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MULTI-STATE TRANSITION FOR THE THERMAL UNFOLDING OF CERTAIN GLOBULAR PROTEINS AS EVALUATED FROM THE ANALYSIS OF DSC CURVE

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ABSTRACT

Differential scanning calorimetry(DSC) has been performed on the thermally induced transitions of soybean trypsin inhibitor(STI) and of Taka-amylase A(TAA) in buffers by using the adiabatic differential heat capacity calorimeter. Thermodynamic parameters have been derived from the excess heat capacity curves and found to be ΔH = 19.8 ± 0.2 J/g and ΔC_p = 0.51 ± 0.02 J/(K g) for STI and ΔH = 37.4 ± 0.7 J/g and ΔC_p= 0.62 ± 0.09 J/(K g) for TAA.

The theoretical DSC curves for the unfolding were drawn on the basis of van't Hoff relation and compared with the excess heat capacity curves observed. The unfolding of both the proteins may be characterized by a three-state process rather than a simple two-state process. This was best evident for TAA, but less evident for STI.

INTRODUCTION

Recent advances in the precise measurement of the changes in heat capacity of dilute aqueous solution of biopolymers enabled the quantitative description of protein denaturation. Using these techniques thermodynamic properties of various proteins have been determined with respect to their stability in aqueous solutions. Many compact globular proteins are believed to undergo thermal unfolding *via* a simple two-state process(refs. 1 & 2). This unfolding is characterized by a reversible transition between initial(folded) and final(unfolded) states. These conclusions were derived on the basis of comparison between calorimetric and van't Hoff enthalpies which were both obtained from the measurement of excess heat capacity by differential scanning calorimetry(DSC).

According to the analysis made on DSC results by Privalov(refs. 1 & 2), the values of the van't Hoff and the calorimetric enthalpies are very close for most compact globular proteins such as myoglobin, lysozyme, and cytochrome *c*. This led to the implication that these proteins are reasonably considered to unfold *via* a simple two-state process. The unfolding of some other proteins, however, such as papain(ref. 3), aspartate transcarbamylase(ref. 4), and the λ-repressor protein(ref. 5) which have domain or subunit structures were shown to involve at least one more intermediate state.

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This paper reports the excess heat capacity measurements for the thermal unfolding of two globular proteins: Soybean trypsin inhibitor and Taka-amylase A. Our data indicate that these proteins undergo a three-state transition, even though they show the excess heat capacity curves with a single peak.

MATERIALS AND METHODS

Materials

The six-times recrystallized soybean trypsin inhibitor(STI) was purchased from Sigma Chemical Co. The protein was dissolved in buffer solutions of pH 7.0 and used for the DSC measurements after appropriate dilutions. Its concentration was determined spectrophotometrically by using an absorptivity of 0.994 cm^2/mg at 280 nm and at pH 2.0.

Taka-amylase A(TAA) from *Aspergillus oryzae* was prepared from Taka-diastase of Sankyo Co., Tokyo. The protein was purified and crystallized by the method of Akabori *et al.*(ref. 6) with slight modification using DEAE column chromatography. The protein was dissolved in pH 7.0 buffer. Its concentration was determined on the basis of absorptivity of 2.21 cm^2/mg at 280 nm and at pH 5.4.

Sample solutions used for DSC measurements had a protein concentration of about 0.5 to 4.0 mg/ml. The buffers(pH 7.0) employed were 10 mM-phosphate, 17 mM-HEPES, 17 mM-ACES, 17 mM-MOPS, 13 mM-Triethanolamine, and 10 mM-Tris. They were prepared in doubly deionized water. All other chemicals were of reagent grade.

Differential scanning calorimetry

The DASM-1M differential heat capacity calorimeter designed by Privalov(ref. 7) with some electronic modifications, was used. A scan rate of 1 K/min was in general employed. Calorimetric enthalpies were evaluated from the DSC curves of excess heat capacity according to the method described previously(refs. 8-10).

RESULTS AND DISCUSSION

The results of DSC measurements are summarized in Table 1. For each protein, the specific enthalpy and heat capacity changes of unfolding are approximately the same as those reported for many compact proteins(refs. 1 & 2). This result implies that both STI and TAA belong to the family of typical globular proteins.

The van't Hoff enthalpies for the unfolding of these proteins were also evaluated and are shown in the column 5 of Table 1. The ratio of the van't Hoff to calorimetric enthalpies are given in the column 6. The ratio is very close to 1 and is comparable to the ratio found for many globular proteins(0.95). This result seems to indicate that the unfolding of STI involves no thermodynamically distinguishable intermediate. However, if we assume that the above difference in the van't Hoff and the calorimetric enthalpies is significant, then there

TABLE 1 Summary of DSC data for STI and TAA at pH 7.0

Protein	t_p	ΔH_{cal}		ΔC_p		ΔH_{vH}	ΔH_{vH}
	°C	kJ mol^{-1}	J g^{-1}	kJ K^{-1} mol^{-1}	J K^{-1} g^{-1}	kJ mol^{-1}	ΔH_{cal}
STI	62.50	425 ±4	19.8 ±.2	11.0 ±.5	0.51 ±.02	411 ±3	0.97
TAA	63	1982 ±37	37.4 ±.7	32.8 ±4.6	0.62 ±.09	587 ±27	0.30

t_p = temperature of maximal excess heat capacity in degree Celcius : ΔH_{cal} = the calorimetric enthalpy obtained by integrating the DSC curve : ΔC_p = the heat capacity change due to unfolding at t_p : ΔH_{vH} = the van't Hoff enthalpy of unfolding calculated from eqs. $4RT_p^2 c_{max}/h_{cal}$ for STI and $5.83RT_p^2 c_{max}/h$ for TAA, where T_p is the temperature of maximal excess heat capacity in degree Kelvin, c_{max} the maximal excess specific heat and h_{cal} the specific enthalpy change of unfolding.

should be an intramolecular interaction involved during the transition and that the process should have one or more intermediate steps. Using the thermodynamic parameters obtained the excess heat capacity values during the transition were calculated on the basis of a simple two-state process(refs. 8 & 9)

$$A \rightleftharpoons B$$

The theoretical DSC curves thus obtained is shown in Fig. 1. The open circles in the figure are the data actually observed in the present calorimetric measurement corrected for baseline change. The actual data deviate from the theoretical curve in the regions of 320 to 326 and 340 to 346 K.

The experimental values were then used to deconvolute into two components using the method developed by Spink *et al.*(ref. 11) on the basis of a sequential three-state transition model

$$A \rightleftharpoons B \rightleftharpoons C$$

The two components resolved are shown in the bold lines in Fig. 2. The curve given in an ordinary line is a sum of the two which fits the experimental

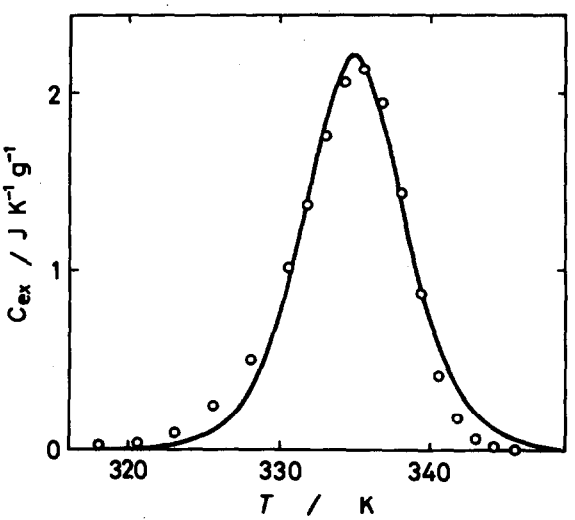

Fig. 1. Theoretical and actual excess heat capacity curves for the unfolding of STI. The theoretical curve(bold line) was drawn on the basis of a two-state process using the thermodynamic parameters given in Table 1. The open circles are the observed excess heat capacity values.

value(the open circles) better than the curve shown in Fig. 1. It is too risky to conclude from the above deconvolution result alone that the transition of the STI molecule proceeds *via* a three-state process. However, the result seems to suggest that there is a possibility that the process under consideration involves more than two states.

The van't Hoff enthalpy for the unfolding of TAA was found to be much smaller than the corresponding calorimetric enthalpy as shown in Table 1. This result also implies the involvement of an intermediate state during the transition. The experimental excess heat capacity curves were deconvoluted on the basis of a three-state transition. TAA is known to contain a Ca^{2+} ion in the molecule which is dissociated from the protein during the thermal denaturation. Taking into account the dissociation of Ca^{2+} ion, the DSC curve was deconvoluted into two components on the basis of a three-state transition

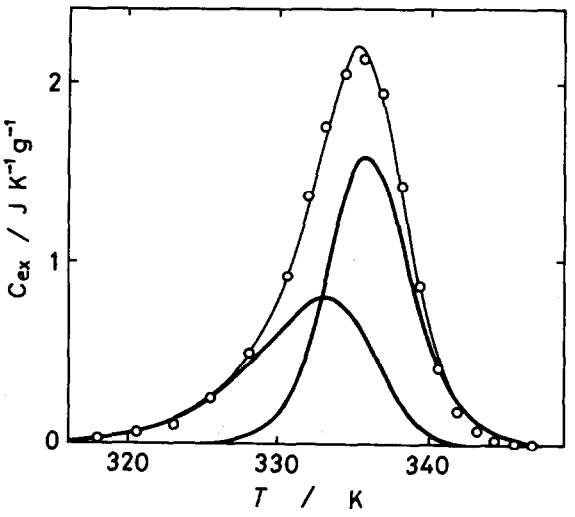

Fig. 2. Curve deconvolution for thermal unfolding of STI. The excess heat capacity curve observed was resolved into two components(bold lines). The curve given in the ordinary line is a sum of the two resolved curves. The open circles are the observed excess heat capacity values.

$$A \rightleftharpoons B \rightleftharpoons C + Ca^{2+}$$

The results are shown in Fig. 3. The sum of the two components fits the actually observed DSC curve better than the curve drawn on the basis of a two-state model(not shown). It will be clear that the curve for the two-state model does not fit well the experimental values, since the raio of van't Hoff to calorimetric enthalpies very much smaller than 1 as shown in the last column of Table 1. X-ray crystallography data(refs. 12-14) have shown that the TAA molecule contains two distinct domains. Proteins having domain structures like hexokinase(ref. 8) and papain(ref. 3) are found to have a van't Hoff enthalpy considerably smaller than its calorimetric enthalpy, which suggests that the two domains unfold with some degree of independence. In the case of TAA, it is most likely that the two domains of the protein also unfold more or less independently.

Complete papers on the thermodynamics of the unfolding of STI and TAA are

in preparation.

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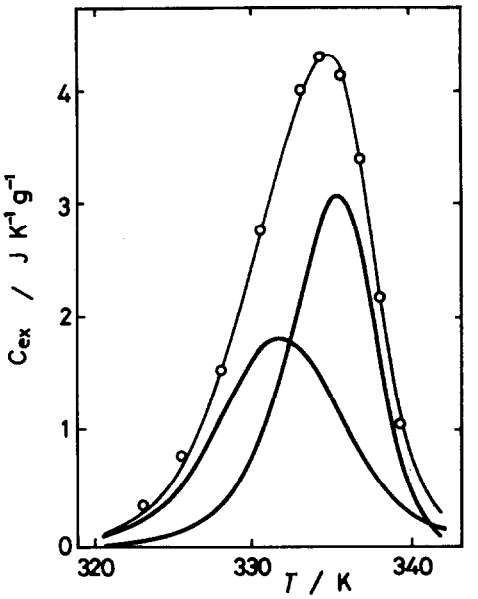

Fig. 3. Curve deconvolution for thermal unfolding of TAA. The excess heat capacity curve observed was resolved into two components(bold lines). The curve given in the ordinary line is a sum of the two resolved curves. The open circles are the observed excess heat capacity values.

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