based suspensions as the manifestations of the common phenomenon. Fig. 4 summarizes all the to date obtained calorimetric data on the enthalpy changes on immersion of

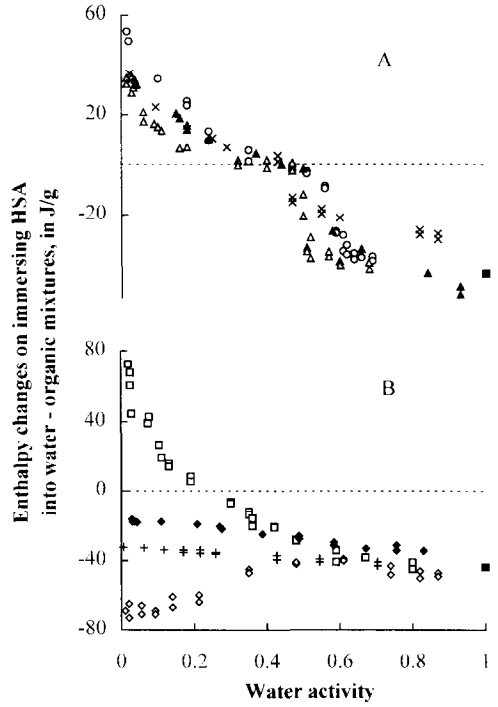


Fig. 4. Enthalpy changes $\Delta H$ on immersing HSA into water–organic mixtures plotted against the equilibrium water activity. (A) *n*-butanol (▲); *n*-propanol (×); pyridine (△), data from Ref. [9]; 1,4 dioxane (○), data from Ref. [19]. (B) Methanol (+), ethanol (◆), acetonitrile (□), data from Ref. [10]; dimethyl sulfoxide (◇), data from Ref. [11]. ■ refers to the solution enthalpy of HSA in water [32].

HSA in various water–organic mixtures. The $\Delta H$ values are plotted against the water activity. Fig. 4A shows data for systems in which there is the 'jump' stage for the $\Delta H$ values. No additional contribution was found for the $\Delta H$ values in the pre-'jump' region under the conditions where there is no change in the quantity of adsorbed water on HSA (i.e. no const values). To the contrary, all dependences shown in Fig. 4B are more smooth, and such const values at low water contents were demonstrated in each case. As it can be seen from Fig. 4, the behaviour of the HSA suspensions based on two highest alcohols and two lowest alcohols under study is different. It is significant that independently on the nature of an organic component the final status of HSA corresponds to a some common level of the enthalpy change that is close to the solution enthalpy in water.

Summarizing the consideration presented in detail in Ref. [9], both these 'jump' stages and the non water sorption contribution on immersion of HSA were explained in terms of the rupture of the protein–protein contacts in the solid HSA phase. In first case shown in Fig. 4A such a rupture is induced by water at a some its activity. Rupture of the protein–protein contacts occurring at high water activity is likely followed with the additional hydration of HSA which results in big exothermic enthalpy changes.

It was noted also [9] that the sharp changes of the caloric and sorption properties in a close water concentration range cannot be interpreted as different kinds of water–water interactions (i.e. multilayer water sorption or the water condensation on the protein surface). Despite of the well known ability of albumins to bind organic molecules [33], we assume that the sharp changes of the $\Delta H$ values in Fig. 4A cannot be explained also on the only basis of such a HSA–organic component binding. So, starting from the identical initial state, the HSA preparation achieves the similar enthalpy level corresponding to its hydrated status in organic solvents of different structure. This fact and the above sharp changes of the enthalpy occurring in a similar water activity range in different organic solvents are not consistent with the hypothesis of the protein–organic component binding which should be sensitive to the molecular structure of an organic component.

However, such an interpretation does not exclude the protein–solvent interactions. Quite the reverse, in second case shown in Fig. 4B the disruption of the interactions in the solid HSA preparation is affected by the protein–organic component interactions that make itself evident in a specific case of methanol and ethanol through the additional enthalpy change const non related to the water sorption.

That is a concluding point of the present study that immersion of the HSA preparation in aliphatic alcohols that are nearest neighbours in a homologous series may involve dramatic differences in the energetics of formation of the suspensions based on these alcohols.

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