Thermochimica Acta 522 (2011) 205–210

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Hydration of α-chymotrypsin: Excess partial enthalpies of water and enzyme

Vladimir A. Sirotkin*, Aigul V. Khadiullina

A.M. Butlerov Institute of Chemistry, Kazan (Volga Region) Federal University, Kremlevskaya Str., 18, Kazan 420008, Russia

ARTICLE INFO

Article history:
Available online 2 March 2011

Keywords:
Hydration of enzymes
Glass transition
Excess enthalpy
Isothermal calorimetry

ABSTRACT

A novel method has been developed for studying simultaneously the excess partial enthalpies of water and the enzyme in the entire range of water content. Bovine pancreatic α-chymotrypsin was used as a model enzyme. The proposed method includes the measurements of the enthalpies of solution of the dried and hydrated enzyme in water at 25 °C. From these thermochemical data the excess partial enthalpies of water and α-chymotrypsin were calculated. The partial quantities are very sensitive to the changes in the state of water and α-chymotrypsin. A transition from the glassy to the flexible state of α-chymotrypsin is accompanied by significant changes in the excess partial enthalpies of water and α-chymotrypsin. This transition appears at water weight fraction (w_1) of 0.06 when charged groups of α-chymotrypsin are covered. Excess partial quantities reach their fully hydrated values at $w_1 > 0.4$ when coverage of both polar and weakly interacting surface elements is complete.

1. Introduction

The hydration of enzymes is a phenomenon of considerable fundamental importance and practical interest. It is well known that water bound to enzymes (hydration or biological water) plays a key role in determining their stability, dynamics and functions [1–3].

Water can act as a plasticizer of protein conformation [1,4]. Dehydrated proteins are rigid and glassy. In the glassy state, the dehydration-induced conformational changes and restrictions on conformational transitions cause the protein to become frozen into a broad distribution of conformational states.

Proteins undergo a glasslike transition at 25 °C and water content of about 10% (w/w) [1]. The transition from the glassy (rigid) to the flexible (elastic) state is accompanied by significant changes in the thermodynamic and structural properties. As the protein crosses the glass transition region into the flexible state, segmental motions and conformational rearrangements become possible and thermal expansivity is greatly increased.

Thermochemical studies have traditionally been of great importance in ascertaining a better understanding of enzyme–water interactions. Below a short review of the studies of hydration of enzymes is given. Since our paper presents a calorimetric study of the enzyme hydration, we have focused mostly on thermochemical results. More comprehensive reviews have been given in [1,2].

Yang and Rupley [5] studied apparent heat capacity of lysozyme as a function of water content. They identified four regions in the hydration process. Region I (dilute solution to 0.38 g water g^{-1} enzyme) corresponds to the addition of water to the fully hydrated protein. Region II (0.38–0.27 g g^{-1}) represents the condensation of water over weakly interacting surface elements. Region III (0.25–0.07 g g^{-1}) corresponds to the addition of water to main chain carbonyls and other polar surface groups. Region IV (0.07–0 g g^{-1}) corresponds to hydration of charged groups.

Luscher-Mattli and Ruegg [6,7] calculated the enthalpy of water sorption by lysozyme and α-chymotrypsin. The hydration enthalpies were calculated from the temperature dependence of the water vapor pressure in the range 25–40 °C. Bone studied the water sorption by lysozyme in the range 1.5–19% (g g^{-1}) [8]. Calculations were done using the temperature dependence of the water vapor pressure in the range 6–46 °C. From the temperature dependence of the water sorption isotherms in the range 17–57 °C, Hnojewyj and Reyerson calculated differential heats of water sorption [9]. The most important assumption of this method is that the hydration enthalpy does not depend on the temperature. However, in strict manner, this is not correct because the heat capacities of the components of hydration process (water and enzyme) depend significantly on the temperature.

Calorimetry is one of the effective methods for obtaining reliable thermochemical information on the interactions of enzymes with water in various environments. Smith et al. in particular have calorimetrically measured the heats of water adsorption by lysozyme in the range of relative water vapor pressures from 0 to 0.895 [10]. They obtained both the sorption isotherm and the enthalpy of hydration of the protein in the water content range 0–18% (g g^{-1}) at 25 °C. Sorption calorimetry has been used to measure the adsorption isotherm of water by lysozyme and the corresponding heat effects in the entire range of water activities [11]. Our research group has developed an experimental method

* Corresponding author.
E-mail address: vsir@mail.ru (V.A. Sirotkin).

0040-6031/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2011.02.030

for measuring the heat effects of hydration–dehydration of proteins over the whole range of thermodynamic water activities [12–14]. The interaction enthalpy was found to depend significantly on the initial water content and hydration history.

These enthalpies [6–14] (estimated from the temperature dependencies of water sorption and the calorimetrically measured heat effects) contain total information on the binary water–enzyme systems including the corresponding conformational changes in the enzyme structure and glass transition. However, no attempt has been undertaken to estimate simultaneously the enzyme and water contributions to the enthalpy of binary enzyme–water systems in the entire range of water contents.

Similar situation was observed for other thermodynamic functions. For example, thermodynamic properties of BPTI (bovine pancreatic trypsin inhibitor) were studied by molecular dynamics simulation and normal-mode analysis [15]. Partial internal energies and entropies of BPTI and water were only computed for dry and fully hydrated protein. [15]. Apparent heat capacities of lysozyme [5] and BPTI [15] were calculated in the water content range from the dried enzyme to the fully hydrated limit. However, apparent heat capacity of water was only estimated for dry and hydrated proteins.

For the separate estimation of the enzyme and water contributions to the thermodynamic functions of binary water–enzyme mixtures it is convenient to use the excess partial functions. The thermodynamic properties of real binary system can be expressed in terms of the excess functions (Z^E): the difference between the observed thermodynamic function of mixing (Z^m) and the function for an ideal binary mixture (Z^m_{id}), Eq. (1):

$$Z^E = Z^m - Z^m_{id} \tag{1}$$

Deviations of the excess functions from zero indicate the extent to which the studied binary system is nonideal due to strong specific interactions between components (first of all, hydrogen bonding and charge–charge interactions).

The Z^E values are composed of two components (Eq. (2)):

$$Z^E = w_1\bar{Z}^E_1 + w_2\bar{Z}^E_2 \tag{2}$$

where $\bar{Z}_1$ is the excess partial function for component 1 (water); $\bar{Z}^E_2$ is the excess partial function for component 2 (enzyme); w_1 and w_2 are the mass fractions of water and enzyme, respectively.

The aim of this work was to develop a novel experimental method for studying simultaneously the excess partial enthalpies of water and enzymes in the entire range of water content. A major focus of our work of enzyme hydration aims to find the excess partial enthalpies of water and the enzyme and show how these quantities correlate with coverage of the enzyme by the water molecules.

Bovine pancreatic α-chymotrypsin was used as a model enzyme. It is one the most studied and applied in biochemistry, molecular biology and enzymology [16,17]. Physiological role of CT is to hydrolyze peptide bonds [16,17]. α-Chymotrypsin is an example of a predominantly β-sheet protein.

2. Experimental

2.1. Materials

Bovine pancreatic -chymotrypsin (Sigma, No. C 4129, essentially salt free; EC 3.4.21.1; specific activity of 52 U/mg of solid) was used without further purification. Purity of enzyme samples was proved by electrophoresis to be approximately 97%. Water used was doubly distilled.

2.2. Calorimetry

Calorimetric experiments were conducted following the procedures described in detail elsewhere [18–21]. The enthalpy changes on the immersion of the dried protein into pure liquid water were measured at 25 °C with a Setaram BT-2.15 calorimeter according to the described procedure [18,19]. Typically, the sample of 8–10 mg of enzyme preparation contacted with 4.0 ml of water in the calorimetric cell. A typical time of a calorimetric experiment was about 40 min. A typical heat evolution curve recorded upon dissolution of solid α-chymotrypsin in pure liquid water is given in [18]. Calorimeter was calibrated using the Joule effect and tested with dissolving sodium chloride in water according to the recommendations [22].

The dried enzyme preparation (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 enzyme was estimated as $0.003 \pm 0.002\,g\,g^{-1}$ by the Karl Fischer titration method according to the recommendations [22].

At the lowest water activity (a_w) values, enzyme samples for the determination of the interaction enthalpies with water were equilibrated at 25 ± 0.5 °C for 5 days in tightly closed desiccator over saturated salt solutions (the isopiestic method). Water activities over saturated salt solutions were taken from [23,24]. The following salts were used: LiBr ($a_w = 0.064$), KOH ($a_w = 0.078$), LiCl ($a_w = 0.11$), $CaBr_2$ ($a_w = 0.17$), 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 ($a_w = 0.75$), KCl ($a_w = 0.84$), KNO_3 ($a_w = 0.94$). 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. Transfer of enzyme samples from dessicator into calorimetric cell was performed in the closed box with the varied water activity.

At higher a_w values, the enzyme and water samples were mixed in the calorimetric cell at various water weight fractions and 25 °C. The masses of enzyme samples used in the equilibration were in the range 8–10 mg. Water content of the samples after equilibration was measured by drying under vacuum using a microthermoanalyzer "Setaram" MGDTD-17S at 25 °C and 0.1 Pa until the constant sample weight was reached.

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:

(1) Initially, the solid enzyme phase does not contain water.

(2) The transfer of water from the gas phase to the enzyme phase occurs at 25 °C and atmospheric pressure. Then, the enthalpy change corresponding to the mixing of the dried enzyme with water is given by Eq. (3):

$$\Delta H_{Solid-Gas}(\text{dried}) = [\overline{H}_w m_w]_{final\,solid} + [\overline{H}_w m_w]_{final\,gas} + [\overline{H}^h_E m_E]_{final\ \ solid} - [\overline{H}_w m_w]_{initial\,gas} - [\overline{H}^0_E m_E]_{initial\ \ solid} \tag{3}$$

$\overline{H}_E$ and $\overline{H}_w$ are the partial enthalpies of the enzyme and water, respectively; and where m_E, m_W are mass amounts of the enzyme and water, respectively. Phases (gas or solid) and states (final or initial) are specified by subscripts. The amount of water, m^{tr}_W, transferred from the gas phase to the enzyme phase is defined as in Eq. (4):

$$m_w^{tr} = [m_{w,\,final} - m_{w,\,initial}]_{solid} = [m_{w,\ initial} - m_{w,\,final}]_{gas} = m_{w,\,final\ \ solid} \quad (4)$$

The partial enthalpy of water in the gas phase is not changed during the interaction with the enzyme. Therefore, Eq. (3) can be transformed into Eq. (5):

$$\Delta H_{Solid-Gas}(\text{dried}) = [\overline{H}_{E,\,final} - \overline{H}_{E,\ initial}]_{\ solid} * m_E + [\overline{H}_w m_w]_{\ solid} + \overline{H}_{w,\,gas}[m_{w,\,final\ \ gas} - m_{w,\,initial\ \ gas}] = [\overline{H}^h_{E,\,final} - \overline{H}^0_{E,\,initial}]_{\ solid} * m_E + [\overline{H}_{w,\,solid} - \overline{H}_{w,\,gas}]m_w^{tr} \quad (5)$$

The hydration of enzymes can be characterized by two different methods.

Method 1. This is a typical way. The $\Delta H_{Solid-Gas}$(dried) value is related to the amount of water bound to the enzyme, m_w^h (Eq. (6)):

$$\Delta H_{hydr}^{H_2O/gas} = [\overline{H_E^h} - \overline{H_E^0}]_{solid}\frac{m_E}{m_w^h} + [\overline{H}_{w,\ \ solid} - \overline{H}_{w,\ \ gas}] \quad (6)$$

The $\Delta H_{hydr}^{H_2O/gas}$ value is called sometimes the enthalpy of enzyme hydration. As can be concluded from Eq. (6), *Method 1* does not allow studying separately the enzyme and water enthalpic contributions to the calorimetrically measured heat effects. According to Eq. 6, the $\Delta H_{hydr}^{H_2O/gas}$ value contains two components. The first component $[\overline{H_E^h} - \overline{H_E^0}]_{solid}(m_E/m_w^h)$ is due to changes in the enzyme state during hydration (including conformational rearrangements and glass transition). The second component $[\overline{H}_{w,\,solid} - \overline{H}_{w,\,gas}]$ is due to the difference between the water state in the gas phase and the bound state.

Method 2. The $\Delta H_{Solid-Gas}$(dried) value observed during the addition of water to enzyme can be considered as an excess quantity of the mixing of water with enzyme (Z^E, Eqs. (1) and (2)). From this quantity the excess partial enthalpies can be calculated using Eqs. (7) and (8):

$$\bar{Z}_1^E = Z^E - w_2\left(\frac{\partial Z^E}{\partial w_2}\right)_{T,P} = [\overline{H}_{w,\ \ solid} - \overline{H}_{w,.gas}] = \overline{H}_1^E \quad (7)$$

$$\bar{Z}_2^E = Z^E - w_1\left(\frac{\partial Z^E}{\partial w_1}\right)_{T,P} = [\overline{H_E^h} - \overline{H_E^0}]_{solid} = \overline{H}_2^E \quad (8)$$

where $\overline{H}_1^E$ and $\overline{H}_2^E$ are the excess partial enthalpies of water and enzyme, respectively; w_1 and w_2 are the mass fractions of water and enzyme, respectively. As can be concluded from Eqs. (7) and (8), *Method 2* allows studying separately the enzyme and water contributions to the calorimetrically measured heat effects.

Eq. (5) can be transformed into an expression useful for experimental applications. When the transfer of water from the liquid phase to the enzyme phase occurs, Eq. (5) can be transformed into Eq. (9):

$$f\phi H_{Solid-Liquid}(\text{dried}) = [\overline{H^h_{E,.final}} - \overline{H}^o_{E,.initial}]_{\ solid} m_E + [\overline{H}_{w,\,solid} - \overline{H}_{w,\,liquid}]m_w^{tr} \quad (9)$$

The enthalpy change corresponding to introducing of some amount of enzyme in pure liquid water (the solution enthalpy, $\Delta_{sol}H$) can be defined by Eqs. (10) and (11). Let's consider two variants. In both cases, the final state of the enzyme is a diluted solution in water at 25 °C and atmospheric pressure.

Variant 1. Initially, the solid enzyme does not contain water

$$\Delta_{sol}H(\text{dried}) = [\overline{H}^b_E m_E]_{final\ \ liquid} + [\overline{H}^b_w m^b_w]_{final\ \ \ enzyme} - \ [\overline{H}_w m^b_w]_{initial\ \ \ liquid} - [\overline{H}^o_E m_E]_{initial\ enzyme} \quad (10)$$

where $\overline{H}^b_w$ is the partial enthalpy of water bound to the enzyme in the solution; $\overline{H}^b_E$ is the partial enthalpy of the enzyme in the solution. m^b_w is the amount of water bound to the enzyme in the solution.

Variant 2. The initial enzyme phase may contain water

$$\Delta_{sol}H(\text{hydrated}) = [\overline{H}_w m^h_w]_{final\ \ \ liquid} + [\overline{H}^b_E m_E]_{final\ \ \ liquid} + [\overline{H^b_w} m^b_w]_{final\ \ \ enzyme} - \ \ [\overline{H}_w m^b_w]_{initial\ \ \ liquid} - [\overline{H^h_E} m_E + \overline{H}_w m^h_w]_{initial\ enzyme} \quad (11)$$

where $\overline{H}_E$ and $\overline{H}_w$ are the partial enthalpies of the enzyme and water; m^h_w is the amount of water bound to initial solid enzyme. m^b_w is the amount of water bound to the enzyme in the solution.

The amount of water transferred from the liquid phase to the enzyme phase is defined by Eq. (12), when the initial enzyme does not contain water:

$$m_w^{tr} = m_w^b \quad (12)$$

The amount of water transferred from the liquid phase to the enzyme phase is defined by Eq. (13), when the initial enzyme contains water:

$$m_w^{tr} = [m^b_{w,.final.enzyme} - m^h_{w,.initial.enzyme}] \quad (13)$$

The difference between the solution enthalpies for the dried and hydrated enzyme is defined by Eq. (14). It is expected that the partial enthalpy of water in liquid phase does not change significantly during the formation of the diluted enzyme solution.

$$\frac{\Delta_{sol}H(\text{dried}) - \Delta_{sol}H(\text{hydrated})}{m^h_w} = \overline{[H^h_E - H^0_E]}_{solid}\frac{m_E}{m^h_w} + [\overline{H}_{w,.solid} - \overline{H}_{w,.liquid}] = \frac{\Delta H_{Solid-Gas}(\text{dried})}{m^h_w} + [\overline{H}_{w,.gas} - \overline{H}_{w,.liquid}] = \frac{\Delta H_{Solid-Gas}(\text{dried})}{m^h_w} + \Delta H^{H_2O}_{vap} \quad (14)$$

where $\Delta H^{H_2O}_{vap}$ is the enthalpy of vaporization of water (43.7 kJ mol^{-1}) [23].

As can be concluded from Eq. (14), the Z^E value (Eqs. (1), (2), (7), and (8)) can be calculated from the enthalpies of solution of the dried, $\Delta_{sol}H$(dried), and hydrated, $\Delta_{sol}H$(hydrated), enzyme in water measured by isothermal calorimetry.

Eq. (15) is similar to Eq. (2). It allows calculating the excess partial quantities from the calorimetrically measured heat effects.

$$\frac{\Delta_{sol}H(\text{dried}) - \Delta_{sol}H(\text{hydrated})}{m^h_w + m_E} = \overline{[H^h_E - H^0_E]}_{solid}\frac{m_E}{m_E + m^h_w} + [\overline{H}_{w,.solid} - \overline{H}_{w,.liquid}]\frac{m^h_w}{m_E + m^h_w} = \overline{[H^h_E - H^0_E]}_{solid} w_2 + [\overline{H}_{w,.solid} - \overline{H}_{w,.liquid}]w_1 \quad (15)$$

3.2. Excess partial enthalpies of water and α-chymotrypsin

Fig. 1 shows how the $(\Delta_{sol}H(\text{hydrated}) - \Delta_{sol}H(\text{dried}))/(m^h_w + m_E)$ values (excess function of mixing per unit mass of the mixture) depend on the hydration level of α-chymotrypsin. The initial state (zero hydration level) for studying the enthalpy of solution of

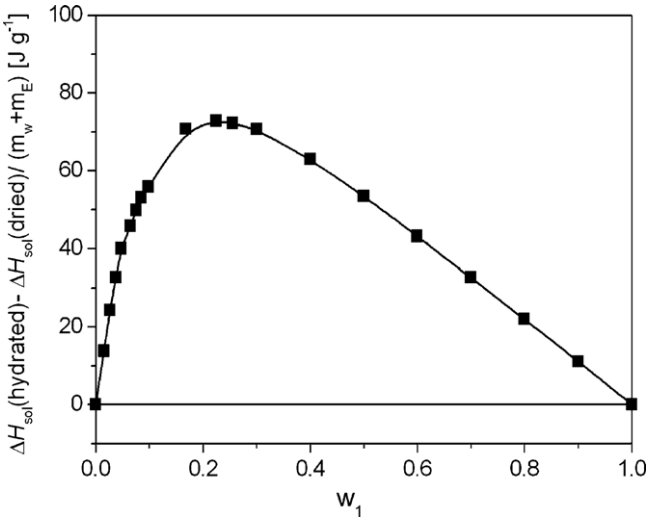

Fig. 1. The $(\Delta_{sol}H(\text{hydrated}) - \Delta_{sol}H(\text{dried}))/(m_w^h + m_E)$ value as a function of the weight fraction of water, w_1, at 25 °C.

the dried α-chymotrypsin in pure liquid water, $\Delta_{sol}H$(dried), was obtained by drying in air at water activity less than 0.01. On maintaining this "dry" sample in equilibrium with a vacuum of 0.1 Pa and at 25 °C for three hours, it lost about 0.3% of its weight, which for α-chymotrypsin implies that at the zero hydration level there are about four water molecules strongly bound to each enzyme molecule. The $\Delta_{sol}H$(dried) value is −2790 (50) kJ mol^{-1} or −111.6 (2.0) J g^{-1} α-chymotrypsin at 25 °C.

Figs. 2 and 3 present the excess partial enthalpy of water, $\overline{H}_1^E$, and the excess partial enthalpy of α-chymotrypsin, $\overline{H}_2^E$, as functions of the weight fraction of water. These thermochemical quantities were calculated using Eqs. (7), (8), and (15). The enthalpy curves presented in Figs. 2 and 3 can be divided into three parts.

Part 1. w_1 = 0–0.06 ($h \sim 0$ to 0.07 g g^{-1}). The main features of part 1 can be described as follows. At the lowest water contents, the enzyme is in a glassy (rigid) state [1,11]. In the low water content range ($w_1 < 0.06$), the strong hydration sites are preferentially occupied [2,5]. These water binding sites are chemically diverse and include a variety of charged enzyme groups. Significant changes in the amide I region of proteins were observed using Fourier transform infrared spectroscopy [25–28].

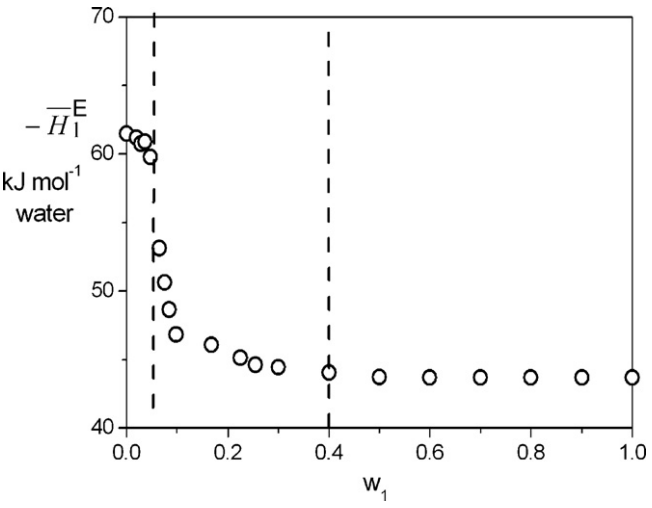

Fig. 2. Excess partial enthalpy of water, $\overline{H}_1^E$, as a function of the weight fraction of water, w_1, at 25 °C. Excess partial enthalpy of water was corrected for the enthalpy of condensation of water at 25 °C.

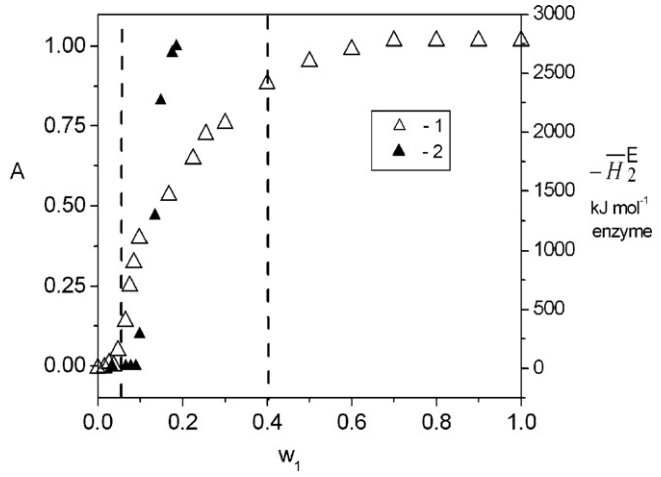

Fig. 3. (1) Excess partial enthalpy of α-chymotrypsin, $\overline{H}_2^E$, as a function of the weight fraction of water, w_1, at 25 °C. (2) Solid-state solvent-free hydrolysis of N-succinyl-L-phenylalanine-p-nitroanilide. The ordinate, $A = D_{416}/D_{357}$, is a measure of the nitroaniline product. Modified data from [29]. Water contents of α-chymotrypsin were taken from [25].

Khurgin et al. [29] measured the chymotrypsin-catalyzed hydrolysis of the amide substrate N-succinyl-L-phenylalanine-p-nitroanilide at low and medium hydration levels. No enzymatic activity was observed at $w_1 < 0.06$ (Fig. 3).

Hutchens et al. [30] studied the heat capacities of chymotrypsinogen A at w_1 of 0 and 0.096, from −263 to 37 °C. No phase transition corresponding to the ice–liquid water transition was observed at low hydrations.

The excess partial enthalpy of water, $\overline{H}_1^E$, is highly exothermic (negative). The $\overline{H}_1^E$ value does not depend noticeably on the water content and equals approx. −61.5 kJ mol^{-1} water (Fig. 2). The excess partial enthalpy of α-chymotrypsin, $\overline{H}_2^E$, is close to zero (Fig. 3). The $\overline{H}_2^E$ value does not depend noticeably on the water content. The fact that α-chymotrypsin is in the glassy state explains this feature of part 1. The $\overline{H}_2^E$ value is rather constant due to the fact that all the enzyme molecules came into contact mainly with the same enzyme molecules during this range of water contents.

Part 2. w_1 = 0.06–0.4. The main features of part 2 are the following. Part 2 corresponds to the addition of water to main chain carbonyls and other polar surface groups [2,5]. During isothermal sorption of water a glasslike transition results in a step on the excess partial enthalpy of water (enzyme) curve (Figs. 2 and 3). $\overline{H}_1^E$ decreases sharply from highly negative to moderate values. $\overline{H}_2^E$ increases from very low values to highly exothermic values. At $w_1 > 0.06$, the catalytic activity of α-chymotrypsin is sharply increased (Fig. 3) [29].

Apparent heat capacity of the enzyme lysozyme, ϕC_{p2}, determined from isothermal experiments using a drop calorimeter increases from very low values to high values in this water content interval [5]. This strong increase of the heat capacity was interpreted in terms of a transition of the enzyme molecules from a glassy (rigid) state to a flexible (elastic) state [11].

The results obtained for proteins and biopolymers by several experimental methods were summarized in Ref. [1]. It was concluded that proteins undergo a glasslike transition at water content of about 10 wt% at 25 °C. This water content is within part 2 in this work.

Part 3. $w_1 > 0.4$. The main features of part 3 can be described as follows. At the highest water contents ($w_1 > 0.4$), the enzyme is in a flexible (elastic) state [1,11]. The structural rearrangements are largely completed [1,25]. Excess partial quantities reach their

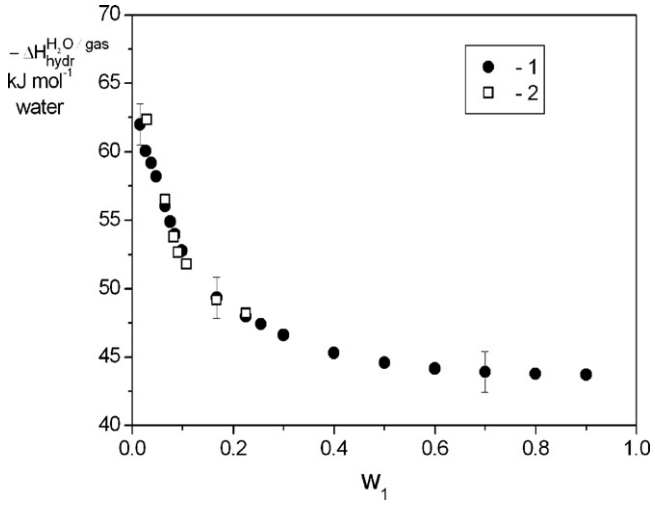

Fig. 4. (1) Enthalpy of hydration of α-chymotrypsin, $\Delta H_{hydr}^{H_2O/gas}$, as a function of the weight fraction of water, w_1, at 25 °C. (2) Enthalpy of hydration of human serum albumin, $\Delta H_{hydr}^{H_2O/gas}$, as a function of the weight fraction of water, w_1, at 25 °C. Adapted data from [31].

fully hydrated values at water weight fraction more than 0.4 when coverage of both polar and adjacent weakly interacting surface elements no longer changes appreciably upon further hydration. The $\overline{H}_1^E$ value is close to the enthalpy of condensation of pure water at 25 °C and atmospheric pressure (−43.7 kJ mol^{-1}). This implies that the average state of water in the hydrated α-chymotrypsin is close to that in the pure liquid water. Apparent heat capacity of lysozyme, ϕC_{p2}, [5] and excess partial enthalpy of α-chymotrypsin, $\overline{H}_2^E$, (Fig. 3) reach their maximal values. This means that all the enzyme molecules are in the flexible state.

3.3. Enthalpy of hydration of α-chymotrypsin

Fig. 4 shows how the $\Delta H_{hydr}^{H_2O/gas}$ values depend on the weight fraction of water. The enthalpy of hydration of α-chymotrypsin, $\Delta H_{hydr}^{H_2O/gas}$ was calculated using Eqs. (6) and (14). As can be concluded from Fig. 4, the $\Delta H_{hydr}^{H_2O/gas}$ function is a smooth curve. The most significant $\Delta H_{hydr}^{H_2O/gas}$ values were observed at the lowest water weight fractions ($\Delta H_{hydr}^{H_2O/gas} \sim -$ 62.0 (1.5) kJ mol^{-1} water). These values are close to the $\overline{H}_1^E$ values (Fig. 2) at the lowest water contents. This result is due to the fact that at the lowest water contents the $[\overline{H_E^h} - \overline{H_E^0}]_{solid}$ component in Eq. (6) is close to zero because the $\overline{H_{E\,solid}^h}$ and $\overline{H_{E\,solid}^0}$ values do not differ significantly. This agreement between the $\Delta H_{hydr}^{H_2O/gas}$ and $\overline{H}_1^E$ values supports the reliability of our calculations.

At $w_1 > 0.4$, the $\Delta H_{hydr}^{H_2O/gas}$ values reach saturation and are close to the enthalpy of condensation of water (−43.7 kJ mol^{-1} water). This saturation range is consistent with that observed for the excess partial quantities (Figs. 2 and 3).

To show the generality of our findings, the hydration enthalpies for α-chymotrypsin were compared with the $\Delta H_{hydr}^{H_2O/gas}$ values for other globular protein, human serum albumin [31]. As can be concluded from Fig. 4, a good agreement was observed between the $\Delta H_{hydr}^{H_2O/gas}$ values for α-chymotrypsin and human serum albumin. Similar hydration enthalpies were observed for lysozyme [10]. These results are indicative of reliability of our experiments.

One should stress that the $\Delta H_{hydr}^{H_2O/gas}$-water content curves are well reproducible in different experiments and at different conditions. However, the changes on the $\Delta H_{hydr}^{H_2O/gas}$-water content curves describing a transition from the glass (rigid) to the flexible (elastic) state are not pronounced.

One should explain why the thermochemical functions presented in Figs. 1–4 have different profiles. The partial enthalpies, $\overline{H}_j^E$, which contain the second derivatives of G, can be defined as follows:

$$\overline{H}_j^E = \left(\frac{\partial H^E}{\partial h_j}\right) = \left(\frac{\partial G}{\partial h_j}\right) - T\left(\frac{\partial^2 G^E}{\partial T\,\partial h_j}\right) \quad (16)$$

where n_j is the molar amount of jth component.

This second derivative quantities signify the actual thermodynamic situation of the target jth component. This contrasts with what is contained in the excess functions (for example, H^E), the first derivative quantities, which provide the respective global averages. In the Gibbs (P, T, n_j) variable system, there are three functions, C_p (heat capacity), α_P (thermal expansion coefficient) and k_T (isothermal compressibility), that are also second derivatives of G.

As can be concluded from our work, the published apparent heat capacities of proteins [2,5] and the partial quantities obtained in our work (Figs. 2 and 3) show similar profiles and a glasslike transition in the w_1 range from 0.06 to 0.4. Fig. 1 shows an excess function of mixing (Z^E). It is the first derivative of G. Therefore, there is no transition on this curve. Hydration enthalpy presented in Fig. 4 is a complicated combination of the enzyme and water partial quantities. Therefore, a smooth curve was observed in this case. These facts are indicative of the reliability of our calculations.

4. Conclusions

Isothermal batch calorimetry was applied to study the hydration of α-chymotrypsin. The hydration process was characterized by two methods. The first method is traditionally used. It includes the calculation of the hydration enthalpy of the enzyme: the calorimetrically measured heat effects of the enzyme–water interaction are related to the transfer of water from the gas phase to the enzyme phase. This method does not allow studying separately the enzyme and water contributions to the heat effects of the enzyme–water interaction.

A novel method based on the analysis of the excess partial thermodynamic quantities was proposed in this work. This second method allows studying separately the enzyme and water excess partial enthalpies in the entire range of water content. It was shown that the excess partial enthalpies are very sensitive to the changes in the state of water and α-chymotrypsin. Two well-pronounced stages were observed on the excess partial enthalpy–water content curves. A transition from the glassy to the flexible state of α-chymotrypsin is accompanied by significant changes in the excess partial enthalpies of water and α-chymotrypsin. This transition appears in the calculated quantities when charged groups of α-chymotrypsin are covered, which occurs at water weight fraction of 0.06 and 25 °C. Excess partial quantities reach their fully hydrated values at water weight fraction more than 0.4 when coverage of both polar and adjacent weakly interacting surface elements no longer changes appreciably upon further hydration.

Acknowledgement

V.S. thanks the German Academic Exchange Service (Referat 325, Kennziffer A/09/03651) for financial support.

References

[1] R.B. Gregory, Protein hydration and glass transition behavior, in: R.B. Gregory (Ed.), Protein–Solvent Interactions, Marcel Dekker, New York, 1995, pp. 191–264.
[2] J.A. Rupley, G. Careri, Adv. Protein Chem. 41 (1991) 37–172.

[3] A. Oleinikova, N. Smolin, I. Brovchenko, A. Geiger, R. Winter, J. Phys. Chem. B 109 (2005) 1988–1998.
[4] S. Bone, R. Pethig, J. Mol. Biol. 181 (1985) 323–326.
[5] P.-H. Yang, J.A. Rupley, Biochemistry 18 (1979) 2654–2661.
[6] M. Luscher-Mattli, M. Ruegg, Biopolymers 21 (1982) 403–418.
[7] M. Luscher-Mattli, M. Ruegg, Biopolymers 21 (1982) 419–429.
[8] S. Bone, Phys. Med. Biol. 41 (1996) 1265–1275.
[9] W.S. Hnojewyj, L.H. Reyerson, J. Phys. Chem. 65 (1961) 1694–1698.
[10] A.L. Smith, H.M. Shirazi, S.R. Mulligan, Biochim. Biophys. Acta 1594 (2002) 150–159.
[11] V. Kocherbitov, T. Arnebrant, O. Söderman, J. Phys. Chem. B 108 (2004) 19036–19042.
[12] V.A. Sirotkin, D.V. Korolev, A.E. Silakova, Russ. J. Phys. Chem. A 81 (2007) 1341–1345.
[13] V.A. Sirotkin, D.V. Korolev, Thermochim. Acta 432 (2005) 246–253.
[14] V.A. Sirotkin, D.A. Faizullin, Thermochim. Acta 415 (2004) 127–133.
[15] X. Yu, J. Park, D.M. Leitner, J. Phys. Chem. B 107 (2003) 12820–12829.
[16] A. Fersht, Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, Freeman & Co, New York, 1999.
[17] A.L. Lehninger, D.L. Nelson, M.M. Cox, Principles of Biochemistry, Worth, New York, 1993.
[18] V.A. Sirotkin, A.N. Zinatullin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, Biochim. Biophys. Acta 1547 (2001) 359–369.
[19] V.A. Sirotkin, A.N. Zinatullin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, Thermochim. Acta 151 (2002) 151–160.
[20] M.D. Borisover, V.A. Sirotkin, B.N. Solomonov, Thermochim. Acta 254 (1995) 47–53.
[21] V.A. Sirotkin, M.D. Borisover, B.N. Solomonov, Thermochim. Acta 256 (1995) 175–183.
[22] M.D. Borisover, V.A. Sirotkin, D.V. Zakharychev, B.N. Solomonov, Calorimetric methods in evaluating hydration and solvation of solid proteins immersed in organic solvents, in: E.N. Vulfson, P.J. Halling (Eds.), Enzyme in Nonaqueous Solvents, Humana Press, Totowa, 2001, pp. 183–202.
[23] B.P. Nikol'skii, Chemist's Handbook, vol. 1, Goskhimizdat, Leningrad, 1963.
[24] L. Greenspan, J. Res. Natl. Bur. Stand. 81A (1977) 89–96.
[25] V.A. Sirotkin, Biochim. Biophys. Acta 1750 (2005) 17–29.
[26] V.A. Sirotkin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, V.D. Russ, J. Phys. Chem. A 76 (2002) 2051–2057.
[27] S.J. Prestrelsky, N. Tedeschi, T. Arakawa, J.F. Carpenter, Biophys. J. 65 (1993) 661–671.
[28] M. van de Weert, P.I. Harvez, W.E. Hennink, D.J.A. Crommelin, Anal. Biochem. 297 (2001) 160–169.
[29] Y.I. Khurgin, N.V. Medvedeva, V.Y. Roslyakov, Biofizika 22 (1977) 1010–1014.
[30] J.O. Hutchens, A.G. Cole, J.W. Stout, J. Biol. Chem. 244 (1969) 26–32.
[31] V.A. Sirotkin, A.V. Sukhov, E.V. Dudkina, Eng. Life Sci. 9 (2009) 74–81.