lac-REPRESSOR HEADPIECE CONSTITUTES A REVERSIBLY UNFOLDING DOMAIN

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1. Introduction

lac-Repressor of *Escherichia coli* is a tetrameric protein composed of 4 identical subunits of M_r 38 600. Each of the 4 identical subunits consists of 2 functionally specialized domains which may be dissected by limited proteolysis [1,2]. Trypsin treatment of native *lac*-repressor yields a tetrameric core-protein (residues 60–360 of *lac*-repressor) which binds inducer while the long (residues 1–59) and short headpieces (residues 1–51) bind to DNA [3,4]. NMR spectroscopic studies of repressor, core and headpieces provided direct evidence for the existence of tertiary structure in the headpieces of the native, tetrameric *lac*-repressor, in the separated, monomeric headpieces, and in the tetrameric cores [5,6]. Since the *lac*-repressor headpieces have a tertiary structure which constitutes an independent domain of the molecule, it is an intriguing question, whether the energetics of unfolding of dissected headpieces in solution follows the characteristics of other small proteins [7]. To obtain quantitative energy parameters we studied unfolding of *lac*-repressor headpieces by highly sensitive scanning microcalorimetry.

2. Materials and methods

lac-Repressor was isolated from *E. coli* strain BMH 74-12 as in [8] using the buffers of [9]. Phenylmethylsulfonylfluoride which inactivates the inducer binding capacity was not used as buffer additive. Limited tryptic digestion of native *lac*-repressor was performed essentially as in [3]. Core and headpieces were separated on phosphocellulose under the conditions in [10]. The headpiece fraction was rechromatographed on Sephadex G-50 superfine (2 × 90 cm bed for 5 mg headpieces) and concentrated by ultrafiltration (Diaflo membranes UM02, Amicon Corp., Lexington). The mixture of short and long headpieces was employed for the studies since there is no indication of structural differences between the two different headpieces [5,6]. Headpiece concentration was determined using an absorption coefficient of $A^{1\%}_{1\,cm,\,280\,nm} = 8.80$.

Measurements were made in 0.01 M phosphate buffer, 0.7 M KCl, 0.02% NaN_3, 2 mM EDTA (pH 8.0 at 25°C). All compounds used for the buffers were at the highest purity available. Calorimetric measurements were made using a DASM 1 M adiabatic microcalorimeter [11] at a heating rate of 1 K/min. Sedimentation equilibrium measurements were performed, employing a Beckman-Spinco model E analytical ultracentrifuge.

3. Results and discussion

Fig.1 shows the variation with temperature of the apparent specific heat capacity of a 2.38 mg/ml solution of *lac*-repressor headpieces in 0.01 M potassium phosphate buffer, 0.7 M KCl, 0.02% NaN_3, 2 mM EDTA (pH 8.0). Curves 1a and 1b refer to two successive scans on the same sample and demonstrate perfect reversibility of the unfolding transition within experimental error. The heat capacity *vs* temperature curve is fairly symmetric, the transition starting at ~40°C, exhibiting a maximum at ~65°C with a change in heat capacity at the transition temperature of 0.21 J/g.K) (0.05 cal/(g.K)) [1.3 kJ/(6000 g.K) (0.3 kcal/(6000 g.K)] and vanishing near 90°C. The specific transition enthalpy is 19.7 J/g (4.7 cal/g) and the molar quantity is 118 ± 13 kJ/mol (28 ± 3 kcal/mol) employing 6000 g/mol as the molar mass. A second run using a lower headpiece concentration of 0.54

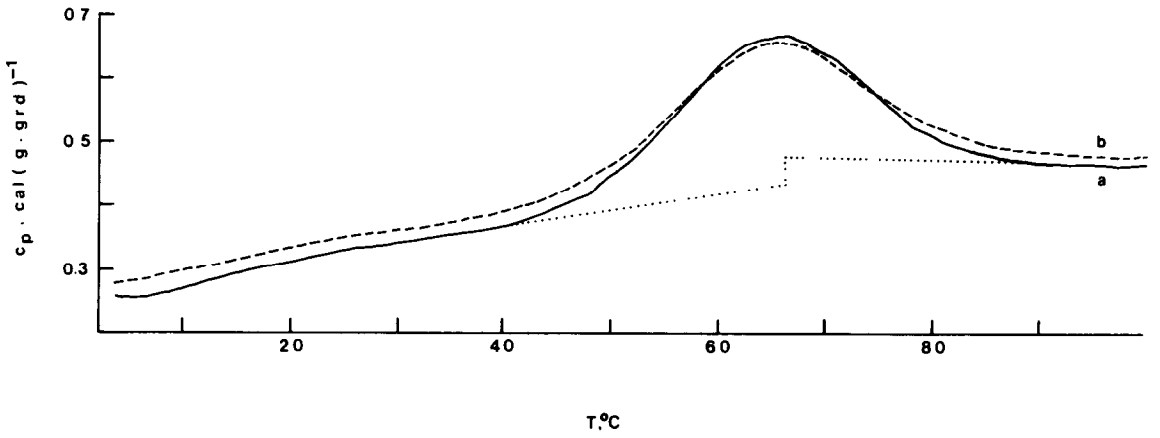

Fig.1. Variation with temperature of the apparent specific heat capacity, c_p, of *lac*-repressor headpieces (2.38 mg/ml, 0.01 M potassium phosphate buffer, 0.7 M KCl, 0.02% NaN_3, 2 mM EDTA, pH 8.0); heating rate: 1 K/min. (a) First heating (——); (b) second heating (– – –).

mg/ml yielded a specific enthalpy of transition of 20.8 J/g (5.0 cal/g) [125.0 kJ/mol (29.9 kcal/mol)]. Since calorimetric measurements employing such a low concentration are on the borderline of sensitivity of the instrument the intrinsic error of the enthalpy values is larger than in runs using higher concentrations of the sample. Therefore the ΔH-values of these measurements can be considered to be identical. The enthalpy values for unfolding headpieces are remarkably small in comparison to those of other proteins. Typical specific enthalpies for compact small globular proteins were 25–34 J/g (6.0–8.1 cal/g) at transition temperatures around 65°C [7]. Thus our results suggest that *lac*-repressor headpieces have weaker energetic interactions of the amino acids in the tertiary or secondary structure than 'normal' small globular proteins. The small change of specific heat capacity on denaturation, Δc_p = 0.21 J/(g.K) (0.05 cal/(g.K)), is also consistent with this interpretation, since other small globular proteins exhibit Δc_p-changes of 0.34–0.59 J/(g.K) (0.08–0.14 cal/(g.K)) [7]. Calculating the Gibbs' free energy of stabilization $-\Delta G(298)$, at 25°C according to the equation:

$$-\Delta G(298) = -\Delta H_{cal} \cdot (1 - \frac{298}{T_m}) - \Delta c_p \cdot 298 \cdot \ln \frac{T_m}{298} + \Delta c_p (T_m - 298)$$

using T_m = 338 K (65°C) as transition temperature, Δc_p = 1255 J/(mol.K) (300 cal/(mol.K)) for the molar heat capacity change at T_m and 118 kJ/mol (28 kcal/mol) for the calorimetric transition enthalpy, ΔH_{cal}, one obtains $-\Delta G(298) = -10.8$ kJ/mol (–2.6 kcal/mol). This value is also smaller than the Gibbs' free energies of stabilization obtained for other small globular proteins studied so far even if one takes the molecular mass differences into account [7]. Thus all thermodynamic evidence supports the notion that *lac*-repressor headpieces assume a less compact structure than other small proteins. This conclusion agrees well with NMR studies which suggested, on the basis of the sharpness of the resonance lines, relatively high mobility of the amino acids in the headpiece.

Our results qualitatively and quantitatively disagree with DTA measurements on unfolding *lac*-repressor headpieces [12]. Headpiece solutions (2.4 mg/ml, 20 mM K_2HPO_4, 960 mM KCl, 0.2 mM cacodylic acid, pH 6.9) were reported to yield a molar transition enthalpy of 799 kJ/mol (191 kcal/ mol). Since the molar value was also based on M_r 6000 the ΔH-value of 799 kJ/mol (191 kcal/mol) would correspond to a specific transition enthalpy of 133 J/g (31.8 cal/g). This unusually high value is so far above any other specific transition enthalpy measured up to now [7] that it appears to be very difficult to rationalize, particularly, since it is apparently inconsistent with the conclusions derived from the NMR studies. A highly mobile protein is difficult to picture as comprising more hydrogen bonds than a rigid one.

It was also suggested in [12], that the enthalpy of

interaction of phosphate buffer with the headpieces could be included in the overal unfolding enthalpy. However, this possibility seems somewhat remote, since it is hard to understand how binding of phosphate ions to 51 amino acids, even if it existed, could account for 669 kJ/mol (160 kcal/mol). Thus our conclusion would be that the large quantitative difference between our ΔH-values and those in [12] is probably due to the admitted low accuracy of the instrument used [12].

It is well known that the ratio of van't Hoff enthalpy, $\Delta H_{\text{v.H.}}$, to calorimetric enthalpy, ΔH_{cal}, provides a measure of the cooperativity of the transition [13–18]. Since the van't Hoff enthalpy can be directly obtained from the calorimetric heat capacity measurements, we analysed the curves in terms of a two state transition and obtained $\Delta H_{\text{v.H.}} = 172$ and 163 kJ/(mol cooperative unit) (41 and 39 kcal/(mol cooperative unit)) for the low and high concentration measurements, respectively. Since the ratios are >1, a reasonable explanation would be that intermolecular cooperation occurs as a result of association. Therefore high-speed sedimentation equilibrium measurements were performed, which gave $M_r \sim 5000$, when employing, in the absence of better information, $\bar{\nu} = 0.73\ \text{cm}^3/\text{g}$ as the partial specific volume of the headpieces. Due to the looser structure of *lac*-repressor headpieces $\bar{\nu}$ could be somewhat larger, which would bring the low M_r up to 6000. In any case, there was no indication in the high-speed sedimentation equilibrium measurements of association of headpiece molecules. This leaves us with the yet unanswerable question of how the van't Hoff enthalpy could be larger than the calorimetric enthalpy in the absence of intermolecular cooperation. The fact that a mixture of 51 and 59 residue headpieces was used here does not provide an explanation either, since there is good evidence [3,5,6] that the structural features of these two headpieces are identical.

Under these experimental conditions, *lac*-repressor headpieces show one reversible transition with all thermodymanic and NMR evidence suggesting a well-defined but less compact structure than normal small globular proteins.

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