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COMPARISON OF THE E. COLI LAC REPRESSOR AND ITS TRYPTIC CORE BY HYDROGEN EXCHANGE STUDIES. EFFECT OF INDUCER BINDING

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Summary

Using the hydrogen exchange technique we have compared the E. Coli Lac repressor and its tryptic core. The effect of isopropyl-β-D-thiogalactoside (IPTG) binding has also been examined. Within the limits of experimental errors the hydrogen exchange curves for both the repressor and the core are the same. This supports the conclusion that "headpieces" (N-terminal fragments released by limited tryptic hydrolysis) and core are rather independent structural units. The binding of IPTG to the core does not affect its hydrogen exchange curve whereas the binding of IPTG to the repressor results in the blocking of a least ten protons. This result demonstrates that the headpiece is involved in the conformational change induced by the binding of IPTG.

Introduction

Lac repressor controls the expression of the lactose operon by binding to the lac operator. Sugarslike allolactose or isopropyl-β-D-thiogalactoside (IPTG) act as inducers of the operon by binding to the protein and releasing it from the DNA.

Genetic experiments have shown that the binding sites for IPTG and for the operator are located on different regions within the protein (for review and discussion see ref. 1-2). This conclusion is also supported by the results of experiments using a biochemical approach. The action of trypsin on lac repressor is very restricted and under appropriate experimental conditions digestion of the lac repressor produces a mixture of monomeric

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amino-terminal fragments called "headpieces" accounting for residues 1-51 and 1-59 together with a trypsin-resistant tetrameric core (3). The tetrameric core has full inducer binding activity but is devoid of any detectable DNA binding activity (4). On the other hand, it has been shown recently by Jovin et al. that the headpiece of E. coli lac repressor binds to DNA (5). It is thus very likely that the amino-terminal region is involved in the non specific as well as in the specific DNA binding whereas inducer binding activity is located within the tetrameric core.

How the binding of IPTG can influence the binding of the operator is still poorly understood. The fact that both binding sites are located within different regions of the protein rules out any direct competition effect. Thus the most plausible explanation is that IPTG is an allosteric effector whose binding switches the conformation of the protein to a new one with lower affinity for the operator. Several techniques like absorption, circular dichroism and fluorescence spectroscopy have been used in order to provide evidence for such conformational changes (6-9). Although changes are seen which might reflect a versatility of the protein structure a clear correlation between a conformational change of the lac repressor and the allosteric inducer effect of IPTG has still not been demonstrated. In connection with this problem we report here a hydrogen exchange (HX) study of the lac repressor and of its tryptic core. The effect of IPTG binding has also been examined.

Materials and Methods

E. coli lac repressor from strain BMH 493 was purified according to the method described by Müller-Hill et al. (14). Tryptic digestions were performed in 1 M Tris-HCl (pH 7.5) 30 % glycerol. The amino terminal segment and the core were separated according to Geisler and Weber (15). Concentrations of lac repressor and core were determined from UV absorption measurements using ε_{280} = 21,400 and 16,600 per protomer for the lac repressor and the tryptic core, respectively.

The tritium Sephadex method of Englander was used (16). All exchange experiments were carried out at 0°C in KPD buffer (0.2 M potassium phosphate pH 7.25, 0.1 mM dithioerythritol (DTE)). Exchangeable hydrogen sites were labelled with tritium by incubating 0.5 ml of the protein solution (0.5 ml, 2×10^{-4} M protomer) with tritiated water (10 µl, 1 Ci/ml) for 16 hours and a half. To initiate the exchange of protein-bound tritium the sample was passed through a Sephadex column (G 25 fine grade, 1 cm x 7 cm) previously equilibrated with KPD buffer. This column removed the free tritium and the protein peak was collected in a test tube. In order to measure the amount of tritium still bound at any given time t aliquots of 250 µl were

passed through a second Sephadex column (G 25, 1 cm x 4 cm) equilibrated with KPD buffer. This column removed the tritiated water formed during time t. The protein peak was collected in four test tubes and for each tube the concentration of protein and the radioactivity were measured. The number of hydrogens per protomer remaining unexchanged at time t was calculated from the ratio of tritium counts per protomer (16).

Radioactivity was measured by pipetting 0.5 ml of the aqueous samples into 5 ml of liquid scintillation counting mixture (BRAY) and counting in an Intertechnique SL 30 spectrometer. Protein concentrations were determined from fluorescence intensity measurement. Fluorescence intensities were measured with a Jobin Yvon spectrofluorimeter modified to correct for fluctuations in lamp intensity. When complexes with IPTG were investigated experimental conditions were chosen to ensure saturation of the IPTG binding sites during the whole exchange of the protein bound tritium. For this purpose the first and second columns were equilibrated with KPD buffer containing 2 mM IPTG and 0.1 mM IPTG, respectively.

Results and Discussion

The HX curves for the lac repressor and its tryptic core in the absence as well as in the presence of IPTG are presented on Fig. 1. The following observations can be made : i) within the limits of experimental errors there is no difference between the HX curves of the lac repressor and of its tryptic core ; ii) the comparison of the HX curves of lac repressor and its tryptic core in the absence and in the presence of IPTG shows that IPTG binding does not affect the hydrogen exchange behavior of the core whereas the hydrogen exchange kinetics of the lac repressor is slowed down upon IPTG binding.

Before discussing these results it is worth recalling briefly some general aspects of hydrogen exchange in a structured protein. Peptidic hydrogens of a structured protein exchange with grossly differing rates depending on the accessibility of the solvent and on the extent to which these hydrogens are exposed to solvent by structural fluctuations (10). Freely exposed peptidic hydrogens exchange very rapidly and under our pH and temperature conditions their half times are in the second time range (11) and thus they do not appear in the curves of fig. 1. On the contrary when these hydrogens are involved in internal hydrogen bonds their exchange rate is slowed down by one to six orders of magnitude (11). It has been shown that the exchange rates of peptidic hydrogens are very sensitive to the conformation of the protein (10).

As we have already mentioned the lac repressor and the tetrameric core have identical HX curves. Unless there are unlikely compensa-

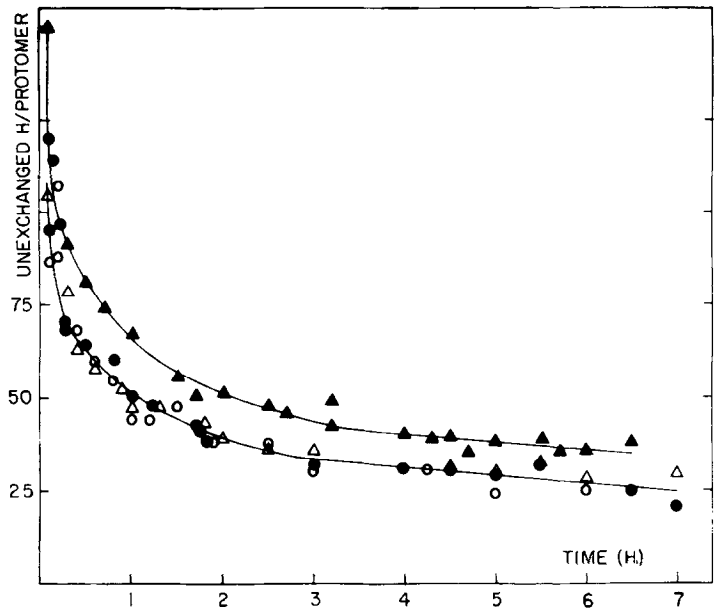

Figure 1 : Absolute unexchanged proton per protomer as a function of time for the lac repressor (●), the core (o), the complex IPTG-lac repressor (▲) and the complex IPTG-core (Δ). Buffer : 0.2 M potassium phosphate, pH 7.25, 0.1 mM dithioerythritol (DTE). Incubation time 16 hours and a half. Temperature 0°C.

ting effects (for example the disappearance of some labile hydrogens is cancelled by the appearance of the same number of new exchangeable hydrogens with exactly the same kinetic properties) this result means that i) all the labelled hydrogens of the lac repressor are found unaffected in the core, e.g. their number and their rates of exchange are not modified by the excision of about sixty amino acids ; ii) the hydrogens of the "headpieces" do not contribute significantly to the HX curves of the lac repressor either because they exchange too fast or because they exchange so slowly that they were not replaced by tritium in the sample of lac repressor which was incubated in ^3H_2O for 16 hours and a half at 0°C. Our data seem to indicate that the presence of the "headpiece" plays no role in determining the structure of the rest of the lac repressor molecule. In other words the "headpiece" does not contribute to the stability of the core structure and this suggests that "headpieces" and core must have only a few points of interaction. If the "headpieces" are not tangled into the core they must be easily accessible to solvent and its peptidic hydrogens are then expected to exchange too rapidly to be measured by our technique. Thus the fact that the peptidic hydrogens of the headpieces do not

appear in the HX curve of the lac repressor can be readily accounted for by the absence of effect of the headpieces on the core structure.

Techniques like fluorescence, circular dichroism or difference absorption did not show any differences when comparing the binding of IPTG to the lac repressor and to its tryptic core (12, 13). The data of figure 1 reveals a marked difference : the binding of IPTG does not change the HX curve of the core whereas its binding to the intact repressor shifts the HX curve upwards. From the essentially parallel curves obtained with the lac repressor in presence and absence of IPTG it is seen that the binding of IPTG prevents or blocks about 10 hydrogen atoms from exchanging. This number can only represent a lower limit of the number of blocked hydrogens because i) we do not know if the lac repressor is fully labelled under our experimental conditions of incubation ; ii) this difference might be entirely due to blocking or to a superposition of blocking and release of hydrogen atoms. Whatever the exact difference it is reasonable to attribute the blocking of some hydrogen atoms to a conformational change of the lac repressor upon IPTG binding. The fact that no blocking takes place when IPTG binds to the core demonstrates that the "headpieces" are involved in this conformational change. The blocked hydrogen atoms may result either from a structural reorganization of the headpieces or from a conformational change of the whole lac repressor molecule involving the formation of new internal hydrogen bonds between the "headpieces" and the core. Such an interpretation is in line with the conclusion of Geisler and Weber (3). The high selectivity of proteolytic cleavage between amino acid residues 50 and 60 suggests that this region forms a hinge between the "headpiece" and the core.

In conclusion our study suggests that : i) the "headpieces" are not involved to a large extent in the structuration of the core : ii) the "headpieces" are in good contact with solvent allowing fast hydrogen exchange ; iii) IPTG binding induces a conformational change of the lac repressor in which the "headpieces" play a central role. This last conclusion may be relevant to the allosteric inducting effect of IPTG.

References

(1) Bourgeois, S. and Pfahl, M. (1976) Adv. Prot. Chem., 30, 1-99
(2) Müller-Hill, B. (1975) Prog. Biophys. Molec. Biol., 30, 227-252
(3) Geisler, N. and Weber, K. (1978) FEBS Letters, 87, 215-218
(4) Platt, T., Files, J.G. and Weber, K. (1973) J. Biol. Chem., 248, 110-121

(5) Jovin, J.M., Geisler, N. and Weber, K. (1977) Nature, 269, 668-672
(6) Laiken, S.L., Gross, C.A. and Von Hippel, P.H. (1972) J. Mol. Biol., 66, 143-155
(7) Ohshima, Y., Matsuura, M. and Horiuchi, T. (1972) Biochem. Biophys. Res. Commun., 47, 1444-1450
(8) Matthews, K.S., Matthews, H.R., Thielmann, H.W. and Jardetzky, O, (1973) Biochim. Biophys. Acta, 285, 159-165
(9)Brochon, J.C., Wahl, P., Charlier, M., Maurizot, J.C. and Hélène, C. (1977) Biochem. Biophys. Res. Commun., 79, 1261-1271
(10)Englander, S.W., Downer, N.W. and Teitelbaum, H. (1972) Ann. Rev. Biochemistry, 41, 903-924
(11) Englander, S.W. and Staley, R. (1969) J. Mol. Biol., 45, 277-285
(12) Matthews, K.S., (1974) Biochim. Biophys. Acta, 359, 334-340
(13) Maurizot, J.C. and Charlier, M., unpublished results
(14) Müller-Hill, B., Beyreuther, K.T. and Gilbert, W. (1974) in "Methods in Enzymology", XXI, part D, L. Grossman and K. Moldave eds, pp. 483-487, Acad. Press.
(15) Geisler, N. and Weber, K., (1977) Biochemistry, 16, 938-943
(16) Englander, S.W. and Englander, J.J. (1972) in "Methods in Enzymology", XXVI, part C., C.H.W. Hirs and S.N. Timasheff eds, pp. 406-413, Acad. Press.