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Effect of Hydration on the Thermal Denaturation of Lysozyme as Measured by Differential Scanning Calorimetry

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Synopsis. The thermal properties of the lysozyme–water system were investigated by differential scanning calorimetry under varied water contents, ranging from 0.05 to 1.6 g per g of protein. The temperature, T_d, and the enthalpy change, ΔH_d, of denaturation at water contents below 0.75 g/g showed strong dependencies on the hydration. At higher water contents, however, T_d and ΔH_d were almost independent of the degree of hydration, showing values almost identical to those in solution.

Recent studies have revealed that water plays a prominent role in the structure and biological function of proteins, but there are many problems remaining unsolved. The hydration process of proteins have been extensively studied by various techniques, such as gravimetric measurements (sorption isotherms),[1,2] IR[3] and NMR spectroscopy.[4] Calorimetric measurements of the hydrated proteins can also provide instructive information on the interaction between protein and water.

This paper will describe the effect of hydration on the thermal denaturation of lysozyme as measured by differential scanning calorimetry.

Experimental

Materials. The hen egg-white lysozyme used in the present study was a three-times-recrystallized sample(lot 74C—8040) from Sigma Chemicals Co. All the chemicals employed for the control of the relative humidities were of a reagent grade and were used without further purification.

Methods. The thermal denaturation of lysozyme was measured with a Rigaku Denki standard-type differential scanning calorimeter. The samples(5—10 mg) were packed in sealable pans and kept in humidostates at appropriate relative humidities in order to adjust the water contents by conditioning for 5 days, then the pans were sealed. The relative humidity was varied by placing saturated aqueous solutions in contact with an excess of various solutes at 293 K.[5] The samples with water contents higher than 1 g/g were prepared by adding twice-distilled water.

For calorimetric measurements, the heating rate was 2.5 K/min. This heating rate was found to be preferable in terms of the sharpness of the resulting thermograms and their reproducibility.

The water content and the dry mass of the sample were gravimetrically determined by drying the punctured sample pan at 378 K *in vacuo* for 24 h. The molecular weight of lysozyme was taken as 14300.[6]

Results and Discussion

Figure 1 shows typical thermograms measured at three different water contents. The thermal denaturation was accompanied by an endothermal heat effect. The temperature, T_d, and the enthalpy change, ΔH_d, of the denaturation were estimated

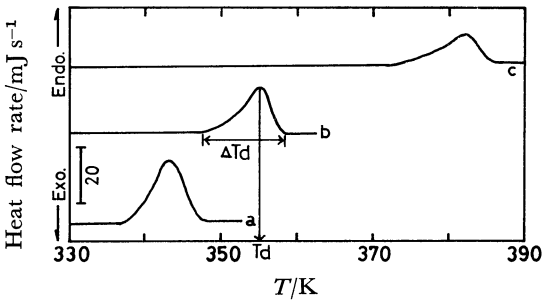

Fig. 1. DSC thermograms of lysozyme.
Water contents and sample weights are (a) 1.13, 6.2; (b) 0.27, 4.8; (c) 0.10 g/g, 4.2 mg.

from the temperature of the peak and the peak-area respectively in the thermogram obtained. The temperature width of the denaturation peak, ΔT_d, was also estimated as the temperature difference between the onset and the outset of the endothermal peak in the thermogram. Unfortunately, these values could not estimated at water contents lower than about 0.05 g/g, because these thermal decomposition occurred instead of thermal denaturation.

The T_d, ΔT_d and ΔH_d are plotted as a function of the water content in Figs. 2 and 3 respectively. These values similarly showed marked dependencies on the water content. At water contents above 0.75 g/g, both T_d and ΔH_d were slightly dependent on the water content. The mean values in this region were 341.7 K and 313 kJ/mol, almost identical to those measured for lysozyme in solution.[7,8] It seems reasonable to assume that a similar conformational change which takes place in solution occurs in a solid state containing much water; the interaction between protein

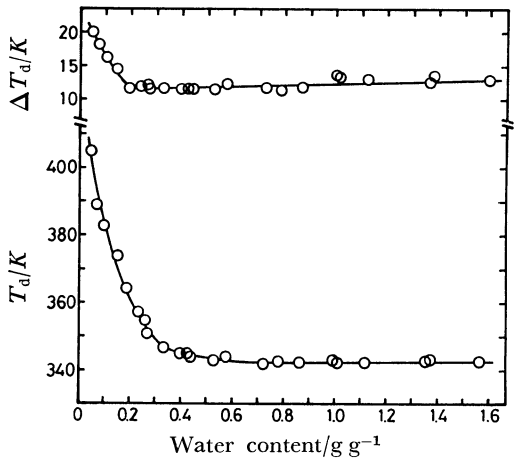

Fig. 2. Temperature of denaturation, T_d, and temperature width of denaturation peak, ΔT_d, of lysozyme as a function of water content.

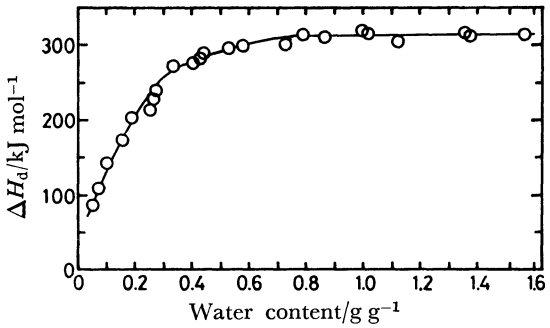

Fig. 3. Enthalpy change of denaturation, ΔH_d, of lysozyme as a function of water content.

molecules does not influence the unfolding of the molecule. In addition, this indicates that the hydration of lysozyme, an essential process for stabilizing its spatial structure in water, is completed at about 0.75 g of water per g of protein, equivalent to about 600 moles of water per mol of protein.

Below the water content of 0.75 g/g, the T_d increased gradually with a decrease in the water content, until the increase became much more marked at water contents lower than about 0.3 g/g. The ΔH_d, on the other hand, decreased with a decrease in the water content in the same region of water content. The ΔT_d showed an almost constant value at water contents higher than about 0.15 g/g, but increased markedly at water contents lower than this value.

To analyze the experimental data we assumed that the observed enthalpy change of the thermal denaturation of lysozyme, ΔH_d, was expressed as follows:

$$\Delta H_d = \Delta H_{comp} + (600-h)\Delta H_{hyd}$$

where ΔH_{comp} is the enthalpy change of the denaturation of the completely hydrated protein, h is the degree of hydration, as expressed by the number of moles of water adsorbed per mole of protein, and ΔH_{hyd} is the contribution made by the removal of the water from the hydrated protein, as expressed on the basis of one mole of water per mol of protein. As is shown in Fig. 3, the hydration of lysozyme was completed at about 600 moles of water per mol of protein, and ΔH_d showed an almost constant value at water contents higher than 600 mol/mol, therefore, ΔH_{comp} was regarded as identical to ΔH_d, 313 kJ/mol, in this region. The plot of ΔH_{hyd} against the degree of hydration is shown in Fig. 4. The ΔH_{hyd} decreased with an increase in the h, this relationship could be represented by two segments of the straight line. This indicates that the effect of hydration on the stabilization of protein decreases with an increase in the degree of hydration; the interaction between protein and water decreases with an increase in the degree of hydration, and the dependence of protein–water interaction on the degree of hydration changes at about 240 mol/mol, equivalent to a water content of 0.3 g/g.

It is interesting to note that a break in the dependence of the protein–water interaction on the water content is seen near 0.3 g of water per g of protein. Harvey and Hoekstra[9] have reported that the dielectric properties of the adsorbed water on lysozyme changed at the same water content. A recent calorimetric

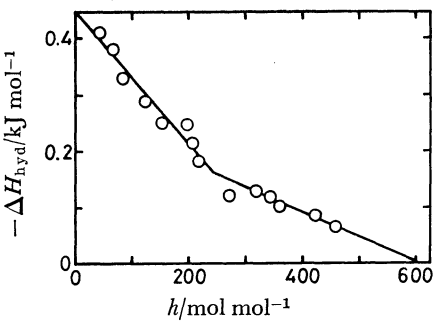

Fig. 4. The plot of the contribution by removal of the water from the hydrated protein, ΔH_{hyd}, to the enthalpy change of denaturation *vs.* the degree of hydration, h.

study of frozen protein solutions[10] has revealed that the threshold water contents for the unfreezable and freezable water fractions with heats of fusion different from that of the bulk water are about 0.3 and 0.7 g/g respectively. The water content of 0.3 g/g corresponds to about 3.5 molecules of water per ploar amino acid residue in the lysozyme molecule. Below the water content of 0.3 g/g, the water molecules are probably bound by hydrogen bonds to the polar amino acid residues on the surface of protein. It seems likely that the exhaustive removal of these associated water molecules brings about changes in the structure of protein[11] and in the mechanism of denaturation, as suggested by the curve for ΔT_d in Fig. 2.

At water contents above 0.3 g/g, the protein–water interaction was weaker than that at lower water contents. In the range of water content between 0.3 and 0.75 g/g, some water molecules may ambivalently interact not only with the water strongly bound to hydrogen-bonding sites of the protein, but also with the normal bulk water, which probably paly an essential role in stabilizing the native conformation of the protein.

It will be interesting to study the contribution of hydration to the stabilization of protein in further detail.

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