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The lac repressor and its N-terminal headpiece can bind a mini-operator containing a hairpin loop made of a hexaethylene glycol chain

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The binding of the lac repressor and the lac repressor N-terminal headpiece to a mini-operator with a hairpin loop made of a hexaethylene glycol chain was investigated using circular dichroism spectroscopy. The lac repressor's headpiece binds to the modified mini-operator with the same affinity as to a mini-operator of the same sequence without the hexaethylene glycol loop. The conformational effect due to the binding is not affected by the presence of the hexaethylene loop. It is also shown that the entire lac repressor binds to this modified mini-operator inducing a conformational change.

Lac repressor; Headpiece; Modified oligonucleotide; Hairpin; DNA–protein interaction

1. INTRODUCTION

Over the past few years a great number of studies on oligodeoxyribonucleotides having the ability to fold back into hairpin structures have been carried out [1–19]. Recently we have shown, using circular dichroism spectroscopy, that an oligodeoxyribonucleotide of sequence d(GCTCACAAT-X-ATTGTGAGC), where X represents a hexaethylene glycol chain can form a hairpin looped structure [20]. It was shown that due to the intramolecular character of the melting process, a large increase (by about 35°C in our experimental conditions) of the melting temperature is observed when compared to its natural duplex analog [20].

As the fragment studied shows a sequence which is part of a half operator of the lactose operon (Fig. 1) we have studied its interaction with the lac repressor as well as with its N-terminal headpiece. We show here that both proteins can bind to this type of hairpin structure.

2. MATERIALS AND METHODS

The modified oligonucleotide bearing the hexaethylene glycol chain was prepared as previously described [20]. The solutions were prepared with a buffer containing 10 mM potassium phosphate pH 7.2. The concentration was calculated using the UV absorption spectrum as previously indicated [20].

The lac repressor from *E. coli* BMH493 was purified as described by Rosenberg et al. [21]. The N-terminal headpiece was prepared by incubation of the lac repressor with 1% (w/w) clostripain in a buffer containing 1 M Tris-HCl, pH 7.5, 30% glycerol, 2×10^{-3} M DTE, 1×10^{-3} M $CaCl_2$ at 22°C for 4 h. The reaction was stopped by addition of EDTA (10^{-2} M) and cooling in ice. The products were fractionated on a G50 column followed, after dialysis, by a phosphocellulose column. The concentration of the headpiece was determined from absorption measurements using an extinction coefficient at 280 nm of 4800 $l\cdot mol^{-1}\cdot cm^{-1}$.

Circular dichroism measurements were carried out on a Jobin Yvon Mark II dichrograph in a cell with pathlength of 0.1 cm. Data acquisition and analysis were performed on a computer interfaced to the spectrometer. Each CD spectrum was run at least twice and we checked for possible base line shift.

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3. RESULTS AND DISCUSSION

3.1. *Binding of the modified oligonucleotide to the headpiece*

The sequence of the oligonucleotide bearing the hexaethylene glycol loop is part of the left half of the lac operator (Fig. 1). This side was chosen since it is known to interact with the repressor more strongly than the other part [22,23].

First, experiments were performed using the N-terminal headpiece which is known to selectively bind a half site of the lac operator [24–32].

The binding experiments using the modified and unmodified mini-operator were performed at 0°C to ensure that the natural oligonucleotide was in the full duplex form, since its melting temperature in the buffer used (10^{-2} M potassium phosphate, pH 7.2) is 25°C whereas that of the looped compound is 62°C.

Fig. 2 shows the effect of the addition of headpiece on the CD spectra of both natural and modified mini-operators. An important increase of the positive band of the CD spectrum is observed. Under our experimental conditions the contribution to the CD spectrum of the headpiece for wavelengths larger than 250 nm is two orders of magnitude smaller than the contribution of the oligonucleotide and can be neglected. We did not

Fig. 1. Sequence of the wild-type lac operator and of the modified oligonucleotide used in this study.

use the measurements at shorter wavelengths where the DNA shows a negative band, because the CD signal of the headpiece, which is due to the peptide chromophore, is of the same order of magnitude as that of the DNA and makes the interpretation of the data difficult.

Clearly the CD results demonstrate that the headpiece binds to both the natural and the looped mini-operator and in both cases change the conformation of the DNA fragment. CD changes are of the same type (an increase of the positive band) as those previously observed for the specific repressor-operator or headpiece-operator interactions [26,28], although quantitative comparison would be hazardous owing to the difference of size of the DNA sequence used. NMR experiments have shown that the binding to the N-terminal headpiece to the natural mini-operator leads to a specific complex with contacts similar to those observed using longer operator sequence (Lancelot and Maurizot, unpublished results, [30-32]). We therefore conclude that the headpiece can also form a specific complex with the modified oligonucleotide.

With both minioperators the binding curves obtained using the CD change at 280 nm, assuming a 1 half operator/1 headpiece complex, could be fitted with a binding constant $K = 1 \times 10^5\ M^{-1}$ (Fig. 3).

As expected, increasing the ionic strength by the addition of KCl led to a decrease of the observed effect that disappears at 0.2 M KCl (data not shown).

3.2. *Binding of the modified oligonucleotide to the lac repressor*

Lac repressor was added to the looped mini-operator at the same ionic strength (10^{-2} M potassium phosphate). As for the headpiece an increase of the CD signal was observed in the wavelength range 260–300 nm indicating that an interaction between the protein and the DNA occurs. This increase was smaller than that observed with the headpiece and precipitation, occurring when the ratio repressor to mini-operator reached a value close to one (Fig. 4). This precipitation as well as the presence of a contribution to the CD signal of the lac repressor did not allow us to quantitatively analyse the binding process of the protein under these conditions. Nevertheless, these results clearly demonstrate that the entire lac repressor is able to bind the looped oligonucleotide.

3.3. *Conclusion*

Previous studies have shown us that the presence of the hexaethylene loop does not modify the conformation of the duplex as compared to the unlooped compound but increases its stability. The results presented here further show that this presence does not perturb the binding of the headpiece and that this binding is qualitatively and quantitatively similar to that of the unmodified duplex. The modified mini-operator is also able to interact with the lac repressor. The large increase in the melting temperature of the looped oligo-

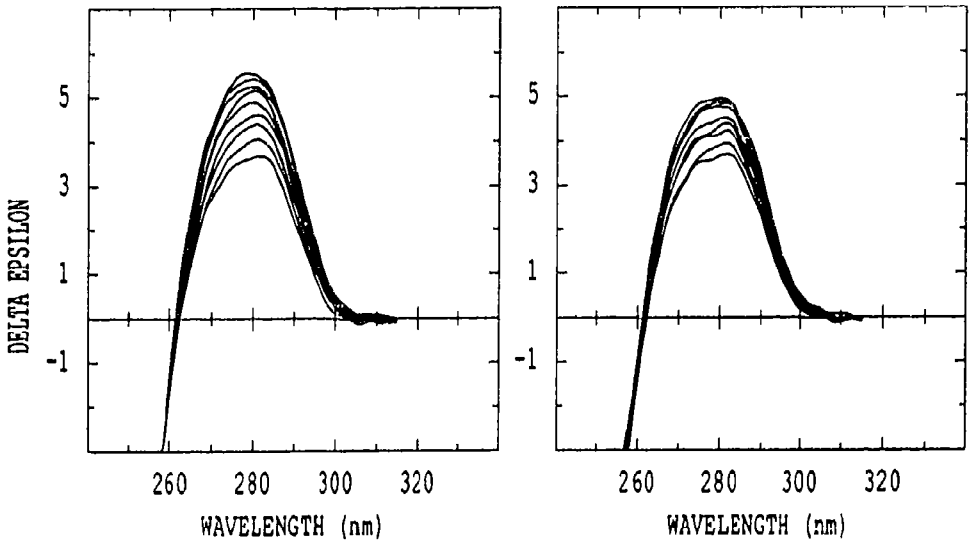

Fig. 2. Circular dichroism spectra of the looped minioperator (left) and of the natural minioperator (right) in the presence of increasing amounts of headpiece at 0°C in a buffer containing 10^{-2} M potassium phosphate. In order of increasing CD intensity the ratio [Headpiece]/[DNA] varied from 0 to 8.

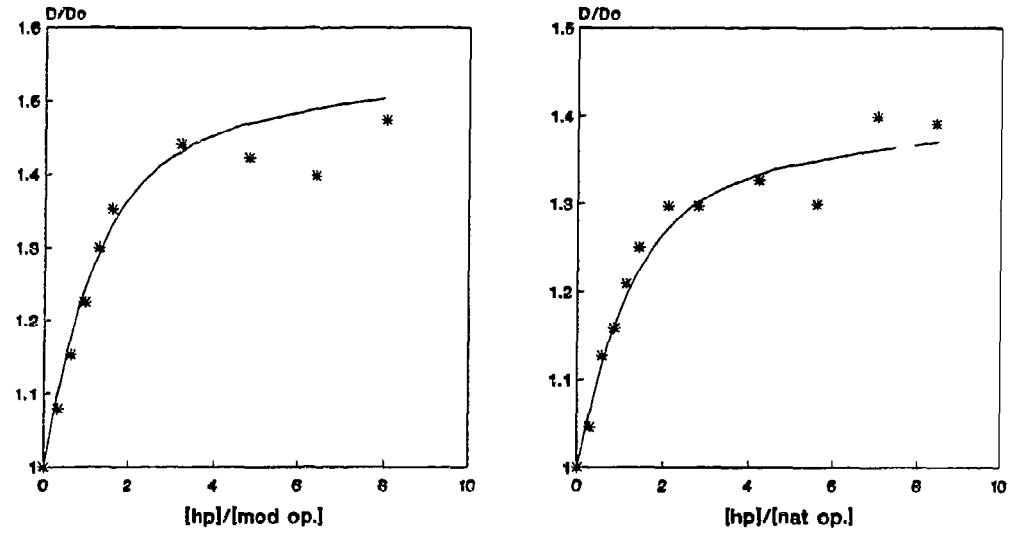

Fig. 3. Variation of the CD intensity at 280 nm as a function of the added headpiece to the modified (left) and natural (right) mini-operator. The lines were calculated assuming a binding constant of 1×10^5 M^{-1}. Same conditions as in Fig. 2.

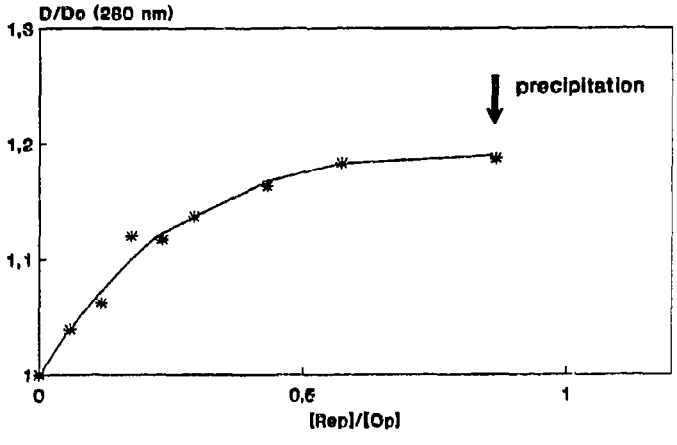

Fig. 4. Increase of the CD intensity of the modified mini-operator in the presence of increasing concentration of lac repressor. The contribution to the CD signal of the lac repressor has been taken into account. Experimental conditions are as those of Fig. 2.

nucleotide, the fact that its duplex structure does not depend on the concentration, allowing its use at very high dilution and the insensitivity of the loop to single-strand nuclease opens new possibilities for the use of such oligonucleotides for numerous molecular biology purposes.

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