PAPAIN DENATURATION IS NOT A TWO-STATE TRANSITION

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Received 28 April 1978

1. Introduction

Denaturation of a number of small globular proteins with a known three-dimensional structure can be regarded as a two-state transition [1]. Even proteins such as lysozyme [2] which has a cleft in the middle and parvalbumin [3] which consists of two distinct domains are denatured without stable intermediate states. This led to a tempting conclusion that all small globular proteins built of one polypeptide chain represent a single cooperative system. In this connection it was very interesting to examine papain in which two structural domains are separated by a deep cleft [4]. These parts of the molecule are linked by a number of secondary bonds and seem to be tightly fastened. However, consideration of the course of the chains in each part suggests that they fold independently [5]. Then the question arises whether these parts are indeed independent.

Thermodynamic analysis of the calorimetric data on papain thermal denaturation carried out in this paper has shown that papain denaturation is not a two-state transition and the two parts of this macromolecule represent quasi-independent cooperative systems. Thus it follows that both parts of papain are folded independently.

2. Materials and methods

A commercial preparation of papain (Fluka AG, Buchs SG, Switzerland) was twice recrystallized according to [6]. Papain was activated by reducing with 0.01 M dithiothreitol in 0.1 M Tris–HCl (pH 8.0) for 2 h at room temperature. Then the SH-group was blocked by iodacetamide treatment for 1 h at +4°C. Homogeneity, purity and intactness of the preparation obtained was tested by disk electrophoresis in 7.5% polyacrylamide gel as well as by electrophoresis in sodium dodecylsulfate. The molecular weight of papain estimated by electrophoresis in sodium dodecylsulfate was 23 500 ± 1000 which does not differ greatly from the known value.

Concentration of papain solutions was determined by A_{280} taking $E^{1\%}_{280} = 25$ [7]. For calorimetric measurements, 0.1–0.15% solutions in 0.05 M glycine buffer were used. Calorimetric measurements were done on the scanning microcalorimeter DASM-1 M [8] at a heating rate of 1 K/min. The procedure for the calorimetric determination of the partial heat capacity of proteins and of the real enthalpy of denaturation was as in [1,8].

3. Results and discussion

Figure 1 represents the temperature dependencies of papain partial heat capacities at different pH values. If we compare them with those reported for other proteins [1–3] we can see that they differ little in appearance. The increase in protein stability resulting from a change of pH is also accompanied here by an increase of the area of the peak of heat absorption Q_d, i.e., by an increase of the calorimetric or real enthalpy of denaturation which is $\Delta_d H^{cal} = M Q_d$, where M is the molecular weight. This increase in enthalpy proceeds at the expense of the peak height, thus it is connected with an increasing transition sharpness. From the transition sharpness or the peak height in the middle of transition $\Delta C_{p½}$ it is

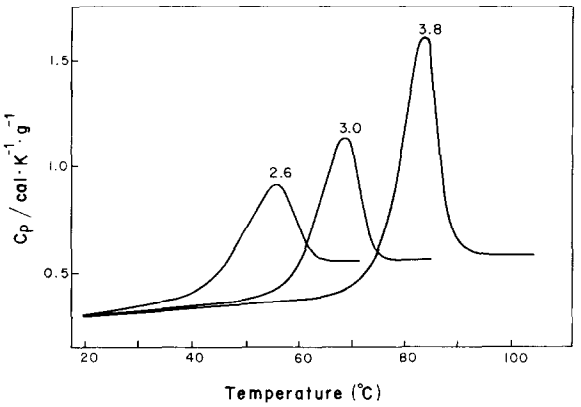

Fig.1. Temperature dependence of partial specific heat capacity of papain in solutions with different pH.

possible to determine Van't Hoff or effective enthalpy of the transition assuming that the denaturation process is a transition between two states [1]:

$$\Delta_d H^{eff} = \frac{4R \cdot T_d \Delta C_{p½}}{Q_d}$$

The values of the real and effective enthalpies of papain denaturation determined for different pH of the solution are given in table 1. It is seen that they differ by a factor of two. This divergence means that the assumption that papain denaturation is a transition between two states is incorrect. Papain has a thermodynamically stable intermediate state and the transition into this state and the transition from this state are rather similar and independent. If such transitions were completely independent, the ratio $\Delta_d H^{cal}/\Delta_d H^{eff}$ would be equal exactly to two and for a one-step process, i.e., when both stages are strongly dependent, it would approach a unit. In the case of papain it is evident that quasi-independent transitions correspond to transitions of separate papain parts. Thus the papain parts represent quasi-independent cooperative systems and, if they were isolated, their stability presumably would be only slightly lower than that of the whole molecule. This result substantiates the hypothesis that folding of papain parts proceeds independently.

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Table 1
Thermodynamic characteristics of papain denaturation

pH	T_d/°C	$\Delta_d H^{cal}$ (kcal . mol^{-1})	$\Delta_d H^{eff}$ (kcal . mol^{-1})	$\frac{(\Delta_d H^{cal})}{(\Delta_d H^{eff})}$
2.60	56.0	122.6	67.5	1.82
2.75	61.0	142.0	78.0	1.82
2.85	65.3	144.0	80.0	1.81
3.00	69.3	168.0	84.0	2.00
3.12	75.0	182.0	106.6	1.71
3.35	77.5	194.0	109.0	1.78
3.50	80.0	196.0	115.0	1.70
3.80	83.8	216.0	117.0	1.85