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Unfolding of *lac* Repressor and Its Proteolytic Fragments by Urea: Headpieces Stabilize the Core within *lac* Repressor†

Manfred Schnarr and Jean-Claude Maurizot*

ABSTRACT: Circular dichroism measurements were used to compare the urea-induced unfolding transition of the *lac* repressor with those of its separated tryptic fragments, the tetrameric core, and the N-terminal headpiece. The presence of the headpieces covalently linked to the core in the intact repressor leads to a stabilization against urea denaturation as compared to that for the isolated core. This results in a shift of the midpoint of the transition by about 0.5 M urea. When the inducer isopropyl β-D-thiogalactoside is bound, the core is stabilized more than the entire repressor. The isolated headpiece is considerably more stable against urea denaturation than the tryptic core or the *lac* repressor. The reversible denaturation process of the headpiece was quantitatively analyzed, and the free energy of unfolding in the absence of urea was found to be 2.4 or 2.9 kcal/mol, depending on the method of calculation used. Comparison between the circular dichroism spectra of the *lac* repressor, the tryptic core of the *lac* repressor, and the headpiece supply further evidence that there are no major conformational differences between the structural domains (core and headpieces) before and after proteolytic cleavage of the *lac* repressor. These results are discussed with respect to the contacts between the different domains of the protein. It is concluded that relatively weak interdomain contacts are probably responsible for the stabilization of the core by the covalently linked headpieces and that these contacts might be weakened upon binding of the inducer.

The *lac* repressor is a tetrameric protein which controls the expression of the structural genes of the *lac* operon by binding to the *lac* operator (Müller-Hill, 1975; Bourgeois & Pfahl, 1976). There is no doubt now that this protein, like many other proteins, is composed of several domains. These domains, which may be obtained by limited proteolysis of the *lac* repressor at high ionic strength, are a tetrameric core (4 × residues 60–360) and four headpieces (residues 1–51 or 1–59, depending on the time of the hydrolysis). The tetrameric core

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0006-2960/81/0420-6164$01.25/0

exhibits full inducer binding activity, whereas the N-terminal headpiece is involved in the binding to nucleic acids (Platt et al., 1973; Geisler & Weber, 1977; Ogata & Gilbert, 1978, 1979; Jovin et al., 1977). However, there is no doubt that these domains are not fully independent since the binding of the inducer to the protein influences its binding to the *lac* operator DNA. Hence, studies on the relationships between these domains of the protein are of great interest to understand the structural organization of the *lac* repressor.

In this paper, we present results on this organization obtained by comparing the denaturation of the intact *lac* repressor, its tryptic core, and its N-terminal headpiece. Attempts were made to study the thermal denaturation, but unfortunately, thermal denaturation of the *lac* repressor and of its tryptic core is accompanied by precipitation of the proteins. Therefore, we used urea as the denaturing agent. It was chosen instead of guanidine hydrochloride, which has a more efficient denaturing power, because the denaturations were followed by the circular dichroism of the peptide chromophores. In this wavelength region, urea absorbs less than does guanidine hydrochloride and allows more precise measurements. Furthermore, it has been shown in many cases that the mechanism of denaturation is similar for the two denaturants (Pace, 1975).

One possible way for the inducer to influence the binding on *lac* operator is to change the structural organization of the various domains of the protein. This is why we have also investigated the effect of the gratuitous inducer isopropyl β-D-thiogalactoside (IPTG)[1] on the urea denaturation of the *lac* repressor and its tryptic core.

Experimental Procedures

The *lac* repressor from *Escherichia coli* BMH 493 (a gift of Dr. B. Müller-Hill and Dr. K. Beyreuther) was purified as described by Müller-Hill et al. (1971), with the modifications proposed by Rosenberg et al. (1977). The phosphocellulose column step was followed by gel filtration on Ultrogel AcA 34. The *lac* repressor was typically more than 98% pure as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis. It showed four binding sites per tetramer for IPTG as determined by equilibrium dialysis, and it was fully active in binding to the operator as determined by the membrane filtration technique (Riggs et al., 1968), assuming that there is one operator binding site per tetramer. The tryptic core of the *lac* repressor was prepared by incubation of the proteins in a buffer containing 1 M Tris–HCl, pH 7.5, 30% glycerol, and 0.01 M Me with trypsin (beef, treated with TPCK) as described by Geisler & Weber (1977). Proteolysis was stopped after 3 h by the addition of soybean trypsin inhibitor, and the products were fractionated on a Sephadex G-150 column. Tryptic digestion for 3 h produces pure short headpiece 1–51 as shown by Ogata & Gilbert (1978). The headpiece fraction was concentrated by lyophilization and further purified on a Sephadex G-50 column. Purity of the core and the headpice was assessed by $NaDodSO_4$–polyacrylamide electrophoresis. The core exhibited an affinity for IPTG identical with that of *lac* repressor. By circular dichroism, it was shown that the headpiece could bind to poly[d(A-T)].

The ionic strength of the core and the repressor solutions was adjusted by dialysis, that of the headpiece by appropriate dilution of a concentrated stock solution. Concentrations were determined from absorption measurements by using molecular extinction coefficients of ϵ_{280} = 21 400 L·mol^{-1}·cm^{-1} (Charlier et al., 1977) for the *lac* repressor, ϵ_{280} = 16 600 L·mol^{-1}·cm^{-1} for the core, and ϵ_{275} = 5600 L·mol^{-1}·cm^{-1} for the headpiece. The samples were prepared in 0.2 M potassium phosphate buffer, pH 7.25, 10^{-4} M DTE, and the appropriate urea concentration. Urea was recrystallized twice from ethanol before use, and the urea concentration of each sample was determined by measuring its refractive index. Samples were stored for 6 h at 22 °C and for about 12 h at 4 °C before CD spectra were taken.

The CD measurements were made with a Jobin Yvon Mark III dichrograph at 20 °C by using cells with a path length of 0.1 cm or, occasionally, of 0.5 cm for diluted samples in the study of renaturation. Each spectrum was run at least 2 times. The ellipticity, [θ], is expressed per residue.

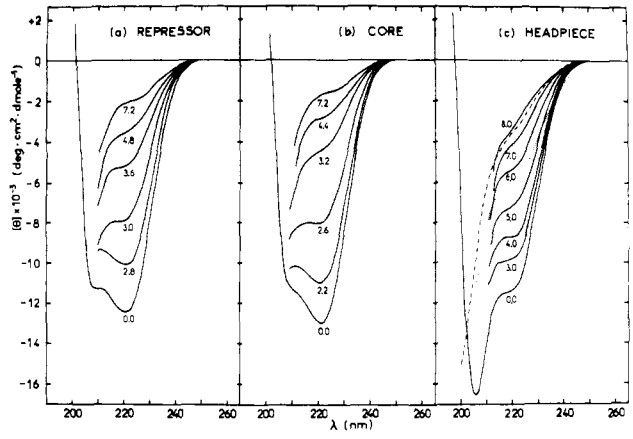

FIGURE 1: CD spectra of *lac* repressor (a), tryptic core (b), and short headpiece in 0.2 M potassium phosphate buffer, pH 7.25, and 10^{-4} M DTE at the indicated molar concentrations of urea at 20 °C. In (c) are included the CD spectra of the headpiece, thermally denatured (···) and fully cleaved by trypsin (---). [θ] is the ellipticity expressed per residue.

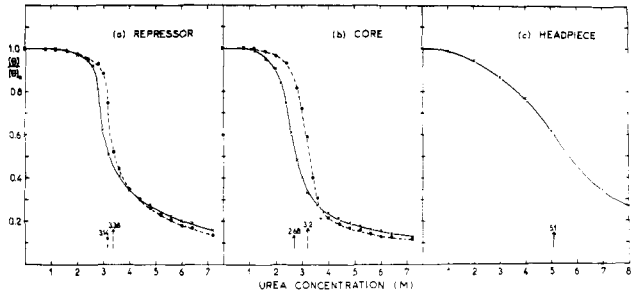

FIGURE 2: Variation of the relative CD intensity for the *lac* repressor (a), its tryptic core (b), and the headpiece (c) at 222 nm as a function of the urea concentration (×) without IPTG and (●) with 1.8 × 10^{-3} M IPTG at 20 °C. The arrows indicate the midpoints of the transition without (solid arrow) and with (broken arrow) IPTG.

Results

Denaturation of the Headpiece. Figure 1c shows the decrease of the CD signal of the headpiece upon unfolding by increasing concentrations of urea. For comparison, we have also included the final spectra of the headpiece after thermal denaturation (which is reversible in the case of the headpiece) and of its complete tryptic hydrolysate. The intensity of the CD signal in 8 M urea is smaller than that of the thermally denaturated state, indicating a higher degree of unfolding by urea than by heat as observed for several other proteins (Pace, 1975). Nevertheless, it can be seen in Figure 2c that even at 8 M urea the unfolding of the headpiece is not complete. The transition plotted as the relative decrease of the intensity of the CD signal is broad as compared with those generally

[1] Abbreviations used: IPTG, isopropyl β-D-thiogalactoside; Tris, tris(hydroxymethyl)aminomethane; ME, 2-mercaptoethanol; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; CD, circular dichroism; DTE, dithioerythritol; $NaDodSO_4$, sodium dodecyl sulfate.

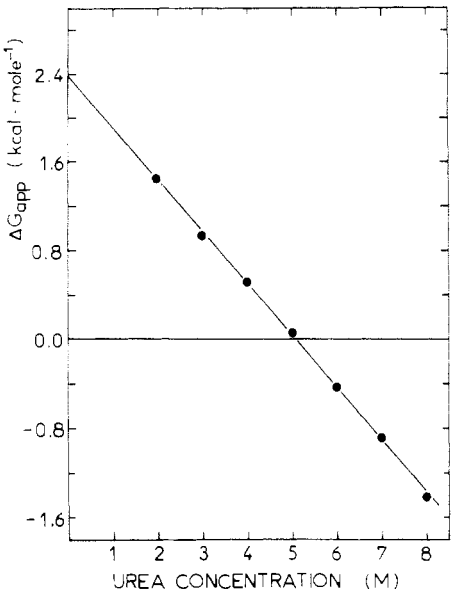

FIGURE 3: Variation of the free energy of unfolding, ΔG_{app}, of the headpiece, calculated from eq 1 as a function of urea concentration. The straight line was fitted to eq 2, giving values of $\Delta G_{app}^{H_2O}$ = 2.4 kcal/mol and m = 0.47 kcal L mol^{-2}.

observed with other proteins [for example, ribonuclease or lysozyme (Greene & Pace, 1974)]. A 5-fold dilution of a 8 M urea sample with buffer restored the original CD intensity, indicating that the urea denaturation of the headpiece is reversible.

A quantitative analysis of the denaturation curve yields thermodynamic parameters related to the stability of the native state of the protein and allows comparison with other proteins. We have used the data treatment proposed by Greene & Pace (1974). With the assumption of a two-state unfolding mechanism, the equilibrium constant, K, and the free energy of denaturation, ΔG_{app}, can be determined from the experimental data by the relationship

$$K = \exp[-\Delta G_{app}/(RT)] = \frac{[\theta]_N - [\theta]}{[\theta] - [\theta]_D} \quad (1)$$

where $[\theta]$ is the observed ellipticity for a given concentration of urea and $[\theta]_N$ and $[\theta]_D$ are the ellipticities of the native and denatured states, respectively. ΔG_{app} for the unfolding of the headpiece varies linearly with the urea concentration (Figure 3). A least-squares analysis was used to fit these data to eq 2 proposed by Greene & Pace (1974):

$$\Delta G_{app} = \Delta G_{app}^{H_2O} - m[\text{urea}] \quad (2)$$

We find values of m = 0.47 kcal L mol^{-2} and $\Delta G_{app}^{H_2O}$ = 2.4 kcal/mol. These values are influenced very little by the choice of the final ellipticity $[\theta]_D$, which has been extrapolated from the transition curve in Figure 2c. The small value of m, as compared with the values obtained by Green & Pace (1974) for several proteins, reflects quite well the unusual broadness of the transition curve. Analysis of the thermal denaturation in the same buffer (M. Schnarr and J. C. Maurizot, unpublished experiments) yields comparable values of the free energy change of denaturation, $(\Delta G_{app})_{20°C}$ = 2.0 ± 0.8 kcal/mol.

Denaturation of the Tryptic Core. With increasing concentrations of urea, the tryptic core of the *lac* repressor exhibits a rather sharp decrease of the CD signal with a midpoint of the transition at 2.68 M urea (Figures 1b and 2b). At the highest concentration of urea, the CD signal is very close to that usually observed for the random coil conformation of proteins.

The same denaturation process was followed in the presence of a saturating (1.8×10^{-3} M) concentration of the inducer IPTG. The denaturation curve is then shifted to higher concentrations of urea, the midpoint being at 3.2 M instead of at 2.68 M. At the highest concentrations of urea, the CD signals in the presence and in the absence of IPTG are very similar.

Denaturation of the lac Repressor. As for the tryptic core, urea induces a very large decrease of the CD signal of the *lac* repressor. A very sharp decrease with a midpoint of 3.14 M is observed first and is followed by a slight decrease between 3.5 and 7.2 M urea (Figures 1a and 2a). This second step is not observed in the case of the core. It might represent the unfolding of the four headpieces of the *lac* repressor after the denaturation of the main part of the protein.

It is to be noticed that the midpoint of the transition for the *lac* repressor is at a higher concentration of urea than that of the core (3.14 M instead of 2.68 M).

As for its tryptic core, addition of saturating concentrations of IPTG to the *lac* repressor leads to a shift of the transition toward a higher concentration of urea (3.36 M instead of 3.14 M), but the shift observed for the core is clearly larger than that for the intact repressor.

Neither for the *lac* repressor nor for its tryptic core is the denaturation fully reversible upon dilution. A 5-fold dilution of a sample containing 6.0 M urea with 0.2 M phosphate buffer restored only 60% of the initial CD intensity of both proteins. Therefore, the results obtained for the core and for the *lac* repressor could not be used for the same quantitative thermodynamic analysis as those of the headpiece. Nevertheless, they can be used to obtain qualitative information on the relative stability of the proteins as for example in the case of the irreversible heat inactivation of the β_2 subunit of *E. coli* tryptophan synthetase (Zetina & Goldberg, 1980).

Discussion

Reversible Unfolding of the Headpiece. The urea denaturation curve of the headpiece indicates a relatively high resistance of its structure to the action of this perturbant. With the assumption of a value of $[\theta]/[\theta]_0$ equal to 0.2 for the completely unfolded state by urea, as done for the calculation of ΔG_{app} (Figure 3), the midpoint of the transition appears at 5.1 M urea.

The weak cooperativity of the transition, as reflected by the small value of m (0.47 kcal L mol^{-2}), is remarkable but is difficult to explain unambiguously. One possible explanation could be that the low value of m may reflect a deviation from the assumed two-state mechanism as observed, for example, in the case of the unfolding of human immunoglobulin light chains by guanidine hydrochloride (Rowe & Tanford, 1973). Another explanation could be that the low value of m simply reflects the small size of the peptide (M_r = 5700). As Greene & Pace (1974) have pointed out, m should decrease if the molecular weight of the protein decreases. To test this hypothesis, we used the approach developed by Tanford (1970) based on model compound data. Tanford has shown that the free energy of unfolding can be calculated if the number and type of residues which are freshly exposed to denaturant by unfolding are known:

$$\Delta G_{app} = \Delta G_{app}^{H_2O} + \sum \Delta\alpha_i n_i^0 \delta g_{tr,i} \quad (3)$$

where $\Delta G_{app}^{H_2O}$ is the free energy of unfolding in water, $\Delta\alpha_i$ is the average fractional change in the degree of exposure to solvent of groups of type i, $\delta g_{tr,i}$ is the free energy of transfer of a group of type i from water to denaturant, and n_i^0 is the total number of groups of type i present in the protein. The

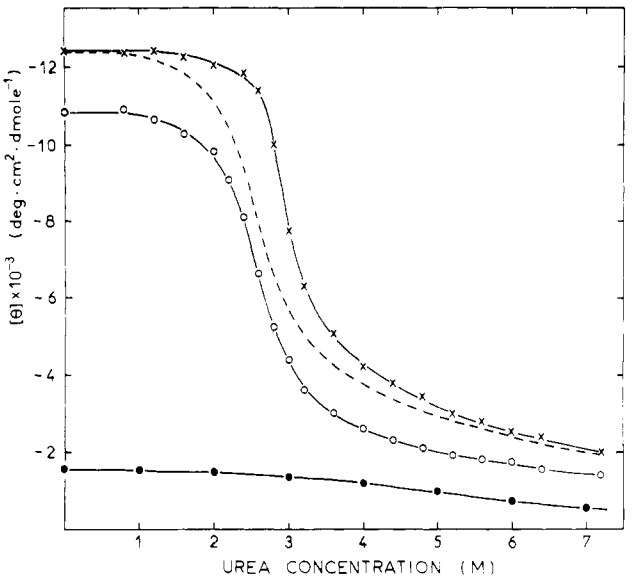

FIGURE 4: Comparison between the experimental (×) and the calculated (---) (according to eq 4) CD intensity at 222 nm as a function of urea concentration: (○) represents the contribution of the core and (●) the contribution of the headpiece to the calculated transition curve (see text).

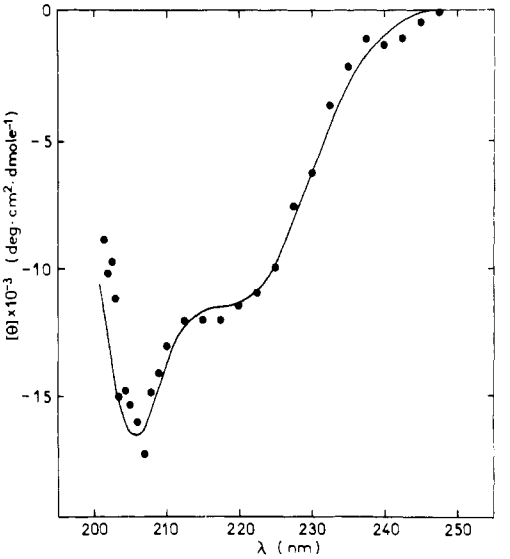

FIGURE 5: Comparison between the ellipticities (expressed per residue) of the headpiece, experimentally determined (—) and calculated from the spectra of the *lac* repressor and its tryptic core according to eq 5 (●).

values of $\delta g_{tr,i}$ have been determined from solubility measurements on amino acids and related derivatives.

We have used the values of $\delta g_{tr,i}$ compiled by Pace (1975) to determine a single average value $\Delta\bar{\alpha}$, corresponding to all the groups, which leads to a calculated value of m identical with the value determined experimentally. We find $\Delta\bar{\alpha} = 0.26$, which is comparable to values usually determined for proteins. For example, Greene & Pace (1974) found values of 0.34 for ribonuclease, 0.21 for lysozyme, 0.21 for α-chymotrypsin, and 0.34 for β-lactoglobulin.

The fact that the value of $\Delta\bar{\alpha}$ is found within this range does not prove that the unfolding of the headpiece is a two-state process, but it indicates that the low value of m may be expected for the headpiece just from its small size and its amino acid composition. From the relationship in eq 3, it is also possible to determine $\Delta G_{app}^{H_2O}$. A value of 2.9 kcal/mol was determined. This is slightly larger than the value obtained from eq 2 as it is generally observed for the Tanford method (Pace, 1975).

Headpieces Stabilize the Core within the lac Repressor. If the tryptic core and the headpieces were fully independent domains in the *lac* repressor, it should be possible to calculate the denaturation curve of the *lac* repressor, $[\theta]_{Rep}^{cald}([u])$, from the denaturation curves of these domains, using the relationship

$$[\theta]_{Rep}^{cald}([u]) = \frac{300}{360}[\theta]_{core}^{exptl}([u]) + \frac{51}{360}[\theta]_{HP}^{exptl}([u]) \quad (4)$$

where $[\theta]_{Core}^{exptl}([u])$ and $[\theta]_{HP}^{exptl}([u])$ are the measured ellipticities per residue of the tryptic core and the headpiece at the concentration of urea ([u]). This relationship is only a approximate one since it does not take into account the contribution of the peptide 52–59 which would be $(8/360)[\theta]_{52-59}^{exptl}$. In any case, the weight of this contribution is very small, about 2%, if the peptide 52–59 has the same ellipticity per residue as that of the whole repressor, and it can be neglected without a major effect on the calculation.

Figure 4 shows the comparison between the ellipticities at 222 nm calculated, according to eq 4, and determined experimentally as a function of the urea concentration. The two curves coincide quite well for low and high urea concentrations, but around the midpoint of the transition, the curve determined experimentally is shifted to a higher concentration of urea by about 0.4 M when compared to the calculated curve. Thus, it is clear that the presence of the headpiece, covalently linked to the core, enhances the stability of the *lac* repressor against denaturation by urea.

This stabilization against denaturation may be explained by a conformational change of one of the domains (or both) in the presence of the other and or by contacts between these domains. We will now discuss these two possibilities, taking into account our results and previously published results on the *lac* repressor.

If there is no major conformational change of the domains when included in the *lac* repressor, one can calculate the CD spectrum of the headpiece within the *lac* repressor from the CD spectrum of the *lac* repressor and that of the core according to the relationship

$$\frac{51}{360}[\theta]_{HP}^{cald}(\lambda) = \frac{360}{360}[\theta]_{Rep}^{exptl}(\lambda) - \frac{300}{360}[\theta]_{Core}^{exptl}(\lambda) \quad (5)$$

where $[\theta]_{Rep}^{exptl}(\lambda)$ and $[\theta]_{Core}^{exptl}(\lambda)$ are the ellipticities per residue of the repressor and the core. As in the case of eq 4, the contribution of the peptide 52–59 may be neglected. Figure 5 shows that the experimental and calculated CD spectra of the headpiece coincide quite well. Of course, we cannot exclude the possibility that a conformational change of the headpiece when included in the *lac* repressor is exactly compensated by a conformational change of the core, but this seems very unlikely. Thus, these CD measurements strongly support the idea that the presence of the headpiece in the *lac* repressor does not induce a major conformational change of the core and vice versa.

For the tryptic core, this was already well established by the use of local probes such as the fluorescence of the tryptophan residues (J.C. Maurizot and M. Charlier, unpublished experiments), the differential absorption of aromatic residues (Matthews, 1974), and the induced circular dichroism of bound effector (Maurizot & Charlier, 1977) and chemically bound reporter groups (Burgum & Matthews, 1978).

More global methods like hydrogen exchange (Ramstein et al., 1979) and infrared spectroscopy (Schnarr & Maurizot, 1980) also indicated the lack of conformational change in the core when isolated. For the headpieces, recent NMR results

(Buck et al., 1978; Wade-Jardetzky et al., 1979) showed similar chemical shifts of the proton resonances of the four tyrosines of the isolated and in situ headpieces, suggesting the similarity of the structures. This was further supported indirectly by the experiments showing that the pattern of chemical modification of the *lac* operator by dimethyl sulfate obtained in the presence of the headpieces was similar to that obtained in the presence of the *lac* repressor (Ogata & Gilbert, 1979).

We can thus exclude the hypothesis that the stabilization effect against urea denaturation is due to a major conformational change of either the core or the headpieces when included in the intact *lac* repressor. We can also exclude the existence of strong noncovalent contacts between the different domains of the protein. Recent hydrogen-exchange experiments (Ramstein et al., 1979) showed the same kinetics of exchange for the *lac* repressor and for its tryptic core in the absence of inducer. Strong contacts should increase the number of relatively slowly exchanging hydrogen atoms. Furthermore, NMR results (Wade-Jardetzky et al., 1979) suggested a high relative mobility of the headpieces within the *lac* repressor which could not be observed in the presence of strong interdomain contacts.

Thus, we have to explain the stabilization of the core in the presence of the covalently linked headpieces probably by the existence of relatively weak interdomain contacts which could not be detected by the methods used so far.

Influence of IPTG on the Denaturation. The presence of saturating concentrations of the inducer IPTG stabilized the *lac* repressor and its tryptic core by shifting the denaturation curves to higher concentrations of urea. However, it is interesting to note that the stabilizing effect is more pronounced for the core, with a shift of the midpoint of the transition from 2.68 to 3.2 M urea, than for the repressor (from 3.14 to 3.36 M urea). Thus, in the presence of inducer, *lac* repressor is only slightly more stable against urea denaturation than its tryptic core.

It has been reported that the addition of high concentrations of sugar may lead to a stabilization of proteins by solvent effects (Back et al., 1979). The low concentration of sugar we have used excludes this possibility, and the observed effects must be due to the binding of IPTG to its site.

We have found that the unfolded state does not exhibit any IPTG binding activity; therefore, since the inducer molecule has a higher affinity for the native conformation, a stabilization effect is expected (Pace & McGrath, 1980). But with the equilibrium constants of association of IPTG to *lac* repressor and to its tryptic core being identical (Platt et al., 1973; Friedman & Matthews, 1979), a similar stabilization should be observed for both proteins. In fact, the stabilization shifts the midpoint of the transition by about 0.5 M urea for the core and by only 0.2 M for the repressor.

This difference in stabilization upon binding of IPTG is probably due to a modification of the interaction between the domains "core" and "headpieces" in the presence of the inducer. Based on the presumed existence of weak interdomain contacts, we propose that the binding of inducer may weaken or abolish these contacts and counterbalance in part the stabilization effect due to the higher affinity of IPTG for the native conformation.

Several hypotheses have been formulated to explain the effect of the inducer on the repressor–operator interactions. Ogata & Gilbert (1979) have proposed that the decrease of the binding constant of the *lac* repressor for its operator is due to an increase of the mobility of the headpieces in the presence of inducer. Decreased contacts between the core and the headpieces could in fact lead to an increased motional freedom of the headpieces.

Based on the fact that the core binds the *lac* operator in an inducer-sensitive fashion, it has been proposed that the well-established conformational change of the core domain upon binding the inducer results in a decreased affinity of this core region for the central symmetrical base sequence of the *lac* operator (Matthews, 1979; O'Gorman et al., 1980). This might very well be accompanied by a change in the contacts between the core and the headpiece as we propose here. In any case, it should be kept in mind that the overall mass distribution in space of the *lac* repressor must be maintained, since the radius of gyration of the protein remains unchanged upon addition of IPTG as shown by neutron-scattering experiments (Charlier et al., 1980).

Acknowledgments

We thank A. Gervais for his help in the preparation of *lac* repressor and Dr. M. Charlier and Professor C. Hélène for numerous stimulating discussions. We thank Professor A. Jonas for critical reading of this manuscript.

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Nuclear Magnetic Resonance Study of Dihydrofolate Reductase Labeled with [γ-^{13}C]Tryptophan†

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ABSTRACT: Dihydrofolate reductase isozyme 2 of *Streptococcus faecium* has been labeled with ^{13}C in the C_γ position of tryptophan residues by growing the organism on a defined medium containing L-[γ-^{13}C]tryptophan (90% ^{13}C). The ^{13}C nuclear magnetic resonance (NMR) spectrum of the enzyme shows four well-resolved resonances which have nuclear Overhauser enhancements of 1.1–1.3. Values of T_1 (spin–lattice relaxation time) and T_2 (spin–spin relaxation time) are significantly less than predicted for an isotropically rotating, rigid sphere with no intermolecular dipole–dipole interactions. Three of the resonances have chemical shifts downfield from the ^{13}C resonance of urea-denatured enzyme by amounts up to 1.43 ppm. The chemical shift of resonance 4 in the spectrum is 4.0 ppm upfield from Trp C_γ of urea-denatured enzyme. This large upfield shift is attributed to electric field effects generated by polar side chains. The two more upfield peaks both provide evidence that the corresponding tryptophan residues, W_C and W_D, each undergo chemical exchange between alternative microenvironments. In the case of W_C, which gives a resonance with two components, exchange is slow (ν_e, exchange rate $\ll 55$ s^{-1}), and the relative populations of the two stable states are in the ratio 2:3. W_D is apparently in intermediate to fast exchange on the NMR time scale. With a two-state model, ν_e increases from approximately 90 to 150 s^{-1} as the temperature is increased from 5 to 25 °C. This increase in temperature is also accompanied by an increase in the fractional population of the minor downfield state(s), from about 0.062 at 5 °C to 0.24 at 25 °C. However, the data may also be interpreted as a temperature-dependent equilibrium between a continuum of many states. W_D is tentatively identified with Trp-22 since comparison of the sequences of *Lactobacillus casei* dihydrofolate reductase and *S. faecium* dihydrofolate reductase and inspection of the crystal structure of the *L. casei* enzyme indicate that Trp-6, Trp-115, and Trp-160 are probably all involved in regions of β sheet whereas Trp-22 is in a loop joining βA to αB. Earlier crystallographic evidence for the *Escherichia coli* reductase suggests that in the methotrexate complex with this enzyme the corresponding loop has a good deal of flexibility. It is probable that in the uncomplexed *S. faecium* reductase the motion of this loop is the major mechanism for the exchange process involving Trp-22. The upfield chemical shift of resonance 4 is attributed to electric field effects on C_γ of Trp-22 produced by the carboxylate groups of Asp-27 and Asp-9. On the basis of the small difference between the chemical shift of resonance 3 and that of tryptophan C_γ in urea-denatured reductase, it is suggested that W_C may be identified with Trp-6.

Fourier transform nuclear magnetic resonance (NMR), because it allows the direct observation of the behavior of specific atomic nuclei in proteins, has proved a powerful tool for obtaining information about many aspects of the solution behavior of proteins. These include the interaction of specific side chains of the protein with ligands or metal ions, conformational transitions induced in the protein by ligand binding or by changes in temperature or ionic strength, and particularly the dynamics of various motions of the protein (London, 1980). Dihydrofolate reductase (EC 1.5.1.3, DHFR) is especially suitable for such studies because of its rather small size, and reductase from *L. casei* has been investigated by ^{1}H NMR observation of the protein (Feeney et al., 1977; Birdsall et al., 1977; Feeney et al., 1980; Gronenborn et al., 1981a) and by ^{19}F NMR after incorporation of 3-fluorotyrosine and 6-fluorotryptophan (Kimber et al., 1977). However, in these studies relatively little information has been obtained about the dynamics of the motion of this protein until recently (Gronenborn et al., 1981b).

^{13}C NMR has certain advantages in such studies, particularly when the isotope is highly enriched in specific positions of specific side chains. Under such conditions, the very low sensitivity encountered in NMR of natural abundance ^{13}C is largely circumvented, and a relatively simple spectrum is obtained which under ideal conditions shows a single, unsplit resonance for each ^{13}C-enriched side chain in the protein. The

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0006-2960/81/0420-6169$01.25/0