LAC REPRESSOR BINDING TO POLY [d(A-T)] . CONFORMATIONAL CHANGES

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SUMMARY : The binding of lac repressor to poly [d(A-T)] at low ionic strength has been investigated by circular dichroism, fluorescence and light scattering. Poly [d(A-T)] undergoes an important conformational change upon binding to lac repressor. The maximum number of binding sites corresponds to about one tetrameric repressor per 11 base pairs of poly [d(A-T)] . The inducer isopropyl β-D-thiogalactoside (IPTG) does not affect the binding of lac repressor to poly [d(A-T)] . It binds equally well to free and poly [d(A-T)] -bound repressor.

INTRODUCTION

Escherichia coli lac repressor binds very tightly to the lac operator which is a DNA segment 24 base pairs long with two-fold symmetry (1). It is now possible to obtain repressor in quantities large enough to do physico-chemical studies (2). However the isolation of large amounts of operator is not yet solved. Lac repressor can also bind to the alternating synthetic polymer poly [d(A-T)] with a remarkably high affinity (3, 4). We have undertaken a study of the interactions between poly [d(A-T)] and lac repressor aimed at obtaining information on the mode of binding of this repressor to lac operator which is an A-T rich segment of DNA. We report here the results of circular dichroism, fluorescence and light scattering experiments showing that the binding of lac repressor to poly [d(A-T)] induces a conformational change of the polynucleotide secondary structure, and that this interaction does not affect the binding of the inducer IPTG to lac repressor.

EXPERIMENTAL METHODS

E. coli lac repressor from strain BMH 461 was purified

by ammonium sulfate fractionation and column chromatography on phosphocellulose as described by Müller-Hill et al (5).

Poly[d(A-T)] was purchased from Boehringer, Mannheim, and IPTG from Sigma. [C^{14}]IPTG was obtained from CEA, Saclay.

Stock solutions of repressor were prepared in a buffer containing 0.2 M phosphate, pH 7.25 and 0.1 mM dithioerythritol (DTE). Poly[d(A-T)] was dissolved in a 2 mM phosphate buffer containing 0.1 mM DTE. Lac repressor is not soluble under such low ionic strength conditions except when it forms a complex with poly[d(A-T)] (see below). Small volumes of the concentrated repressor solutions at high ionic strength were added to the polynucleotide sample.

Circular dichroism (CD) spectra were recorded with a Roussel-Jouan apparatus in a thermostated cell at 0°C.

Fluorescence measurements were recorded with a Jobin Yvon spectrofluorimeter which has been modified to correct for fluctuations in lamp intensity. Light scattering measurements were performed on the same spectrofluorimeter whose emission monochromator was set at the same wavelength as the excitation monochromator (280 nm).

RESULTS AND DISCUSSION

Binding of lac repressor to poly[d(A-T)]

The CD spectra of poly[d(A-T)] in the wave length range 260-300 mm in the absence and the presence of lac repressor are shown in figure 1. At the concentrations of repressor used in these experiments the CD spectrum of lac repressor in the same wavelength range is indistinguishable from that of the buffer. Thus the increase of ellipticity shown in figure 1 should be due to some conformational change of poly[d(A-T)]. Increase of the phosphate concentration from 2×10^{-3} to 1.7×10^{-2} M when repressor is added to poly[d(A-T)] cannot explain this change, since increasing the ionic strength leads rather to a small decrease of the amplitude of the poly[d(A-T)] CD spectrum in this wavelength range. The observed effect is very similar

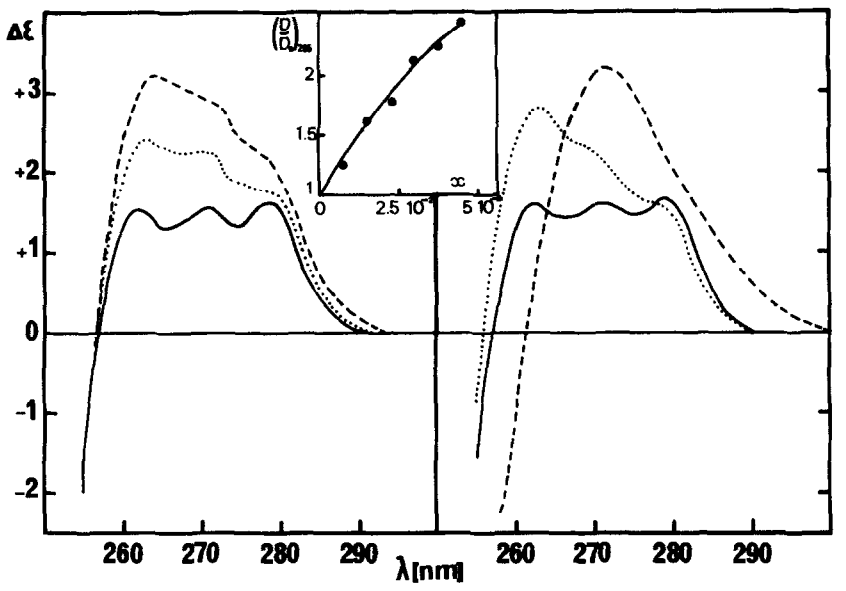

Figure 1 :

Left CD spectrum of 2 x 10^{-4} M poly[d(A-T)] alone (——) ; (. . . .) same plus lac repressor (one tetrameric repressor per 45 base pairs) ; (- - - -) one tetrameric repressor per 22 base pairs. Phosphate concentrations were 2 mM, 9 mM and 18 mM respectively (see experimental methods). All the spectra were recorded at 0°C.

Right CD spectra of poly[d(A-T)] at different temperatures in 2 mM phosphate buffer (——) 1°C ; (. . . .) 18°5 C ; (- - - -) 49°5 C. At this ionic strength the melting temperature was 39°C.

Inset The relative variation of the CD spectrum intensity at 265 nm upon addition of lac repressor ; x is the ratio of lac repressor (tetramer) to base pair concentration of poly[d(A-T)].

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to that observed upon increasing the temperature. Poly[d(A-T)] shows a very marked premelting effect (6, 7) which is illustrated on figure 1. The CD spectrum obtained in presence of lac repressor clearly differs from that of the melted form of poly[d(A-T)] . It should be noted that in the range of concentration used in CD experiments (up to one repressor tetramer per 20 base pairs) the increase of the CD signal does not reach a plateau (see figure 1).

Raguet and Brahms (7) have reported that the amplitude of the CD spectrum of poly[d(A-T)] decreases in the presence of DNA polymerase. This was interpreted as a stabilization of the poly[d(A-T)]

structure by the enzyme. In contrast, the effect observed with lac repressor could be ascribed to a destabilization of poly[d(A-T)] upon binding to repressor. However it should be noted that the shape of the CD spectrum in presence of repressor shows small differences (particularly around 280 nm where there is a broadening of the band) with that of the premelted form and that the addition of repressor does not decrease the melting temperature of poly[d(A-T)] as would be expected from a destabilization effect.

When lac repressor was added to poly[d(A-T)] in a 0.2 M phosphate buffer no change was observed in the CD spectrum, which might indicate either that the binding is greatly reduced or that the conformation of the polynucleotide is not affected. The first explanation seems more likely as we could show that upon heating the solution in 0.2 M phosphate buffer precipitation of the repressor occured around 40°C as observed for lac repressor alone whereas such a precipitation was not observed for lac repressor - poly[d(A-T)] mixtures at low ionic strength.

The fluorescence spectrum of lac repressor exhibits a maximum around 340 nm which is characteristic of the tryptophan emission (8). In the presence of poly[d(A-T)] under low ionic strength conditions where the CD spectrum of the polynucleotide is markedly changed (see figure 1), the shape and maximum wavelength of the fluorescence spectrum of lac repressor are not modified. This result indicates that the environment of the tryptophyl residue(s) responsible for fluorescence emission is not affected on complex formation.

The maximum number of binding sites of lac repressor on poly[d(A-T)] was determined by measuring the intensity of light scattered by the samples in the spectrofluorimeter. As a result of its insolubility in 2×10^{-3} M phosphate buffer, addition of lac repressor leads to a large increase in scattered light intensity. When poly[d(A-T)] is present in the sample, due to the solubility of the complex, the scattered intensity is greatly reduced (figure 2). If more repressor is added to the sample there is a rapid increase of the scattered intensity when poly[d(A-T)] is saturated by the lac repressor. As shown in figure 2 the maximum number of binding sitesis about one tetrameric

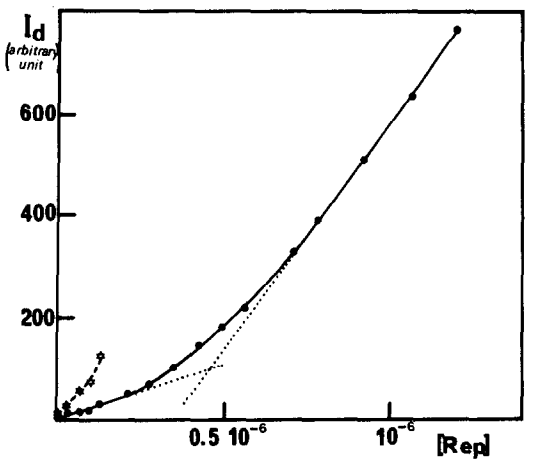

Figure 2 :

(•) Intensity of scattered light at 280 nm when lac repressor is added to 10^{-5} M poly[d(A-T)] in 2 mM phosphate buffer.

(✡) Intensity of light scattered in the absence of poly[d(A-T)]

Repressor concentrations are expressed in tetramer

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repressor per 11 base pairs, which represents about half the number of base pairs involved in the specific operator-repressor interaction (1).

Effect of the inducer IPTG on the binding of lac repressor to poly[d(A-T)]

Addition of IPTG to a mixture of lac repressor and poly[d(A-T)] (1 tetramer per 22 base pairs) does not induce any change in the CD spectrum which indicates that there is no dissociation of the complex. This is in agreement with the results of Lin and Riggs (9) showing that repressor binding to non-operator DNA is sensitive to ionic strength but is not affected by IPTG.

Addition of IPTG to the repressor alone shifts the fluorescence maximum to shorter wavelengths (8). The binding of IPTG to repressor can be followed by plotting the change in fluorescence intensity at 360 nm. Figure 3 shows that there is essentially no difference between the binding curves obtained in the presence and in the absence of poly[d(A-T)]. This result and the lack of conformational change of poly[d(A-T)] upon addition of IPTG demonstrate that there is no interference on the repressor molecule between the binding sites for poly[d(A-T)] and for IPTG.

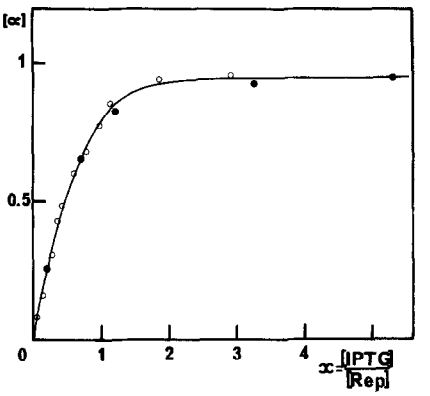

Figure 3 : Relative fluorescence intensity change at 360 nm upon binding of IPTG to lac repressor

(o) 2×10^{-5} M repressor in 0.2 M phosphate buffer at pH 7.25

(•) 1.7×10^{-5} M repressor in the presence of 1.85×10^{-4} M poly[d(A-T)] in 2 mM phosphate buffer at pH 7.25.

Repressor concentrations are expressed in monomer and x is the ratio of IPTG and repressor concentrations.

CONCLUSION

Our results demonstrate that the binding of lac repressor to poly[d(A-T)] induces a conformational change in this polynucleotide, whereas the conformation of the repressor does not seem to be drastically modified as indicated by the lack of fluorescence change. The maximum number of binding sites is about one tetrameric repressor molecule per eleven base pairs. Such a segment of poly[d(A-T)] is about 40 Å in length. This length seems too small to allow for a conformational change of poly[d(A-T)] to a branched conformation as proposed for repressor-operator interactions by Gierer (10) and Sobell (11). The binding of repressor to poly[d(A-T)]is non-specific as indicated by the lack of effect of the inducer IPTG. Nevertheless some features of this binding may also be involved in the interaction between operator and repressor and especially it is possible that lac operator does not adopt a B-type conformation when it interacts with lac repressor.

Acknowledgments

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