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Heat effects and water sorption by human serum albumin on its suspension in water–dimethyl sulphoxide mixtures

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Abstract

The heat effects on suspending solid human serum albumin (HSA) in various water–dimethyl sulphoxide (DMSO) mixtures were measured calorimetrically at 298 K. The isotherm of the water sorption for HSA suspended in the water–DMSO mixtures was also measured. The recording of the calorimetric heat effects exhibits endothermic and exothermic peaks. The endothermic heat effects were estimated graphically from the calorimetric curves. These values are shown to obey the Langmuir isotherm of the water sorption. The quasi-thermodynamic constant of water adsorption (1.2 ± 0.3 M^{-1}) and the monolayer formation energy (-20.1 ± 1.0 J g^{-1}) were estimated from the calorimetric data with the Langmuir model. The adsorption constant (0.16 ± 0.08 M^{-1}) was evaluated from fitting the water sorption isotherm by the Langmuir model also. There is a divergence between the latter constant and the adsorption constant obtained from the calorimetric data. It appears that the processes accompanying the exothermic heat evolution influence the HSA's ability to bind water. The surface area of the water monolayer was also calculated from the fitting of the water sorption isotherm. It essentially exceeds the recognised values for proteins estimated from the data for water vapour sorption. The aqueous solubility of the protein after the exposure of the HSA preparation in the water–DMSO mixtures is also essentially decreased. Hence, changes in the protein–protein interactions of a diverse nature might accompany the exothermic heat evolution on suspending HSA in water–DMSO mixtures.

Keywords: Dimethyl sulphoxide; Human serum albumin; Sorption; Thermodynamics; Water

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1. Introduction

Interest in the study of proteins suspended in organic solvents is dictated mainly by the progress of enzymatic catalysis in media with low water contents [1–3]. In addition, the response of a protein preparation on suspension in unusual environments is interesting itself as a characteristic property of the inter- (and/or intra-)molecular interactions governing the state of proteins.

Calorimetry might be a good method to estimate quantitatively this response. Earlier we applied the calorimetric method to obtain the enthalpies corresponding to suspending solid protein (human serum albumin, HSA) in a number of nearly anhydrous organic solvents and water–organic mixtures [4–6]. These enthalpies were shown to depend essentially on the nature of the organic solvent and its water content. The influence of the low water concentration in the solvents on the enthalpies was described within the framework of an isotherm similar to the Langmuir isotherm [5,6]

In the present report, we measured the heat effects corresponding to suspending solid HSA in water–dimethyl sulphoxide (DMSO) mixtures. We also determined the sorption of water by HSA suspended in water–DMSO mixtures.

DMSO is the good solvating solvent that can form strong hydrogen bonds with various hydrogen donors [7,8]. It is also able to solve some proteins [9], in contrast to many other solvents. In this case, DMSO can disturb the protein molecular structure and inactivate the dissolved enzymes [1,9–11]. Hence, DMSO can interact strongly with protein molecules. In this study, we attempted to observe the features of formation enthalpies of the "protein + DMSO + water" suspensions in comparison with the heterogeneous systems containing other organic components. The second goal of the study was to estimate the contribution of the water sorption by HSA to the enthalpies and to compare it with data on the amount of water bound to HSA.

2. Experimental

Human serum albumin (HSA) was obtained from Sigma (product No. A1887). DMSO was purified and dried by refluxing over barium oxide followed by distillation over calcium hydride according to the recommended method [12]. Water used for preparation of the water–DMSO mixtures was doubly distilled.

The initial HSA sample contained 9.0% (w/w) of water (weight to weight of dry HSA). It was measured on a Setaram microthermoanalyser from the weight loss of the protein sample at 298 K and 1×10^{-3} Torr. This corresponded to the value of the water content obtained by Karl Fischer titration, mentioned below.

HSA was insoluble in water–DMSO mixtures containing less than 25 M water. This was confirmed by measurements on a Specord M-40 spectrophotometer at 280 nm. No noticeable variation in the absorbance of the liquid phase was observed after exposing the HSA sample for 24 h to the water–DMSO mixtures.

The calorimetric heat effects on suspending solid HSA in the water–DMSO 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 determined to check the accuracy of the calorimeter. The solution enthalpy measured at 0.0347 M corresponded to the recommended value [13].

The experimental procedure included placing the sample of the initial solid HSA (4–6 mg) in the water–DMSO mixtures (4.0 ml) at various water contents in the solvent. The technique for the determination of the heat was described earlier [5,6]. The measured heat effects corresponded to the enthalpies of the heterogeneous system formation (ΔH^{total}). The enthalpy values obtained were expressed in J g^{-1} of dry HSA.

The concentration of water in the liquid phase, containing less than 1.2 M water, was determined after carrying out a calorimetric experiment, using Karl Fischer titration, see below. The water content in the liquid phase containing water exceeding 1.2 M was considered to be the sum of the water concentration in the initial "dry" DMSO and the added amount of water.

The amount of water bound to the suspended protein and the equilibrium water contents in the solvent were also determined in separate experiments. These determinations were carried out electrochemically in the Fischer reagent medium according to the recommendations [14]. Previously, the suspension of HSA in water–DMSO mixture was thermostated at 298 K for 6 h. This time period exceeded the time corresponding to the completion of the heat evolution in the calorimetric experiments. In general, the technique of determination was similar to that described earlier [15], except that the bulk of the liquid phase was separated from the solid phase with a syringe.

We checked the influence of the time of suspension thermostating on the measured amounts of bound water. To do this, some determinations were performed after exposing the HSA preparation for 24 h in water–DMSO mixtures at 0.16, 0.63, 1.00, 2.19, 5.10 M water. No dependence of the amount of the bound water on the thermostating time was observed.

The water amount on HSA (A) was expressed in weight to weight percentage of dry protein (w/w). The amounts of water bound to HSA and the water concentrations in the equilibrium liquid phase (C_W) are presented in Table 1.

Table 1
Amount of water bound to HSA (A) at various water concentrations (C_W) in the water–DMSO mixtures at 298 K

C_W/M	A/(%, w/w)	C_W/M	A/(%, w/w)
0.16	0.3	2.8	7.3
0.50	1.4	4.3	8.3
1.0	1.6	4.4	9.0
1.3	2.6	4.9	9.8
1.6	4.0	5.3	9.6
2.6	6.8		

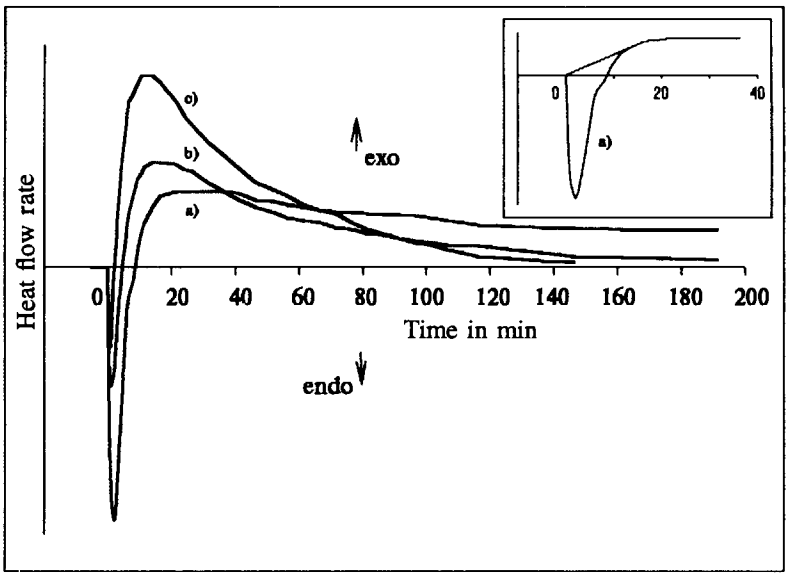

Fig. 1. Calorimetric curves recorded on suspension of HSA sample in water–DMSO mixtures containing (a) 0.2 M water, (b) 1.0 M water, (c) 3.0 M water. Inset: the area enclosed between the experimental calorimetric curve and the direct line is considered as corresponding to the endothermic heat effect. The direct line connects the initial point of the heat effect with the calorimetric curve as the tangent to the latter.

The solubility of HSA in water after exposing the HSA samples for 24 h in various water–DMSO mixtures was also determined. To do this, after thermostating the suspension, the liquid phase was carefully separated from the solid using a syringe. Then, all solid HSA (3–10 mg) containing nearly the same amount of the entrapped liquid phase was placed in 10 ml of water. The concentration of dissolved protein was determined using the Specord M-40 spectrophotometer at 280 nm.

3. Results and discussion

3.1. Calorimetry of introducing the protein preparation into water–DMSO mixtures

A typical recording of the calorimetric heat effects on suspension of HSA in water–DMSO mixtures is presented in Fig. 1 (curves a, b, c). In Fig. 1, there are endothermic and exothermic peaks. Such complex profiles of the experimental curves were not observed earlier for measurements of enthalpies in other organic solvents, namely 1,4-dioxane, pyridine, and 1-butanol [5,6]. The total heat effects (ΔH^{total}) corresponding to the sum of the endo- and exothermic heat evolution on suspending HSA in various water–DMSO mixtures are presented in Table 2.

Rapid endothermic heat evolution occurs first. The visible area of this heat effect peak depends very critically on the water concentration in the water–DMSO

mixture. One can see in Fig. 1 that an increase in the water concentration in the solvent involves a considerable decrease in the area of the endothermic heat effect peak. At some water contents in the solvent, the endothermic peak disappears. Similar endothermic peaks were also observed earlier on recording the heat evolution on introducing HSA into various organic solvents [4–6]. Such heat effects were interpreted in terms of water desorption from the initial HSA preparation to the organic solvent [5,6]. Hence, we assumed that the endothermic heat effects on suspending HSA in water–DMSO mixtures might also be attributed to water desorption from the initial HSA sample to the environment. An increase in the water concentration in the solvent must involve the replacement of water desorption by water adsorption from the environment to the solid and the disappearance of the observable endothermic heat effect. We attempted to estimate an area of the curve corresponding to this process. This area was taken empirically as indicated on the inset in Fig. 1. The calculated endothermic enthalpies (ΔH^{endo}) proportional to such areas are presented at various water contents in the solvent in Table 2.

The second exothermic heat evolution is slow (Fig. 1, curve a). At low water contents in the solvent, there is no completion of the exothermic heat effect. An increase in the water concentration in the solvent substantially accelerates this heat evolution (Fig. 1, curves b and c). The nature of the exothermic heat effect is still elusive. However, it is of interest that the aqueous solubility of the protein after exposure of the HSA preparation in a water–DMSO mixture is essentially de-

Table 2
Enthalpies of the heterogeneous system formation (ΔH^{total}) and the ΔH^{endo} values at various water concentrations (C_W) in the water–DMSO mixtures at 298 K [a]

C_W/M	ΔH^{total}/(J g^{-1})	ΔH^{endo}/(J g^{-1})	C_W/M	ΔH^{total}/(J g^{-1})	ΔH^{endo}/(J g^{-1})
0.17		15.7	10	−64	
0.26		12.1	15	−47	
0.27		13.5	15	−45	
0.31		12.5	20	−42	
0.53	−69	12.6	20	−41	
0.83		9.7	20	−38	
1.10	−73	6.0	20	−36	
1.10	−65	6.4	25	−39	
1.17		6.4	25	−40	
3.0	−66	2.7	25	−40	
3.0	−71	2.3	25	−41	
5.0	−69	0.7	30	−43	
5.0	−71	1.9	30	−48	
6.0		0.8	35	−46	
6.0		0.9	35	−50	
7.0	−61	0.5	40	−47	
7.0	−67	0.6	40	−49	
10	−60				

[a] At low water contents in the solvent there is no completion of heat evolution. At high water contents there are no visible endothermic peaks in the calorimetric recording.

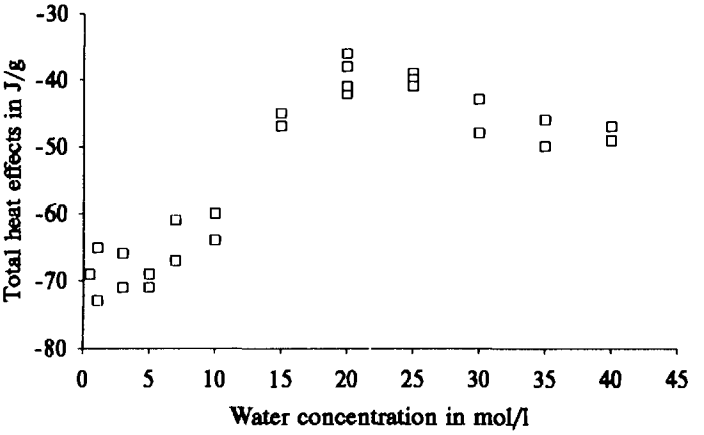

Fig. 2. The dependence of the total heat effects (ΔH^{total}) of suspension formation on the water concentration (C_W) in the water–DMSO mixtures at 298 K.

creased (for details, see Experimental). In accordance with results obtained in Ref. [16], the decrease in the aqueous solubility of the protein can be caused by HSA aggregation. Hence, the exothermic heat evolution on suspending HSA in water–DMSO mixtures is likely to be accompanied by changes in protein–protein interactions that are diverse in nature.

Thus, two observable kinds of processes as revealed by the heat effects, opposite in sign, may be considered to be features of formation of the "HSA + water + DMSO" heterogeneous system in comparison with other organic solvents studied previously [5,6]. The sum of the heat effects determines the complex shape of the dependence of the total heat effects on the water concentration in the solvent. This dependence, exhibiting a slight maximum, is shown in Fig. 2.

3.2. Endothermic contribution to the measured enthalpies

We considered the endothermic heat evolution in greater detail. Earlier the water sorption isotherm similar to the Langmuir model was used to describe the influence of low water contents in organic media on the formation enthalpies of the "HSA + water + organic solvent" suspensions [5,6]. And now we also applied the model

$$\Delta H^{endo} = A_0 \Delta h \left[\frac{K_c C_W}{1 + K_c C_W} - \Theta_0 \right] + \text{constant} \quad (1)$$

to represent the ΔH^{endo} values collected in Table 2. K_c corresponds to the Langmuir adsorption constant (M^{-1}), Δh is the adsorption enthalpy (J mol^{-1}), A_0 is the number of sorption sites in the Langmuir monolayer on one g of the dry protein, Θ_0 corresponds to the population of such a Langmuir monolayer in the initial HSA preparation, and constant represents the possible non-sorption contribution to the measured enthalpies.

The approximation of the ΔH^{endo} values with Eq. (1) by the non-linear regression method is presented graphically in Fig. 3 as the solid curve. The endothermic

enthalpies are also plotted against the equilibrium water concentration C_w in water–DMSO mixtures in Fig. 3. From the fitting, the model parameters were obtained as follows: $K_c = 1.2$ (0.3) M^{-1}, $A_0\Delta h = -20.1$ (1.0) J g^{-1}, $-A_0\Delta h\Theta_0 +$ constant = 18.3 (1.3) J g^{-1}, where the values in parentheses are the standard errors of estimation.

The solid curve demonstrates that the simple equilibrium scheme underlying Eq. (1) is a good approximation for the evaluated endothermic contributions to the enthalpies of heterogeneous system formation. This approximation also lends support to the validity of estimating the endothermic enthalpies as shown in the inset in Fig. 1.

However, the heat effects considered in Fig. 3 are followed by the exothermic effects. Consequently, in the time period corresponding to the completion of the endothermic heat evolution, the system did not reach the equilibrium state (relative to the second exothermic process). Hence, it is of interest to compare the ΔH^{endo} values with the water sorption isotherm determined after the completion of the exothermic heat evolution.

This water sorption isotherm is also presented in Fig. 3. We fitted it with the equation

$$A = A_0 \times 18 \times 100\left[\frac{K_c C_w}{1 + K_c C_w}\right] + A' \qquad (2)$$

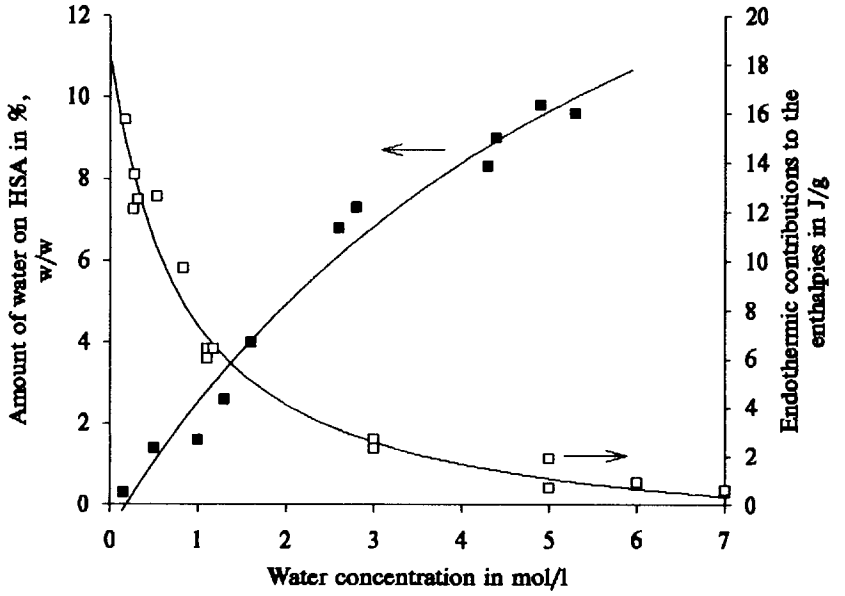

Fig. 3. The ΔH^{endo} values (□) and the amount of water bound to HSA (■) plotted against the equilibrium water concentration (C_w) in the water–DMSO mixtures at 298 K. The solid lines were fitted according to the model equations, Eqs. (1) and (2). Residual standard deviations of fitting are 0.8 J g^{-1} and 0.6%, w/w, respectively.

where A is the amount of water on HSA (%, w/w), and 18 is the molecular weight of water; A' was introduced to test the experimental data for a zero offset.

The calculated curve is depicted in Fig. 3 as the solid curve. Because of the difficulties of measuring the water sorption at high water contents in the solvent, we could not reach the saturation region of water on HSA. Hence, the model parameters of Eq. (2) were estimated with large uncertainties: $K_c = 0.16$ (0.08) M^{-1}, $A_0 = 0.013$ (0.003) mol g^{-1} and $A' = -0.64$ (0.62)%, w/w.

Nevertheless, there is a divergence between the sorption equilibrium constant calculated from the water sorption isotherm and the K_c value estimated from the calorimetric data.

Thus, the following conclusions may be derived from the data fitted in Fig. 3.

(1) Introducing the HSA preparation into water–DMSO mixture containing a relatively small amount of water results in the desorption of water from the HSA sample to the solvent. This desorption makes itself evident in the endothermic peaks in the calorimetric recording. The influence of the water concentration in the solvent on the area of such peaks obeys the Langmuir scheme.

(2) It appears that the subsequent exothermic heat evolution in the calorimetric recording is accompanied by processes influencing the ability of the protein to bind water in the organic solvent.

(3) In a strict sense, the protein state described by the K_c value in Eq. (1) is a doubly non-equilibrium state. Firstly, it is the non-equilibrium state relative to the next exothermic heat evolution. Secondly, the hysteresis phenomenon was shown to occur for water vapour sorption on proteins [17,18]. Therefore, the amount of water on HSA on desorption can differ from the value determined on water adsorption. Therefore, the sorption constants in Eqs. (1) and (2) should be considered as the certain effective values. Nevertheless, the quasi-thermodynamic K_c values will determine the water-binding ability of the HSA in the different states. The use of such constants is similar to the application of water activities in the thermodynamic predictions for biocatalysis in non-conventional media. Thus, it was assumed that the thermodynamic activity of water "will determine the tendency of the enzyme molecules to adsorb or desorb water, even if this process shows hysteresis" [19].

(4) The $A_0\Delta h$ value in Eq. (1) corresponds to the formation enthalpy of the water monolayer on the HSA suspended in DMSO before the second exothermic process has occurred. This value is essentially more positive than the analogous values obtained earlier in the other organic solvents, i.e. dioxane, pyridine, and 1-butanol [5,6]. It is likely that such a decrease of the $A_0\Delta h$ value in absolute size is caused by the change in the solvation enthalpy of water in different organic solvents. Therefore, the solvation enthalpy of water in DMSO is more negative in comparison with many other organic solvents [20]. This has to result in more positive values of the transfer energy of the water molecules from the solvent to the HSA preparation.

(5) The A' value does not essentially differ from zero. This means that all water molecules bound to HSA are involved in an equilibrium described by the simple adsorption scheme. The evaluated weight percentage of water in the filled mono-

layer ($A_0 \times 18 \times 100 = 23.4$ (5.4)%, w/w) is essentially more than the similar value calculated for bovine serum albumin from the data on water vapour sorption within the framework of the multilayer sorption isotherm (6.7%) [21]. Most likely this increase in the accessible surface area of the protein is associated with the exothermic process that takes place slowly on suspending HSA in water–DMSO mixtures.

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