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Research Article

Heat Effects of Dehydration of Human Serum Albumin in Hydrophilic Organic Solvents

A thermochemical model for describing the transfer of water from the protein phase to the organic solvent liquid phase and for determining how the solvation ability of organic solvents affects this process was developed. Enthalpy changes on the interaction of dried and hydrated human serum albumin (HSA) with hydrophilic organic solvents (dimethyl sulfoxide, formamide, ethanol, methanol and acetic acid) and water were measured by isothermal calorimetry at 25 °C. The initial hydration level of human serum albumin was varied in the entire water content range from 0–30 % [g water/g HSA]. The dependence of the interaction enthalpies on the initial water content is complex. The interaction enthalpies of the dried HSA with organic solvents are exothermic. At low water contents (less than 0.1 g/g), there is a sharp increase in the interaction enthalpy values. At the highest water contents (more than 0.2 g/g), the interaction enthalpies are endothermic for acetic acid and formamide and exothermic for DMSO, methanol, and ethanol. These thermochemical data were analyzed in conjunction with the results for the water adsorption in organic solvents to calculate the molar enthalpies of dehydration of HSA in organic liquids. It was found that the dehydration enthalpy changes may be endothermic or exothermic depending on the initial water content and the water solvation enthalpy value. From the results obtained, it can be concluded that: (*i*) only the solvation of water by hydrophilic organic solvent determines the changes in the dehydration enthalpy values, and (*ii*) the data for the enthalpies of solvation of water by the solvent at infinite dilution reflect this effect.

Keywords: Heat effect of dehydration, Isothermal calorimetry, Water solvation

Received: March 31, 2008; *revised* (second revision): September 4, 2008; *accepted:* October 14, 2008

DOI: 10.1002/elsc.200800039

1 Introduction

It is well known that water plays a key role in the biological functions of proteins [1, 2]. The interaction of proteins with water is also of importance for the formation of their native spatial structure. Many properties of proteins depend on their water content. In particular, a new area of biotechnology, i.e., enzymatic catalysis in nonaqueous media (including organic solvents, ionic liquids, and supercritical fluids), has been intensively developed [3–5]. Studies in this area strongly suggest that biocatalysts in organic media can catalyze industrially important synthetic reactions, e.g., peptide synthesis or esterification, and protein adsorbents can selectively bind low-molecular-weight compounds. However, the efficiency of these catalysts and adsorbents depends markedly on the hydration level [3–8]. Consequently, studying the interactions 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 knowledge about protein macromolecules and the role that water plays in protein functioning. In general, this means that there is a clear need for experimental methods by which the biothermodynamic characteristics of the protein hydration/dehydration in organic solvents may be obtained.

A short review of the studies of hydration-dehydration of proteins is given below. Since the current paper presents a

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calorimetric study of protein dehydration, the main focus is on the thermochemical results. More comprehensive reviews are available elsewhere [1, 2].

Thermochemical studies are traditionally of great importance in ascertaining a better understanding of protein-water interactions. Calorimetry is one of the effective methods for obtaining reliable thermochemical information on the interactions of proteins with water in various environments, including organic solvents. Thus, in particular, Amberg [9] performed the direct calorimetric determination of the heat effects of adsorption of pure water vapor on bovine serum albumin in the water content range from 0–12 % (g water/g protein) at 20 °C. Smith et al. [10] calorimetrically measured the heats of water adsorption on lysozyme in the range of relative water vapor pressures from 0–0.895. They obtained both the sorption isotherm and the enthalpy of hydration of the protein in the water content range 0–18 % [g/g] at 25 °C. Kocherbitov et al. [11] used sorption calorimetry to measure the adsorption isotherm of water on lysozyme and the corresponding heat effects in the entire range of water activities. The present research group developed an experimental method for measuring the heat effects of hydration-dehydration of proteins over the whole range of thermodynamic water activities [12]. The interaction enthalpy was found to depend significantly on the initial water content and hydration history.

However, similar information on the thermochemistry of the protein-water interactions is more limited and is extremely pertinent to the understanding of the functioning of proteins in non-aqueous organic solvents. The enthalpy changes and structural rearrangements accompanying the interaction of dried α-chymotrypsin and albumin with pure organic solvents including hydrocarbons, alcohols, and hydrogen bond accepting solvents have been studied by isothermal calorimetry and FTIR spectroscopy [13, 14]. It was found that the enthalpy and integral structural changes depend cooperatively on the solvent hydrophilicity. The solvent hydrophilicity was characterized by the Gibbs energy of dissolution of water in an organic solvent. Based on this solvent hydrophilicity parameter, all the solvents were divided into two groups. The first group included hydrophilic solvents, e.g., methanol, ethanol, and DMSO. Considerable structural rearrangements were observed in this group of solvents. The interaction enthalpies of the dried proteins with hydrophilic liquids were strongly exothermic. The second group included the hydrophobic and medium hydrophilic liquids, e.g., benzene, dioxane, butanol-1, and propanol-1. The enthalpy and structural changes in the second group of solvents were close to zero.

The heat effects released/consumed on the interaction of the initially hydrated (10 % of water) human serum albumin with water-organic mixtures have been studied by isothermal calorimetry [15]. The organic solvents mainly included the medium hydrophilic solvents from the second group [13, 14]. The interaction heat effects were shown to contain substantial contributions from the protein-water and protein-organic solvent interactions. Thermodynamic parameters of the water sorption (including the water binding enthalpies) were evaluated using the Langmuir model. The effect of organic solvents on the thermodynamic parameters of the water sorption can be approximately described by the thermodynamic data on the solvation of water at infinite dilution. This result is possible due to the fact that the protein-solvent interactions are "frozen" in the second group of liquids at room temperature and low humidities [13, 14]. This implies that the equilibrium thermochemical rules, e.g., Hess's rule, may not be effective in this case.

According to the results of the current authors [13, 14], no kinetic limitations are expected in the hydrophilic solvents of the first group. Therefore, the effect of hydrophilic liquids on the thermochemical parameters of the water sorption by proteins may only be described using the equilibrium water solvation thermodynamics. However, to the best of the current authors' knowledge, no attempt has been made to study the protein hydration/dehydration enthalpies in hydrophilic liquid solvents in the entire range of water contents and to test this hypothesis.

Considering the combination of calorimetric and water sorption measurements as an informative tool to study the protein-water interactions, new data on the interaction enthalpies of the dried and hydrated protein with anhydrous hydrophilic organic solvents, are reported in this work. The initial hydration level was varied in the entire range of water contents from 0–30 % (g water/g protein). These thermochemical results were compared with the water sorption data to calculate the molar enthalpy of the protein dehydration in organic liquids. The aim of this work was to develop a thermochemical model for describing the transfer of water from the protein phase to the organic solvent liquid phase and to determine how the solvation ability of hydrophilic organic liquids affects this process.

Human serum albumin (HSA) was used as a model protein. Human serum albumin is the most abundant protein in blood serum and plays a number of important biological roles, including divalent cation transport, fatty acid and drug complexation and transport [16]. It is widely used in biological and physicochemical studies of the behavior of proteins in both aqueous solutions and organic environments. The hydrophilic organic solvents used were dimethyl sulfoxide (DMSO), formamide, acetic acid, methanol, and ethanol. These water-miscible organic solvents represent a series of liquids in which the enthalpy of solvation of water is gradually changed over a wide range.

2 Experiments and Methods

2.1 Materials

Human serum albumin (Sigma, Product No. A 1887, essentially fatty acid free) was used without further purification. The molecular weight of human serum albumin was taken as 66,000 Da. Organic solvents (reagent grade, purity > 99 %) were purified and dried according to general recommendations available elsewhere [17]. The water used was doubly distilled.

2.2 Calorimetric Measurements

The enthalpy changes, ΔH_{tot}, on the immersion of the dried and hydrated protein preparations into pure liquid water or binary water-organic mixtures were measured at 25 °C with a Setaram BT-2.15 calorimeter according to the procedure described elsewhere [18–20][1)]. Typically, the sample consisted of 5–10 mg of HSA contacted with 4.0 mL of a solvent in the calorimetric cell. Typical heat evolution curves recorded on the interaction of proteins with pure liquid water or organic solvents are given elsewhere [13, 18]. The heat evolution was completed for 30 min. The calorimeter was calibrated using the Joule effect and tested by dissolving sodium chloride in water according to the recommendations available elsewhere [18].

The dried protein preparations (zero hydration level) were obtained by drying under vacuum using a microthermoanalyzer "Setaram" MGDTD-17S at 25 °C and 0.1 Pa until a constant sample weight was reached. The water content of the dried protein was estimated as 0.2 ± 0.1 % (g of water/g of protein) by the Karl Fischer titration method according to the recommendations [18]. Samples for the determination of the interaction enthalpies of the hydrated proteins with water were equilibrated at 25.0 ± 0.5 °C for 5 days in tightly closed desiccators over saturated salt solutions. The following salts were used: LiCl, CH_3COOK, $CaCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaCl, $BaCl_2$. The water activities over saturated salt solutions were taken from the work of Nikol'skii [21]. The masses of protein samples used in the equilibration were in the range of 8–10 mg. The water content of the samples after equilibration was also measured by drying using a microthermoanalyzer "Setaram" MGDTD-17S at 25 °C and 0.1 Pa until a constant sample weight was reached. Salts for the conditioning of the samples were of analytical pure grade. The conditioned samples were then taken from the desiccators and equilibrated in the calorimetric cell at 25 °C before each experiment.

2.3 Water Sorption Measurements

The residual water content of the HSA preparations immersed in organic solvents, h_{res}, was determined from water adsorption measurements using the Karl Fisher method, and the results are summarized in Tab. 1. The procedure for determination of the water content bound to HAS has been described previously elsewhere [18, 22]. Typically, it involves 5–10 mg of solid HSA preparation contacted in the sorption cell with 4.0 mL of a solvent. The amount of residual water on the HSA was determined after maintaining the suspensions for 2–3 h in closed vials at 25 °C. This time period exceeded the time corresponding to the completion of the heat effect in all calorimetric experiments. It can be concluded from Tab. 1 that the h_{res} values do not depend markedly on the initial water content of the HSA. The equilibrium water concentrations in organic solvents after immersion of the HSA preparations did not exceed 0.02 mol/L for ethanol, 0.07 mol/L for formamide, 0.1 mol/L for DMSO, 0.05 mol/L for methanol, and 0.09 mol/L for acetic acid. The corresponding water activities calculated using Eq. (1) did not exceed 0.01 for all of the solvents studied.

Table 1. Residual water content of the HSA preparations immersed into organic solvents at 25 °C.

No.	Solvent	h_{res} [% (g water/g protein)] h_{ini} = 0.2 %	h_{res} [% (g water/g protein)] h_{ini} = 10.0 %	h_{res} [% (g water/g protein)] h_{ini} = 29.0 %
1	Formamide	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)
2	Ethanol	0.2 (0.1)	0.2 (0.1)	0.2 (0.2)
3	Methanol	0.1 (0.1)	0.1 (0.1)	0.3 (0.2)
4	Acetic acid	0.2 (0.1)	0.3 (0.2)	0.3 (0.2)
5	DMSO	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)

$$a_w = \gamma_w x_w \quad (1)$$

where x_w is the mole fraction of water in the solution and γ_w is the activity coefficient of water (in mole fraction, the standard state is pure water). The water activity coefficients, γ_w, were calculated using literature data [23] on the vapor-liquid equilibrium according to Eq. (2).

$$\gamma_w = \frac{y_w P_{tot}}{x_w P_w^o} \quad (2)$$

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

2.4 Enthalpies of Solvation of Water in Organic Solvents

The enthalpies of salvation of water in organic solvents were calculated by using Eq. (3):

$$\Delta H_{solv}^{H_2O/S} = \Delta H_{sol}^{H_2O/S} - \Delta H_{vap}^{H_2O} \quad (3)$$

where $\Delta H_{sol}^{H_2O/S}$ is the enthalpy of solution of water in a solvent at infinite dilution and 25 °C [kJ/mol], and $\Delta H_{vap}^{H_2O}$ is the enthalpy of vaporization of water (43.7 kJ/mol) [21]. Consequently, the $\Delta H_{solv}^{H_2O/S}$ value for water is equal to –43.7 kJ/mol. The $\Delta H_{solv}^{H_2O/S}$ values for DMSO, methanol, ethanol, formamide, and acetic acid were found to be –49.0, –47.1, –45.8, –42.7 and –40.9 kJ/mol, respectively. The enthalpies of solution of water in organic solvents at infinite dilution and 25 °C were taken from Borisover et al. [24] and Belousov and Morachevski [25].

1) List of symbols at the end of the paper.

3 Results and Discussion

3.1 Definition of the System under Study

The correct analysis of calorimetric data requires the definition of the system under study. The first consideration is the case when:
(*i*) Initially, the solid protein phase does not contain organic component. The initial protein phase may contain water;
(*ii*) The protein immersed in a low water organic mixture forms a two-phase system including the protein phase and liquid solution;
(*iii*) There is no significant dissolution of a protein in the liquids under study (organic solvents with low water content) [13], and
(*iv*) The final protein phase may contain water and organic components. Then, the enthalpy change, ΔH_{tot}, corresponding to an introduction of some amount of protein in a water-organic mixture is given by Eq. (4):

$$\Delta H_{tot}(hydrated) = [\overline{H}_{\mathrm{w}} m_w + \overline{H}_{\mathrm{S}} \cdot m_S]_{final\ liquid} + [\overline{H}_{\mathrm{P}} \cdot m_P + \overline{H}_w m_w + \overline{H}_{\mathrm{S}} \cdot m_S]_{final\ solid} - [\overline{H}_w m_w + \overline{H}_{\mathrm{S}} \cdot m_S]_{initial\ liquid} - [\overline{H}_{\mathrm{P}} \cdot m_P + \overline{H}_w m_w]_{initial\ solid} \quad (4)$$

where $\overline{H}_{\mathrm{P}}$, $\overline{H}_{\mathrm{w}}$, $\overline{H}_{\mathrm{S}}$ are the partial enthalpies of the protein, water, and organic components, respectively, and m_{P}, m_{w} and m_{s} are mass amounts of the protein, water, and organic component, respectively. Phases (liquid or solid) and states (final or initial) are specified by the subscripts. The amounts of water, $m_{\mathrm{w}}^{\mathrm{tr}}$, and organic component, $m_{\mathrm{s}}^{\mathrm{tr}}$, transferred from the liquid phase to the protein phase during suspension of the protein samples are defined in Eqs. (5) and (6):

$$m_{\mathrm{w}}^{\mathrm{tr}} = [m_{\mathrm{w,final}} - m_{\mathrm{w,initial}}]_{\mathrm{liquid}} = [m_{\mathrm{w,initial}} - m_{\mathrm{w,final}}]_{\mathrm{solid}} \quad (5)$$

$$m_{\mathrm{s}}^{\mathrm{tr}} = [m_{\mathrm{s,final}} - m_{\mathrm{s,initial}}]_{\mathrm{solid}} = [m_{\mathrm{s,initial}} - m_{\mathrm{s,final}}]_{\mathrm{liquid}} = m_{\mathrm{s,\ final\ solid}} \quad (6)$$

When the solid-liquid ratio is small enough (in the current work the molar ratio of HSA/organic solvent is ca. $2 \cdot 10^{-6}$), the partial enthalpies of water and organic component in the liquid phase are not changed during the protein sample immersion. Therefore, Eq. (4) can be transformed into an expression useful for experimental applications, given by Eq. (7):

$$\Delta H_{tot}(hydrated) = \overline{H}_w m_{w,liquid}^{tr} + \overline{H}_w m_{w,final\ solid} - \overline{H}_w m_{w,initial\ solid} + m_P[\overline{H}_{P,final\ solid} - \overline{H}_{P,initial\ solid}] + m_S^{tr}[\overline{H}_{S,solid} - \overline{H}_{S,liquid}] \quad (7)$$

where ΔH_{tot} (hydrated), $m_{\mathrm{w}}^{\mathrm{tr}}$, $m_{\mathrm{s}}^{\mathrm{tr}}$ are now related to the unit mass amount of protein. When the transfer of water from the protein to the liquid phase is complete, i.e., $m_{\mathrm{w,final\,solid}} = 0$ and $m_{\mathrm{w}}^{\mathrm{tr}} = m_{\mathrm{w},initial\ solid}$), Eq. (7) can be transformed into Eq. (8):

$$\Delta H_{tot}^{o}(hydrated) = m_{\mathrm{w}}^{\mathrm{tr}}[\overline{H}_{w,liquid} - \overline{H}_{w,solid}] + m_P[\overline{H}_{P,final\,solid} - \overline{H}_{P,initial\ solid}] + m_{\mathrm{S}}^{\mathrm{tr}}[\overline{H}_{S,final\ solid} - \overline{H}_{S,initial\ liquid}] \quad (8)$$

In the presence of water in the initial liquid phase, $m_{\mathrm{w,\ final\,solid}} = 0$, Eq. (7) can be transformed into Eq. (9):

$$\Delta H_{tot}(dried) = \overline{H}_w m_{w,liquid}^{tr} + \overline{H}_w m_{w,final\ solid} + m_P[\overline{H}_{P,final\ solid} - \overline{H}_{P,initial\ solid}] + m_S^{tr}[\overline{H}_{S,final\ solid} - \overline{H}_{S,initial\ liquid}] \quad (9)$$

In the absence of water in the protein and liquid phase, the $\Delta H_{tot}^{o}(dried)$ values immediately demonstrate the energetics of protein-organic solvent interactions, Eq. (10). Depending on the specific mechanism, these enthalpy changes may be considered as wetting heats (if solvent molecules solvate the surface of the protein sample) or swelling heats (if the solvent molecules penetrate into the protein bulk).

$$\Delta H_{tot}^{o}(dried) = m_P[\overline{H}_{P,final\ solid} - \overline{H}_{P,initial\ solid}] + m_{\mathrm{S}}^{\mathrm{tr}}[\overline{H}_{S,final\ solid} - \overline{H}_{S,initial\ liquid}] \quad (10)$$

In order to characterize the protein-water interactions in the presence of organic solvent, the $\Delta H_{tot}^{o}(dried)$ and ΔH_{tot}^{o} *(hydrated)* values should be compared with water sorption data obtained independently, i.e., $m_{\mathrm{w}}^{\mathrm{tr}}$, Eq. (11). The ΔH_{deh} value has a complex nature. It includes contributions resulting from (*i*) the difference between the partial enthalpies of the initially dried and hydrated protein, and (*ii*) the difference between the partial enthalpies of water in the solid protein and liquid phases.

$$\Delta H_{deh} = \frac{\Delta H_{tot}^{o}(hydrated) - \Delta H_{tot}^{o}(dried)}{m_w^{tr}} = \frac{m_P}{m_w^{tr}}[\overline{H}_P^{o}, solid - \overline{H_{P,solid}^{h}}] + [\overline{H}_{w,liquid} - \overline{H}_{w,gas}] + [\overline{H}_{w,gas} - \overline{H}_{w,solid}] = \Delta H_{desorp}^{H_2O/gas} + \Delta H_{solv}^{H_2O/S} \quad (11)$$

where $\overline{H}_P^{o}$ is the partial enthalpy of the initially dried protein, $\overline{H}_P^{h}$ is the partial enthalpy of the initially hydrated protein, and $\Delta H_{desorb}^{H_2O/gas}$ is the molar enthalpy of transfer of water from the protein phase to the gas phase.

Eq. (11) is based on the assumption that the transfer of water from the protein phase to the anhydrous organic solvent is complete. However, in general, co-adsorption of both organic solvent and water may occur. This implies that the component corresponding to the transfer of organic solvent molecules from the liquid phase to the protein phase, $m_{\mathrm{S}}^{\mathrm{tr}}$, may not be identical in Eqs. (8) and (10). The maximum value of this deviation is estimated using data from Tab. 1, e.g., the residual water content in methanol (h_{ini} = 29.0 %) is 0.3 %. When this h_{ini} value corresponds to the total number of the competition sites, the fraction of the water-occupied co-adsorption sites is ca. 1 %. This value does not exceed the limits of experimental errors. When organic solvent molecules have independent binding sites, the fraction of the water-occupied co-adsorption sites is lower than 1 %.

3.2 Interaction Enthalpies of the Dried and Hydrated HSA with Organic Solvents

Fig. 1 shows how the interaction enthalpies, ΔH_{tot}, (dried) of the initially dried HSA depend on the concentration of water in organic solvents. Fig. 1 also demonstrates that the interaction enthalpies show a linear dependence on the concentration of water in organic solvents. Similar results were obtained for the initially hydrated HSA preparations. The interaction enthalpies of the initially dried and hydrated protein preparations with an anhydrous organic solvent, ΔH^0_{tot}, were calculated from these thermochemical data. Fig. 2 shows how the ΔH^0_{tot} values for the dried and hydrated protein preparations depend on the initial water content of HSA. The interaction enthalpies of the dried and hydrated HSA with pure water were taken from Sirotkin and Korolev [26].

The dependence of the interaction enthalpies on the initial water content is complex. The interaction enthalpies, ΔH^0_{tot}, of the dried HSA with organic solvents are exothermic (negative). Immersion of the dried HSA in the organic liquids under study results in significant structural changes [13], e.g., methanol and ethanol result in an absorbance increase at 1658 cm^{-1}, which indicates an increased helical content. DMSO strongly reduces the initial helical content. The increase in the absorbance at 1628 and 1690 cm^{-1} was assigned to the formation of an intermolecular β-structure. At low water contents (h_{ini} = 0–0.1 g/g), there is a sharp increase in the interaction enthalpies values. It is well known that at a hydration level between 0 and 0.1 g/g, water is strongly bound to ionizable and polar groups on the protein [1, 2]. This implies that the primary dehydration process (the dehydration of polar and ionizable protein groups) is sufficient for the expression of the interaction enthalpies in this water content range. At high water contents, the water-organic solvent interactions play a dominant role in the interaction enthalpies, e.g., at the highest water contents ($h_{\mathrm{ini}} > 0.2$ g/g), the interaction enthalpies for acetic acid and formamide are endothermic (positive, see Fig. 2, curves 2 and 3). This result is due to the endothermic enthalpies of solution of water in these organic solvents. 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 [25]. The interaction enthalpies for methanol, ethanol, and DMSO are exothermic at high water contents ($h_{\mathrm{ini}} > 0.1$ g/g) and are shown in Fig. 2, curves 1, 4, 5. This result is due to the exothermic enthalpies of solution of water in these organic solvents [24, 25].

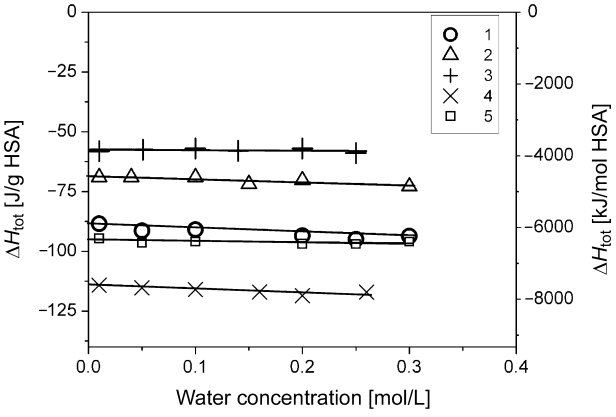

Figure 1. Interaction enthalpies of the initially dried HSA (initial water content h_{ini} = 0.2 % [g/g]) as a function of water concentration in organic solvents: (1) DMSO; (2) Methanol; (3) Ethanol; (4) Acetic acid; (5) Formamide.

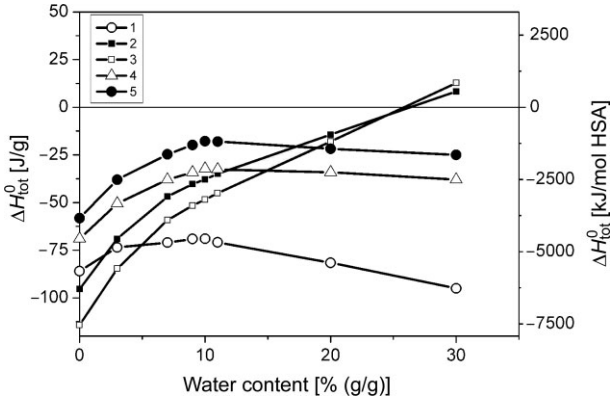

Figure 2. Interaction enthalpies of the dried and initially hydrated HSA with anhydrous organic solvents (ΔH^0_{tot}) as a function of water content: (1) DMSO, (2) formamide, (3) acetic acid (4) methanol, (5) ethanol. Standard errors of estimation of the ΔH^0_{tot} values were 1–2 J/g HSA.

3.3 Heat Effects of Dehydration of Human Serum Albumin in Organic Solvents

The next step of this work was to calculate the heat effects of dehydration of HSA in organic solvents and to determine the effect of the solvation ability of organic solvents on the heat effects of dehydration. Two types of heat effects were considered, i.e., the interaction enthalpies for the dried HSA ($\Delta H^0_{tot}(dried)$) and initially hydrated HSA ($\Delta H^0_{tot}(hydrated)$) with anhydrous organic media. The molar enthalpies of dehydration of HSA in organic solvents, ΔH_{deh}, were calculated by a modified version of Eq. (11), as given in Eq. (12):

$$\Delta H_{deh} = \frac{(\Delta H^0_{tot}(hydrated) - \Delta H^0_{tot}(dried)) \cdot 18 \cdot 100}{h_{ini}} \qquad (12)$$

where h_{ini} is the initial water content of HSA (%, g water/g protein), and the number 18 is the molecular weight of water (g/mol). The ΔH_{deh} values are summarized in Tab. 2.

A thermochemical model was proposed to explain the effect of organic solvents on the ΔH_{deh} values. This model is based on a thermochemical scheme, which suggests that the interaction enthalpies of the hydrated protein, $\Delta H^0_{tot}(hydrated)$, include contributions from only two processes, i.e., (1) the transfer of water from the protein phase to the organic solvent phase, and (2) the interaction of the protein with organic solvent. Consequently, subtracting $\Delta H^0_{tot}(dried)$ from $\Delta H^0_{tot}(hydrated)$, one obtains the ΔH_{deh} values. Here, it is assumed that the value of $\Delta H^0_{tot}(dried)$ is the same for the dried

Table 2. Heat effects of dehydration HSA in organic solvents at 25 °C (kJ/mol water).

Solvent	h_{ini} = 3.0 %	h_{ini} = 7.5 %	h_{ini} = 9.0 %	h_{ini} = 10.0 %	h_{ini} = 11.0 %	h_{ini} = 19.9 %	h_{ini} = 29.0 %
Formamide	15.9 (0.6)	11.6 (0.5)	10.6 (0.4)	10.4 (0.4)	9.4 (0.3)	7.3 (0.2)	6.2 (0.2)
Ethanol	13.1 (0.5)	8.2 (0.3)	7.8 (0.2)	7.3 (0.3)	6.7 (0.2)	4.5 (0.2)	3.0 (0.1)
Methanol	11.6 (0.5)	7.1 (0.2)	6.6 (0.2)	6.3 (0.2)	5.4 (0.2)	3.2 (0.1)	2.0 (0.1)
Acetic acid	17.8 (0.6)	13.3 (0.5)	12.4 (0.4)	11.9 (0.4)	11.3 (0.4)	8.8 (0.3)	7.6 (0.3)
DMSO	9.0 (0.4)	4.7 (0.2)	4.0 (0.2)	3.8 (0.1)	3.1 (0.1)	0.7 (0.1)	–0.4 (0.1)

and hydrated HSA. This means that both the hydrated and dried protein samples must eventually pass into a state with the same enthalpy. In turn, the ΔH_{deh} value consists of contributions from two processes, i.e., (*i*) the transfer of water from the protein into the gas phase, $\Delta H_{desorb}^{H_2O/gas}$, and (*ii*) the solvation of water by the organic solvent, $\Delta H_{solv}^{H_2O/S}$. Hence, the ΔH_{deh} value must depend linearly on the enthalpy of solvation of water, $\Delta H_{solv}^{H_2O/S}$, with the slope being close to unity, which is indeed the case, as shown in Fig. 3. The parameters of the linear correlations, as outlined in Eq. (13), are given in Tab. 3.

$$\Delta H_{deh} = \Delta H_{desorp}^{H_2O/gas} + \text{slope} \cdot \Delta H_{solv}^{H_2O/S} \quad (13)$$

The intercept of Eq. (13) corresponds to the molar enthalpy of transfer of water from the protein phase to the gas phase, $\Delta H_{desorb}^{H_2O/gas}$. It is well known that the thermodynamic and structural properties of proteins in the presence and absence of organic solvents depend markedly on the hydration level [1, 2, 27, 28]. The hydration of proteins in the absence of organic solvents occurs in two well-resolved stages. In the low water activity range ($a_w < 0.5$, $h_{ini} < 0.1$ g of water/g of protein), the strong hydration sites are preferentially occupied. These water binding sites are chemically diverse and include a variety of polar functional protein groups. This step induces pronounced structural rearrangements in the dried proteins [1, 2, 27, 28]. The most significant $\Delta H_{desorb}^{H_2O/gas}$ values were observed in this range, Fig. 4A. At higher water activities ($a_w > 0.5$, $h_{ini} > 0.1$ g/g), these structural rearrangements are largely completed. In this water content range, the conformation of proteins is close to the solution conformation. Therefore, the $\Delta H_{desorb}^{H_2O/gas}$ values do not depend markedly on the water content. At $h > 0.1$ g/g, the $\Delta H_{desorb}^{H_2O/gas}$ values reach saturation and are close to the enthalpy of vaporization of water (43.7 kJ/mol).

To test the reliability of the current findings, the $\Delta H_{desorb}^{H_2O/gas}$ values determined in this work were compared with previously published results on the interaction enthalpies of the dried and hydrated HSA with pure water [26]. The $\Delta H_{desorb}^{H_2O/gas}$ values were calculated using Eq. (14):

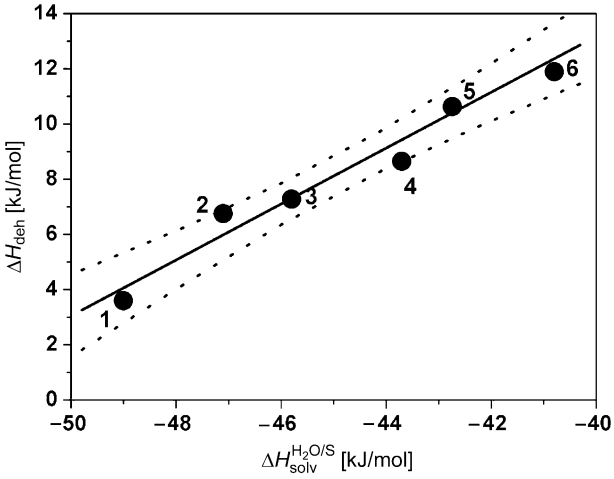

Figure 3. The enthalpy of dehydration (ΔH_{deh}) of human serum albumin (initial water content h_{ini} = 10.0 % [g/g] as a function of the enthalpy of solvation of water $\Delta H_{solv}^{H_2O/S}$ at infinite dilution and 25 °C: (1) DMSO, (2) methanol, (3) ethanol, (4) water, (5) formamide, (6) acetic acid. The dashed lines show the 95 % confidence interval.

$$\Delta H_{desorp}^{H_2O/gas} = \frac{(\Delta H_{tot}^0(hydrated) - \Delta H_{tot}^0(dried)) \cdot 18 \cdot 100}{h_{ini}} + \Delta H_{vap}^{H_2O} \quad (14)$$

Table 3. Parameters of Eq. (13).

h_{ini} [% g/g]	$\Delta H_{desorb}^{H_2O/gas}$ [kJ/mol]	Slope	R	N	Standard error of estimation [S_o]
3.0 (0.1)	61.9 (2.1)	1.1 (0.1)	0.99	6	0.30
7.5 (0.2)	56.0 (2.5)	1.0 (0.1)	0.99	6	0.38
9.0 (0.2)	53.0 (2.5)	1.0 (0.1)	0.98	6	0.38
10.0 (0.2)	51.7 (2.7)	1.0 (0.1)	0.98	6	0.40
11.0 (0.2)	51.1 (2.2)	1.0 (0.1)	0.98	6	0.33
19.9 (0.3)	48.5 (2.4)	1.0 (0.1)	0.99	6	0.36
29.0 (0.4)	47.6 (2.1)	1.0 (0.1)	0.99	6	0.30

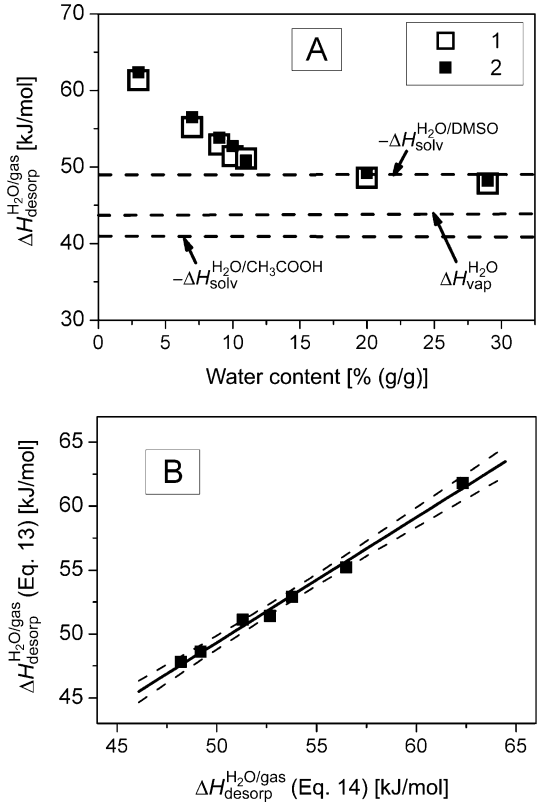

Figure 4. A) The enthalpy of transfer of water from the initially hydrated HSA to the gas phase as a function of initial water content: (1) This work (see Eq. 13). (2) Calculated from the interaction enthalpies of the dried and hydrated HSA with pure water [26] using Eq. 14. B) Correlation between the molar enthalpy of transfer of water from the protein phase to the gas phase calculated using Eq. 13 (1) and Eq 14 (2).

$$\Delta H_{desorp}^{H_2O/gas.}(1) = (-1.2\pm1.5) + (1.02\pm0.03)\cdot\Delta H_{desorp}^{H_2O/gas.}(2)$$

The number of experimental points is N = 7, the standard error of estimation is $S_o = 0.56$, and the correlation coefficient is $R = 0.991$. The dashed lines show the 95 % confidence interval.

where $\Delta H_{vap}^{H_2O}$ is the enthalpy of vaporization of water at 25 °C and atmospheric pressure [kJ/mol water], h_{ini} is the initial water content of HSA [%, g water/g protein], the number 18 is the molecular weight of water [g/mol], and $\Delta H_{tot}^{0}(hydrated)$ is the interaction enthalpy of the hydrated HSA with pure liquid water at 25 °C [J/g protein]. It can be concluded from Figs. 4A and 4B that the molar enthalpies of transfer of water from the initially hydrated HSA to the gas phase calculated using Eq. (13) agree well with those obtained using Eq. (14).

It can be concluded from Tab. 2 that the ΔH_{deh} values may be endothermic or exothermic depending on the initial water content and the water solvation enthalpy value. For example, the ΔH_{deh} values for acetic acid, formamide, methanol, and ethanol are endothermic. This result is due to the fact that the $\Delta H_{desorb}^{H_2O/gas}$ value is higher than the enthalpy of desolvation of water in these liquids over the entire range of water contents. In order to illustrate this situation, a line corresponding to the enthalpy of desolvation of water in acetic acid is given in Fig. 4A.

DMSO has the most exothermic water solvation enthalpy value of the solvents under study. The ΔH_{deh} values for DMSO are endothermic at low water contents. However, the ΔH_{deh} values for DMSO are exothermic at h_{ini} =29.0 % [g/g]. In order to illustrate this situation, a line corresponding to the enthalpy of desolvation of water in DMSO is also shown in Fig. 4A. This result is due to the fact that at the highest water contents, the $\Delta H_{desorb}^{H_2O/gas}$ value is lower than the enthalpy of desolvation of water in DMSO. Overall, this means that the average water binding affinity in the hydrated proteins may be lower than in the good solvating liquids such as DMSO, where the proteins can form strong hydrogen bonds with various hydrogen donors.

4 Conclusions

A novel method based on the combined calorimetric and water adsorption measurements was proposed to determine the molar enthalpies of dehydration of proteins in organic solvents. A special thermochemical scheme was proposed to predict the effect of the solvation ability of a medium on the molar enthalpy of transfer of water from the solid protein phase to the organic solvent liquid phase. This scheme shows that the enthalpies of protein dehydration in organic solvents may be predicted on the basis of the enthalpies of solution of the hydrated, $\Delta H_{tot}^{0}(hydrated)$, and dried, $\Delta H_{tot}^{0}(dried)$, protein preparations in pure water and the enthalpies of solvation of water in the corresponding solvents at infinite dilution, $\Delta H_{solv}^{H_2O/S}$. The results obtained give strong support to the ideas that: (*i*) the changes in the dehydration enthalpies are mainly determined by the solvation of water in hydrophilic solvents, and (*ii*) the data of the enthalpies of solvation of water by the solvent, $\Delta H_{solv}^{H_2O/S}$, at infinite dilution and room temperature reflect this effect.

Acknowledgments

This research was partly supported by the "Michail Lomonosov" Program (a grant of the Ministry of Education and Sciences of the Russian Federation and the German Academic Exchange Service, Referat 325, Kennziffer A/04/38429). The comments of the reviewers were very helpful and assisted in the clarity of the presentation.

Symbols used

$\Delta H_{tot}^{0}(dried)$	[J/g protein]	interaction enthalpy of the initially dried HSA with anhydrous organic solvent
ΔH_{tot} (dried)	[J/g protein]	interaction enthalpy of the initially dried HSA with water-organic mixture
$\Delta H_{tot}^{0}(hydrated)$	[J/g protein]	interaction enthalpy of the initially hydrated HSA with anhydrous organic solvent
$\Delta H_{tot}(hydrated)$	[J/g protein]	interaction enthalpy of the initially hydrated HSA with water-organic mixture

$\Delta H_{sol}^{H_2O/S}$	[kJ/mol water]	enthalpy of solution of water in a solvent at infinite dilution and 25 °C
$\Delta H_{vap}^{H_2O}$	[kJ/mol water]	enthalpy of vaporization of water at atmospheric pressure and 25 °C
$\Delta H_{solv}^{H_2O/S}$	[kJ/mol water]	enthalpy of solvation of water in organic solvent at infinite dilution and 25 °C or enthalpy of solvation of water in pure liquid water at 25 °C
h_{ini}	[% (g water/g protein)]	initial water content of HSA
h_{res}	[% (g water/g protein)]	residual water content bound to HSA immersed in organic solvent
ΔH_{deh}	[kJ/mol water]	enthalpy of dehydration of the initially hydrated HSA in organic solvents (enthalpy of transfer of water from the protein phase to the organic solvent liquid phase)
$\Delta H_{desorb}^{H_2O/gas}$	[kJ/mol water]	enthalpy of transfer of water from the initially hydrated HSA to the gas phase

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DOI: 10.1002/elsc.200800039

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Eng. Life Sci. **2009**, *9 (1)*, ■ ... ■