The Effect of Hydration on the Thermal Stability of Ovalbumin as Measured by Means of Differential Scanning Calorimetry

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Synopsis. The thermal denaturation of ovalbumin (egg albumin) was investigated by means of differential scanning calorimetry in the water-content range from 0.11 to 1.15 g of water per g of protein. At water contents above 0.76 g/g, the temperature, T_d, and the enthalpy, ΔH_d, of denaturation were scarcely dependent at all on the water content. At lower water contents, however, both T_d and ΔH_d showed a marked dependence on the water content. The degree of the hydration dependence of the T_d and ΔH_d suggested that the amount of water required to hydrate ovalbumin was 0.76 g/g and that at least two types of the hydration phase contributed to the thermal stability of the protein.

It is well known that water is an essential element in many biological processes. In spite of the importance of hydration in the maintaining of protein structure some important questions on protein hydration remain unresolved. As reviewed by Kuntz and Kauzmann,[1] approximately 0.3—0.5 g of water per g of protein is bound to globular protein; its properties are evidently different from those of pure water, *e.g.*, a lowered vapor pressure, a reduced mobility, and a much-reduced freezing point.

In previous papers,[2-4] the effect of hydration on the thermal stability of lysozyme and chymotrypsinogen A was investigated by means of differential scanning calorimetry (DSC). The essential hydration values for stabilizing the spatial structure of lysozyme and chymotrypsinogen A were considerably larger than those estimated by other techniques. It was also suggested that at least two types of the hydration phase contributed to the thermal stability of the proteins.

The present work was undertaken in order to investigate the effect of hydration on the thermal stability of ovalbumin and at the same time to confirm the above suggestions.

Experimental

Materials. The chicken ovalbumin (egg albumin) used in the present study was a salt-free, five-times-crystallized sample from Miles Laboratories, Inc. The molecular weight of ovalbumin was taken as 45000.[5]

Methods. The water content of the sample was adjusted by conditioning in a constant-humidity apparatus at the appropriate relative humidity for 7 d. Higher water contents were adjusted by placing the sample in saturated vapor at 293 K for an appropriate period. The water content was determined gravimetrically by drying at 378±5 K *in vacuo* for 24 h.

The calorimetric measurements were performed with a Rigaku Denki differential scanning calorimeter and a hermetic aluminum pan at a heating rate of 2.5 K/min.

Results and Discussion

The thermal denaturation of ovalbumin was measured by means of DSC in the water-content range from 0.11 to 1.15 g of water per g of protein. The DSC curves showed an endothermic peak, having a temperature width 10—15 K. The temperature, T_d, and the enthalpy, ΔH_d, of denaturation were estimated from the peak temperature and the peak area of the DSC curve here obtained respectively. The T_d and ΔH_d are plotted as functions of the water content in Fig. 1.

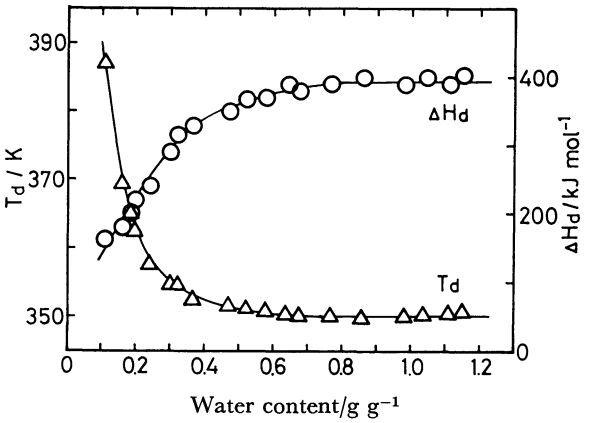

Fig. 1. The temperature, T_d, and the enthalpy, ΔH_d, of denaturation of ovalbumin as functions of the water content.

At water contents above 0.76 g/g, both T_d and ΔH_d were scarcely dependent at all on the water content. The mean values in this region were 350.5 K and 393 kJ/mol, values which were in rough agreement with those for ovalbumin in aqueous solutions.[6] Below the water content of 0.76 g/g, however, the T_d increased with a decrease in the water content. The increase became much more marked at water contents lower than about 0.35 g/g. On the other hand, the ΔH_d decreased gradually with a decrease in the water content in the same region, until the decrease became more pronounced at water contents below 0.34 g/g.

It is interesting that the T_d and ΔH_d values at water contents higher than 0.76 g/g hardly differ from those for the protein in an aqueous solution. From heat-capacity measurements on ovalbumin in the solid state with different amounts of water and in solution, Suurkuusk[7] has suggested that, between 0.1 g/g and a dilute solution, there can be no substantial change in the protein structure. It appears reasonable to assume that a conformational change similar to that which takes place in solution occurs in the solid state. In addition, it is suggested that the hydration for ovalbumin, an essential process for stabilizing its native structure, is completed at about 0.76 g/g. The hydration value is considerably higher than those determined by other techniques.[1]

According to Kauzmann,[8] the most important

contributor to the increase in heat capacity on protein denaturation is the interaction of nonpolar groups with water. At water contents below 0.76 g/g, the large decrease observed in ΔH_d may be substantially attributed to the reduction of the interactions between the exposed nonpolar groups with water, *i.e.*, the reduction of the hydrophobic hydration in the denatured state. In addition, it may be expected that, at a very low water content, the protein molecule does not completely unfold, because water is a competitor of the protein polar groups in creating "hydrogen bond." This causes a decrease in the enthalpy and entropy of protein denaturation.

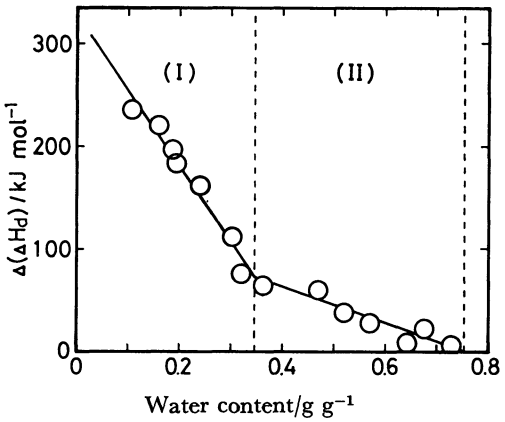

Fig. 2. The reduction in the denaturation enthalpy of ovalbumin by dehydration, $\Delta(\Delta H_d)$, as a function of the water content. The denaturation enthalpy at the full hydration is regarded as 393 kJ/mol.

Assuming that the denaturation enthalpy of ovalbumin at the full hydration is 393 kJ/mol, which is the mean at the water contents above 0.76 g/g, the reduction in the enthalpy of denaturation by dehydration, $\Delta(\Delta H_d)$, is expressed as follows:

$$\Delta(\Delta H_d) = 393 - \Delta H_d$$

where ΔH_d is the observed enthalpy of denaturation at the partial hydration. The $\Delta(\Delta H_d)$ is plotted as a function of the water content in Fig. 2. The relationship is represented by two segments of a straight line, with the break occurring at 0.34 g/g. This suggests that at least two types of phase exist in the hydration of the protein. The increase in T_d due to dehydration became much more marked at water contents below about 0.35 g/g. In an operational way, the hydration phase can be classified into two phases as follows: (I) the primary hydration phase, *i.e.*, the water contents below 0.34 g/g, and (II) the secondary hydration phase, *i.e.*, the water-content range of 0.34—0.76 g/g.

The primary hydration phase is completed at 0.34 g/g. This value is comparable to the amount of unfreezable water determined by NMR[9] and calorimetric studies,[10] which is barely sufficient to constitute a monolayer and which corresponds to 850 mol of water per mol of protein. These water molecules may be selectively arranged in the vicinity of the polar regions of a protein surface by hydrogen bonds and thus form part of a first hydration monolayer.

The secondary hydration phase is completed at 0.76 g/g. The real nature of the secondary hydration water is not yet clear, though it may be expected to play an important role in determining the structure and biological function of protein. In a recent calorimetric study of a frozen α-chymotrypsin solution, Luscher *et al.*[11] have suggested that the heat, entropy, and temperature of fusion of the secondary hydration water are lower in value than those of bulk water. This can be explained by hydrogen-bonding defects in the ice lattice. As has been pointed out by Kuntz and Kauzmann,[1] it is considered that the secondary hydration water has rotational properties only slightly different from those of bulk water and can not be readily differentiated by NMR and dielectric dispersion techniques, as there is little experimental evidence for the secondary hydration phase using either of the dispersion techniques.

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