Binding of *lac* repressor induces different conformational changes on operator and non-operator DNAs

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1. INTRODUCTION

Binding of *lac* repressor on a *lac* operator sequence included in a DNA fragment of 25 basepairs (bp) induces a conformational change of this fragment [1]. This conformational change as reflected by the circular dichroism spectrum modification is different from that observed in the 'non-specific' binding of *lac* repressor to non-operator DNA [2–5]. The most likely explanation of this fact is that the mode of binding of *lac* repressor is different for operator and non-operator DNA. However, in these experiments one could not exclude the possibility that the difference observed between the two conformational changes arises from the small size of the fragment of operator DNA as compared to the large size of non-operator DNA previously used. The pertubated length on the DNA might be larger than the length of the operator fragment (25 bp) or/and the lack of base-pairing in the segments adjacent to the operator might modify the conformational change, even if it is restricted to the operator part of the DNA. To get further insight into this problem, we have made use of a longer DNA fragment, a 203 bp *Eco*RI fragment containing the entire *lac* promotor–operator region [6]. In this fragment, the *lac* operator is located at −50 bp from the nearest extremity, excluding that the interaction could be modified by end effects. Furthermore, the length of the DNA fragment allows other binding sites than the specific one on the *lac* operator, giving thus an internal control of the conformational change. In [7], at least 8 *lac* repressors could be bound on this DNA fragment. Here, we report circular dichroism measurements on the binding of *lac* repressor on this fragment.

EXPERIMENTAL

2.1. *Isolation of the restriction fragment*

The *E. coli* strain carrying the plasmid pMB9 with the 203 bp insert was the gift of O. Mercereau-Puijalon. Large amounts of plasmid DNA were prepared by the standard cleared lysate [8] and purified on hydroxyapatite column [9]. Restriction enzyme *Eco*RI was prepared according to [10]. The operator fragment was generated by treatment of the plasmid with *Eco*RI endonuclease and purified away from plasmid vehicle by gel filtration (in preparation).

2.2. *Formation of repressor–DNA complexes*

Repressor–operator complexes were studied in a buffer containing 10 mM Tris, 10 mM KCl, 0.1 mM DTE (pH 7.5). As solubility of *lac* repressor is very limited in this buffer, stock solutions of *lac* repressor were made in 0.2 M potassium phosphate, 0.1 mM DTE (pH 7.25). Aliquots of concentrated repressor solution were added to the solution of *lac* operator. The increase of ionic strength resulting from this addition was negligible for the repressor to operator ratios used in this study. Unspecific binding experiments were performed using the 1100 bp *Eco*RI fragment of the plasmid pBR345 [11] prepared as in [1].

The concentrations of *lac* repressor were determined using ε_{280} (protomor) = 21 400 $M^{-1} \cdot cm^{-1}$.

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The concentrations of DNA were determined using ε_{260} = 13 000 $M^{-1} \cdot cm^{-1}$ (/mol/bp).

Circular dichroism spectra were taken with a dichrograph III Roussel Jouan CWRS, in thermostatted cells at 20°C. Each spectrum was run at least twice and baseline was run between each spectrum. [DNA fragment] was ~2 × 10^{-7} M.

3. RESULTS AND DISCUSSION

The CD spectrum of the 203 bp fragment is shown in fig.1. Its position and intensity are indicative of the classical structure of the DNA in double strand in solution. It does not differ from that of a DNA fragment which does not bear the *lac* operator sequence as for example a 1100 bp *Eco*RI fragment of the plasmid pBR345 (fig.1).

Upon addition of *lac* repressor the signal of both DNA fragments increases in the wavelength range 260–300 nm as a result of the binding of *lac* repressor on the DNA. As discussed in [1–5] one can exclude that a possible conformational change of the protein makes a significant contribution to the observed CD change in this wavelength range and we therefore subtracted the small contribution of

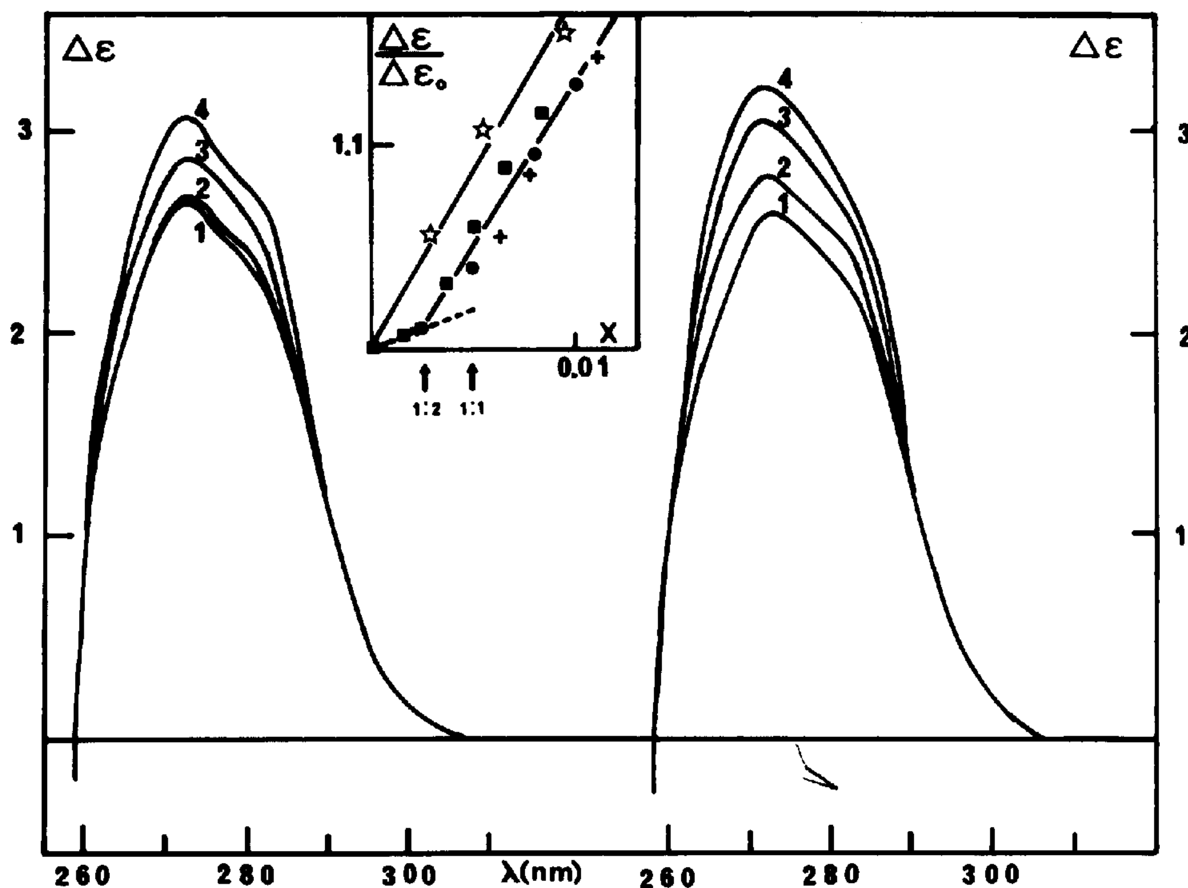

Fig.1. CD spectra of the 203 bp *lac* operator fragment (left) and of the 1100 bp non-operator fragment (right) in the absence of *lac* repressor (curves 1) and in presence of one repressor tetramer for 380 bp (curves 2), for 129 bp (curves 2), for 85 bp (curves 4). Insert: Relative variation of the CD intensity at 275 nm, as a function of tetrameric to DNA basepair ratio (X); non-operator DNA (•••); (+ + +, ▪▪▪) operator DNA; (•) and (▪) correspond to two sets of experiments performed with different preparations of operator and repressor. The various lines were drawn using least square fitting of the data. The arrows indicate the stoichiometries corresponding to 1 operator–1 repressor and 2 operators–1 repressor.

the *lac* repressor. Looking in detail at the change of the CD signal as a function of *lac* repressor added clearly shows that for the fragment with the *lac* operator sequence the increase of intensity in the very beginning of the curve is lower than for the non-operator fragment. In a second step the CD signal increased more strongly. In this second step the slope of the increase of the CD signal is identical to that observed in the non-specific binding with non-operator DNA. In a third part (not shown on the figure) the intensity of both signals reaches a plateau.

The simplest interpretation of our results is that in the first step the *lac* repressor binds on the *lac* operator changing its conformation. Then it binds on the other sites inducing another type of conformational change. This second step corresponds to the non-specific binding of *lac* repressor on DNA. This result implies that the mode of binding to *lac* operator is sufficiently different from that on non-operator DNA to induce a different conformational change.

This result is in agreement with that previously obtained with a short DNA fragment of 25 bp [1]. However, the use of the 203 bp fragment excludes, as explanation of the difference between specific and non-specific binding, the effect of the end of the fragment first because the *lac* operator sequence is far from the extremity of the DNA, secondly because after the specific binding, non-specific binding is observed which exhibits the same conformational change as in a long DNA.

With a shorter operator DNA fragment we have found that the CD difference spectrum upon binding is different in magnitude and in shape from that induced upon non-operator binding [1]. With the 203 bp fragment the *lac* operator accounts only for ~ 10% of the length of the DNA fragment; it is therefore difficult to accurately obtain the CD difference spectrum corresponding to the *lac* operator binding. Clearly, this difference spectrum is smaller than that of the non-specific binding but we cannot determine whether it is only different in magnitude or whether it also changes in shape. In any case even if it is only a difference of magnitude it demonstrates different conformational changes for the 2 types of binding.

One important point of this study deals with the stoichiometry of the interaction. With a shorter DNA fragment it was found that complexes of 1 repressor–2 operators could be formed [1,13]. Winter and Von Hippel [14] have extensively considered the 1:1 *vs* 1:2 repressor:operator problem as a function of operator-containing DNA fragment length, and have concluded that for fragments $\gtrsim$ 100 bp only 1:1 stoichiometry can be observed. To analyse this point we have followed the relative variation of the CD signal at 275 nm. This wavelength was selected since it allows the more sensitive measurements in the non-specific binding and since the CD signal is quite insensitive to small errors in wavelength. A least square fitting of the 2 parts of the plot $\Delta\varepsilon/\Delta\varepsilon_0$ as a function of the repressor to operator ratio indicates that the end of the first binding process occurs from a stoichiometry of 1 repressor:2 operators. Although the precision on the first slope of the titration is low, our data does exclude a pure 1:1 stoichiometry.

The 203 bp fragment we have used contains a secondary binding site for the *lac* repressor, the pseudo-operator 03 [15,16] located at the end of the *i* gene. It has been found that the binding on this site is 15–18-fold weaker than operator binding [7]. If the binding on the pseudo-operator site would induce the same conformational change as that on operator one should see the end of the first step of the titration curve shifted towards larger repressor to operator ratios. Therefore, from our data we can conclude:

(1) That from a conformational point of view the binding on the pseudo-operator 03 is clearly distinguishable from that on the operator;

(2) That in our experimental conditions a complex of 1 repressor–2 operators can be formed even if the operator is included in a 203 bp fragment.

The difference between this result and that in [14] may be explained taking into account the fact that in our experiments the concentration of *lac* operator fragment is several orders of magnitude larger than those used in the nitrocellulose filter experiments. The finding of a complex containing 2 operators is in perfect agreement with the model of *lac* repressor proposed in [17,18].

Our results definitively demonstrate that the binding of *lac* repressor on *lac* operator DNA induces a conformational change of the nucleic acid which is different from that observed upon binding on non-operator DNA. One may think that additional interactions in the specific binding, such as

hydrogen bonding, not only increase the binding constant but also impose new constraints on the DNA.

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