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Thermodynamics of water binding by human serum albumin suspended in acetonitrile

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Abstract

Heat effects resulting from the introduction of solid human serum albumin (HSA) into various water–acetonitrile mixtures were measured calorimetrically at 298 K. The amount of water bound to the suspended HSA as a function of the water content of the solvent was also determined. Introducing HSA into water–acetonitrile mixtures involves water binding according to the Langmuir isotherm with an adsorption constant $K_c = 1.0 \pm 0.1$ M^{-1}, enthalpy $\Delta h = -9.0 \pm 1.5$ kJ mol^{-1} and entropy $\Delta S = -30 \pm 6$ J mol^{-1} K^{-1}. Placing HSA in the solvent has an additional heat effect of 46 ± 19 J g^{-1}, which is attributed to an unknown transformation of the protein preparation.

Keywords: HSA; Human serum albumin; Organic solvent; Thermodynamics; Water sorption

1. Introduction

The layer of water bound to proteins is assumed to be essential for protein functioning in media of low water content [1]. The state of this layer depends on the ability of the solvent to strip water from the protein [2,3]. Quantitative information on the enthalpies and entropies of water binding by proteins in organic solvents is thus important. Such thermodynamic values are available for water vapour adsorption by proteins [4–6]. Isotherms for water sorption by proteins suspended in organic solvents are also known [7,8]. However, there are no experimental data on the enthalpies and entropies of water adsorption by proteins from organic liquids.

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We have therefore studied the adsorption of water by human serum albumin (HSA) suspended in water–acetonitrile mixtures. Data on water sorption by HSA and calorimetric heat effects corresponding to the introduction of HSA into water–acetonitrile mixtures are reported, and the thermodynamics of water binding by the suspended protein is estimated.

2. Experimental

Human serum albumin was obtained from Sigma (Product No. A1887). Acetonitrile was purified and dried by refluxing over phosphorus pentoxide followed by distillation over calcium hydride according to the recommended method [9]. Water used for preparation of the water–acetonitrile mixtures was doubly distilled.

Calorimetric heat effects corresponding to the introduction of solid HSA into the water–acetonitrile mixtures were measured with a SETARAM BT-215 calorimeter at 298 K. The calorimeter was calibrated using the Joule effect. The solution enthalpy of potassium chloride in water was also determined in order to check the accuracy of the calorimeter. The solution enthalpy measured at 0.0347 M corresponded to the literature value [10].

The experimental procedure involved placing the sample of solid HSA (2–8 mg) in water–acetonitrile mixtures (4.0 ml) having various water contents in the solvent. The technique for determination of heat was described earlier in more detail [11]. Measured heat effects corresponded to the enthalpies of formation of the heterogeneous system (ΔH^{HSA}). The enthalpy values obtained were expressed in J g^{-1} of dry HSA. The ΔH^{HSA} values and the corresponding water concentrations in the equilibrium liquid phase (C_w) are presented in Table 1.

Table 1
Enthalpies (ΔH^{HSA}) of formation of the heterogeneous system at various water contents (C_w) in the water–acetonitrile mixtures at 298 K

No.	C_w/M	ΔH^{HSA}/(J g^{-1})	No.	C_w/M	ΔH^{HSA}/(J g^{-1})
1	0.07	72.4	15	2.00	−12.1
2	0.09	67.4	16	2.00	−13.8
3	0.12	60.3	17	2.11	−16.1
4	0.42	44.5	18	2.11	−20.5
5	0.42	39.2	19	2.50	−20.9
6	0.45	42.8	20	2.50	−21.3
7	0.61	26.2	21	3.00	−28.3
8	0.72	19.1	22	3.00	−28.7
9	0.80	15.8	23	4.00	−34.1
10	0.80	14.0	24	4.00	−40.8
11	1.10	8.2	25	4.80	−38.3
12	1.10	5.3	26	7.00	−38.5
13	1.70	−3.4	27	7.00	−41.0
14	1.70	−4.2			

Table 2
Amount of water A bound to the HSA suspended in water–acetonitrile mixtures at various water contents C_w at 298 K

No.	C_w/M	A/wt%	No.	C_w/M	A/wt%
1	0.02	4.95	5	1.06	19.0
2	0.16	6.99	6	2.31	23.8
3	0.45	9.15	7	4.55	25.9
4	1.02	21.3			

The C_w values in a liquid phase containing no more than 1 M water were determined after carrying out a calorimetric experiment by the volumetric method [12]. A known volume of the liquid phase was treated with calcium hydride and the volume of hydrogen released was measured. No noticeable amounts of gas-generating impurities were observed in the calcium hydride, which was tested by measuring the water concentration in solutions with precisely added amounts of water. The equilibrium water content in liquid phases containing water concentrations exceeding 1 M was estimated from the added amount of water.

HSA was insoluble in all the studied water–acetonitrile mixtures, as confirmed by measurement on a Specord M-40 spectrophotometer at 280 nm.

A special-purpose experiment was performed in order to determine the water sorption isotherm. First, the suspension of HSA in water–acetonitrile mixtures was thermostated at 298 K for 2–3 h. Then the amount of water bound to the suspended protein was measured electrochemically in Karl Fischer reagent medium according to the recommendations [12]. The equilibrium water content in the solvent was also measured by the Karl Fischer method. In general, the technique of determination was similar to that described earlier [7], except that the bulk of the liquid phase was separated from the solid phase with a syringe. The amount of water on the HSA (A) was expressed in wt% relative to the dry protein. The values for the amount of water bound to HSA and the water concentrations in the equilibrium liquid phase (C_w) are presented in Table 2.

The initial HSA sample contained 10.1 wt% of water (relative to the weight of dry HSA), as found by the Karl Fischer method [12].

3. Results and discussion

The heat effects (ΔH^{HSA}) for suspension formation as a function of the equilibrium water concentration in the water–acetonitrile mixtures are presented in Fig. 1. Fig. 1 also shows the dependence of the amount of water bound to HSA (A) on the water concentration. At low C_w values, the sorption isotherm and the heat effects correspond to desorption of water to the solvent from the initial HSA preparation containing 10.1 wt% of water. At high water contents in the water–acetonitrile mixtures, water is adsorbed from the solvent by the suspended protein.

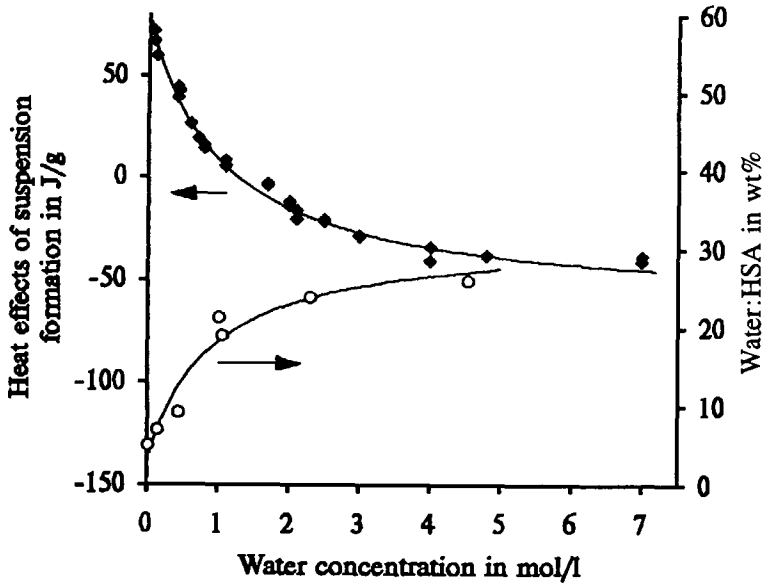

Fig. 1. The heat effects of suspension formation (◆) and the amount of water bound to HSA (○) plotted vs. the equilibrium water concentration C_w in water–acetonitrile mixtures at 298 K. The solid lines were fitted according to the model Eqs. (1) and (2).

Formation enthalpies of HSA + water + dioxane suspensions at low water contents in the liquid phase have been shown [11] to obey the Langmuir model for water adsorption by the protein. Therefore the calorimetric data in Fig. 1 were treated according to the Langmuir model in the form

$$\Delta H^{HSA} = A_0 \Delta h \left(\frac{K_c C_w}{1 + K_c C_w} - \theta_0 \right) + \text{const} \quad (1)$$

where K_c is the adsorption constant (M^{-1}) corresponding to the Langmuir equilibrium sorption site + water $\rightleftharpoons$ sorption complex, Δh is the enthalpy of this reaction (J mol^{-1}), A_0 is the number of sorption sites taking part in Langmuir equilibrium (in mol per g of dry protein), C_w is the equilibrium concentration of water in the solvent, θ_0 is the fraction of the sorption sites occupied by water molecules in the initial HSA preparation, and const corresponds to the possible non-sorption contribution to the measured heat effect.

To describe the change in the water amount on HSA on interaction with the solvent, we used

$$A - 10.1 = A_0 \times 18 \times 100 \times \left(\frac{K_c C_w}{1 + K_c C_w} - \theta_0 \right) \quad (2)$$

where A is the amount of water bound to HSA (wt%), 10.1 is the amount of water on the initial HSA sample (wt%), and 18 is the molecular weight of water. It should be borne in mind that, in general, the product $A_0 \times \theta_0 \times 18 \times 100$ does not correspond to the water content of the initial HSA preparation.

Table 3
Parameters of Eqs. (1) and (2) fitted to the experimental dependences in Fig. 1

Eq. (1)	$K_c/(M^{-1})$	$A_0\Delta h/(J\ g^{-1})$	$-A_0\Delta h\theta_0 + \text{const}/(J\ g^{-1})$	$s_0/(J\ g^{-1})$	n
	1.0(0.1)	−142.8(3.0)	79.9(2.8)	3.5	27
Eq. (2)	$K_c/(M^{-1})$	$A_0/(\text{mol}\ g^{-1})$	θ_0	s_0/wt%	n
	1.1(0.6)	$1.59(0.23) \times 10^{-2}$	0.24(0.11)	2.0	7

Note: n is the number of the experimental points included in the fitting procedure; s_0 is the residual standard deviation of fitting; the values in parentheses are the standard errors of estimation of the parameters in Eqs. (1) and (2).

The data in Fig. 1 were fitted to Eqs. (1) and (2) by a nonlinear regression procedure. The evaluated parameters of the model are summarized in Table 3 and the fitted curves are shown as the solid lines in Fig. 1. From Fig. 1 and Table 3 it can be concluded that the single equilibrium successfully represents both the enthalpy of heterogeneous system formation and the water adsorption on HSA in the studied water concentration range.

Water vapour sorption by proteins exhibits sorption hysteresis [6,13,14]. It is thus surprising that the adsorption and desorption data in Fig. 1 can be described simultaneously with the simple equations (1) and (2). There is no substantial hysteresis of water sorption on HSA in water–acetonitrile mixtures. To explain this effect, we venture a hypothesis that acetonitrile, as a good solvating medium, decreases the conformational rigidity of the protein and enhances the molecular motion in HSA, and thus eliminates the sorption hysteresis phenomenon. The ability of acetonitrile to decrease protein conformational rigidity has been discussed earlier in explaining the influence of the solvent on subtilisin's enantioselectivity in anhydrous media [15].

In general, considering the experimental data and the curve fitting, we must accept that HSA binds water in acetonitrile in accordance with an isotherm superficially similar to the Langmuir isotherm for monolayer adsorption. It is of interest that such an "ideal" effect is observed for water sorption on a sorbent protein with a complex structure and a number of different sorption sites.

Let us consider in greater detail the evaluated parameters in Table 3. The adsorption constants obtained from fitting of the calorimetric and adsorption dependences are in good agreement with each other (Table 3). This agreement lends support to the above conclusion that binding of water by HSA suspended in water–acetonitrile mixtures obeys the Langmuir isotherm. It can also be concluded that both the described technique of sorption determination and the calorimetric measurement of the suspension formation enthalpies may be applied to estimate the thermodynamic parameters of water adsorption by proteins.

The product $A_0 \times \theta_0 \times 18 \times 100$ in Eq. (2) was found to be 6.9 ± 2.3 wt%, which differs slightly from the amount of water on the initial HSA sample (10.1 wt%). This means that some fraction of water in the initial HSA preparation ($\approx$3 wt%) is bound to HSA rather tightly and does not take part in the adsorption equilibrium in the water concentration range studied.

The constant in Eq. (1) differs significantly from zero. Correlation of the heat effects (ΔH^{HSA}) and the change in the amount of water on HSA on suspending the protein sample (ΔA) yields a satisfactory linear dependence (3)

$$\Delta H^{HSA} = (44.7 \pm 4.9) - (4.7 \pm 0.5)\ \Delta A \quad (3)$$

where the ΔH^{HSA} values are the data from Table 1 averaged for close water concentrations in the solvent. The number of experimental points $n = 7$, the correlation coefficient $r = 0.973$, and the residual standard deviation of the linear dependence $s_0 = 10.5$. This linearity also means that the enthalpy of adsorption of water from the environment to all sorption sites on HSA (i.e. the slope of the dependence (3)) is the same. The intercept of the dependence (3) corresponds to the constant in Eq. (1). This value was estimated more accurately from the parameters in Table 3 as 46 ± 19 J g^{-1}. The value of const means that the heat effect of water adsorption by HSA is not the only contribution to the heat effect of placing HSA in the solvent. Interestingly, constant substantially exceeds the reported heats of protein denaturation [16]. On the other hand, it should also be borne in mind that the evaluated percentage of water in the filled monolayer ($A_0 \times 18 \times 100 = 28.6$ wt%) exceeds the value estimated for bovine serum albumin on the basis of adsorption data from the gas phase (6.73 wt%) [4]. Hence it can be assumed that the HSA preparation can undergo some transformation in acetonitrile, although the nature of this change is still obscure. The value of the intercept may thus be considered to be the energy of this transformation.

The enthalpy of water adsorption on HSA was calculated by two methods. First, this enthalpy was evaluated from the slope of the line (Eq. (3)) (-470 ± 50 J per g water, or -8.5 ± 0.9 kJ mol^{-1}). Secondly, the more correct value $\Delta h = -9.0 \pm 1.5$ kJ mol^{-1} was found by dividing the $A_0\ \Delta h$ value by the A_0 value.

The thermodynamics of water binding by HSA in acetonitrile at 298 K can thus be summarized as follows: the Gibbs free energy $\Delta G = 0.0 \pm 0.3$ kJ mol^{-1} ($\Delta G = -RT \ln K_c$, where K_c is the calorimetric value from Table 3), the enthalpy of the water adsorption equilibrium $\Delta h = -9.0 \pm 1.5$ kJ mol^{-1}, calculated from the evaluated model parameters, and the entropy of the adsorption equilibrium $\Delta S = -30 \pm 6$ J mol^{-1} K^{-1}.

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