A HEAT DENATURATION STUDY OF SEVERAL RIBOSOMAL PROTEINS FROM *ESCHERICHIA COLI* BY SCANNING MICROCALORIMETRY

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Received 11 September 1978

1. Introduction

An important problem in the study of individual ribosomal proteins is to obtain information concerning their tertiary structure in solution. One method to show the existence of a tertiary structure in proteins is to study the disruption of the structure during the process of heat denaturation, using scanning microcalorimetry [1]. This technique reveals conformational transitions of the 'order–disorder' type which are displayed as heat absorption peaks, their presence being a direct indication of the existence of a tertiary structure in the proteins under investigation [2].

Here a microcalorimetry study of isolated ribosomal proteins S4, S7, S8, S15, S16, S18 and L11 has been done. It has been shown that proteins S4, S7, S8, S15, S16 and L11 display conformational transitions of the 'order–disorder' type which is evidence that they possess a cooperative tertiary structure in solution.

2. Materials and methods

Ribosomal proteins S4, S7, S15 and S18 were prepared at the Institute of Protein Research, Poustchino, by extraction with 4 M urea–3 M LiCl with subsequent phosphocellulose column chromatography and Sephadex G-100 gel filtration in the presence of 6 M urea [3]. The preparations of individual proteins were kept in solution, without lyophilization and, if required, were stored frozen in 50 mM K^+-phosphate buffer, pH 5.8, containing 200 mM KCl, 15 mM methylamine, 10 mM β-mercaptoethanol and 6 M urea. Renaturation was done immediately before the microcalorimetry experiments by gradual removal of urea and its substitution with the corresponding buffer (see lower) by careful dialysis.

Ribosomal proteins S8, S16 and L11 were prepared at the Max-Planck-Institut für Molekulare Genetik, Berlin (Dahlem), in the absence of denaturing agents by chromatography on CM–Sephadex C-25 and Sephadex G-100 in the pH 5.5–8.0 range [4,5]. They were transported to the Institute of Protein Research, Poustchino, in the frozen state in dry ice.

The purity of the proteins was checked by two-dimensional electrophoresis in polyacrylamide gels [6].

In the experiments we used different buffer solutions resulting in the best solubility of the corresponding ribosomal proteins:

(A) Na^+-acetate buffer, 50 mM (pH 5.6) with 600 mM LiCl and 1 mM dithioerythritol, for protein S8;

(B) K^+-phosphate buffer, 50 mM (pH 7.0) with 350 mM KCl and 1 mM dithioerythritol, for proteins S4 and L11;

(C) K^+-phosphate buffer, 2 mM (pH 7.0) with 10 mM KCl, for protein S18;

Buffer (B), but without dithioerythritol, was used for proteins S7, S15 and S16.

Before the experiment the protein solutions were dialyzed against the corresponding buffer for 10–12 h and clarified from aggregates by centrifugation at 20 000 × *g* for 30 min.

Calorimetric studies were done in the DASM-1M automatic differential scanning microcalorimeter [7] with a 1.0 ml cell operational volume, heating at 1°C/min. Proteins were from 0.5–1.2 mg/ml.

Elsevier/North-Holland Biomedical Press

3. Results and discussion

The calorimetric curves of heat denaturation of the ribosomal proteins S4, S7, S8 and L11 in solution are presented in fig.1. Distinct heat absorption peaks are observed for these proteins at temperatures of 45°C, 43°C, 54°C and 53°C, respectively. In the buffer solutions indicated above, the heat denaturation process of the proteins S4, S7, S8 and L11 is irreversible due to aggregation which is apparent after the middle of the transition. The phenomenon of aggregation which results in the distortion of the curve shapes complicates the thermodynamic analysis and the quantitative interpretation for the melting process. Nonetheless, the existence itself of the peak and its intensity suggest that the structure of the proteins studied is cooperative.

High reversibility of the denaturation process is observed for proteins S15 and S16 (fig.2). This permits the calculation of the enthalpy of the transition per mole of the cooperative region. It is known [1] that:

$$\Delta H = 4RT_t^2 \frac{\Delta C_p}{Q}$$

where R (cal.deg^{-1}.mol^{-1}) is the universal gas constant; T_t (K) is the temperature of transition; C_p (cal/°C) is the heat capacity value at transition temperature; Q (cal) is the heat absorption peak area. It follows from here that the enthalpy value of heat absorption for proteins S15 and S16 is 60 kcal/mol and 50 kcal/mol of cooperative region, respectively. However, the data obtained in the present work are insufficient to evaluate the size of the cooperative regions undergoing the conformational transitions and require additional experimental material.

The high thermostability in the case of proteins S15 and S16 is noteworthy; it is evidence that their cooperative structure in solution is very firm.

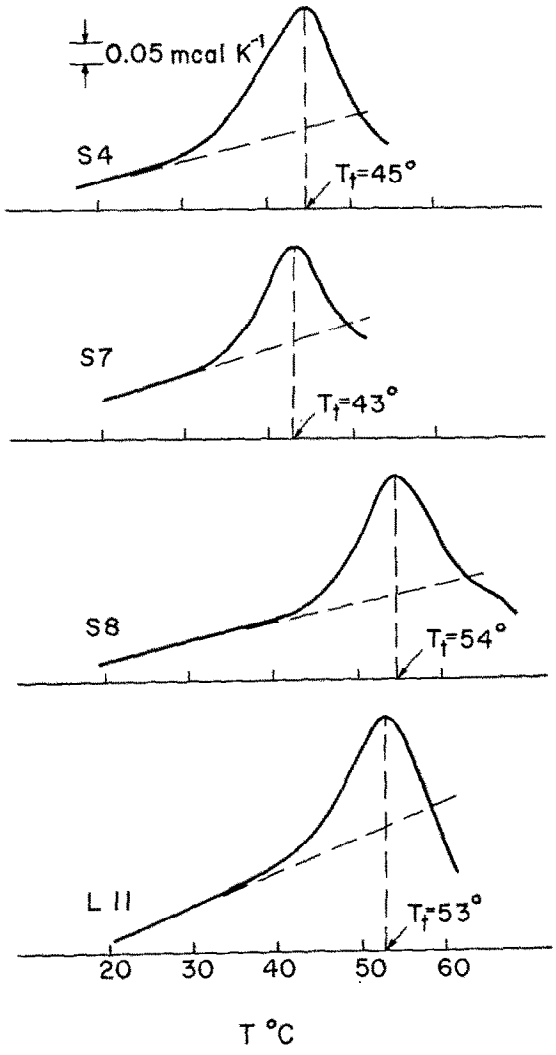

Fig.1. Calorimetric curves of the heat denaturation process of ribosomal proteins S4, S7, S8 and L11 in solution.

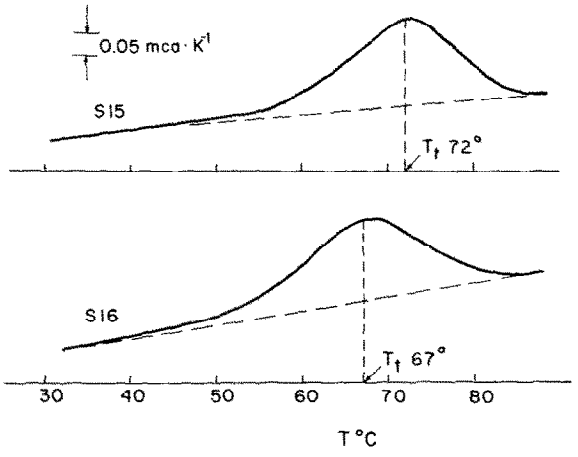

Fig.2. Calorimetric curves of the heat denaturation process of ribosomal proteins S15 and S16 in solution.

In the case of protein S18, no conformational transitions in the range of 10–100°C could be found. We can conclude from this that under the given conditions the polypeptide chains of this protein in solution are disordered and do not form a cooperative structure.

Acknowledgements

The authors are grateful to Professors P. L. Privalov, A. S. Spirin and H. G. Wittmann for support and discussion.

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