UNFOLDING OF THE *lac*-REPRESSOR HEADPIECE

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1. Introduction

The small, N-terminal fragment (headpiece, HP) of the *lac*-repressor protein consists of 51 amino acid residues and possesses extensive structure in solution [1,2]. The small size, single subunit structure and lack of disulfide bridges makes this polypeptide very suitable for studies of its folding and unfolding. The chemical shift changes in the HP NMR spectra have been described as a function of temperature [3]. In contrast to the majority of proteins examined thus far, HP shows weak cooperativity. Unfolding with increasing temperature proceeds gradually with the folded and unfolded forms in rapid exchange. The characteristic transition temperature is dependent upon ionic strength. To understand the relationship of these changes to different stages of the unfolding process, it is necessary to correlate spectroscopic data with calorimetric measurements. The results of these measurements are reported here.

2. Materials and methods

Headpiece samples were prepared as described for the NMR experiments [1,2]. Protein samples were studied in H_2O with Tris and phosphate buffers and in D_2O with the same phosphate buffer. Aluminium sample pans used in most experiments were filled with 20 μl solution and sealed under pressure. These buffers do not interact with aliminium even at pH > 9 [8]. Buffer samples gave no heat effects under conditions used for the protein solutions. In a few cases, golden sample pans were used and gave essentially the same results. A DuPont 990 thermal analyzer with a sensitivity of 10 μcal . s^{-1} . $in.^{-1}$ was used. Due to the small sample volume and in spite of the high sensitivity of the calorimeter, the heat capacity measurements were an order of magnitude less precise than those reported in [7]. The corresponding buffer was used as a reference sample. The heating rate was 10°C/min. Cooled and thermostated dry nitrogen gave good baseline stability and a slight increase in sensitivity. The melting points of mercury, benzene and indium were used for calibration of temperature and heat capacity, and Al_2O_3 (sapphire) was used for heat capacity measurements between 280–400 K.

3. Results and discussion

The temperature dependence of the partial heat capacity of HP is shown in fig.1. The results are very different from those reported for globular proteins. The heat capacity of HP in H_2O changes in a broad temperature range of 60°C starting at 35°C. In phosphate buffer (fig.1A), 2 broad, poorly resolved endothermic peaks at 50°C and 68°C can be easily distinguished. In Tris buffer (fig.1B), 3 peaks are well resolved and appear at 50°C, 60°C and 73°C. The process of cooling and heating was repeated 4 times with all peaks of the same intensity being obtained each time. The calorimetric and NMR data available thus far are consistent with the generally held view that HP exists as a monomer [9]. The transitions at lower temperatures can not be attributed to micro-unfolding or to predenaturation phenomena because they are fully reversible with an apparent specific heat of the same order of magnitude as the main transition at 70°C. It has also been partially confirmed by CD and absorption spectroscopy (unpublished). (Concerning the possibilities of buffer influence on

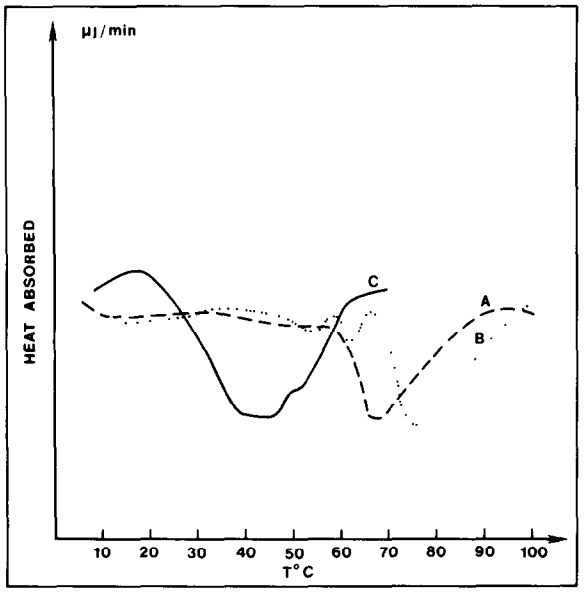

Fig.1. Denaturation of HP peptide. (A) 2.4 mg HP/ml in 20 mM K_2HPO_4–960 mM KCl–0.2 mM cacodylic acid in H_2O (pH 6.9). Heating rate 10°C/min. *Y*-scaling factor = 1. (B) 2.88 mg HP/ml in 50 mM Tris–HCl–950 mM KCl–0.2 mM cacocylic acid in H_2O (pH 6.8). Heating rate 10°C/min. *Y*-scaling factor = 2. (C) 2.8§ mg HP/ml in 20 mM K_2HPO_4–960 mM KCl–0.2 mM cacodylic acid in D_2O (pH 6.8). Heating rate 10°C/min. *Y*-scaling factor = 4.

headpiece structure, 'slight' differences in CD spectra were mentioned in [9].)

HP unfolding in phosphate buffer in D_2O is represented in fig.1C. Although the broad general pattern of unfolding is similar, the specific features are different. The unfolding temperature is ~20°C lower with 60% of the intensity. This destabilization effect is quite inexplicable at present, considering the stronger hydrogen bonds in D_2O. Unfolding begins at >20°C with two distinguishable steps with greater intensity at the lower temperature. Although the calorimetric results in D_2O can not be compared in detail with NMR results [3] because of different pD values and phosphate concentrations (for NMR, pD = 7.7 and 300 mM K_2HPO_4/KH_2PO_4, 200 mM KCl), they are consistent with the NMR finding of a broad unfolding transition of the HP peptide. The calorimetric transitions at 1.0 M ionic strength correspond best to the NMR results at 0.1 M ionic strength, each having phosphate concentrations (~30 mM) which suggests a dominant role of phosphate in stabilizing the structure.

From the peak areas, the enthalpy for each transition was calculated using 6000 for the molecular weight of HP. In phosphate buffer in H_2O, the two peaks were unresolved and gave a total enthalpy (ΔH) of ~799 kJ/mol (191 kcal/mol). In Tris buffer in H_2O, the enthalpies were 50 kJ/mol (12 kcal/mol), 79 kJ/mol (19 kcal/mol) and 439 kJ/mol (105 kcal/mol) for the 3 respective transitions, or 569 kJ/mol (136 kcal/mol) for the total transition. In phosphate buffer in D_2O, the total enthalpy (ΔH) is 477 kJ/mol (114 kcal/mol). Although these transition enthalpies must be regarded as estimates because corrections due to peptide ionization have not been made, they are, nevertheless, high compared to those reported for most globular proteins [7] or a similar fragment from the λ-repressor [10]. Two explanations can be proposed:

1. The phosphate buffer could play a specific role in stabilizing the tertiary/secondary structure of the peptide such that the enthalpy of interaction between HP and phosphate is included in the total enthalpy.
2. The number of internal hydrogen bonds in HP could be higher per unit weight than in globular proteins which have 4–7 hydrogen bonds/1000 daltons [4,7].

Although no quantitative estimates of the extent of secondary structure are as yet available, the high value of $[\theta]$ obtained in CD measurements suggests the existence of an extensive secondary structure.

It is apparent that HP unfolding is not a simple two-state transition. The best term for this unfolding is 'gradual' since different transition temperatures can be seen for different NMR spectral lines [3].

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