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Interaction enthalpies of solid human serum albumin with water–dioxane mixtures: comparison with water and organic solvent vapor sorption

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Abstract

Enthalpy changes (ΔH_{tot}) on the immersion of dehydrated human serum albumin (HSA) into water–dioxane mixtures have been measured using a Setaram BT-2.15 calorimeter at 298 K. Thermodynamic activity of water was varied from 0 to 1. Calorimetric results are discussed together with the FTIR-spectroscopic data on water and organic solvent vapor adsorption/desorption isotherms on solid HSA. Dioxane sorption exhibits a pronounced hysteresis. Calorimetric and dioxane desorption dependencies consist of two parts. No dioxane sorption was observed in low water activity region ($a_w < 0.5$). At low water activities, the ΔH_{tot} values are close to zero. At water activity about 0.5 the sharp exothermic drop of the interaction enthalpy values was observed. This exothermic drop is accompanied by the sharp increase in the amount of sorbed dioxane and additional water sorption (compared with that for pure water). Dioxane adsorption branch resembles a smooth curve. In this case, solid HSA binds more than 300 mol dioxane/mol HSA at low water activities. By using a water activity-based comparison we distinguished between dioxane-assisted and dioxane-competitive effect on water sorption. The obtained results demonstrate that the hydration "history" of solid protein is an important factor that controls as the state of protein macromolecule as well as the sorption of low-molecular organic molecules.

Keywords: Interaction enthalpy; Solid protein; Water and organic solvent sorption; Hysteresis phenomenon

1. Introduction

Protein–water [1–5] and protein–organic solvent [6–12] interactions are well known to play an important role in the state and functions of proteins. Knowledge of processes occurring on hydration or dehydration of proteins (hysteresis phenomenon) in the presence of organic liquids is also very important in biotechnological applications of proteins (the catalysts in low water organic solvents [13–16] and use of imprinted serum albumin and some other proteins as selective adsorbents [17]). Hence, the analysis of the thermodynamic characteristics of intermolecular interactions that occur on immersing solid proteins in water–organic mixtures appears necessary in explaining various protein activities.

The interaction enthalpies of proteins with organic liquids might be a very informative property of the intermolecular interactions in such systems. Calorimetry is a reliable method to determine quantitatively this thermodynamic property. For example, based on calorimetric measurements we proposed two different mechanisms of interaction of the hydrated human serum albumin (HSA) with organic solvents [4,18–20]. It was found that in low water pyridine, dioxane, propanol-1, and butanol-1, the water sorption is the only process contributing to the heat effects of interaction of solid HSA with water–organic mixtures. The additional exothermic process was observed when some critical water content was reached. This process was considered to include the rupture of the protein–protein contacts in the solid phase induced by protein–organic component and/or protein–water interactions. On the immersion of solid HSA in binary mixtures of water with acetonitrile, dimethyl sulfoxide, methanol or ethanol, these two

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processes occur simultaneously over a whole water activity range.

One of the methods capable of yielding the thermodynamic and structural characteristics of sorbent–sorbate interactions in solid–liquid and solid–gas systems is infrared spectroscopy [21]. This method has been successfully used in studying the secondary structure of solid proteins in various states, including solid preparations with various degrees of humidity [1,2,22–28] and solid proteins immersed into pure organic solvents and water organic mixtures [29–31].

Infrared spectroscopy is also effective in studying the thermodynamic properties of proteins in organic environments. For example, we studied using IR-spectroscopic measurements the relationships between the HSA hydration, acetonitrile vapor sorption by solid protein and changes in the secondary structure [12].

The combination of the calorimetric and IR-spectroscopic data has a great potential in understanding factors governing the solid protein–solvent interactions. For example, by means of the combined calorimetric and IR-spectroscopic measurements we recently examined the structure and stability of bovine pancreatic α-chymotrypsin and human serum albumin in a variety of anhydrous organic liquids (including hydrocarbons, monoatomic aliphatic alcohols, and hydrogen bond accepting solvents) [10,11]. We showed that hydrogen bonding ability of a solvent could be regarded as a main factor controlling the solid protein–organic solvent interactions in those media.

In the present work, we want to combine the calorimetric heat effects on interactions of dried human serum albumin with water–organic mixtures and IR-spectroscopic data on water and organic solvent vapor adsorption/desorption on solid protein. The aim of this combined study is to elucidate the mechanism of intermolecular processes that occur on hydration or dehydration of solid protein in the presence of organic media.

Dioxane was selected as a probe organic compound because it is capable of forming strong hydrogen bonds with various hydrogen donors. However, in contrast to water, it has no evident hydrogen bond donating ability. Serum albumin is widely used in studying the molecular basis of the phenomenon of “molecular memory” in organic solvents [17,32,33]. Besides, serum albumin and dioxane were subjects of our previous thermochemical studies of protein behavior in nonaqueous solvents and water-organic mixtures [4,10,12,18–20].

2. Experimental

2.1. Materials

Human serum albumin (Sigma, product no. A 1887, essentially fatty acid free) was used without further purification. The molecular mass of the protein was assumed to be 66 000 g mol^{-1}. Dioxane (reagent grade, purity >99%) were purified and dried according to the recommendations [34] and than were stored over dry 3 Å molecular sieves for at least 24 h prior to use. Water used was doubly distilled.

2.2. Calorimetric measurements

The enthalpy changes (ΔH_{tot}) on the immersion of the dehydrated HSA into mixtures of a solvent and water were measured at 298 K with a Setaram BT-2.15 calorimeter according to the described procedure [20,35]. Protein preparation was dried under vacuum using a microthermoanalyzer (“Setaram” MGDTD-17S) at 298 K and 0.1 Pa until the constant sample weight was reached. Water content of the dried protein was estimated as 0.002 ± 0.002 g of water per gram of protein by the Karl Fischer titration method according to the recommendations [20,35].

Typically, the sample of 5–10 mg of HSA contacted with 4.0 ml of a given solvent in the calorimetric cell. Calorimeter was calibrated using the Joule effect and tested with dissolving sodium chloride in water according to the recommendations [36].

2.3. FTIR-spectroscopic measurements

FTIR-spectrometry has been carried out on Vector 22 (Bruker) FTIR-spectrophotometer at 4 cm^{-1} resolution. Absorption spectra were obtained with a glassy like protein films casted from 2% (w/v) water solution onto the CaF_2 window at room humidity and temperature. After mounting the window onto the sample cell, the film was dehydrated by flushing the air dried over P_2O_5 powder. Relative vapor pressure over P_2O_5 at 298 K does not exceed 0.01 [37]. The protein film was flushed until no further spectral changes were detected in 3450 cm^{-1} water absorbance region and amide A contour in this side represented a smooth line without any visible shoulders. Spectrum of this sample was used as a standard for calculation of difference spectra.

Then, the sample was in situ exposed to pure water vapor or water–organic vapors. In the first case, air consecutively flowed through the saturator and the cell containing the protein sample. The water activity (a_w) in vapor phase was adjusted by changing the difference between the temperature of the saturator and the cell. The data on the water vapor pressure at various temperature were taken from [38].

In the second case, dry air flowed through the saturator filled with a water–organic mixture and then through the measuring cell. The temperature of the cell and saturator were 298 K. The relative pressure of water vapor was adjusted by changing the water activity in the liquid water–organic mixture.

Water sorption by protein was controlled in the region of OH stretching vibration band at 3450 cm^{-1}. Dioxane sorption by solid HSA was controlled at 1121 cm^{-1} (stretching vibration band of the CO group).

Supposing that during water sorption the protein film swells predominantly due to increasing thickness we can

calculate the protein hydration or adsorption of organic sorbate from Eq. (1):

$$A = \frac{2.3\, S_{solv}\, \varepsilon_P}{B_{solv}\, D_P}, \quad (1)$$

where A is the protein hydration or adsorption of organic sorbate, mole per mole protein, S_{solv} is area of water or organic solvent absorbance band, ε_P is protein molar extinction at the maximum of amide I band, B_{solv} is water or organic solvent integral molar extinction coefficient, and D_P is optical density at the maximum of amide I band. For pure water, it was taken that $B_W = 96000 \pm 1000\,l\,mol^{-1}\,cm^{-2}$ [39,40]. The amounts of sorbed dioxane were calculated using a formula similar to Eq. (1). The integral absorption extinction coefficient for dioxane ($B_{dioxane}$) was calculated from the area of the absorption band of the pure substance in a cell with layer thickness of 10 μm: $B_{dioxane} = 19100 \pm 15\,l\,mol^{-1}\,cm^{-2}$.

The molar absorption extinction coefficient of HSA was determined measuring the amide I spectra of protein solutions in heavy water: $\varepsilon_P = 237600 \pm 300\,l\,mol^{-1}\,cm^{-1}$.

2.4. Thermodynamic water activity in organic solvent

Water activities a_w in organic solvent were calculated using the Eq. (2):

$$a_w = \gamma_w x_w, \quad (2)$$

where x_w is the mole fraction of water in the solution and γ_w is activity coefficient of water (in mole fractions; the standard state of pure water). Water content in dioxane (x_w) was measured using Karl Fisher method according to the recommendations [20,35].

Water activity coefficients in organic solvent γ_w were calculated from literature data on the vapor–liquid equilibrium [41] by the Eq. (3):

$$\gamma_w = \frac{y_w \times P_t}{x_w \times P_o}, \quad (3)$$

where y_w is the measured mole fraction of water in vapor phase, P_t is total pressure, P_o is saturated vapor pressure of pure water at the same temperature and x_w is mole fraction of water in the liquid phase.

3. Results and discussion

3.1. Enthalpy changes on the immersion of solid human serum albumin in water–dioxane mixtures

Fig. 1 shows the ΔH_{tot} values plotted against the water activity a_w in dioxane. The starting point for the measured enthalpy changes was the solid protein dehydrated by air at relative humidity no more than 0.01 plus water–organic mixtures. Therefore, the measured calorimetric curve may be considered as an adsorption branch of the adsorption/desorption calorimetric cycle.

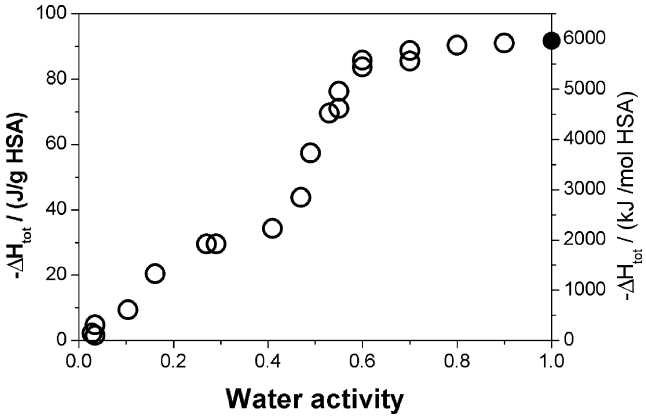

Fig. 1. Enthalpy changes ΔH_{tot} on immersing solid HSA into water–dioxane mixtures plotted against the water activity a_w in mixtures. Closed circle is the solution enthalpy of dried HSA in water.

In most cases the enthalpy changes were observed as one heat evolution exothermic peak completed for 30–40 min. In the interval of water activities in dioxane from 0.4 to 0.55, the heat evolution was completed for 1.5–2 h.

The calorimetric curve (Fig. 1) represents a complicated dependence including the sharp drop of the enthalpy values in a relatively narrow water activity range. Similar calorimetric dependencies were previously obtained on the immersion of hydrated (10% of water) HSA preparation into binary mixtures of water with pyridine, butanol-1, and propanol-1 [4,18,20]. At low water activities, the ΔH_{tot} values are close to zero. This result is in close agreement with the data obtained in [10], where no significant heat evolution and structural rearrangements were found on the interaction of dehydrated HSA with anhydrous dioxane. At high water activities the ΔH_{tot} values reach a some level which is close to the solution enthalpy of dried HSA in water ($-91.2\,J\,g^{-1}$of protein). This ΔH_{tot} value is in a good agreement with the solution enthalpy found previously in [10]: $-91.8 \pm 2.8\,J\,g^{-1}$ of HSA.

3.2. Dioxane vapor sorption

Fig. 2 shows the adsorption and desorption branches of the isotherm of dioxane vapor sorption on solid HSA from water–organic mixtures. The initial state of the protein film for studying organic sorbate desorption was obtained by drying in air at a relative humidity of 0.01 (Film A). The initial state for studying dioxane adsorption was prepared by hydrating the Film A at a relative humidity of 0.98 (Film B). For the repeated adsorption/desorption cycles of water–organic vapors, the initial state was prepared by dehydrating the Film B in vapors of water-free dioxane (Film C).

As can be seen from Fig. 2, the sorption isotherms for dioxane show a pronounced hysteresis. The desorption branch consists of two parts. No sorbed dioxane is observed in low water activity region ($a_w < 0.5$). At water activity about 0.5, the sharp exothermic drop of the ΔH_{tot} values

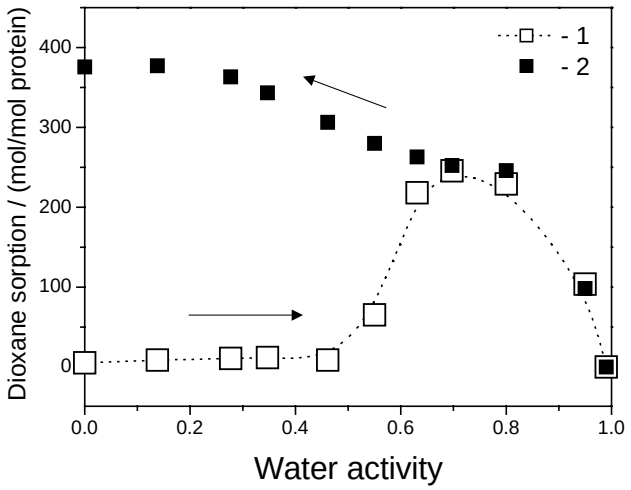

Fig. 2. Sorption of dioxane vapor on solid HSA (mol water/mol HSA) as a function of the water activity: (1) desorption (□) and (2) adsorption (■) branches (relative to dioxane sorption).

(Fig. 1) is accompanied by the significant increase in the dioxane uptake (Fig. 2, curve 1).

The adsorption branch resembles a smooth curve. At low water activities, solid HSA binds about 370 mol dioxane/mole protein. No more than 5% of this value was removed by flushing the dry air for 48 h. For the subsequent adsorption/desorption cycles, when the state of the protein is achieved without removing dioxane (Film C), the organic sorbate sorption becomes independent of the direction of the process and is determined only by the water activity value. These results lend support to the idea that the state of dehydrated HSA is a non-equilibrium state relative to the sorption of dioxane at low water activities.

3.3. Water vapor sorption

Fig. 3 shows the adsorption and desorption branches of the isotherms of water vapor sorption on solid HSA from pure water and water–organic mixtures. The initial state of the protein for studying water adsorption was a Film A (Section 3.2). The initial state for studying water desorption was a Film B.

As can be seen from Fig. 3, in all cases the water sorption isotherms resemble typical sigmoidal curves. However, the presence of organic molecules markedly affects the ability of the solid protein to bind water. The water sorption isotherms for proteins usually exhibit a marked hysteresis; i.e. the desorption branch lies above the sorption one [1,42,43]. Our data demonstrate that a pronounced hysteresis indeed occurs in the case of pure water (Fig. 3, curves 1 and 2). However, in the presence of organic sorbate the hysteresis loop is essentially narrowed.

The effect of dioxane on the water sorption was characterized by the differences in water uptake for the corresponding branches in the presence and absence of organic solvent vapors (Fig. 4). Two different organic solvent effects were observed: organic solvent-assisted effect and organic solvent-competitive effect on water sorption by solid protein. At water activities more than 0.6, both water the desorption and adsorption branches lie above the corresponding branches for pure water. This result corresponds to the organic solvent-assisted effect on water sorption.

Significant decrease in the water uptake is observed for desorption branch in low water activity region ($a_w < 0.5$). The value of this decrease is much lower for water adsorption branch. This effect may be interpreted as a competition of dioxane for water binding sites on protein.

3.4. Analysis of water sorption isotherms

The BET model [44] is widely used for describing the sorption ability of various solids, including proteins [1,3,42,43] (Eq. (4)):

$$h = h_m \left[\frac{Ka_w}{1 + Ka_w} + \frac{a_w}{1 - a_w} \right] \quad (4)$$

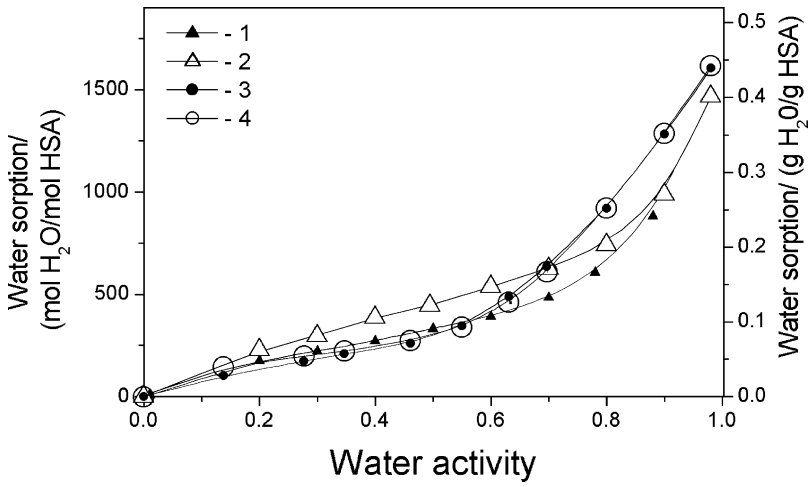

Fig. 3. Water sorption isotherms on human serum albumin: (1) desorption and (2) adsorption branches in the absence of dioxane vapor; (3) desorption and (4) adsorption branches in the presence of dioxane vapor. Solid lines were fitted by a set of polynomials.

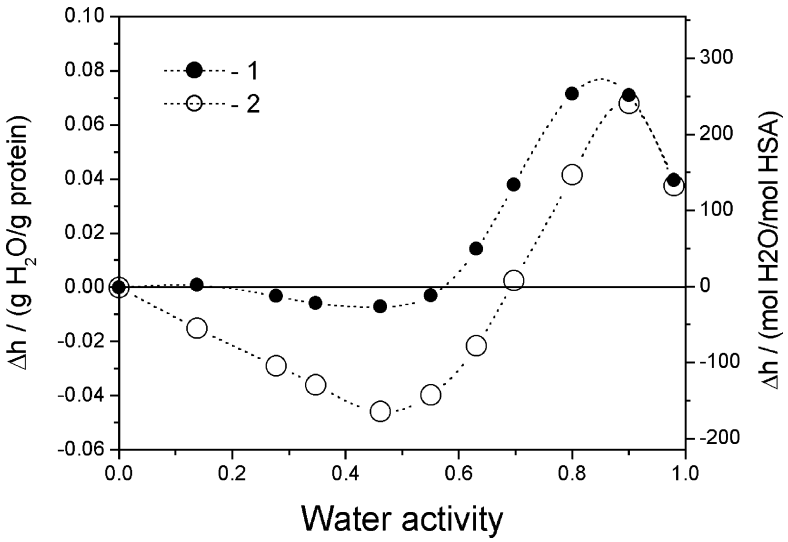

Fig. 4. Differences in the water uptake in the presence of dioxane for water (1) adsorption and (2) desorption branches.

where h is the hydration of solid protein, mole water per mole protein, h_m is number of water binding sites, mole water per mole protein, K is equilibrium water sorption constant.

We found that the BET equation (Eq. (4)) describes the isotherms displayed in Fig. 3 up to a water activity no more that 0.6. This result is in close agreement with the data obtained in previous studies on serum albumin and other proteins [1,3,42,43].

The water sorption parameters estimated from Eq. (4) are presented in Table 1. As can be seen from Table 1, the value of sorption constant K for water desorption branch is markedly smaller in the presence of dioxane than in the absence of organic sorbate. This means that dioxane suppresses the ability of the protein to bind water at relatively low water activities. No significant organic solvents effect on the value of K was found for water adsorption branch. These results are in close agreement with the observations from Section 3.3.

Table 1
Parameters of water vapor sorption by solid HSA estimated by Eq. (4)[a]

Type of sorption branch	h_m (mol water/mol protein)	K	s_o[b]
In the absence of dioxane			
Desorption	249.4 (11.0)[c]	10.9 (3.9)	12.6
Adsorption	177.1 (1.4)	15.3 (1.3)	1.8
[43][d]	240.7 (3.4)	13.0 (0.9)	4.3
In the presence of dioxane			
Desorption	212.8 (10.2)	2.1 (0.7)	20.2
Adsorption	168.9 (5.8)	13.3 (5.2)	14.6

[a] The water activity range of applicability is 0–0.6.
[b] s_o is the residual standard deviation.
[c] The values of the confidence interval of the parameters calculated by Eq. (4) are given in parenthesis.
[d] In [43], no adsorption/desorption cycle measurements were performed.

The values of h_m for desorption branches as in the absence as well in the presence of dioxane are higher than the similar values for adsorption branches. Most likely this decrease in the h_m values is associated with the lower availability of water sorption sites for adsorption process compared with that for desorption one.

Water binding by serum albumin was determined in many studies [43,45–47]. To confirm the reliability of our sorption data we also applied Eq. (4) to approximate the water vapor adsorption isotherm of horse serum albumin measured by Bull [43]. As it can be deduced from Table 1, a good agreement is observed between the Bull's [43] and our data.

3.5. Solvent-assisted effect on water and dioxane sorption

An explanation for solvent-assisted sorption behavior may be provided on the basis of earlier hypothesis on water and/or organic solvent disruption of polar contacts in the solid protein phase [4,18] and soil organic matter [48,49].

On partial or complete dehydration of human serum albumin, like in many other proteins [1,30], the protein-protein contacts are formed mainly due to hydrogen bonding and/or bridging via ion pairing between polar functional groups. These processes result in a shrunken, rigid, condensed structure in the dehydrated state. Therefore, certain moieties of dry protein are unavailable for sorption due to strong interactions between them resulting in sorption hysteresis (Figs. 2 and 3).

It was shown previously that the potential of a solvent to form hydrogen bonds is an important factor that controls the stability of dehydrated proteins at room temperature [10,11]. Hence, H-donating and H-accepting properties of a solvent is expected to be important to estimate the possible effect of organic molecules on protein structure. When a hydrogen bond mediated protein–protein contact (formed, for example, by carboxylic, alcoholic, phenolic or amide groups) is

disrupted, solvent (water or dioxane) molecules may differentiate between H-donating and H-accepting fragments of the disrupted contact. Water (H-donor and H-acceptor) is able to solvate both H-accepting and H-donating groups of the protein. An H-accepting sorbate molecule (in our case, dioxane) is expected to prefer H-donating groups, while the remaining H-bond accepting partner will be solvated by water more effectively. Hence, it is expected that dioxane molecules are not effective in disrupting H-bond mediated protein–protein contacts alone. Therefore, no dioxane sorption was observed at the lowest water activity values for desorption branch (Fig. 2, curve 1).

Penetration of dioxane occurs together with the hydration of all the protein moieties. By penetrating into the dried protein structure, water molecules hydrate the polar moieties of protein–protein contacts creating new sorption sites at the solvated contacts. "The driving force for the solvent-assisted sorption is solvation of the partner of the disrupted polar contact that does not directly interact with the sorbate rather than sorbate-site interactions" [49]. The sharp increase in dioxane uptake at water activity about 0.5 (Fig. 2, curve 1) provides an example of the hydration-assisted effect on organic sorbate sorption.

From the other hand, dioxane molecules consist of four hydrophobic CH_2-groups. Therefore, it is expected that by penetrating into the hydrated HSA, the dioxane molecules (in contrast to water) are able to solvate polar moieties not only in hydrophilic, but also in hydrophobic regions of the protein creating new sites for water sorption. Additional water uptake observed at relatively high water activities (Fig. 4) provides an example of the dioxane-assisted effect on water sorption.

According to this model, there will be a tradeoff between solvent-assisted penetration into polar contacts, versus water/dioxane competition for new sites at those disrupted contacts. Interplay between a hydration-assisted effect and hydration-competitive effect may be interpreted from the maximum in dioxane sorption at $a_w = 0.7$ for desorption branch (Fig. 2, curve 1). The difference curve 2 (Fig. 4) provides an example of the change in dominance between the dioxane-assisted effect at high relative water activities versus competition at low relative water activities.

Calorimetric enthalpy changes on the interaction of the protein with the water–organic mixtures are also sensitive to the changes in mechanism of water sorption. Solvent-assisted rupture of the protein–protein contacts occurring at relatively high water activity in dioxane is followed by the additional hydration of HSA compared with that for pure water (Fig. 4) which results in big exothermic drop of the enthalpy changes (Fig. 1).

4. Conclusions

The obtained results demonstrate that the state of human serum albumin in water–dioxane mixtures depends markedly on how solid protein has been hydrated—whether via sorption or desorption of water–organic vapor mixtures.

The combined study based on calorimetric and IR-spectroscopic measurements provides an informative tool in estimating molecular processes that occur on hydration of solid protein in the presence of organic solvent.

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