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

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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

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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):