Thermal Denaturation of the Core Protein of *lac* Repressor†

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ABSTRACT: The thermal denaturation of the core protein of *lac* repressor was studied alone and in the presence of the inducer isopropyl β-D-thiogalactoside (IPTG) and the antiinducer *o*-nitrophenyl β-D-fucoside (ONPF) by means of high-sensitivity differential scanning calorimetry. The denaturation that takes place at about 65 °C is apparently irreversible; i.e., a rescan of a previously scanned sample of protein solution shows no denaturational endotherm. Despite this irreversibility, the denaturation appeared to follow quantitatively the dictates of equilibrium thermodynamics as embodied in the van't Hoff equation. The results obtained indicate clearly that the tetrameric protein dissociates to monomers during denaturation and that the ligands are not dissociated until denaturation takes place. The enthalpy of denaturation of the protein is 4.57 ± 0.25 cal g^{-1} and is independent of temperature. The enthalpies of dissociation of IPTG and ONPF at the denaturation temperature are very large, 37 and 42 kcal (mol of ligand)$^{-1}$, respectively.

The study of the thermal denaturation of proteins by means of differential scanning calorimetry (DSC)[1] has led to many interesting results (Privalov, 1979, 1982). In the case of the reversible denaturation of many small globular proteins such as ribonuclease A (Tsong et al., 1970; Privalov & Khechinashvili, 1974a) and lysozyme (Pfeil & Privalov, 1976), DSC has afforded the most convincing evidence that the process takes place with a surprising degree of cooperativity involving the entire molecule in an all-or-non, or two-state, process, with no intermediate states between the native and unfolded states being significantly populated at equilibrium. The demonstration of two statedness involves the use of the van't Hoff equation

$$\mathrm{d} \ln K/\mathrm{d}T = \Delta H_{\mathrm{vH}}/(RT^2) \qquad (1)$$

which expresses the variation with temperature of the equilibrium constant, K, for the denaturation process. For any reversible two-state reaction, ΔH_{vH}, the van't Hoff, or apparent, enthalpy, must equal ΔH_{cal}, the calorimetric or true enthalpy. Procedures for evaluating ΔH_{vH} and ΔH_{cal} from DSC data will be outlined below.

In the cases of some proteins it has been found that ΔH_{vH} is significantly smaller than ΔH_{cal}. In such cases the denaturation may involve two or more sequential two-state steps or the more or less independent unfolding of two or more separate domains in the protein molecule. In these cases the observed DSC curve, which gives the temperature variation of c_{ex}, the apparent excess heat capacity, can be expressed as the composite of appropriate component curves. This decomposition of the DSC curve into component curves assumes reversibility of the process under study.

The usual criterion for complete reversibility that is applied in DSC studies is that a reheating of the scanned material after cooling should give a curve that is superimposable on the original curve. On this basis, the denaturation of many proteins turns out to be partially or completely irreversible. However, this criterion for reversibility may be too restrictive, as is suggested by the familiar example of the unwinding of double-helical polynucleotides. The thermal denaturation of a polynucleotide such as poly[d(AT)] is fully reversible, demonstrating the fundamental reversibility of the unwinding process. On the other hand, the denaturation of calf thymus DNA appears to be irreversible. This process is rendered reversible if a very few cross-links are introduced between the two chains (Becker et al., 1964). These cross-links serve to prevent actual chain separation with loss of register between the chains. It is thus apparent that the denaturation of the DNA is energetically reversible but that the renaturation is extremely slow. This is further indicated by partial renaturation by annealing at a temperature slightly below the denaturation temperature range.

The lactose repressor protein regulates the transcription of the *lac* metabolizing enzymes in *Escherichia coli* by specific interaction at the operator site in the genome (Miller & Reznikoff, 1980). This binding is modulated by the presence of sugar ligands bound to the tetrameric protein of M_r 150000; inducers decrease the affinity of the protein for operator DNA, while antiinducers stabilize the repressor–operator complex (Miller & Reznikoff, 1980). Two domains within the repressor, amino terminus and core protein, can be isolated by mild proteolytic digestion (Platt et al., 1973). The amino terminus binds to nonspecific DNA and operator-containing DNA (Jovin et al., 1977; Ogata & Gilbert, 1979), while the core protein binds to inducer and to operator DNA (Platt et al., 1973; Matthews, 1979).

In this paper we report DSC measurements of the thermal denaturation of the chymotryptic core protein of the *lac* repressor of *E. coli*. This reaction appears to be irreversible by the usual DSC criterion in that the denaturational endotherm is completely missing from a rescan of the denatured protein, which appears to have been rendered completely insoluble by the denaturation process. We shall show here that this denaturation, nevertheless, appears to follow quantitatively, both in the absence and in the presence of various ligands, the dictates of equilibrium thermodynamics as expressed in the

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[1] Abbreviations: DSC, differential scanning calorimetry; IPTG, isopropyl β-D-thiogalactoside; ONPF, *o*-nitrophenyl β-D-fucoside; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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Table I: Experimental Thermodynamic Data for the Thermal Denaturation of Core Protein

protein concn (mg mL^{-1})	t_m (°C)		Δh (cal g^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}(1)$ (kcal mol^{-1})[a]	c_{max} (cal K^{-1} g^{-1})
1.35	63.80		5.55	721	414	1.130
1.43	63.70		5.71	742	432	1.214
2.44	64.95		4.78	621	532	1.243
3.13	64.97		4.47	581	517	1.129
5.74	65.10		4.30	559	545	1.145
11.5	67.00		4.46	580	518	1.115
15.3	66.65		4.31	560	571	1.190
		mean	4.80	623	504	1.167
		SE[b]	±0.26	±34	±26	±0.022

[a] $\Delta H_{vH}(1) = 9.01RT_m^2c_{max}/\Delta h$ (see text). [b] SE, standard error.

van't Hoff equation. We take this as evidence that the denaturation is fundamentally reversible under the conditions of the DSC experiments but that we do not know how operationally to achieve renaturation.

Materials and Methods

Isolation of Lactose Repressor. Repressor was isolated from *Escherichia coli* CSH 46 according to the methods described by Rosenberg et al. (1977), as modified by O'Gorman et al. (1980). The isolated repressor was 98% pure by SDS gel electrophoresis. Assays were executed as described previously (O'Gorman et al., 1980).

Isolation of Core Protein. The core repressor was purified as described previously (Matthews, 1979; Hsieh & Matthews, 1981), with digestion of the intact repressor by chymotrypsin to produce the core domain consisting of amino acid residues 56–360. The isolated core protein exhibited inducer-sensitive operator DNA binding. The concentration of the core protein was determined by absorbance at 280 nm ($E_{0.1\%} = 0.51$). The isolated domain was concentrated to 3–15 mg/mL in a Pro-Di-Con vacuum concentrator/dialyzer. Following concentration, the preparation was dialyzed for 48–72 h against the buffer utilized in the DSC experiments: 0.048 M potassium phosphate, pH 7.4, 15% glycerol, 0.1 mM dithiothreitol. The protein solutions were frozen and shipped from Houston to New Haven in dry ice.

Calorimetry. All scanning calorimetric experiments were performed with a DASM-1M microcalorimeter (Privalov et al., 1975). A large exotherm was observed during the denaturational endotherm when a scan rate of 1 K min^{-1} was employed, making it impossible to obtain quantitative data concerning the denaturation. It was found empirically that the exotherm, for completely unknown reasons, was reduced in amplitude by reducing the scan rate and by employing relatively high protein concentrations.[2] All the data reported here were obtained at a scan rate of 0.23 K min^{-1}. A tracing of a typical DSC curve for core protein is given in Figure 1. This curve illustrates the noise level observed in our experiments and the vestigial exotherm at 60 °C.

Results and Discussion

Denaturation of Core Protein in the Absence of Ligands. The thermal denaturation of core protein was studied at concentrations ranging from 1.35 to 15.3 mg mL^{-1}. The observed results are listed in Table I. Column 1 gives the total protein concentration and column 2 the temperature, t_m, at which the excess specific heat, c_{ex}, reaches its maximal value, c_{max}. The specific enthalpies in column 4 were obtained by planimeter integration of the DSC curves, and those in column 5 by multiplying by the molecular weight of the tetrameric protein, 130 000. Column 7 gives the values of c_{max}. The procedure for evaluating the van't Hoff enthalpies in column 5 will be given below.

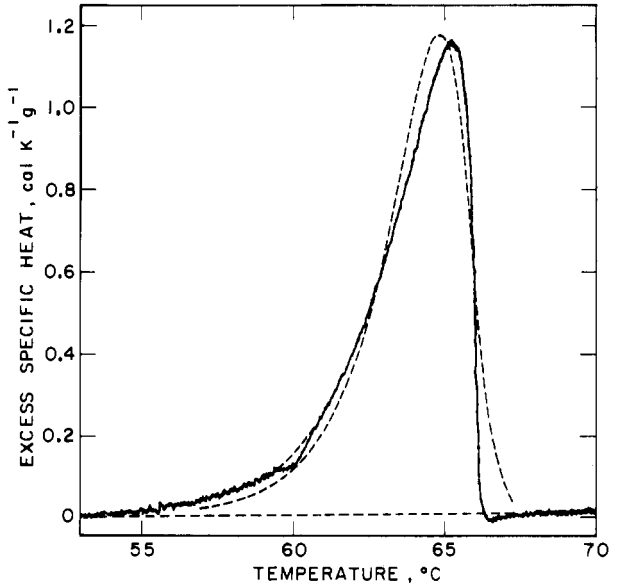


FIGURE 1: Tracing of a typical DSC scan of core protein, showing the noise level typical of the scans obtained in this work. Protein concentration was 5.74 mg mL^{-1}; scan rate was 0.23 K min^{-1}. The dashed curve is the best fit of a theoretical curve for the process $N_4 \rightleftarrows 4D$ to the experimental curve. See text for the method of arriving at this curve.

All the DSC curves showed the pronounced asymmetry evident in Figure 1. This asymmetry is to be expected if the denaturation is of the form

$$N_m \rightleftarrows mD \qquad (2)$$

where N and D represent native and denaturated protein, respectively, with the protein remaining oligomeric up to the denaturational temperature and then undergoing simultaneous denaturation and dissociation. As shown in an earlier paper (Takahashi & Sturtevant, 1981), the excess specific heat, c_{ex}, is given by the expression

$$c_{ex} = \frac{\alpha(1-\alpha)}{m-(m-1)\alpha}\frac{\Delta h \Delta H_{vH}}{RT^2} \qquad (3)$$

where α is the extent of denaturation and Δh is the specific enthalpy in cal g^{-1}. Takahashi & Sturtevant (1981) outlined a procedure for fitting the theoretical curve, eq 3, to the experimental data by varying the adjustable parameter $T_{1/2}$ (the

[2] After the work reported here was completed, it was found that no exotherm appears when the protein is scanned at 1 K min^{-1} in the new version of the Privalov DSC, the DASM-4. In this instrument the cells are helices of small-bore platinum tubing with horizontal axes. The absence of the exotherm shows that it is in some unexplained way due to the pillbox form of the cells in the earlier instrument and is not due to heat evolution by the protein. This conclusion is supported by the fact that the exotherms when observed did not appear to reduce significantly the denaturational endothermic enthalpies.

Table II: Thermodynamic Data for the Thermal Denaturation of Core Protein Derived by Curve Fitting

protein concn (mg mL^{-1})	$t_{1/2}$ (°C)		Δh (cal g^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}(2)$ (kcal mol^{-1})[a]	SD (% c_{max})[b]
1.35	62.34		5.55	722	401	7.7
1.43	62.73		5.18	673	471	7.4
2.44	64.10		4.45	579	556	6.8
3.13	64.07		4.11	534	555	8.8
5.74	64.20		4.20	546	550	6.4
11.5	66.11		4.27	555	559	9.1
15.3	65.71		4.20	546	580	7.3
		mean	4.57	594	525	7.6
		SE[c]	±0.25	±32	±30	±0.4

[a] $\Delta H_{vH}(2)$ is obtained by minimization of the standard deviation of experimental data from a theoretical curve (see text). [b] SD, standard deviation. [c] SE, standard error.

Table III: Values of the Constant A in Equation 6

m	1	2	3	4
A	4.00	5.83	7.47	9.01

absolute temperature at which $\alpha = 0.5$), Δh, and ΔH_{vH} to minimize the standard deviation of the observed values of c_{ex} from the calculated curve. The dashed curve in Figure 1 was obtained by this procedure, with $m = 4$. It is evident that the asymmetry of the curve is well accounted for on the basis of reaction 2. It is also evident that a slightly better fit can be obtained by adding an additional small component centered at about 59 °C. However, since there appears to be no reasonable model that would include this additional step, we have restricted our curve fitting to the single two-state model.

The thermodynamic parameters resulting from curve fitting are presented in Table II. It is seen that Δh and ΔH_{vH} agree well with the values obtained directly from the DSC curves.

Further support for the formulation in eq 2 is given by the fact that the values of t_m given in Table I increase with increasing protein concentration. Fukada et al. (1983) showed that for the more general reaction

$$N_mL_n \rightleftarrows mD + nL \quad (4)$$

in which ligand molecules are also dissociated during denaturation, to a good approximation

$$\Delta H_{vH}/(RT_m) + (m - 1) \ln [N]_0 + n \ln [L]_0 = \text{constant} \quad (5)$$

In this expression $[N]_0$ and $[L]_0$ are the total protein and ligand concentrations, respectively, and it is assumed that $[L]_0 \gg [N]_0$. Thus at constant (including zero) ligand concentration, a plot of ln $[N]_0$ vs. $1/T_m$ should be a straight line if ΔH_{vH} is independent of temperature. Such a plot for the data in Table I is curve C in Figure 2, with the experimental points having a standard deviation from the least-squared line of ±0.37° in t_m. The slope of the plot is −83 600 K, from which one obtains ΔH_{vH} = 498 kcal mol^{-1}.

Additional values for ΔH_{vH} that can be obtained from the individual DSC curves by means of the equation

$$\Delta H_{vH} = ART^2c_{max}/\Delta h \quad (6)$$

where the values of A for varius values of m are given in Table III, are listed in column 5 of Table I.

We have thus obtained three values for ΔH_{vH} that are in close agreement, as summarized in Table IV. The mean of these values is 18% less than ΔH_{cal}, a discrepancy that is not unusual with proteins showing reversible denaturation and presumably indicates a significant contribution from intermediate states. We wish to emphasize that the agreement between the various enthalpy values in Table IV constitutes strong empirical evidence that the thermal denaturation of the core protein follows equilibrium thermodynamics despite the

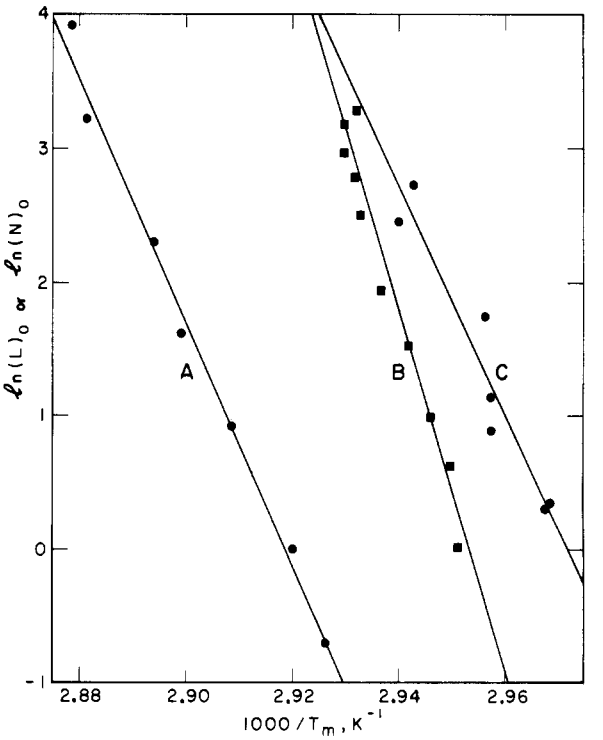


FIGURE 2: van't Hoff plots of the logarithm of the total protein concentration, $[N]_0$, or the total ligand concentration, $[L]_0$, against $1/T_m$, where T_m is the absolute temperature at which the value of the excess specific heat is maximal. (Curve A) IPTG, $[N]_0$ = 3.13 mg mL^{-1}; (curve B) ONPF, $[N]_0$ = 2.68 mg mL^{-1}; (curve C) core protein alone.

Table IV: ΔH (kcal mol^{-1}) for the Denaturation of Core Protein

$\Delta H_{vH}(1)$[a]	$\Delta H_{vH}(2)$[b]	$\Delta H_{vH}(3)$[c]	$\Delta H_{cal}(4)$[d]
504 ± 26	525 ± 30	498 ± 4	623 ± 34

[a] Mean of values in column 6 of Table I. [b] Mean of values in column 6 of Table II. [c] From slope of line C in Figure 2. [d] Mean of values in column 5 of Table I.

fact that it appears to be overall irreversible. DSC results obtained with the regulatory and catalytic subunits of aspartate transcarbamoylase also indicate that the application of the van't Hoff equation to the irreversible denaturations of these proteins may be permissable (Edge et al., 1985).

In order to investigate further the utilization of the van't Hoff equation in cases of irreversible denaturation, we have carried out simulations of the model reaction

$$N_4 \rightleftarrows 4D \qquad D \xrightarrow[E_a]{k} I \quad (7)$$

in which the denaturated protein reacts irreversibly to form a product I with first-order rate constant k and activation energy E_a. We do not propose this model as a realistic rep-

Table V: Thermodynamic Data for the Thermal Denaturation of Core Protein in the Presence of Ligands

(A) Experimental Data

ligand	protein concn (mg mL^{-1})	Δh (cal g^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}(1)$ (kcal mol^{-1})[a]	c_{max} (cal K^{-1} g^{-1})
IPTG	3.13	5.93 ± 0.20	771 ± 26	422 ± 17	1.171 ± 0.026
ONPF	2.44–3.83	6.08 ± 0.31	791 ± 40	410 ± 20	1.185 ± 0.035

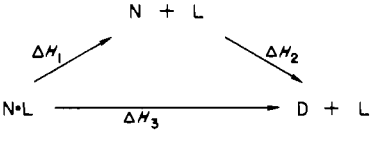
(B) Data Derived from Curve Fitting

ligand	Δh (cal g^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta H_{vH}(2)$ (kcal mol^{-1})[b]	SD (% c_{max})[c]
IPTG	5.13 ± 0.17	677 ± 22	494 ± 18	9.2 ± 0.3
ONPF	5.04 ± 0.16	655 ± 21	484 ± 10	10.4 ± 0.8

[a] Mean of values calculated from $\Delta H_{vH} = 9.01RT_m^2c_{max}/\Delta h$. [b] Mean of values obtained by minimizing the standard deviation of theoretical curve from observed data. [c] SD, standard deviation.

resentation of the irreversibility of the denaturation of core protein but only as a simple formulation that includes irreversibility. As an example of these simulations, the parameters for the reversible step were initially assigned to be $t_{1/2}$ = 62.10 °C, Δh = 5.00 cal g^{-1}, and ΔH_{vH} = 400 kcal mol^{-1}, and k was assigned the value 10^{-4} s^{-1} at 50 °C and E_a the value 30 kcal mol^{-1}. With these values at $[N]_0 = 5$ (any arbitrary units), $[I] = 0.1[N]_0$ when $\alpha = 0.5$, $[I] = 0.16[N]_0$ when $\alpha = 0.75$, and $[I] = 0.47[N]_0$ when $\alpha = 0.998$. At this last value of α, the half-time for the irreversible step is 12 min. Fitting the calculated values of c_{ex} to the theoretical curve for $N_4 \rightleftarrows 4D$ gives $t_{1/2}$ = 61.97 °C, Δh = 5.01 cal g^{-1}, and ΔH_{vH} = 445 kcal mol^{-1}, with a standard deviation equal to 1.3% of c_{max}. A van't Hoff plot of ln $[N]_0$ vs. $1/T)_{1/2}$ for $1 \leq [N]_0 \leq 128$ has a slope of 71.7, which gives ΔH_{vH} = 427 kcal mol^{-1}. We thus see that this irreversible process appears to foliow equilibrium thermodynamics and yields derived data very similar to those that would have been obtained had the reaction been fully reversible.

Denaturation of Core Protein in the Presence of Ligands. We have studied the effects on the thermal denaturation of core protein produced by the addition of an inducer, isopropyl β-D-thiogalactoside (IPTG), at seven concentrations from 0.50 to 50 mM and of an antiinducer, *o*-nitrophenyl β-D-fucoside (ONPF), at 10 concentrations from 1.01 to 26.5 mM. Both ligands cause increases in t_m and in enthalpy, with the effect of IPTG on t_m at a given concentration being greater than that of ONPF. The mean values (±standard errors) for the various thermodynamic parameters are listed in Table VA. The values of ΔH_{cal} are 148 and 168 kcal mol^{-1} higher in the presence of IPTG and ONPF, respectively, than in their absence (Table I). If we consider



it is evident that these differences, $\Delta H_3 - \Delta H_2$, must be attributed to the enthalpy of dissociation of the ligands, ΔH_1, amounting to 37 and 42 kcal (mol of ligand)$^{-1}$ for IPTG and ONPF. Donner et al. (1982) have determined the enthalpy of dissociation of IPTG from *lac* repressor to be 4.06 ± 0.65 kcal mol^{-1} at 25 °C and pH 7.0 in phosphate buffer and the change in heat capacity due to the dissociation to be only 48 cal K^{-1} mol^{-1} (in Tris buffer). Given the similarity in equilibrium and kinetic binding parameters for IPTG with repressor and core proteins (Friedman et al., 1977), it is difficult to understand how the energetics of ligand binding to *lac* repressor can be as different from that to core protein as is indicated by a comparison of our data with those of Donner et al.

The discrepancies between ΔH_{vH} and ΔH_{cal} are much larger for both ligands than observed with core protein in the absence of ligands, amounting to 45–50% as compared with 18% for core protein. A possible explanation for this is that intermediate states are much more significant in the presence of these ligands than in their absence, as also suggested by the relatively poor fit of the theoretical curves to the experimental data (see below).

Table VI: ΔH (kcal mol^{-1}) for the Denaturation of Core Protein in the Presence of Ligands

ligand	$\Delta H_{vH}(1)$[a]	$\Delta H_{vH}(2)$[b]	$\Delta H_{vH}(3)$[c]	$\Delta H_{cal}(4)$[d]
IPTG	422 ± 17	494 ± 18	723 ± 37	771 ± 26
ONPF	410 ± 20	484 ± 10	1073 ± 145	791 ± 40

[a] Mean of values calculated from $\Delta H_{vH} = 9.01RT_m^2c_{max}/\Delta h$. [b] Mean of values obtained by curve fitting. [c] From slopes of lines A (IPTG) and B (ONPF) in Figure 2. [d] Mean of values obtained by integration of the observed DSC curves.

The DSC curves in the presence of ligands showed asymmetry similar to that observed with the core protein alone. Fitting these data to the theoretical curve for reaction 4 by the method outlined above led to the thermodynamic quantities listed in Table VB. It is seen that the fits obtained as judged by the standard deviations were not quite as close as those obtained for the core protein alone, primarily due to the more gradual rise in c_{ex} during the first part of the denaturation, which also accounts for the lower values for Δh and ΔH_{cal} obtained by curve fitting. Here again the values for ΔH_{vH} are smaller than those for ΔH_{cal}, and by wider margins than found with core protein alone.

van't Hoff plots of ln $[L]_0$ vs. $1/T_m$ are given in Figure 2. Line A for IPTG has a coefficient of determination of 0.99 and line B for ONPF a coefficient of 0.93. The values of ΔH_{vH} derived from the slopes of these plots in accordance with eq 5 are given in column 4 of Table VI. Also listed in Table VI are the other enthalpy values derived from the DSC data. It is seen that the value for IPTG is reasonably close to ΔH_{cal} but much larger than the other two values for ΔH_{vH} for this system listed in columns 2 and 3, while in the case of ONPF $\Delta H_{vH}(3)$ is much larger than any of the other enthalpy values. This latter discrepancy would be removed if on average only three or less molecules of ONPF are lost per tetramer molecule during denaturation as a result of either fewer molecules being bound before denaturation or significant binding of the ligand by the denatured protein. The fact that the binding of ONPF to repressor is 100 times weaker than that of IPTG at 25 °C (Maurizot & Charlier, 1977) suggests that the former possibility is the more likely.

The DSC data for the denaturation of core protein in the presence of IPTG and ONPF lend further empirical support to the validity of applying equilibrium thermodynamics to these apparently irreversible reactions. It may also be noted that the good adherence of the data to van't Hoff plots indicates that although some conformational change undoubtedly takes place in each polypeptide chain when a ligand is bound to it, the steady increase in t_m with increase in ligand concentration

is to be attributed to a simple reversal of an equilibrium by addition of one of the products of the reaction, that is, the principle of Le Chatelier, rather than to some mysterious "stabilization" of the protein.

Registry No. IPTG, 367-93-1; ONPF, 1154-94-5.

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Native-like Folding Intermediates of Homologous Ribonucleases†

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ABSTRACT: The mechanism of the slow refolding reactions of four different pancreatic ribonucleases from ox, sheep, red deer, and roe deer has been investigated. Refolding kinetics of these proteins were very similar. In particular, a native-like intermediate, I_N, was shown to be populated on the slow refolding pathway of all ribonucleases. We conclude that, similar to the stability of the folded proteins, the pathway of slow refolding has been conserved despite the differences in amino acid sequence and the varying number of proline residues.

The three-dimensional structure of a folded polypeptide chain as well as the pathway of folding from the unfolded state to the native state is determined by the amino acid sequence of the protein (and by the native environment). The mechanism by which changes in primary sequence influence the pathway of folding can in principle be elucidated by a comparison of different mutant proteins from one species or of homologous proteins from different species, which have a similar three-dimensional structure but vary in the amino acid sequence (Crisanti & Matthews, 1981; Nall, 1983; Elwell & Schellman, 1979; Krebs et al., 1983; Hollecker & Creighton, 1983; Hawkes et al., 1984). The refolding characteristics of RNases[1] from ox, sheep, red deer, and roe deer have been examined recently and found to be very similar, despite the differences in primary sequences, which range from 4 to 17 substitutions (out of a total of 124 amino acids). In addition, the relative amplitudes of the fast and the slow refolding reactions were independent of the number of proline residues in the sequence (Krebs et al., 1983). These results suggested that the pathway of refolding, i.e., the sequence of intermediates and activated states on the pathway of folding, has been conserved during the evolution of these proteins. The mechanism of the major slow refolding reaction of bovine RNase A, which involves both folding and proline isomerization steps, has been characterized in considerable detail. Two intermediates of refolding have been detected on the slow refolding pathway: an early hydrogen-bonded intermediate, I_1 (Nall et al., 1978; Schmid & Baldwin, 1979; Kim & Baldwin, 1980), and a late native-like intermediate, I_N, which is compactly folded but still contains at least one incorrect proline isomer (Cook et al., 1979; Schmid & Blaschek, 1981). I_N accumulates transiently when folding is carried out under strongly native folding conditions (Schmid, 1983).

Here we ask whether the pathway of slow refolding of RNase A has been conserved. The RNases from sheep, red

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[1] Abbreviations: RNase, pancreatic ribonuclease (EC 3.1.27.5) with disulfide bonds intact; U_S^I, U_S^{II}, and U_F, slow- and fast-folding species of unfolded RNase; N, native RNase; I_1 and I_N, folding intermediates; 2′,3′-CMP, cytidine 2′,3′-phosphate; Gdn·HCl, guanidine hydrochloride; τ, time constant of a chemical reaction (reciprocal of the apparent rate constant, k^{-1}).

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