===== BIOPHYSICAL =====
CHEMISTRY

Heat Effects of Dehydration of Human Serum Albumin in Organic Solvents

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Abstract—Based on analysis of our own results concerning the thermochemistry of the interactions of serum albumin in organic media with a low water content and the adsorption of water on the protein in conjunction with the published data on the thermochemistry of solvation of water in organic solvents, we suggest a scheme capable of predicting the influence of the solvation ability of the medium on the enthalpies of dehydration of the protein in organic solvents.

The aim of this work was to study how protein macromolecules interact with the components of aqueous organic mixtures. In recent years, this problem has attracted considerable interest in view of the intense development of enzyme catalysis in organic media with a low water content, a new research field [1–4]. Studies in this field show that protein macromolecules not only retain their catalytic properties in organic media with a low water content but also acquire a number of additional valuable properties, e.g., "molecular memory" and enhanced thermal stability.

It is well known that water plays a key role in the formation of the structure of proteins and their functioning in both aqueous [5–7] and water-deficient media [1–4, 8–1]. Consequently, studying the mechanisms of interaction of proteins with water in organic media may facilitate the development of novel efficient biocatalytic systems. In addition, such fundamental studies make it possible to extend our knowledge about protein macromolecules and the role that water plays in biocatalysis, thereby providing information that is difficult or even impossible to obtain from classical experiments in aqueous media.

Proteins in organic media form heterogeneous systems, which are difficult to study by most of the optical methods. Therefore, as the authors of [12] believe, the emphasis should be placed on calorimetric studies of the thermodynamic and kinetic characteristics of proteins in organic media. Isothermal calorimetry is an efficient method for obtaining reliable thermodynamic information on the interactions of proteins with water in various environments, including organic media. In [13], direct calorimetric measurements of the heat of adsorption of water on bovine serum albumin were carried out. In [14], the enthalpies of sorption of water on lysozyme were measured calorimetrically over the entire range of relative pressures of water vapor. In [15], isothermal calorimetry was used to measure the

sorption and desorption branches of the isotherm of adsorption of water on collagen.

In [16–21], the thermochemistry of the interaction of the hydrated protein preparation with aqueous–organic mixtures of various compositions was studied. The calorimetric experiments were performed in eight organic media (dimethyl sulfoxide (DMSO), acetonitrile, 1.4-dioxane, pyridine, methanol, ethanol, propanol-1, and butanol-1). It was demonstrated that, in all cases, the enthalpies of wetting of the protein included a considerable contribution from the sorption/desorption of water on the protein. However, it remained unknown, what properties of the organic solvent determine the thermochemistry of the adsorption of water on a protein.

Motivated by this, we determined in the present work the thermochemical parameters of the interaction of water with a protein, more specifically, the heat effects of desorption of water from a protein preparation placed into an anhydrous organic medium. The aim of this work was to develop a scheme for describing the desorption of water from a protein into the solvent and to determine what properties of the solvent affect this process. Human serum albumin (HSA) was chosen as the model protein. We used serum albumin in a series of calorimetric studies on intermolecular interactions in protein–organic solvent–water heterogeneous systems [16–22]. Serum albumin was used to study the phenomenon of molecular memory in nonaqueous organic media [23, 24]. The organic solvents used were DMSO, formamide, acetic acid, methanol, and ethanol. These well-studied hydrophilic solvents are widely used in various physicochemical and biological studies, including those on the molecular dynamics of proteins in organic media [25–27].

EXPERIMENTAL

Materials. The HSA preparation (Sigma, A1887) contained not less than 96–99% of HSA (the content of fatty acids was within 0.005%) and was used without additional purification. The organic solvents were purified and dried using the standard procedures [28] and then conditioned over 3 Å molecular sieves.

Calorimetric experiment. The initial lyophilized protein with $10.1 \pm 0.1\%$ of water (g/g dry protein) was dried to a constant weight in an MGDTD-17S SETARAM microthermoanalyzer at 298 K and a pressure of 0.1 Pa. The moisture content of the dried sample was $0.2 \pm 0.1\%$ (g water/g protein). The moisture content of the sample before and after drying was determined by electrochemical titration of water with the use of the Fischer reagent [16, 21].

The heat effects were measured on a BT-2.15 SETARAM calorimeter at 298 K. The instrument was calibrated by electric current. To measure the heat effects of formation of protein–organic solvent–water heterogeneous systems, a HSA sample (5–10 mg) was placed in a titanium container tightened with a Teflon membrane. The hermetically closed container was placed into a calorimetric cell filled with solvent (4.0 ml). After thermal equilibrium was established, the Teflon membrane was punched to bring the solvent and protein in contact. After the calorimetric experiment was complete, the equilibrium concentration of water in the liquid phase was determined by the Fischer electrochemical titration of water. The procedures of calorimetric measurements and water electrochemical titration were described in detail in [16, 20, 21].

Hydrophilicity of the solvents. The measure of hydrophilicity was the Gibbs energy of solution of water in the solvent at infinite dilution and 298 K $\Delta G_{sol}^{H_2O/S}$, which was calculated by the equation

$$\Delta G_{sol}^{H_2O/S} = RT \ln \gamma_w^\infty, \qquad (1)$$

where γ_w^∞ is the activity coefficient of water at infinite dilution (the mole fraction scale; the standard state is the pure substance). The Gibbs energies of solution of water at infinite dilution for the solvents used in this work were calculated in our previous works [22, 29] from data on the vapor–liquid equilibrium in water—organic solvent mixtures [30]. They were found to be 2.3, 2.1, 1.0, and 3.0 kJ/mol for ethanol, acetic acid, methanol, and DMSO, respectively. The value of $\Delta G_{sol}^{H_2O/S}$ for water is equal to 0.

The enthalpies of solvation of water in solvents were calculated by the equation

$$\Delta H_{solv}^{H_2O/S} = \Delta H_{sol}^{H_2O/S} - \Delta H_{vap}^{H_2O}. \qquad (2)$$

The enthalpy of evaporation of water $\Delta H_{vap}^{H_2O} = 43.7$ kJ/mol was borrowed from [31]. The value of $\Delta H_{solv}^{H_2O/S}$ for DMSO was found to be –49.0 kJ/mol [32]. The enthalpies of solvation of water in methanol and ethanol were calculated by Eq. (2) using the enthalpies of solution of water calculated in [33], –47.1 and –45.8 kJ/mol, respectively. The enthalpies of solution and solvation of water in acetic acid and formamide at infinite dilution and 298 K were calculated using the reference data on the heats of mixing of water with acetic acid and formamide [34]. We started with calculating the molar enthalpies of solution of water in water–organic solvent mixtures over the entire range of compositions by the equation

$$H_2^E = H^M - x_1 (dH^M/dx_1)_{p, T}, \qquad (3)$$

where H_2^E is the molar partial excess enthalpy of water, H^M is the enthalpy of mixing of water with the organic solvent, and x_1 is the mole fraction of the organic solvent. Then, we determined the molar partial excess enthalpy of water at infinite dilution $H_2^{E\infty}$ by extrapolating the concentration of water to zero. Next, using the equation [35]

$$\Delta H_{sol}^{H_2O/S} = H_2^{E\infty} - H_2^\circ, \qquad (4)$$

we calculated the enthalpies of solution of water $\Delta G_{sol}^{H_2O/S}$ in acetic acid and formamide at infinite dilution at 298 K; H_2° is the enthalpy of water in the standard state (pure liquid at 298 K and 1.01×10^5 Pa). Thus, the enthalpies of solution of water in acetic acid and formamide at infinite dilution were found to be 2.8 and 1.0 kJ/mol, respectively. The enthalpies of solvation of water in acetic acid and formamide were calculated (by Eq. (2)) to be –40.9 and –42.7 kJ/mol, respectively.

RESULTS AND DISCUSSION

Heat Effects of Wetting of Dry Serum Albumin by Organic Solvents

Figure 1 shows the dependence of heat effects of wetting of dry HSA on the concentration of water in the aqueous-organic solvents. The enthalpy of solution of dry HSA in water was determined previously [29], -91.8 ± 2.8 J/g. An analysis of Fig. 1 shows that the enthalpies of wetting depend linearly on the concentration of water in the solvent. For each solvent, we determined the parameters of the linear dependence (Table 1)

$$\Delta H_{tot} = \Delta H_{tot}^\circ + B c_w. \qquad (5)$$

Here, ΔH_{tot}° is the enthalpy of wetting of a given protein preparation by an anhydrous organic solvent.
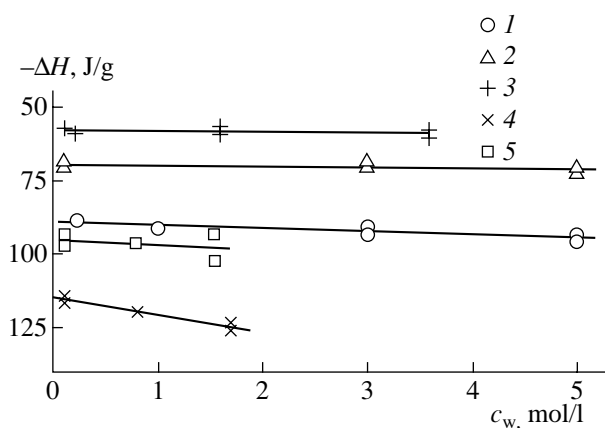
Fig. 1. Dependence of the enthalpies of wetting of dry HSA ΔH (J/g dry protein) in aqueous–organic solvents with various organic components ((*1*) DMSO, (*2*) methanol, (*3*) ethanol, (*4*) acetic acid, and (*5*) formamide) on the concentration of water in these solvents.

In [17, 19], it was shown that the enthalpy of wetting of a protein in an aqueous–organic mixture obeys the Langmuir model of monomolecular adsorption:

$$\Delta H_{tot} = h_0 \Delta H_{ads} \left(\frac{K_c c_w}{1 + K_c c_w} - \Theta_0 \right) + \Delta H_{tot}^{\circ}, \qquad (6)$$

where ΔH_{tot}° (J/g) is the enthalpy of formation of the protein–organic solvent–water system (the enthalpy of wetting); K_c (l/mol) and ΔH_{ads} (J/mol) are, respectively, the adsorption equilibrium constant and enthalpy of the reaction

Sorption site + H_2O ⇌ sorptiom complex ,
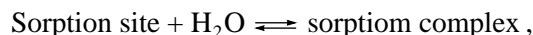
c_w (mol/l) is the equilibrium concentration of water in the solvent; h_0 (mol water/g dry protein) is the amount of water in a complete monolayer; $h_0 \Delta H_{ads}$ (J/g) is the

energy of formation of the monolayer; ΔH_{tot}° is the enthalpy of wetting of the protein by the organic solvent (the contribution from the interaction of the protein with the organic solvent to ΔH_{tot}°, J/g); and θ_0 is the fraction of sorption sites filled with water in the initial protein.

At low water concentrations, Eq. (6) takes the form

$$\Delta H_{tot} = h_0 \Delta H_{ads} K_c c_w - h_0 \Delta H_{ads} \Theta_0 + \Delta H_{tot}^{\circ}. \qquad (7)$$

And, therefore, *B* is the product of the equilibrium constant of adsorption of water on the protein K_c and the enthalpy of formation of a complete Langmuir monolayer, $h_0 \Delta H_{ads}$. To compare, we evaluated the product $h_0 \Delta H_{ads} K_c$ for methanol and ethanol using the values of K_c and $h_0 \Delta H_{ads}$ calculated in [17] for serum albumin. The values of $h_0 \Delta H_{ads} K_c$ for methanol and ethanol were found to be –0.82 and –2.87 J l/(g mol), respectively. These values agree with the values of *B* (Table 1).

Heat Effects of Wetting of Humid Serum Albumin by Organic Solvents

Figure 2 shows how the enthalpies of wetting of HSA containing 10% of water depend on the concentration of water in the aqueous–organic solvents. The enthalpy of solution of the protein in pure water was measured previously [20], –43.8 (3.8) J/(g dry protein). An analysis of data of Fig. 2 shows that the enthalpies of wetting of humid HSA depend linearly on the concentration of water in the solvent, as is the case for the dry protein. For each solvent, we determined the parameters of Eq. (5) (Table 1).

Table 1. Parameters of linear dependence (5) for dry HSA and HSA containing 10% water, the enthalpies of solution of these preparations in water (the two first values in the first row), the enthalpies of dehydration of the protein ΔH_{deh}, and the enthalpies of solvation of water $\Delta H_{solv}^{H_2O/S}$ in the solvents at infinite dilution and 298 K

Solvent	$-\Delta H_{tot}^{\circ}$ (0%), J/g protein	$-B$, J l/(g mol)	$-R$	S_0	N	$-\Delta H_{tot}^{\circ}$ (10%), J/g protein	$-B$, J l/(g mol)	$-R$	S_0	N	ΔH_{deh}, kJ/mol	$-\Delta H_{solv}^{H_2O/S}$, kJ/mol
	Dry HSA					Hydrated HSA						
Water	91.8 (2.8)	–	–	–	–	43.8 (3.8)	–	–	–	–	8.7	43.7
Ethanol	58.2 (0.7)	0.15 (0.2)	0.31	0.81	6	17.8 (0.2)	1.25 (0.1)	0.98	0.23	6	7.3	45.8
Methanol	69.7 (0.7)	0.25 (02)	0.53	0.99	6	32.3 (0.4)	0.8 (0.2)	0.9	0.6	6	6.7	47.1
Acetic acid	114.2 (1.4)	6.1 (0.9)	0.98	1.49	5	48.4 (0.6)	7.4 (0.5)	0.98	0.75	5	11.9	40.9
Formamide	95.4 (2.9)	1.8 (2.8)	0.35	4.0	5	37.8 (0.4)	0.4 (0.2)	0.75	0.75	7	10.4	42.7
DMSO	89.0 (2.0)	1.1 (0.3)	0.87	1.44	6	68.6 (2.2)	0.2 (0.7)	0.14	3.0	7	3.6	49.0

Note: *R* is the correlation coefficient, S_0 is the standard deviation, and *N* is the number of experimental points.

Heat Effects of Dehydration of the Protein in Organic Solvents

The next stage of this work was to determine the heat effects of dehydration of protein in organic solvents and to determine the effect of the solvation activity of the media on the heat effects of dehydration of protein. We compared heat effects of two types (Table 1): the enthalpies of wetting of dry HSA (ΔH_{tot}° (0%)) and HSA containing 10% (g water/g dry protein) (ΔH_{tot}° (10%)) by anhydrous organic media.

The enthalpies of dehydration of protein in organic solvents ΔH_{deh} (Table 1) were calculated on the assumption that all the water (i.e., 10%) is transferred from the protein into organic solvent by the equation

$$\Delta H_{deh} = (\Delta H_{tot}^{\circ}(10\%) - \Delta H_{tot}^{\circ}(0\%))/h_i, \qquad (8)$$

where h_i is the initial content of moisture in the protein, mol/g protein (0.00555 mol water/g dry HSA or (g water)/(g dry protein) × 10%). The assumption that all 10% of the water is transferred from the protein into the solvent was supported by the following data. Table 2 shows the values of the residual water content h_{res} in the HSA sample (initially containing 10% water) placed into anhydrous organic media. The values of h_{res} were calculated by approximating the isotherms of adsorption of water on HSA measured in [17, 19] to the zero concentration of water in the aqueous–organic solvents; the calculations were performed within the framework of the Langmuir model:

$$h = h_0\left(\frac{K_c c_w}{1 + K_c c_w}\right) \times 18 \times 100 + h_{res}, \qquad (9)$$

where h_0 (mol water/g dry protein) is the amount of water in a complete monolayer; h_{res} ((g water/g dry protein) in percent) is the parameter introduced to verify the deviation of the isotherm of adsorption at $c_w = 0$ mol/l from zero (this parameter shows the amount of water
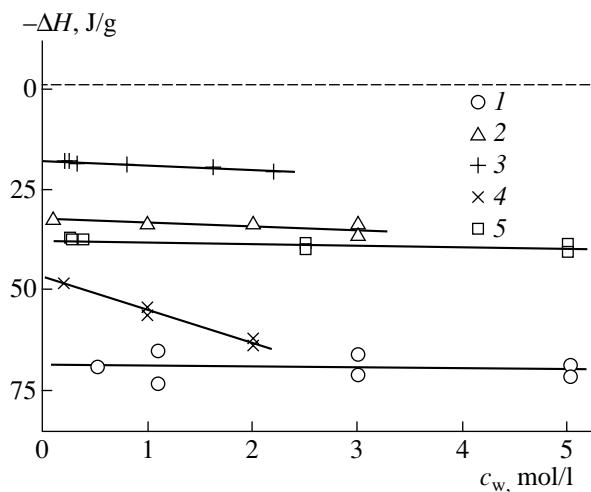

Fig. 2. Dependence of the enthalpies of wetting of serum albumin containing 10% water on the concentration of water in the aqueous-organic solvents (for designations, see Fig. 1).

bound with the protein so tightly that this water does not participate in the sorption equilibrium over the concentration range studied); and K_c, l/mol is the adsorption equilibrium constant for water. Note that the conditions of the adsorption experiment on the determination of the residual water content were similar to those of the calorimetric experiment. Table 2 shows that the desorption of water from the protein into organic solvents with medium and high degrees of hydrophilicity is almost complete: $\Delta G_{sol}^{H_2O/S}$ varied from 4.2 to –3.0 kJ/mol. In this work, we used highly hydrophilic solvents ($\Delta G_{dis}^{H_2O/S} < 2.3$ kJ/mol). Therefore, it is safe to suggest that the transfer of all 10% of the water from the protein in such solvents must be complete as well.

Then, using the thermodynamic data on the solvation of water by various solvents, we examined how the solvation ability of the solvents affects ΔH_{deh}.
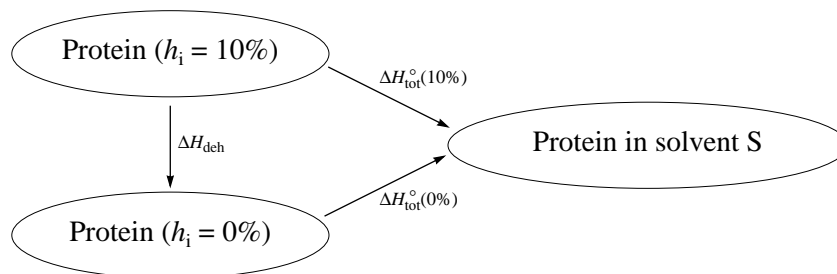
Scheme 1. Interrelation between the enthalpies of wetting of dry ΔH_{tot}° (0%) and hydrated ΔH_{tot}° (10%) HSA by organic solvents.

Table 2. Amounts of residual water (h_{res}, g water/g dry protein) in the HSA sample that initially contained 10% water after its treatment in anhydrous organic solvents as calculated by Eq. (5) from the data of [17–19] and the Gibbs energies of solution of water $\Delta G_{sol}^{H_2O/S}$ in butanol-1, ethanol, and DMSO at infinite dilution and 298 K

Solvent	h_{res}, %	$h_0 \times 18 \times 100$, %	K_c, l/mol	S_0	N	$\Delta G_{sol}^{H_2O/S}$, kJ/mol
Butanol-1	−0.2 (0.7)	12.7 (0.8)	2.6 (0.8)	0.43	0.43	4.2
Ethanol	0.3 (0.6)	26.6 (3.0)	0.2 (0.1)	0.58	8	2.3
DMSO	−0.4 (0.6)	23.6 (6.1)	0.2 (0.1)	0.63	11	−3.0
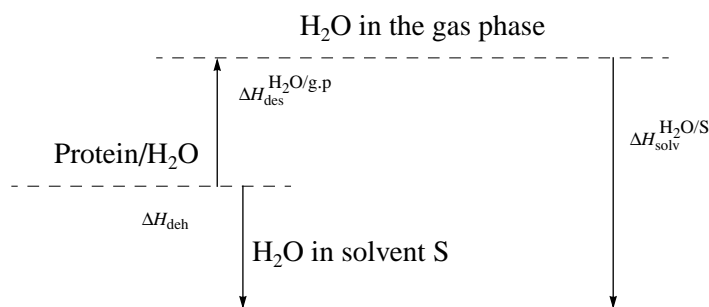
Scheme 2. Separation of the heat effect of dehydration ΔH_{deh} of the initial hydrated protein (protein/H_2O) by organic solvents into the contributions from the desorption of water from the protein into the gas phase and the solvation of water by the solvent ($\Delta H_{des}^{H_2O/g.p}$ and $\Delta H_{sol}^{H_2O/S}$, respectively).

The analysis was based on Scheme 1, which suggests that the enthalpies of wetting of the humid protein ΔH_{tot}° (10%) include the contributions from only two
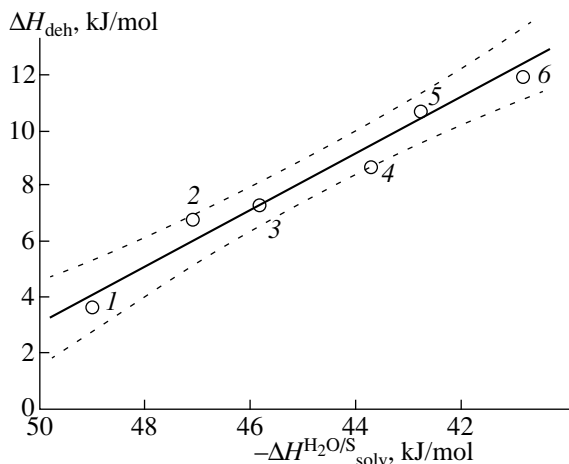

Fig. 3. Dependence of the enthalpy of dehydration of protein on the enthalpy of solvation of water in various organic solvents ((*1*) DMSO, (*2*) methanol, (*3*) ethanol, (*4*) H_2O, (*5*) FA, and (*6*) CH_3COOH) at infinite dilution ($\Delta H_{solv}^{H_2O/S}$) at 298 K. The dashed lines show the 95% confidence interval; $\Delta H_{deh} = 51.4(3.9) + 0.97(0.09) \Delta H_{solv}^{H_2O/S}$. The correlation coefficient, $R = 0.98$; the standard square deviation, $s_0 = 0.58$; and the number of experimental points $N = 6$.

processes: (1) the desorption of water from the protein (Scheme 2) and (2) the interactions of the protein with the organic solvent. Consequently, subtracting ΔH_{tot}° (0%) from ΔH_{tot}° (10%), we obtained the enthalpy of transfer of water from the protein into solvent S, ΔH_{deh}. Here, we assumed that the value of ΔH_{tot}° (0%) is the same for the dry and hydrated HSA samples; i.e., Scheme 1 suggests that both the hydrated and dry HSA samples must eventually pass into a state with the same enthalpy.

In turn, ΔH_{deh} consists of the contributions from two processes: the desorption of water from the protein into the gas phase ($\Delta H_{des}^{H_2O/g.p}$) followed by the solvation of water by the organic solvent ($\Delta H_{solv}^{H_2O/S}$) (Scheme 2). Hence, the enthalpy of desorption of water into the solvent ΔH_{deh} must depend linearly on the enthalpy of solvation of water, with the slope being close to unity, which is indeed the case (Fig. 3). This result suggests that only the solvation of water by the solvent determines changes in ΔH_{deh} and that the data on the enthalpies of solvation of water by the solvent at infinite dilution $\Delta H_{solv}^{H_2O/S}$ reflect this effect.

The free term of the linear dependence (Fig. 3), the enthalpy of desorption of water from protein to the gas phase, is equal to 51.4 kJ/mol. This value agrees with those reported in [7, 13], where the enthalpies of desorption of water vapor from HSA into the gas phase

were determined by analyzing DSC and direct calorimetry data on the adsorption of water vapor on serum albumins of various origins. The calculated values were 50.6 and 52.3 kJ/mol for moisture contents in the protein of 11.9% [7] and 10% [13], respectively.

Thus, analyzing the obtained results and published data, we proposed a scheme for predicting the effect of the solvation ability of a medium on the enthalpy of desorption of water from a protein into an anhydrous highly hydrophobic organic solvent on the basis of the enthalpies of solution of hydrated and dry proteins in pure water and the enthalpies of solvation of water in the corresponding solvents at infinite dilution $\Delta H_{\mathrm{solv}}^{\mathrm{H_2O/S}}$.

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