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A study of the hydration of lysozyme in neat organic solvents using isothermal calorimetry: Effect of water solvation

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ABSTRACT

The interaction enthalpies of the dried and hydrated lysozyme with neat organic solvents (DMSO, formamide, ethylene glycol, and methanol) were obtained at 25 °C. These organic solvents represent a series of liquids in which the water solvation enthalpy is gradually changed over a wide range. The interaction enthalpies of the dried lysozyme with neat organic solvents are exothermic. At high water content, the interaction enthalpies are endothermic for formamide and exothermic for DMSO, methanol, and ethylene glycol. The dehydration enthalpies of lysozyme in organic liquids may be endothermic or exothermic depending on the initial water content and the water solvation enthalpy.

1. Introduction

It is well-known that the enzyme–water interactions play a crucial role in determining the stability, structure and functions of the enzyme molecules [1–6]. Knowledge of processes occurring upon the hydration of enzymes in neat organic solvents is also very important in biotechnological and pharmaceutical applications of enzymes such as their use as biocatalysts [7–11], biosensors [12,13], downstream protein processing in protein-dissolving organic solvents [14,15], transdermal delivery of pharmaceutical proteins [16]. However, it is still unclear which physicochemical parameters control the enzyme–water interactions in neat organic liquids.

Dordick and Gorman [17] studied the desorption of the enzyme-bound T_2O into nonaqueous organic liquids for chymotrypsin, subtilisin Carlsberg, and horseradish peroxidase. They showed that enzymes lost the enzyme-bound T_2O in organic solvents. Polar solvents resulted in the highest degree of T_2O desorption. However, no quantitative correlation was observed between the desorption ability of organic liquids and solvent polarity, hydrophobicity, and the saturated molar solubility of water in a given solvent.

The structure of the model enzyme lysozyme in water and neat organic solvents has been examined using ^{1}H NMR and circular dichroism spectroscopies [18]. No correlation has been observed between the conformational state of lysozyme in organic liquids and their physicochemical characteristics as dielectric constant, dipole moment, and Hildebrand solubility parameter.

Hence, there is a clear need for experimental and theoretical approaches by which the thermodynamic characteristics of the enzyme–water interactions in organic solvents may be determined.

Thermochemical studies have traditionally been very important in ascertaining a better understanding of the enzyme–water interactions. Enthalpy is an important thermodynamic quantity directly associated to the intensity of the intra- and intermolecular interactions in the above mentioned systems. Thus, particular, Smith et al. [19] calorimetrically measured the heats of pure water adsorption on lysozyme. They obtained both the water sorption isotherm and the enthalpy of hydration of the protein in the water content range 0–18% (g water/g enzyme) at 25 °C. The sorption calorimetry has been used to measure the adsorption isotherm of water on lysozyme and the corresponding heat effects in the water content range 0–50 wt% [20]. Our research group developed an experimental method for measuring the heat effects of hydration of proteins [21,22]. The most important observations of these calorimetric studies [19–22] can be summarized as follows:

(i) The hydration enthalpies vary strongly with the hydration level.
(ii) The hydration enthalpies are highly exothermic at low hydration level.
(iii) As the water content increases, the hydration enthalpies approach the enthalpy of condensation of pure water.

Thermochemical studies may be extremely pertinent to the understanding of the enzyme–water interactions in organic liquids. The interaction enthalpies of the dried α-chymotrypsin

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with neat organic solvents including hydrocarbons, alcohols, and hydrogen bond accepting solvents have been studied using isothermal calorimetry [23]. It was found that the interaction enthalpies depend cooperatively on the solvent hydrophilicity. All the solvents were divided into two groups. The first group included the hydrophilic solvents (methanol, DMSO). The interaction enthalpies of the dried enzyme with hydrophilic liquids were strongly exothermic. The second group included the hydrophobic and medium hydrophilic liquids (benzene, dioxane, butanol-1, propanol-1). The enthalpy changes in the second group of solvents were close to zero.

In our previous studies [24], we proposed a novel methodology for estimating the enthalpies of the protein–water interactions in neat organic solvents. This methodology is based on the analysis of the interaction enthalpies of the hydrated and dried protein with organic liquids and the water solvation enthalpies in organic solvents.

To test the predictive ability of the proposed methodology, a system containing, as a model enzyme, hen white-egg lysozyme was studied in this work. We reported new data on the interaction enthalpies of the dried and hydrated enzyme with neat organic solvents. The interaction enthalpies were compared with the water sorption data to calculate the molar enthalpy of the enzyme dehydration in neat organic liquids. The thermochemical quantities for lysozyme were compared with the published data for another globular protein human serum albumin [24]. The present study is aimed at understanding which molecular parameters control the enthalpies of the enzyme–water interactions in neat organic solvents.

Organic solvents are widely used as model substances to induce the amyloid fibril and molten globule formation [25,26]. These protein states are associated with numerous debilitating diseases such as Alzheimer's disease, Parkinson's disease and type II diabetes [27,28]. In perspective, our methodology may be applied to the water–organic mixtures for the thermochemical description of these intermediate protein states.

Hen egg white lysozyme is one of the most well studied and applied in biomolecular investigations [29,30]. Lysozyme is a small monomeric protein of 129 amino acid residues, containing no non-protein components. The physiological role of lysozyme is to hydrolyze polysaccharide chains [29,30].

Hydrophilic organic solvents (dimethyl sulfoxide (DMSO), formamide, methanol, and ethylene glycol) were used as model organic liquids. 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. Methodology

Our calculations are based on the assumption that the transfer of water from the enzyme to neat hydrophilic organic solvents is complete. This assumption was previously supported [24]. The studied systems can be described as follows:

Initial state for the measured enthalpy changes, ΔH_{tot}:

(A) Lysozyme

(i) The enzyme obtained by drying in the absence of organic solvents at water activity less than 0.01 at 25 °C ($\bar{H}_E$(dried));
(ii) The enzyme hydrated with pure water vapor with the varied water activity in the absence of organic solvents at 25 °C ($\bar{H}_E$(hydrated)).

(B) Water. The initial state was the water molecules bound to lysozyme at 25 °C ($\bar{H}_w$(enzyme)).

(C) Organic solvent. The initial state was the organic solvent molecules in neat liquids at 25 °C ($\bar{H}_S$(solvent)).

Final state for the measured enthalpy changes, ΔH_{tot}:

(AA) Lysozyme. The final state was the enzyme dissolved in neat organic solvents or water ($\bar{H}_E$(solvent)) at 25 °C.

(BB) Water. The final state was the water molecules dissolved in neat organic solvents or water ($\bar{H}_w$(solvent)) at 25 °C.

(CC) Organic solvent. The final state was the organic solvent molecules bound to lysozyme ($\bar{H}_S$(enzyme)) at 25 °C. Symbols used in Section 2 are described in Table 1.

The interaction enthalpy of the dried enzyme with neat organic solvent is given by Eq. (1):

$$\Delta H_{tot}(\text{dried}) = n_E\bar{H}_E(\text{solvent}) + n_S\bar{H}_S(\text{enzyme}) - n_S\bar{H}_S(\text{solvent}) - n_E\bar{H}_E(\text{dried}) \quad (1)$$

The interaction enthalpy of the hydrated enzyme with neat organic solvent is given by Eq. (2):

$$\Delta H_{tot}(\text{hydrated}) = n_w\bar{H}_w(\text{solvent}) + n_E\bar{H}_E(\text{solvent}) + n_S\bar{H}_S(\text{enzyme}) - n_S\bar{H}_S(\text{solvent}) - n_E\bar{H}_E(\text{hydrated}) + n_w\bar{H}_w(\text{enzyme}) \quad (2)$$

where $\bar{H}_E$, $\bar{H}_w$, $\bar{H}_S$ are the partial enthalpies of the enzyme, water, and organic component, respectively (Table 1); n_E, n_w, n_S are the moles of the enzyme, water, and organic component, respectively.

The amount of water, n_w^{tr}, transferred from the enzyme to the liquid is defined using Eq. (3):

$$n_w^{tr} = n_w(\text{solvent}) - n_w(\text{enzyme}) \quad (3)$$

Table 1
Symbols used in Section 2.

Symbol	Name	Unit
$\bar{H}_w$(solvent)	Partial enthalpy of water in the liquid at 25 °C	J/mol water
$\bar{H}_w$(enzyme)	Partial enthalpy of water bound to the enzyme at 25 °C	J/mol water
$\bar{H}_w$(gas)	Partial enthalpy of water in gas phase at 25 °C	J/mol water
$\bar{H}_E$(dried)	Partial enthalpy of the dried enzyme at 25 °C	J/mol enzyme
$\bar{H}_E$(hydrated)	Partial enthalpy of the hydrated enzyme at 25 °C	J/mol enzyme
$\bar{H}_S$(solvent)	Partial enthalpy of organic solvent molecules in the liquid at 25 °C	J/mol solvent
$\bar{H}_S$(enzyme)	Partial enthalpy of organic solvent molecules bound to the enzyme at 25 °C	J/mol solvent
ΔH_{tot}(dried)	Interaction enthalpy of the initially dried enzyme with neat organic solvent or water	J
ΔH_{tot}(hydrated)	Interaction enthalpy of the initially hydrated enzyme with neat organic solvent or water	J
$\Delta H_{sol}^{H_2O/S}$	Enthalpy of solution of water in a solvent at infinite dilution and 25 °C	kJ/mol water
$\Delta H_{vap}^{H_2O}$	Enthalpy of vaporization of water at atmospheric pressure and 25 °C	kJ/mol water
$\Delta H_{solv}^{H_2O/S}$	Enthalpy of water solvation in organic solvent at infinite dilution and 25 °C or enthalpy of water solvation in pure liquid water at 25 °C	kJ/mol water
h_{ini}	Initial water content of lysozyme	% (g water/g enzyme)
ΔH_{deh}	Enthalpy of dehydration of the initially hydrated enzyme in neat organic solvents (enthalpy of transfer water from the enzyme to the organic solvent)	kJ/mol water
$\Delta H_{desorp}^{H_2O/gas}$	Enthalpy of transfer of water from the initially hydrated enzyme to the gas phase	kJ/mol water

The amount of organic component, n_S^{tr}, transferred from the liquid to the enzyme is defined using Eq. (4):

$$n_S^{tr} = n_S(\text{enzyme}) - n_S(\text{solvent}) \quad (4)$$

The ΔH_{tot}(dried) values demonstrate immediately the enzyme–organic solvent interactions. The ΔH_{tot}(hydrated) values describe simultaneously the enzyme–water and enzyme–organic solvent interactions. To characterize the enzyme–water interactions in neat organic solvents, the ΔH_{tot}(dried) and ΔH_{tot}(hydrated) values should be compared with water sorption data obtained independently, i.e., n_w^{tr} (Eq. (5)). The dehydration enthalpy, ΔH_{deh}, includes the contributions resulting from (1) the difference between the partial enthalpies of the initially dried and hydrated enzyme and (2) the difference between the partial enthalpies of water bound to enzyme and in the solvent.

$$\Delta H_{deh} = \frac{\Delta H_{tot}(\text{hydrated}) - \Delta H_{tot}(\text{dried})}{n_w^{tr}} =$$
$$= \frac{n_E}{n_w^{tr}}(\bar{H}_E(\text{dried}) - \bar{H}_E(\text{hydrated})) + (\bar{H}_w(\text{solvent}) - \bar{H}_w(\text{gas})) + \quad (5)$$
$$(\bar{H}_w(\text{gas}) - \bar{H}_w(\text{enzyme})) = \Delta H_{desorp}^{H_2O/gas} + \Delta H_{solv}^{H_2O/S}$$

where $\Delta H_{desorp}^{H_2O/gas.}$ is the enthalpy of transfer of water from the enzyme phase to the gas phase; $\Delta H_{solv}^{H_2O/S}$ is the enthalpy of solvation of water in pure liquid water or organic solvents at infinite dilution; $\bar{H}_w$(gas) is the partial enthalpy of water in gas phase at 25 °C and atmospheric pressure.

3. Experimental

3.1. Materials

Hen egg-white lysozyme of the highest commercially available purity was purchased from Sigma Chemical (St. Louis, MO, USA) and used without further purification. The purity of protein samples was verified through electrophoresis and dynamic light scattering measurements (90Plus Particle Size Analyzer, Brookhaven Instruments Corporation, USA) to be more than 95%. The molecular mass of protein was taken as 14 300 Da. The water used was doubly distilled. Organic solvents (reagent grade, purity >99%) were purified and dried according to general recommendations available elsewhere [31].

3.2. Calorimetry

The interaction enthalpies (ΔH_{tot}) of the dried and hydrated enzyme with pure liquid water or organic solvents were measured at 25 °C with a Setaram BT-2.15 calorimeter according to the described procedure [32,33]. Typically, the sample of 5–10 mg of lysozyme contacted with 4.0 mL of a solvent in the calorimetric cell. Typical heat evolution curves recorded on the interaction of lysozyme with pure liquid water or organic solvents are given in Figure 1A and B. Curves may involve exothermic peak, endothermic peak or both together. Endothermic peaks are related to the water desorption from lysozyme to the solvent. The heat evolution was completed for 30–90 min. Calorimeter was calibrated using the Joule effect and tested with dissolving sodium chloride in water according to the recommendations [23].

The dried enzyme (**zero hydration level**) was obtained by drying under vacuum using a microthermoanalyzer 'Setaram' MGDTD-17S at 25 °C and 0.1 Pa until the constant sample weight was reached. Water content of the dried lysozyme was estimated as 0.2 ± 0.1% (g water/g enzyme) by the Karl Fischer titration method according to the recommendations [32,33]. Samples for the determination of the interaction enthalpies of the hydrated lysozyme with water were equilibrated at 25 ± 0.5 °C in tightly closed desiccator over the saturated salt solutions. The following salts were used: LiCl (a_w = 0.11), CH_3COOK (a_w = 0.22), $MgCl_2$ (a_w = 0.33), K_2CO_3 (a_w = 0.44), $Mg(NO_3)_2$ (a_w = 0.53), NaCl (a_w = 0.75), $BaCl_2$ (a_w = 0.88). Water activities of the saturated salt solutions were taken from [34,35]. The masses of enzyme samples used in the equilibration were in the range of 8–10 mg. Water content of the samples after equilibration was measured by drying using a microthermoanalyzer 'Setaram' MGDTD-17S at 25 °C and 0.1 Pa until the constant sample mass was reached. Salts for the conditioning of the samples were of analytical pure grade. The conditioned samples were then taken from the desiccator and equilibrated in the calorimetric cell at 25 °C before the experiment.

3.3. Water content of organic solvents

The water content of organic solvents was determined using the Karl Fisher method [23,32,33]. The equilibrium water concentrations in organic solvents after immersion of the enzyme preparations were lower than 0.04 mol/L for ethylene glycol, 0.05 mol/L for formamide, 0.07 mol/L for DMSO, and 0.02 mol/L for

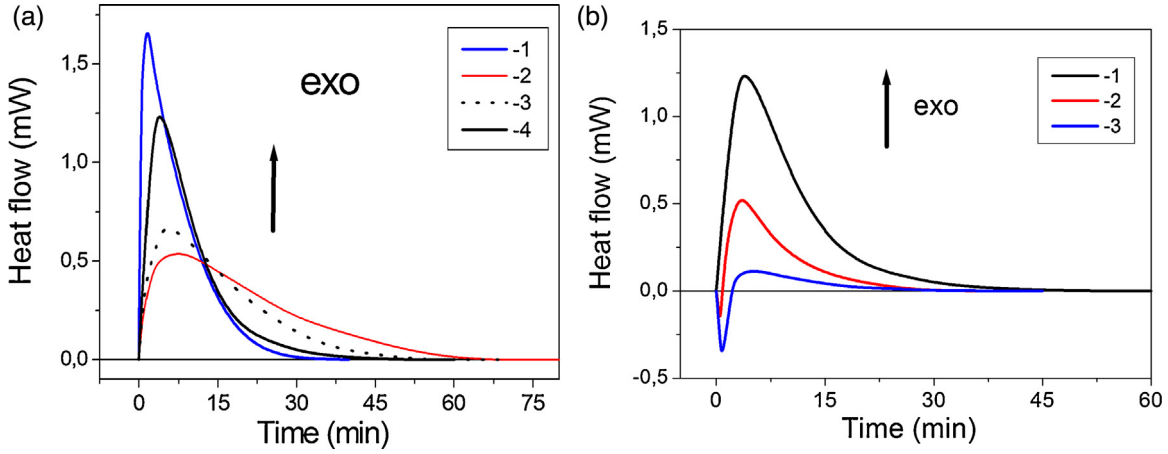

Figure 1. (A) Original calorimetric curves recorded for 10 mg of the dried lysozyme contacting with water or neat organic solvents at 25 °C: (1) water; (2) DMSO; (3) ethylene glycol; (4) formamide. The measured heat effect is −0.9 J. (B) Original calorimetric curves recorded for 10 mg of the initially hydrated lysozyme contacting with neat formamide at 25 °C. Initial water content of lysozyme: (1) h_{ini} < 0.2% (g water/g enzyme); (2) h_{ini} = 9.0% (g water/g enzyme); (3) h_{ini} = 19.9% (g water/g enzyme). Similar heat evolution curves were observed for DMSO, ethylene glycol and methanol.

Table 2
Heat effects of dehydration of lysozyme in organic solvents, ΔH_{deh}, at 25 °C (kJ/mol water).

Solvent	h_{ini} = 3.3%	h_{ini} = 5.5%	h_{ini} = 7.2%	h_{ini} = 9.0%	h_{ini} = 10.9%	h_{ini} = 19.9%	h_{ini} = 28.3%
Formamide	17.5 (0.6)	15.6 (0.5)	12.7 (0.4)	11.0 (0.4)	9.3 (0.3)	6.8 (0.2)	6.1 (0.2)
Ethylene Glycol	15.3 (0.5)	12.7 (0.3)	10.0 (0.2)	8.3 (0.3)	6.5 (0.2)	3.8 (0.2)	3.1 (0.2)
Methanol	13.1 (0.5)	10.9 (0.2)	8.1 (0.2)	6.3 (0.2)	4.7 (0.2)	2.3 (0.1)	1.6 (0.1)
DMSO	11.3 (0.4)	9.1 (0.2)	6.3 (0.2)	4.6 (0.2)	3.1 (0.1)	0.4 (0.1)	−0.4 (0.1)

methanol. The corresponding water activities calculated using Eq. (6) did not exceed 0.01 in all the studied solvents:

$$a_w = \gamma_w x_w \tag{6}$$

where x_w is the mole fraction of water in the solution and γ_w is the activity coefficient of water (in mole fractions; the standard state is pure water). Water activity coefficients (γ_w) were calculated using literature data [36] on the vapor-liquid equilibrium using Eq. (7):

$$\gamma_w = \frac{y_w P_{tot}}{x_w P_w^0} \tag{7}$$

where y_w is the measured mole fraction of water in vapor phase, P_{tot} is the total pressure, P_w^0 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.

3.4. Enthalpies of solvation of water in organic solvents, $\Delta H_{solv}^{H_2O/S}$

These thermochemical parameters were calculated using Eq. (8):

$$\Delta H_{solv}^{H_2O/S} = \Delta H_{sol}^{H_2O/S} - \Delta H_{vap}^{H_2O} \tag{8}$$

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); $\Delta H_{vap}^{H_2O}$ is the enthalpy of vaporization of water (43.7 kJ/mol) [37]. Consequently, the $\Delta \boldsymbol{H}_{solv}^{H_2O/S}$ value for water is equal to −43.7 kJ/mol. The $\Delta \boldsymbol{H}_{solv}^{H_2O/S}$ values for DMSO, methanol, ethylene glycol, and formamide were found to be −49.0, −47.1, −45.8, and −42.7 kJ/mol, respectively. The enthalpies of solution of water in organic solvents at infinite dilution and 25 °C were taken from [37].

4. Results and discussion

4.1. Interaction enthalpies of the dried and hydrated enzyme with organic solvents

Figure 2 presents the dependence of the interaction enthalpies for the dried and hydrated enzyme with organic solvents on the initial water content. The interaction enthalpies of the dried and hydrated lysozyme with pure water are also given in Figure 2.

The interaction enthalpies depend in a complicated way on the initial water content. The interaction enthalpies of the dried lysozyme with organic solvents are exothermic (negative). At low water content (h_{ini} = 0–0.1 g water/g enzyme), there is a sharp change in the interaction enthalpies. It is well known that at a hydration level between 0 and 0.1 g water/g enzyme, water is strongly bound to ionizable and polar groups [1,2,38]. 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 content, the water–organic solvent interactions play a dominant role in the interaction enthalpies. For example, at high water content (h_{ini} > 0.2 g water/g enzyme), the interaction enthalpies for formamide are endothermic (positive) (Figure 2). This result is due to the endothermic enthalpies of solution of water in this organic solvent. The enthalpy of solution of water in formamide at infinite dilution was found to be 1.0 kJ/mol water [37]. The interaction enthalpies for methanol, ethylene glycol, and DMSO are exothermic at high water content (h_{ini} > 0.1 g water/g enzyme): (Figure 2). This result is due to the exothermic enthalpies of solution of water in these organic solvents [37].

4.2. Heat effects of dehydration of lysozyme in organic solvents

We compared the heat effects of two types: the interaction enthalpies for the dried, ΔH_{tot}(dried), and hydrated, ΔH_{tot}(hydrated), enzyme with neat organic media. The molar enthalpies of dehydration of lysozyme in organic solvents, ΔH_{deh}, were calculated by a modified version of Eq. (5), as given in Eq. (9):

$$\Delta H_{deh} = \frac{(\Delta H_{tot}(\text{hydrated}) - \Delta H_{tot}(\text{dried})) * 18 * 100}{h_{ini}} \tag{9}$$

where h_{ini} is the initial water content of lysozyme (%, g water/g enzyme), and the number 18 is the molecular mass of water (g/mol). The ΔH_{deh} values are summarized in Table 2.

A thermochemical model was applied (Figure 3A and B) 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 enzyme, ΔH_{tot}(hydrated), include the contributions from only two processes (Figure 3A), i.e., (1) the transfer of water from the enzyme phase to the organic solvent phase, and (2) the interaction of the enzyme with organic solvent. Consequently, subtracting ΔH_{tot}(dried) from ΔH_{tot}(hydrated), one obtains the ΔH_{deh} values. Here, we assumed that the value of ΔH_{tot}(dried) is the same for the dried and hydrated enzyme. It means that both the hydrated and dried enzyme samples must eventually pass into a state with the same enthalpy. In turn, the ΔH_{deh} value consists of the contributions from two processes (Figure 3B), i.e., (1) the transfer of water from the enzyme phase to the gas phase, $\Delta H_{desorp}^{H_2O/gas.}$, and

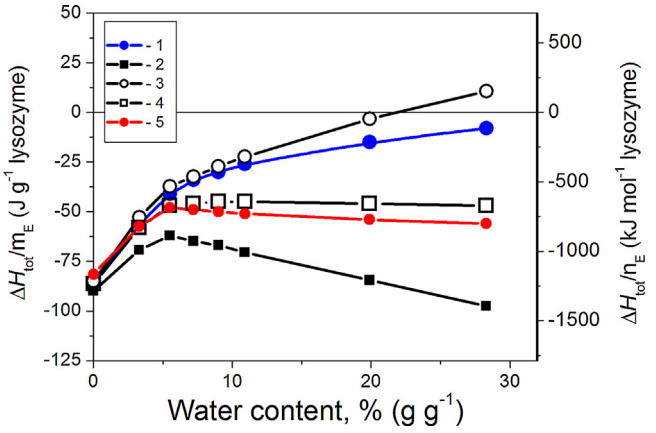

Figure 2. Interaction enthalpies of the initially dried and hydrated lysozyme with water and neat organic solvents as a function of water content: (1) water; (2) DMSO (3) formamide; (4) ethylene glycol; (5) methanol. The standard errors of estimation of the interaction enthalpies 0.8–1.2 J/g lysozyme. Each experiment was performed 3–4 times.

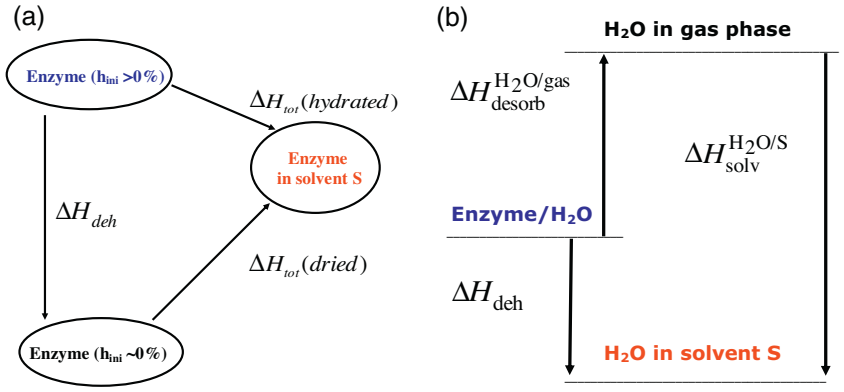

Figure 3. (A) Scheme describing the interrelation between the interaction enthalpies of the dried, ΔH_{tot}(dried), and hydrated, ΔH_{tot}(hydrated), enzyme with neat organic solvents and heat effects of dehydration, ΔH_{deh}. (B) Scheme describing the separation of the heat effect of dehydration, ΔH_{deh}, of the hydrated enzyme (**Enzyme/H_2O**) by organic solvents into two contributions: (1) the transfer of water from the initially hydrated enzyme to the gas phase, $\Delta H_{desorb}^{H_2O/gas}$, and (2) the solvation of water by the solvent, $\Delta H_{solv}^{H_2O/S}$.

Table 3
Parameters of Eq. (10).

h_{ini} % (g water/g enzyme)	$\Delta H_{desorb}^{H_2O/gas}$ kJ/mol water	Slope	R	N	Standard error of estimation, S_o
3.3 (0.1)	61.7 (2.1)	1.0 (0.1)	0.99	5	0.23
5.5 (0.2)	58.0 (1.9)	1.0 (0.1)	0.99	5	0.21
7.2 (0.2)	55.2 (1.7)	1.0 (0.1)	0.99	5	0.19
9.0 (0.2)	53.3 (1.6)	1.0 (0.1)	0.99	5	0.18
10.9 (0.2)	52.1 (1.7)	1.0 (0.1)	0.99	5	0.19
19.9 (0.3)	49.4 (1.6)	1.0 (0.1)	0.99	5	0.18
28.3 (0.4)	48.6 (1.7)	1.0 (0.1)	0.99	5	0.20

N is the number of the experimental points, S_o is the standard error of estimation, R is the correlation coefficient.

(2) 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 Figure 4. The parameters of the linear correlations, as outlined in Eq. (10), are given in Table 3.

$$\Delta H_{deh} = \Delta H_{desorp}^{H_2O/gas} + slope * \Delta H_{solv}^{H_2O/S} \quad (10)$$

The intercept of Eq. (10) corresponds to the enthalpy of transfer of water from the enzyme phase to the gas phase $\Delta H_{desorp}^{H_2O/gas.}$. At low hydration level (h_{ini} < 0.1 g water/g enzyme), the strong hydration sites are preferentially occupied. These water binding sites are chemically diverse and include a variety of ionizable and polar functional protein groups. The most significant $\Delta H_{desorp}^{H_2O/gas.}$ values were observed in this range (Figure 5).

At high hydration level (h_{ini} > 0.1 g water/g enzyme), the $\Delta H_{desorp}^{H_2O/gas.}$ values do not depend markedly on the water content. At h_{ini} > 0.1 g/g, the $\Delta H_{desorp}^{H_2O/gas.}$ values reach saturation and close to the enthalpy of vaporization of water (43.7 kJ/mol water).

To test the reliability of our findings, we compared the $\Delta H_{desorp}^{H_2O/gas.}$ values determined in this study with previously

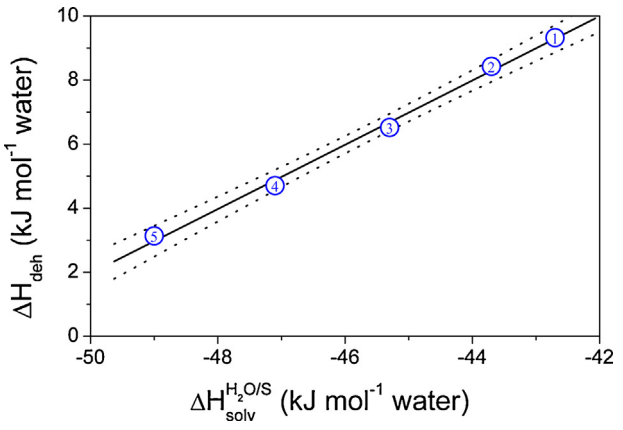

Figure 4. The enthalpy of dehydration of lysozyme, ΔH_{deh}, (initial water content h_{ini} = 10.9%) as a function of the enthalpy of water solvation, $\Delta H_{solv}^{H_2O/S}$, at infinite dilution and 25 °C: (1) formamide; (2) water; (3) ethylene glycol; (4) methanol; (5) DMSO. The dashed lines show the 95% confidence interval.

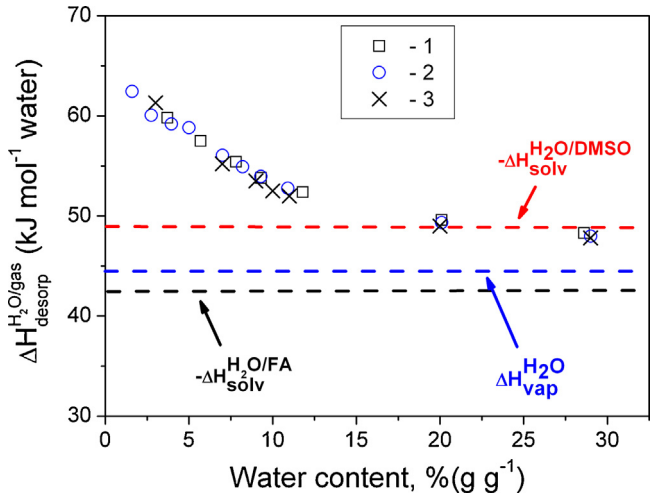

Figure 5. The enthalpy of transfer of water from the initially hydrated protein to the gas phase as a function of initial water content: (1) lysozyme. This study (Eq. (10)). (2) Calculated from the mixing enthalpies of the dried and hydrated lysozyme with pure liquid water [21] using Eq. (11). (3) Human serum albumin. Modified data from [24].

published results [21,22] on the hydration enthalpies of lysozyme obtained in the absence of organic solvents. The $\Delta H^{H_2O/gas}_{desorb}$ values were calculated by Eq. (11):

$$\Delta H^{H_2O/gas}_{desorp} = \frac{(\Delta H_{tot}(hydrated) - \Delta H_{tot}(dried)) * 18 * 100}{h_{ini}} + \Delta H^{H_2O}_{vap} \quad (11)$$

where $\Delta H^{H_2O}_{vap}$ is the enthalpy of vaporization of water at 25 °C and atmospheric pressure (kJ/mol water); h_{ini} is the initial water content of lysozyme (%, g water/g enzyme); the number 18 is the molecular mass of water (g/mol). As shown in Figure 5, the enthalpies of transfer of water from the initially hydrated lysozyme to the gas phase calculated using Eq. (10) agree well with those obtained using Eq. (11).

As concluded from Table 2, the ΔH_{deh} values may be endothermic or exothermic depending on the initial water content and the water solvation enthalpy. For example, the ΔH_{deh} values for formamide, methanol, and ethylene glycol are endothermic. This result is due to the fact that the $\Delta H^{H_2O/gas}_{desorb}$ value is higher than the enthalpy of desolvation of water in these liquids in the entire range of water content. To illustrate this situation, a black line corresponding to the enthalpy of desolvation of water in formamide is given in Figure 5.

DMSO has the most exothermic water solvation enthalpy from the studied solvents. The ΔH_{deh} values for DMSO are endothermic at low water content. However, the ΔH_{deh} values for DMSO are exothermic at h_{ini} = 28.3% (g water/g enzyme). A red line corresponding to the enthalpy of desolvation of water in DMSO is presented in Figure 5. This result is due to the fact that at the highest water content, the $\Delta H^{H_2O/gas}_{desorb}$ value is lower than the enthalpy of desolvation of water in DMSO. These facts imply that the average water binding affinity in the hydrated enzymes may be lower than in the good solvating liquids like DMSO that can form strong hydrogen bonds with various hydrogen bond donors.

To show the generality our findings, we compared the dehydration enthalpies for lysozyme with the published data for human serum albumin [24] in Figure 5. Good correlation was observed between the $\Delta H^{H_2O/gas}_{desorb}$ values for lysozyme and serum albumin. This correlation shows the reliability of our thermochemical calculations.

5. Conclusions

A special thermochemical scheme was proposed for studying the transfer of water from the initially hydrated enzyme to neat organic solvents. This methodology is based on the analysis of the enthalpies of solution of the hydrated, ΔH_{tot}(hydrated) and dried, ΔH_{tot}(dried), enzyme in pure water and the enthalpies of solvation of water in hydrophilic solvents at infinite dilution, $\Delta H^{H_2O/S}_{solv}$. The results obtained give strong support to the ideas that

(i) the changes in the enzyme–water enthalpies in neat organic solvents are mainly determined by the water solvation in hydrophilic organic liquids and
(ii) the enthalpies of water solvation by the solvent, $\Delta H^{H_2O/S}_{solv}$, at infinite dilution and room temperature reflect this effect.

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