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Interactions of water with human serum albumin suspended in water–organic mixtures

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Abstract

Calorimetric enthalpy changes on suspending a partially hydrated preparation of human serum albumin (HSA) in various water–organic mixtures are discussed together with the water sorption isotherms. Experimental data indicate that suspending the HSA preparation is accompanied mainly by two processes. The first is water desorption–sorption which superficially obeys the Langmuir model. The influence of the medium on the thermodynamic parameters of water sorption can be described approximately by thermodynamic data on the solvation of water at infinite dilution. The second effect is a non-sorption process attributed tentatively to rupture of protein–protein contacts in the HSA preparation on suspending it. Depending on the nature of the solvent and its water content, such transformation of the HSA preparation can result in deviations from the Langmuir isotherm of water sorption by the suspended protein. This transformation is accompanied by the corresponding increase in the accessible surface area of the protein preparation and a significant enthalpy change. Experimental data cast doubt on the validity of the traditional opinion that the significant increase in water sorption by proteins at high water activities results from the various kinds of water–water interaction on the protein surface. It appears that the imposition of the transformation of the protein preparation on water sorption–desorption can determine both the calorimetric profile and thermodynamic data on suspending the protein preparation in various solvents.

Keywords: Human serum albumin; Organic solvents; Thermodynamics; Water sorption

1. Introduction

Water–protein interactions have been investigated repeatedly because they are well known to play an essential role in the state and the functions of proteins [1–5]. In

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addition, there are the studies of proteins placed in various unusual environments. Those in reverse micelles [6,7], immobilized proteins in organic solvents [8,9] and those suspended in organic environments [10–13] are examples of such kinds of the system in which the aqueous bulk is replaced by the organic solvent or its mixture with water. Interest in the study of the proteins in organic solvents also demands quantitative information on water–protein interactions.

Thermodynamic study is traditionally of great importance to a better understanding of water–protein interactions. Data have been obtained repeatedly for water vapour sorption by solid proteins [1,4,14–18]. Water–protein interactions have been intensively discussed in calorimetric studies of the denaturation of proteins in aqueous solutions [19,20] and also of solid proteins [21].

Similar information on water–protein interactions is, however, more limited when going to proteins in non-aqueous media. So, water–protein interactions water discussed in a calorimetric study of protein stability in reverse micelles [7]. Isotherms have been obtained for water sorption by proteins suspended in organic solvents [22–24]. The influence of water activity on water binding by the proteins in various environments was also discussed [25,26]. The effects of water have also been observed in calorimetric studies of the thermostability of ribonuclease [27,28] and of human serum albumin [29] in organic solvents.

Nevertheless, as one can see from overviews of the thermodynamics of proteins in unusual environments [26,30], there are not enough data describing water–protein binding over a wide range of water concentrations in different solvents. To the best of our knowledge, there is no information on the heat effects of placing proteins in organic media, nor is there any data on the enthalpies of water sorption on proteins surrounded by organic liquids. Additionally, it would be desirable to discuss a possible model for water sorption by proteins in organic solvents.

In a number of studies [31–36] we have measured calorimetrically the heats evolved on suspending solid human serum albumin (HSA) preparation in various organic solvents containing different amounts of water. In some instances, the sorption isotherms of water by HSA were also determined [34–36].

In the present paper we attempt to compare all these calorimetric and sorption data obtained for the suspensions of HSA in different water–organic mixtures. The aim of this comparison is to discuss the mechanism of the water sorption and the influence of the nature of organic component on the state of the suspended protein.

2. Discussion

2.1. Experimental data

The heat effect on suspending the solid protein preparation corresponds to the enthalpy ΔH of formation of the heterogeneous system. The ΔH values for suspending the HSA preparation in various water–organic mixtures are plotted against the equilibrium concentration of water in the solvent (C_w, mol L^{-1}) in Figs. 1–4. The initial HSA preparation (Sigma) was partially hydrated. Its water content was about 10% *w/w*

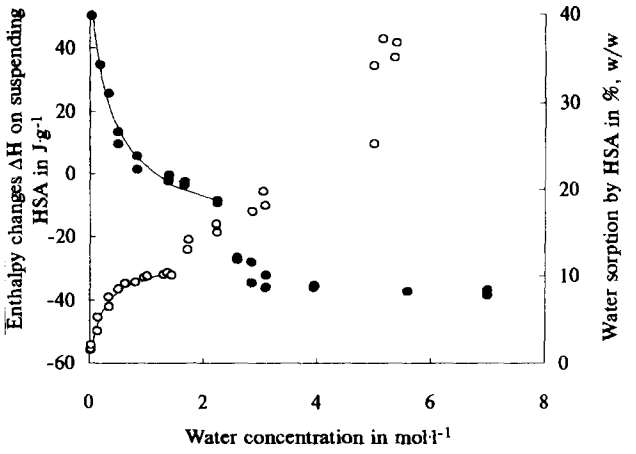

Fig. 1. Enthalpy changes ΔH on suspending HSA (●) and the water sorption by HSA (○) plotted against the equilibrium water concentration C_w in water – 1,4-dioxane mixtures at 298 K. Data from Refs. [32,34].

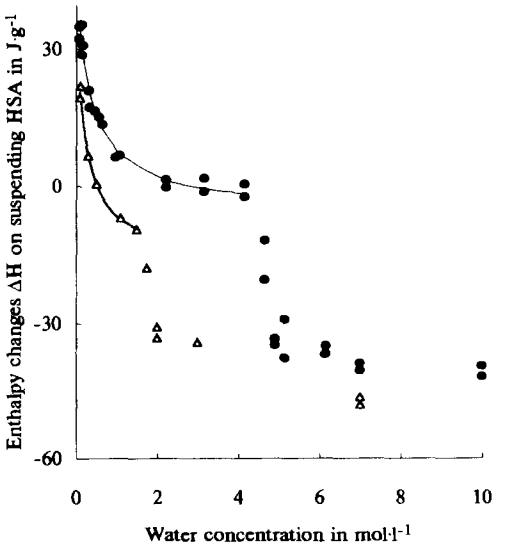

Fig. 2. Enthalpy changes ΔH on suspending HSA plotted against the equilibrium water concentration C_w in water–1-butanol mixtures (Δ) and in water–pyridine mixtures (●) at 298 K. Data from Ref. [33].

(weight to weight of dry HSA). The enthalpy changes are expressed in J g^{-1} of dry protein.

The ΔH values plotted in Figs. 1–3 were observed during calorimetric measurements as one heat evolution peak. The calorimetric recording in water–dimethyl sulfoxide (DMSO) mixtures gave a profile complicated by endothermic and exothermic peaks

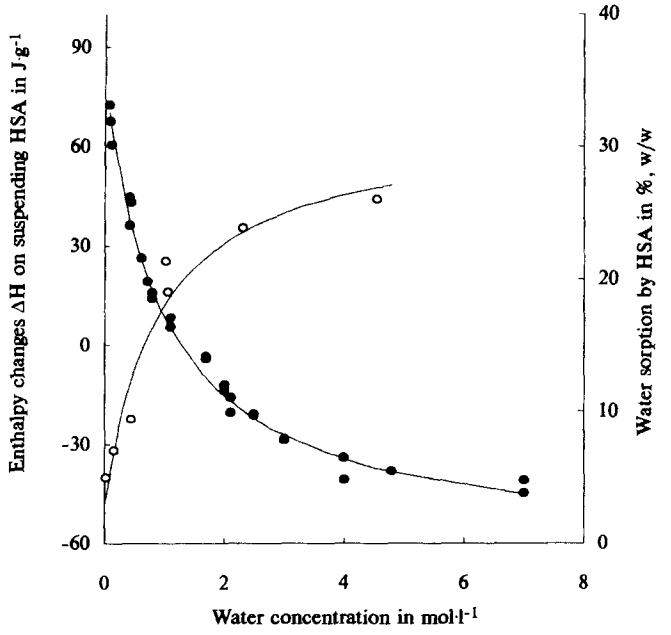

Fig. 3. Enthalpy changes ΔH on suspending HSA (●) and the water sorption by HSA (○) plotted against the equilibrium water concentration C_w in water–acetonitrile mixtures at 298 K. Data from Ref. [35].

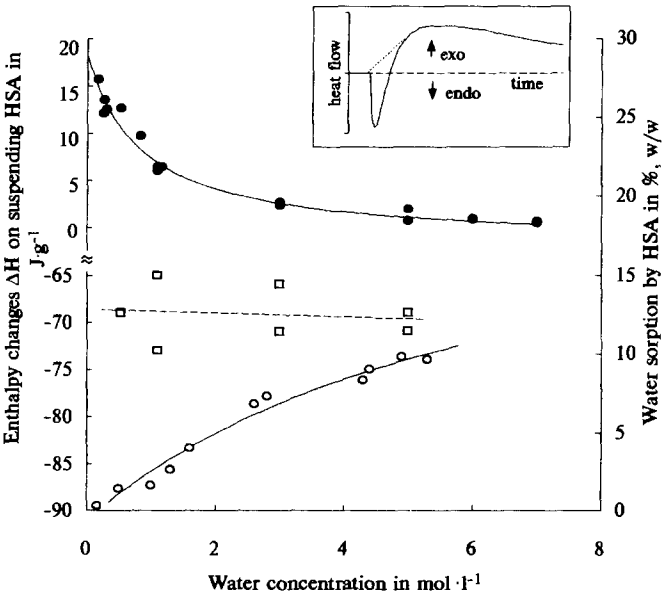

Fig. 4. Total enthalpy changes ΔH on suspending HSA (□), the ΔH^{endo} values (●) and the water sorption by HSA (○) plotted against the equilibrium water concentration C_w in water–DMSO mixtures at 298 K. Data from Ref. [36]. Inset: a calorimetric curve recorded on placing the HSA preparation in a water–DMSO mixture at low water content in the solvent.

[36] as demonstrated on the inset in Fig. 4. Hence, both the total measured ΔH values and the evaluated endothermic contributions (ΔH^{endo}) are plotted in Fig. 4.

The dependences of the amount of water bound to HSA (A, %, w/w) on the C_W value are also presented in Figs. 1,3, and 4. For experimental details see Refs. [34–36].

2.2. *Testing the experimental data for conformity to the Langmuir model of adsorption*

To describe the calorimetric and sorption data presented in Figs. 1–4, the Langmuir model was tested in the following form:

$$A = A_0\left[\frac{K_C C_W}{1 + K_C C_W}\right] \times 18 \times 100 + A' \tag{1}$$

$$\Delta H = A_0 \Delta h\left[\frac{K_C C_W}{1 + K_C C_W} - \Theta_0\right] + const \tag{2}$$

where K_C is the adsorption constant (L Mol^{-1}) corresponding to the Langmuir equilibrium "sorption site + water $\rightleftharpoons$ sorption complex". A' corresponds to the amount of bound water that does not take part in the adsorption equilibrium in the water concentration range studied. A_0 is the amount of water in the filled monolayer (in mol to one gram of dry HSA). Θ_0 corresponds to the population fraction of such a Langmuir monolayer in the initial partly hydrated HSA preparation. Δh is the enthalpy of the Langmuir equilibrium, in J mol^{-1}, subsequently, $A_0\Delta h$ corresponds to the enthalpy of monolayer formation, in J g^{-1}. *const* is the possible non-sorption contribution to the measured ΔH values, in J g^{-1}.

The solid curves in Figs. 1–4 were fitted quite well by Eqs. (1) and (2) by the non-linear regression procedure [32–36]. Adjustable parameters of Eqs. (1) and (2) are K_C, A', A_0, $A_0\Delta h$ and $-A_0\Delta h \times \Theta_0 + const$. All these parameters are collected in Table 1. The *const* values evaluated in Refs. [34, 35] from the simultaneous treatment of the calorimetric and sorption data are also presented in Table 1. It should be borne in mind that the hyteresis phenomenon is known to occur at the water vapour sorption on the solid proteins [16,17]. Therefore, in a strict sense the protein preparation can be in a non-equilibrium state. Hence, the K_C value in Eqs. (1) and (2) should be considered primarily as the quasi thermodynamic value governing the tendency of the water molecules to interact with the suspended protein (with the exception of data in the water–acetonitrile mixtures, for which there was no the observable hysteresis of water sorption on HSA [35]).

One can see from Table 1 that there is good agreement between the sorption constants calculated from the calorimetric data and from the sorption isotherm for the HSA suspension in water–1,4-dioxane mixtures [34]. The sorption constants estimated by Eqs. (1) and (2) for the HSA suspension in the water–acetonitrile mixtures are also in good agreement [35]. One can conclude from such agreement that the technique used for the determination of sorption [34] is consistent with the calorimetric measurements of the ΔH values on suspending the protein preparation [32, 35].

Table 1
Parameters of the Langmuir model fitted to the calorimetric and sorption data for HSA suspensions in water–organic mixtures, and the thermodynamic hydrophilicity of the solvents

N	Organic solvent	Parameters of Eqs. (1) and (2)							γ_w [c]	$-\Delta h_{solv}(W)$ [d]/ kJ mol^{-1}
		K_C [a]/ L mol^{-1}	$-A_0\Delta h$/ J g^{-1}	$-A_0\Delta h\Theta_0 +$ const/J g^{-1}	$A_0 \times 18 \times 100$ [b]/%, w/w	A' %, w/w	const/ J g^{-1}	Ref.		
1	1,4-Dioxane	2.5 ± 0.4 3.8 ± 0.6	77.6 ± 2.9	57.5 ± 2.5	11.7 ± 0.4	0.5 ± 0.3	0.8 ± 1.5	[32–34]	6.3	37.7
2	1-Butanol	4.2 ± 1.0	52.9 ± 2.5	36.2 ± 3.6	–	–	–	[33]	5.5	41.9
3	Pyridine	2.6 ± 0.5	49.4 ± 2.4	43.5 ± 2.8	–	–	–	[33]	2.7	45.6
4	DMSO	1.2 ± 0.3	20.1 ± 1.0	18.3 ± 1.3	–	–	–	[36]	0.3	49.0
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	DMSO	0.16 ± 0.08			23.4 ± 5.4	-0.6 ± 0.6	–			
5	Acetonitrile	1.0 ± 0.1 1.1 ± 0.6	142.8 ± 3.0	79.9 ± 2.8	28.6 ± 4.1	3.2 ± 2.3	44.7 ± 4.9	[35]	7.6	36.4

[a] The first K_C value corresponding to a given organic solvent was obtained by calorimetry; the second K_C value was determined from the water sorption isotherm.

[b] The A_0 values were re-calculated from the weight percentage of water in the filled monolayer.

[c] Refs. [33, 37].

[d] The solvation enthalpies for water at infinite dilution Δh_{solv} (W) were calculated from the difference between the solution enthalpies for water [38,39] and the vaporization enthalpy of water (43.7 kJ mol^{-1} [40]).

However, there is the essential divergence between the sorption constants obtained from the calorimetric ΔH^{endo}values and from the equilibrium sorption isotherm in water–DMSO mixtures (Table 1). On the other hand, one can see from the inset in Fig. 4 that the endothermic heat evolution used for calorimetric evaluation of the K_C value is followed by exothermic heat evolution. A process accompanying this exothermic heat evolution results in the difference between the sorption constants obtained from the calorimetric data and from the equilibrium sorption isotherm [36].

The protein structure is able to demonstrate different sites for interaction with the water molecules. In this sense, the protein preparation is the complex sorbent. Nevertheless, fitting the experimental data in Figs. 1–4 and the above agreement for the sorption constants obtained by the different methods lend support to the conclusion that the Langmuir model has the capability of approximating both the ΔH values and the water sorption by the suspended protein.

However, it is clear from Figs. 1 and 2 that there are also the remarkable deviations from the Langmuir pattern at high water content in the solvent.

2.3. Deviations from the Langmuir model

2.3.1. Water–water interactions as a possible reason for the deviations from the Langmuir model

To discuss the deviations from the Langmuir model, first of all let us consider in greater detail the suspension of HSA in the water–1,4-dioxane mixtures (Fig. 1). Water sorption of the Langmuir type is followed by the necessary increase in the amount of water on HSA. This increase in the water sorption is accompanied by the sharp fall in the ΔH values. On the other hand, there are on the deviations from the Langmuir type for the water sorption isotherm for water–acetonitrile mixtures (Fig. 3). The sharp changes in the ΔH values observed for the water–1,4-dioxane mixtures also disappear for water–acetonitrile mixtures. In addition, these ΔH values reach a saturation common for different mixtures (Figs. 1–3). Therefore, it is reasonable to say that the deviations from the Langmuir model observed in Fig. 1 for both the calorimetric data and the water sorption are related to each other.

The kind of the sorption curve presented in Fig. 1 is well known for the sorption of water vapour by solid proteins [1,4,14–18,25]. An increase in the amount of water at high water activities was considered as the multilayer adsorption of the water vapour by proteins [1,15]. On the other hand, on the basis of IR spectroscopic and calorimetric data for the water vapour sorption by proteins, it was suggested that the significant increase in the amount of sorbed water at high water activities should be considered as its condensation on the less strongly interacting surface elements of the protein [4,18].

One would think that such simple explanations are applicable also for the substantial increase in the amount of water bound to HSA in water–1,4-dioxane mixtures (Fig. 1). Then, the drop in the ΔH values could be interpreted in terms of the association of the water molecules with the Langmuir monolayer of water or as a water condensation on the protein surface. To examine such assumptions, let us consider the following experimental facts:

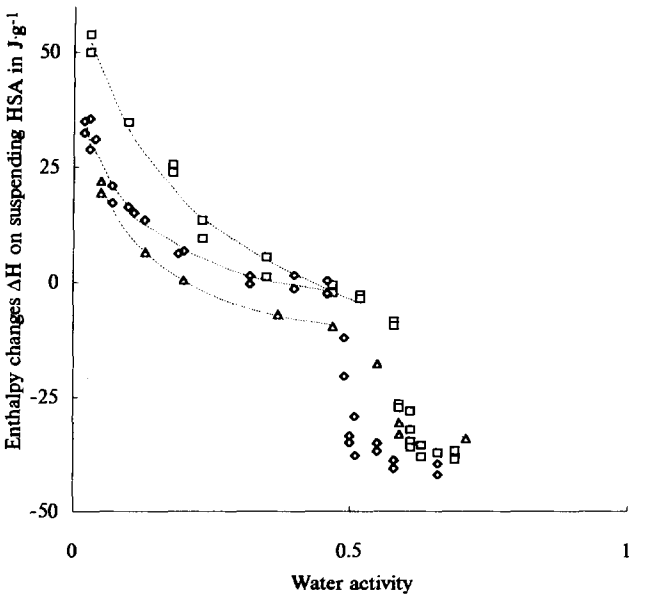

Fig. 5. Enthalpy changes ΔH on suspending HSA in water–1,4-dioxane mixtures (□), in water–1-butanol mixtures (Δ) and in water–pyridine mixtures (◇) plotted against the equilibrium activity of water in the solvents, at 298 K. Water activities were calculated using the water concentrations and the activity coefficients for water in the mixtures studied. Final values were evaluated from data on the vapour–liquid equilibrium [41] on the basis of the interpolating relationship $\ln \gamma_W = a_1 + a_2(1 - x_W)^2$ where x_W is the mole fraction of water in a water–organic mixture.

(1) Calorimetric data presented in Figs. 1 and 2 were plotted together against water activity in Fig. 5. One can see from Fig. 5 that the sharp changes in the ΔH values occur for all three systems in the same water activity range which is 0.5–0.6. Such similarity indicates that the same processes take place on suspending HSA in the above mentioned mixtures. However, it is well known [38] that the solution enthalpy at infinite dilution for water in pyridine equals -1.92 kJ mol^{-1}. Hence, the transfer enthalpy of water from pyridine to water is a positive value. This fact is inconsistent with the interpretation of the exothermic fall of the ΔH values in Figs. 1 and 2 in terms of the association of the water molecules with the Langmuir monolayer or of the self-association of water near the protein surface.

(2) At high water content in the solvent the ΔH values in Figs. 1–3 do not differ significantly from the solution enthalpy for HSA in water. As an example, at $C_W = 7$ mol L^{-1} the ΔH values equal -38, -47, -39 and -40 J g^{-1} for suspensions containing 1,4-dioxane, 1-butanol, pyridine and acetonitrile, respectively [33]. The solution enthalpy for HSA in water equals -43.5 ± 3.8 J g^{-1} at a protein concentration of 1 g L^{-1} [32]. This means that the molecular interactions occurring in the suspensions at high water content are similar to those in aqueous solutions of HSA. The state of the HSA suspended at high water contents in the solvent is common for different suspensions.

This state can be reached by two ways. The first way is presented in Figs. 1 and 2. The second way, shown in Fig. 3, corresponds to the Langmuir model. One can see from Fig. 3 that there is no evidence for multilayer adsorption of water or water condensation on HSA. It would, however, be reasonable to assume that the non-Langmuir process resulting in the deviations in Figs. 1 and 2 has to occur also in the water–acetonitrile mixtures in order to reach the common state at high water content in the solvents. Since the calorimetric and sorption data in Fig. 3 obey Eqs. (1) and (2), the contribution of the non-Langmuir process to the measured ΔH values has to be independent on the water content in the water–acetonitrile mixtures. Hence the non-langmuir process is a non-sorption process.

Thus, some doubts are cast upon the explanation for the significant deviations from the Langmuir type in Figs. 1 and 2 in terms of various water–water interactions near the protein surface.

To demonstrate a non-sorption process on suspending HSA in water–acetonitrile mixtures, A_0 and *const* from Table 1 should be considered. The monolayer surface area A_0 for HSA suspended in water–1,4-dioxane mixtures at low water content does not differ essentially from the surface area that was obtained on the basis of data [1] for water vapour sorption by various proteins. On the other hand, the A_0 value for HSA in water–acetonitrile mixtures significantly exceeds the latter [35]. Hence, placing HSA in acetonitrile results in an increase in the accessible surface area A_0 in comparison with suspending HSA in 1,4-dioxane with low water content.

One can see from Table 1 that the *const* value for HSA in the water–1,4-dioxane mixtures equals zero. Subsequently, there is no non-sorption contribution to the measured ΔH values when HSA is suspended in 1,4-dioxane. In contrast, such *const* for HSA in the water–acetonitrile mixtures is a large positive value. From this one can conclude that a non-sorption process contributes to the enthalpy of suspension of the HSA preparation in acetonitrile.

It is our opinion that the same process is responsible both for the deviations from the Langmuir model in Figs. 1 and 2 and for the non-sorption process on suspending HSA in water–acetonitrile mixtures (Fig. 3). This unknown process results in an increase in the monolayer surface area for HSA in acetonitrile and is attended by a significant endothermic enthalpy change. The addition of water is followed by its sorption according to the Langmuir model. When HSA is placed in mixtures containing 1,4-dioxane, 1-butanol or pyridine (Figs. 1 and 2), some concentration of water is required for this process. Its completion in water–organic mixtures results also in an increase in the monolayer surface area which makes itself evident in an increase in water uptake and in sharp changes in the enthalpies. Because of the hydration of the protein preparation, such sharp changes in the ΔH values are negative. Eventually, the suspensions of the protein in various water–organic mixtures become similar to an aqueous solution of the protein.

2.3.2. "Transformation" of the HSA preparation

Earlier [34] we ventured a hypothesis that the increase in the accessible surface area of the protein preparation on suspension might be resulting from a change in the structure of the protein.

There is however, evidence [4,18] that the protein structure is not changed when going from the aqueous solution of the protein to the hydrated solid protein preparation containing various amounts of water. Since the *const* value for HSA in the water–1,4-dioxane mixtures is zero (Table 1), and the sorption curve in Fig. 1 is similar to that for the water vapour sorption [1,4,14–18,25], it is possible that the structure of the suspended protein is not changed essentially in comparison with that in the solid state and that in the aqueous solution also.

Hence, another possible explanation of the above increase in the accessible surface area is the rupture of protein–protein contacts on suspending the HSA preparation in organic solvents or water–organic mixtures. In a sense, it is worth speaking of a "transformation" of the solid preparation of the protein. Such rupture of protein–protein contacts can be attended by a significant enthalpy change. It is of interest that a similar interpretation was used in Ref. [17] to explain the deviations from the Brunauer–Emmet–Teller isotherm for sorption of water vapour on keratin.

On the other hand, considering the similarity of the water sorption curves for the water–1,4-dioxane mixtures and for the gas phase, we should admit that the unified interpretation is desirable for both the water sorption isotherms. Hence, some contradictions are possible between the interpretation suggested for the HSA suspensions and the known explanations of the water vapour sorption on the solid proteins in terms of various kinds of water–water interaction near the protein surface [1,4,15,18]. This issue needs additional analysis.

We assume that the rupture of the protein–protein contacts makes itself evident also in the calorimetric recording on suspending HSA in water–DMSO mixtures (see the inset in Fig. 4). This rupture is manifested as an exothermic peak following the endothermic peak for water desorption. The weight percentage of water in the filled monolayer $A_0 \times 18 \times 100$ determind from the equilibrium sorption isotherm in water–DMSO mixtures is close to that obtained for HSA suspension in water–acetonitrile mixtures (23.4 ± 5.4 and 28.6 ± 4.1, respectively, see Table 1). This indicates that the same transformation of the HSA preparation occurs in the different water–organic mixtures.

One may assume that such imposition of the transformation of the protein preparation on water sorption–desorption will determine both the calorimetric profile and thermodynamic data on suspending the protein preparation in various solvents.

2.4. Analysis of parameters of Eqs. (1) and (2)

2.4.1. Influence of the medium on the sorption parameters

The parameters referring to the different states of HSA must be considered separately. Hence, all the parameters of Eqs. (1) and (2) in Table 1 should be divided into two groups. The first group of data corresponds to the state of HSA in the suspension when the transformation of the protein preparation (or the non-Langmuir process in Figs. 1 and 2) has not yet occurred. The parameters obtained in the suspensions with 1,4-dioxane, 1-butanol and pyridine refer to this group. The parameters evaluated from the calorimetric ΔH^{endo} values in the water–DMSO mixtures are also in this category. The second group includes the data obtained in the water–acetonitrile mixtures and the parameters evaluated from the equilibrium isotherm of water sorption in water–

DMSO mixtures. The parameters in the second group correspond to the final state after transformation of the HSA preparation. Such division of the parameters of Eqs. (1) and (2) is manifested in Table 1 by the dotted line.

The solvation enthalpies (Δh_{solv}(W)) and the mole fraction-based activity coefficients (γ_W) for water dissolved at infinite dilution in the studied solvents are also presented in Table 1. These thermodynamic values describe the ability of the solvent to dissolve water. One can see from these thermodynamic data that 1-butanol, pyridine and DMSO are more hydrophilic in comparison with 1,4-dioxane. On the other hand, the A' value does not differ essentially from zero for HSA in the water–1,4-dioxane mixtures (Table 1). Hence, all the molecules of water bound to HSA take part in the adsorption equilibrium. Therefore, it is reasonable also to assume that all the amount of water on the initial HSA preparation participates in the sorption equilibrium in the studied water–organic mixtures containing 1-butanol, pyridine or DMSO. This means that data from the first group in Table 1 describe the same equilibrium or the water sorption on HSA.

Thus, the effect of the medium on the parameters of Eqs. (1) and (2) may be compared with the thermodynamic data on the solvation of water in different solvents.

On the basis of data for the water vapour sorption, we attempted to calculate the K_C values included in the first group in Table 1. To do this, Eq. (3) [33] was applied:

$$K_C = C \times \gamma_W \times V_m \tag{3}$$

where C is the constant for the monomolecular adsorption of water vapour. This C value was estimated within the framework of the Brunauer–Emmet–Teller isotherm of adsorption of the water vapour on solid serum albumin [1]. It equals 11.25 in the scale of the water activity. V_m is the molar valume of the solvent. Eq. (3) is based on the assumptions that (i) water solvation mainly influences K_C values and (ii) data on the solvation thermodynamics for water at infinite dilution account this effect. Such assumptions are related to the ideas of Refs. [25, 26]. The calculated K_C values are 6.0, 5.7, 2.4 and 0.24 L mol^{-1} for 1,4-dioxane, 1-butanol, pyridine and DMSO, respectively. The calculated values are in a good agreement with the experimental sorption constants for mixtures containing 1-butanol or pyridine (see Table 1). The sorption constant determined in the water–DMSO mixtures from the calorimetric data considerably exceeds the calculated K_C value. As is evident from Table 1, the sorption constant for water–1,4-dioxane mixtures is an intermediate case between the former and the latter.

There is a certain tendency in the divergence between the experimental sorption constants and the K_C values evaluated by Eq. (3). To stress this, we calculated the ratio B between the evaluated sorption constant and the measured K_C value. The sorption constant for the water–1,4-dioxane mixtures was averaged over the values obtained by two methods. This ratio B was compared with the activity coefficients γ_W for water at infinite dilution in various solvents:

$$\log B = (-0.33 \pm 0.03) + (0.70 \pm 0.05) \log \gamma_W \tag{4}$$

The correlation coefficient is 0.995 and the standard error of estimation is 0.05. One can see from Eq. (4) that there is a good relationship between the deviation of the

experimental K_C values from Eq. (3) and the thermodynamic hydrophilicity of solvents. the non-zero slope of Eq. (4) is likely to arise from the failure of the above assumptions (i) and (ii).

Nevertheless, one can conclude that the influence of solvent on the constants K_C of water sorption by the suspended HSA can be estimated within the framework of Eqs. (3) and (4) from data on the activity coefficients for water at infinite dilution.

We correlated also the monolayer formation enthalpies $A_0\Delta h$ from Table 1 with the solvation enthalpies at infinite dilution for water in the solvents studied. Such a comparison is presented in Fig. 6(a). There are in Fig. 6(a) two points relating to HSA in water–DMSO mixtures. One point corresponds to the $A_0\Delta h$ value estimated from the calorimetric ΔH^{endo} values (i.e. first group of data). The second point is the $A_0\Delta h$ value corresponding to the state of HSA after transformation in water–DMSO mixtures (i.e. second group of data). The last value was taken to be zero since there are no the essential changes in the total measured ΔH values (see Fig. 4).

One can see from Fig. 6(a) that there is a linear dependence of the $A_0\Delta h$ values of the first group on the solvation enthalpies for water. The points corresponding to data from the second group in Table 1 deviate significantly from the straight line in Fig. 6(a) in the opposite directions.

It is reasonable to say that the A_0 value is the same for all four $A_0\Delta h$ values obeying the linear dependence in Fig. 6(a). On the other hand, the variation of the surface area A_0 could result in the deviations of the second group data from the straight line in Fig. 6(a). Hence, the Δh values for water sorption by HSA in various water–organic mixtures should be compared with the solvation enthalpies for water. To do this, the $A_0\Delta h$ values from the first group were divided by the A_0 value measured in water–1,4-dioxane mixtures. Such Δh values are plotted against the solvation enthalpies in Fig. 6(b). The enthalpy change Δh calculated for the HSA suspension in water–acetonitrile mixtures by dividing $A_0\Delta h$ by A_0 is shown also in Fig. 6(b). Similar to Fig. 6(a), there are

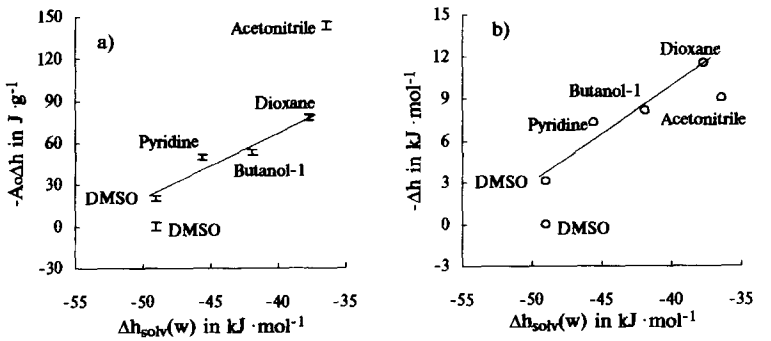

Fig. 6. Correlations of the monolayer formation energy $A_0\Delta h$ (a) and the enthalpy of the water adsorption Δh (b) on the solvation enthalpies Δh_{solv} (W) for water in the studied solvents: (a) $-A_0\Delta h = (253 \pm 41) + (4.7 \pm 0.9)\Delta h_{solv}(W)$, the correlation coefficient is 0.96, the standard error of estimation is 7.9, (b) $-\Delta h = (38.7 \pm 6.3) + (0.71 \pm 0.14)\,\Delta h_{solv}$ (W), the correlation coefficient is 0.96, the standard error of estimation is 1.2.

in Fig. 6(b) also two points corresponding to HSA in the water–DMSO mixtures. The Δh value corresponding to the state of HSA after transformation in the water–DMSO mixtures was taken to be zero like the $A_0\Delta h$ value.

The straight line in Fig. 6(b) is drawn also on points from the first group. Both points corresponding to the adsorption enthalpies Δh from the second group lie slightly below the straight line. It is of interest that the values of deviation for the points from the linear dependence are nearly the same. A decrease of the slope of linear dependence in Fig. 6(b) from unity probably results from the same reasons which cause the non-zero slope of Eq. (4).

In general, it appears that thermodynamic data on water solvation at infinite dilution enable approximate explanation of the influence of the medium on the thermodynamic parameters of water sorption by the suspended HSA.

2.4.2. Non-sorption contributions to the measured enthalpy changes ΔH

const as the non-sorption contribution corresponds to suspending the initial partly hydrated HSA preparation in a solvent without any changes in the amount of water on the HSA. In addition to the determined *const* values in Table 1 we estimated these parameters for other systems.

All the water (10% *w/w*) on the initial HSA preparation participates in the sorption equilibrium in the water–organic mixtures included in the first group. Taking it into account and using the A_0 value determined for HSA in the water–1,4-dioxane mixtures, the *const* values were calculated for 1-butanol, pyridine and DMSO from the adjustable parameters $A_0\Delta h$ and $-A_0\Delta h \times \Theta_0 + const$ of data from the first group. The *const* values were found to be -9 ± 7, 1 ± 6 and 1 ± 3 J g^{-1}, respectively. Considering these *const* values and the same parameter obtained in the water–1,4-dioxane mixtures (Table 1), one should admit that there is no significant contribution from the protein–solvent interactions to the measured ΔH values. This means that for a fixed water content in the protein preparation the energy of the HSA state preceding the transformation does not change essentially when the surrounding medium is varied.

Since we considered the Δh value for the final state of HSA in the water–DMSO mixtures to be zero, so the total ΔH value on suspending HSA in water–DMSO mixtures at low water content can be taken to be the *const* value for this state. According to the data in Ref. [36], it equals -69 ± 2 J g^{-1}. From comparison of this value with the *const* value estimated in the water–acetonitrile mixtures (44.7 ± 4.9 J g^{-1}, Table 1), one can conclude that for a fixed water content of the protein preparation the energy of the HSA state after transformation depends significantly on the nature of the solvent.

Thus, one might also expect a solvent influence "cleaned" from the water desorption–sorption effect on the energy of transformation of the protein preparation.

Conclusions

Suspension of partly hydrated HSA in water–organic mixtures is accompanied by the water desorption–sorption process. Depending on the nature of the solvent and its

water content, an additional process can occur in suspensions. It is most likely that this process is non-sorptive in nature; it is attributed tentatively to the rupture of protein–protein contacts. Such transformation of the protein preparation results in an increase in the surface area accessible for water sorption. It is followed by a significant enthalpy change.

Water sorption on the HSA preparation suspended in water–organic solvents superficially obeys the Langmuir model. The solvation thermodynamics for water at infinite dilution approximately accounts for the influence of the medium on the thermodynamic parameters of water sorption. The trnsformation of the protein preparation can tend to deviate from the Langmuir model. The increase in the accessible surface area resulting from the transformation of the HSA preparation has the capacity to change the water sorption and calorific properties of the studied HSA suspensions.

It would be useful to examine in greater detail the kinetic and thermodynamic reasons for the transformation of the HSA preparation and its dependence on the solvating ability of solvents.

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