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**DNA conformational change in Gal repressor-operator complex: involvement of central G-C base pair(s) of dyad symmetry**

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**ABSTRACT**

Gal repressor dimer binds to two gal operator sites,  $O_E$  and  $O_I$ , which are 16 bp long similar sequences with hyphenated dyad symmetries (11,12). Repressor occupation hinders the reactivity of the N7 atoms in the major groups of guanines, located at positions 1,3 and 8, and the rotational 1', 3' and 8' of the symmetries. We have shown that Gal repressor binding to  $O_E$  or  $O_I$  DNA fragments increases the circular dichroism (CD) spectral peak in the 270 to 300 nm range. The CD change is similar to that observed for Lac repressor binding to its operator site (14). It is consistent with a DNA conformational change during complex formation between Gal repressor and  $O_E$  and  $O_I$  DNA. The CD spectral change was not observed when the central 8,8' G-C base pairs in the DNA-protein complex were replaced by A-T base pairs, whereas substitution of the 1,1' G-C base pairs do show the accompanying increase in the spectra during repressor binding. The absence of CD change of the Gal repressor complex with DNA mutated at the 8,8' base pairs suggest that the central G-C base pairs are required for the repressor induced conformational change.

**INTRODUCTION**

The gal operon of *Escherichia coli* encompasses three structural genes E, T, and K, and is transcribed from one of two overlapping promoters P1 and P2 (1-3). Both gal promoters are negatively controlled by the Gal repressor protein (4-6). Genetic analyses have shown that two DNA operator sites,  $O_E$  and  $O_I$ , are essential elements for gal repression (3,4,7-10). Biochemical studies have demonstrated that Gal repressor binds to both operator sites, and that the protein-DNA complexes are sensitive to D-galactose, an inducer of gal operon (11). The  $O_E$  site is located upstream to P1 and P2, about -60 bp from the startpoint of P1 transcription. The  $O_I$  site is inside the first structural gene, galE, 114 bp distant from  $O_E$ .  $O_E$  and  $O_I$  are 16 base pair similar sequences with hyphenated dyad symmetries (Fig. 1a and b). The complexes between Gal repressor and its DNA operator sites have also been probed by DNase I and dimethyl sulfate protection experiments (12). Figure 1 also displays the segments of DNA sequence at the  $O_E$  and  $O_I$  loci protected from DNase I digestion. The guanines which show reduced methylation at their

N7 group in the presence of repressor are also indicated in Fig. 1. Because repressor binding to  $O_E$  and  $O_I$  does not exclude binding of RNA polymerase and CRP, which is required for stimulation of  $P_I$  promoter, we have proposed other models by which repressor could block transcription and have also suggested potential changes in DNA structure by repressor binding (12,13).

In order to investigate conformational aspects of the Gal repressor-operator complexes we have employed circular dichroism (CD) spectroscopy. This technique provides a sensitive method for monitoring DNA conformational changes. It has been previously applied to study other gene regulatory DNA sites in complexes with their gene regulatory proteins (14-17). We report the CD spectra of the wild type  $O_E$  and  $O_I$  sites in the presence and absence of Gal repressor, and the effects of replacing G-C base pairs, protected from methylation by the repressor, with A-T on the CD of the complexes. Our results indicate that Gal repressor induces a conformational change in both operator sites, and that the central G-C pairs are more important than the outer G-C pairs for inducing this alteration. The N7 edges of the central guanines lie on the same face of a B conformation DNA. The N7 edges of the outer guanines are located on the opposite face (12). Comparison of the results with observations on other protein DNA complexes are discussed.

### EXPERIMENTAL PROCEDURES

#### Materials

Gal repressor protein employed in the experiments was purified and characterized previously (18). It was estimated to be 99% pure based on SDS polyacrylamide gel electrophoresis. The protein was dialyzed to 0.6 M KCl, 10 mM Tris (pH = 8.0), 1 mM DTT and 15% glycerol. It was centrifuged for five minutes at 13,700 g prior to measurements to remove any insoluble material. Concentration of the dimeric protein was determined from its extinction coefficient at 280 nm to be of  $39,600 \text{ M}^{-1}$  (1./mole repressor dimer), evaluated using the procedure of Levine (19). The four synthetic DNA duplexes used in this study are described in figure 1. Complimentary strands of each DNA were synthesized on an Applied Biosystems DNA Synthesizer using cyanoethylphosphoramidite chemistry. After deblocking the oligomers with ammonium hydroxide, DNAs were repeatedly lyophilized, extracted with ether, and eluted from Sephadex G-50 column. Full length strands were further separated from shorter products for  $^{32}\text{P}$  labeling by polyacrylamide gel electrophoresis (20). Equimolar mixtures of complimentary strands were mixed in 0.05 M NaCl, 10 mM Tris, 1 mM EDTA and heated to 80°C for 15 minutes.

They were then slowly cooled to room temperature over a period of 2-3 hours. The duplex DNAs were dialyzed to 10 mM NaCl, 10 mM Tris, and 0.1 mM EDTA.

#### Methods

Circular dichroism. CD spectra were obtained using a Jasco J-500A spectropolarimeter at 25°C. DNA concentrations were about 10 µg/ml (0.8 µM in DNA molecules). Four scans were averaged to produce a CD spectra. Two or three separate CD experiments were carried out on each DNA. The error in CD spectra correspond to about 0.1 Δε. Gal repressor and DNA were mixed to produce the desired molar ratios with a constant DNA concentration. The solvent consisted of 0.2 M KCl, 10% glycerol, 10 mM Tris (8.0) and less than 0.05 mM of EDTA and DTT. Samples were checked for light scattering before and after measurements by examining the UV absorbance of the sample from 340-300 nm. When the ratio of Gal repressor to DNA was 2:1 or greater, the solutions often gave an apparent rise of absorbance in this region indicative of light scattering. These samples were disregarded.

DNA labeling. The DNA duplexes were labeled at the 5' ends using polynucleotide kinase and <sup>32</sup>P-ATP (21). Labeled DNA was separated from unincorporated nucleotides using a G-50 Sephadex column. Small amounts of labeled DNAs were diluted with corresponding unlabeled DNAs of known concentrations and used in the binding assay.

DNA-protein binding assay. Binding of the Gal repressor to operator DNA fragments was assayed using polyacrylamide gel electrophoresis (11,22,23). 25 µl reaction mixtures contained protein and DNA in a solvent of 200 mM KCl, 10 mM Tris (pH = 8.0), 1 mM DTT, 0.1 mM EDTA and 3% ficol. The DNA concentration in each reaction was fixed, and the concentration of Gal repressor was varied. Samples were incubated at 25°C for 25 minutes before being applied to a 7%, 39:1 (w/w) acrylamide: bisacrylamide slab gel. After 2-3 hrs of electrophoresis, the gel was autoradiographed, and the intensities of the bound and free DNA bands determined with a Helena Quicksandensitometer and integrator. In the assays for DNA-protein complex formation in the CD studies, ethidium bromide staining was employed.

#### RESULTS

CD spectra. Fig. 1 shows the four gal operator DNA duplexes used in this study. Line A shows a 22 bp DNA fragment containing wild-type sequence O<sub>E</sub>. Lines C and D show two modified DNA fragments, O<sub>E</sub>M1 and O<sub>E</sub>M2 respectively. O<sub>E</sub>M1 DNA has the two central (8,8') G-C bp of O<sub>E</sub> changed to A-T bp, whereas in O<sub>E</sub>M2, the two G-C bp at the perimeter (1,1') are changed to A-T bp. Line B displays a 30 bp long wild-type O<sub>I</sub> DNA sequence. The

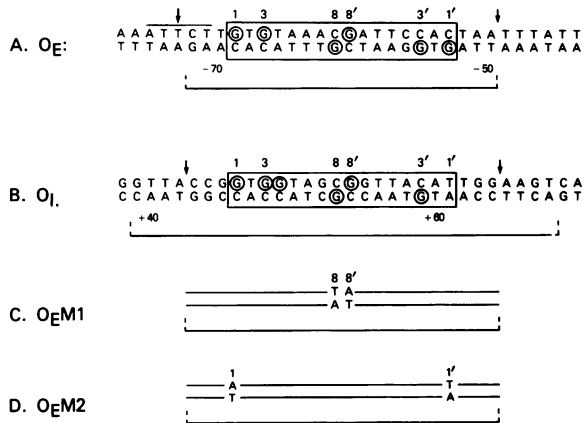


FIG. 1: Gal operator sequences and four DNA fragments employed. Base pair sequences of operators  $O_E$  (A) and  $O_I$  (B) are shown together with previous results from DNase I and dimethyl sulfate (DMS) protection studies (12). The circled guanines were protected from DMS methylation at their N-7 positions by Gal repressor binding. The downward arrows indicate the regions protected by Gal repressor from DNase I digestion. The uncertainty of the upstream protection site for  $O_E$  is given by the line perpendicular to the arrow. Boxed regions correspond to homologous segments of  $O_E$  and  $O_I$  with hyphenated dyad symmetry. Numbers below the sequences indicate base pair positions relative to the transcription startpoint of the P1 promoter. Numbers above the sequences indicate the position of base pairs of the dyad symmetry. The four synthetic DNA fragments,  $O_E$ ,  $O_{EM1}$ ,  $O_{EM2}$  and  $O_I$  are diagrammed below the base pair sequences.  $O_E$  (22 bp) and  $O_I$  (30 bp) maintain the wild-type sequences.  $O_{EM1}$  and  $O_{EM2}$  (both 22 bp) have two GC pairs changed to AT pairs at the positions shown.

effect of Gal repressor on the CD spectra of the 22 bp  $O_E$  DNA is shown in figure 2 (bottom panel). The curve with the lowest intensity peak corresponds to the free  $O_E$  site. The positive peak in the 260-320 nm region as well as a negative peak at 246 nm (not shown) are characteristic of B type DNA conformations. As the ratio of the Gal repressor to DNA increased from 0.0 to 1.0 the intensity of the positive CD spectra increased with a slight shift of the peak to a higher wave length. The CD signal from corresponding concentrations of free Gal repressor was within the noise level of the solvent baseline for the 255-330 nm region. Below 255 nm Gal repressor showed a negative CD signal similar to other DNA binding proteins (24). Gal repressor solutions were used as references in the CD measurements. The changes observed in figure 2 (bottom panel) is consistent with an alteration of the DNA conformation. If the changes were due to the protein, a fifty-fold enhancement of the protein CD signal would be required. This

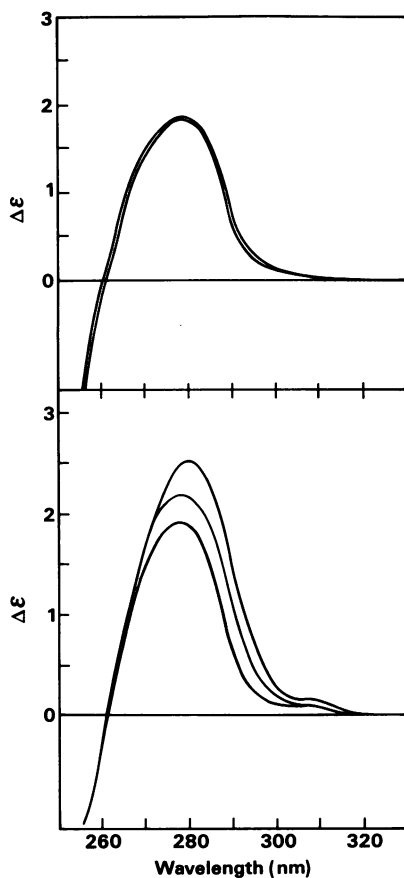


FIG. 2: CD spectra of DNA fragment  $O_E$  (Bottom Panel) and  $O_E M1$  (Top Panel) with Gal repressor. Spectra of DNA-Gal repressor complexes were obtained as described in "Experimental Procedures". Bottom Panel. Three spectra shown are of  $0.8 \mu M$   $O_E$  DNA with Gal repressor concentrations at  $0.0$  (lowest peak),  $0.4 \mu M$  (middle peak) and  $0.8 \mu M$  (highest peak). Top Panel. Spectra of  $0.8 \mu M$   $O_E M1$  DNA with no Gal repressor, with  $0.4 \mu M$  or  $0.8 \mu M$  Gal repressor gave the same lower curve. When  $1.6 \mu M$  repressor was added the slightly higher CD spectra was observed.

seems unlikely since the 19 aromatic amino acids of each polypeptide are distributed throughout each 342 amino acid monomer (25). Similar arguments have been previously made for CD studies on other DNA binding proteins (14-16). At protein:DNA ratios of 2:1 and 3:1, the CD spectral changes were not reproducible due to light scattering. When light scattering was not

indicated, the CD spectra were slightly higher than shown for the 1:1 spectra.

The effect of Gal repressor on the CD spectra of the  $O_{EM1}$  DNA is shown in figure 2 (top panel). Gal repressor no longer had a significant effect on the CD spectra. This DNA has the two central G-C pairs of the  $O_E$  DNA changed to A-T pairs (figure 1). No change is observed at or below 1:1 protein to DNA ratio. A very slight increase is seen at the 2:1 molar ratio. This behavior was similar to non-specific DNA. CD spectra of pUC19 DNA did not change upon the addition of a 2:1 molar ratio of repressor to 22 bp sites. Evidence indicating that the repressor still binds to the  $O_{EM1}$  DNA is shown in lanes 3-5 of figure 4. Aliquots from the circular dichroism experiments were examined for DNA-protein complex formation by the gel binding assay. Approximately one half of the  $O_{EM1}$  DNA no longer migrates as free DNA when the protein/DNA ratio is 1:1 (figure 4, lane 4). At a 2:1 ratio, essentially all of the DNA migrates at the mobility of the complex or as a faint smear above the mobility expected for the free DNA (figure 4, lane 5).

Figure 3 (top panel) shows the effect of Gal repressor binding on the CD spectra of the 22 bp  $O_{EM2}$  DNA site. In this DNA, the two G-C pairs at the perimeter of the  $O_E$  site are replaced by A-T pairs (figure 1). The CD changes are similar though not identical to those observed for the wild-type  $O_E$  site. The small shift of the 270 nm peak to higher wavelength is not observed. The effect of Gal repressor on the CD spectra of the 30 bp  $O_I$  DNA is shown in figure 3 (bottom panel). An intensity increase is again observed; however, it is a smaller increase than observed for the 22 bp  $O_E$  DNA. This may be due to the additional eight base pairs present in the  $O_I$  DNA. DNase protection experiments showed that about 22 bp of the  $O_I$  and  $O_E$  sites are protected by Gal repressor (12). Thus, the fractional change in the CD spectra of the 30 bp  $O_I$  DNA is expected to be smaller than the change observed for the 22 bp  $O_E$  DNA.

Gal repressor-DNA complex formation. The gel electrophoresis assay was employed to examine the formation of complexes between Gal repressor and DNA. Previous studies have shown that site specific complexes between Gal repressor and its specific operator sites can be detected by this method, and that these complexes do not form in the presence of D-galactose, an inducer of the gal operon (11,12). Figure 4 demonstrates that protein-DNA complexes were formed in the samples used in the CD experiments. The  $O_I$ ,  $O_{EM1}$  and  $O_E$  DNA sites are saturated at a 2:1 ratio of repressor to DNA. A similar result was obtained for the  $O_{EM2}$  DNA (results not shown). Since the DNA concentrations in the CD experiments are large with respect to the

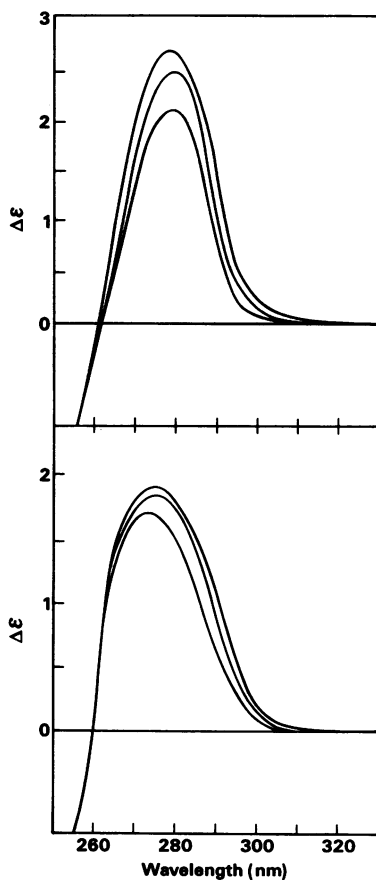


FIG. 3: CD spectra of DNA Fragments  $O_I$  (Bottom Panel) and  $O_E M2$  (Top Panel) with Gal repressor. Spectra were obtained as described in "Experimental Procedures". **Bottom Panel.** Spectra are shown of  $0.8 \mu M$   $O_I$  DNA with Gal repressor concentrations of  $0.0$  (lowest peak),  $0.4 \mu M$  (middle peak), and  $0.8 \mu M$  (highest peak). **Top Panel.** Spectra are shown of  $0.8 \mu M$   $O_E M2$  DNA with Gal repressor concentrations of  $0.0$  (lowest peak),  $0.4 \mu M$  (middle peak), and  $0.8 \mu M$  (highest peak).

equilibrium dissociation constant (see below), figure 4 indicates that about 50% of the repressor dimers are active for operator binding. Experiments with  $^{32}P$  labeled  $O_E$  DNA at  $2 \mu M$  produced values of 40-50%.

An estimate of the equilibrium association constant of Gal repressor with the 22 bp  $O_E$  DNA was made by measuring the amount of free and complexed DNA at different ratios of protein to DNA. Figure 5 shows a gel autoradiograph of the titration of  $20 nM$   $O_E$  DNA with various amounts of Gal repressor. Equilibrium binding between Gal repressor and the  $O_E$  DNA site is

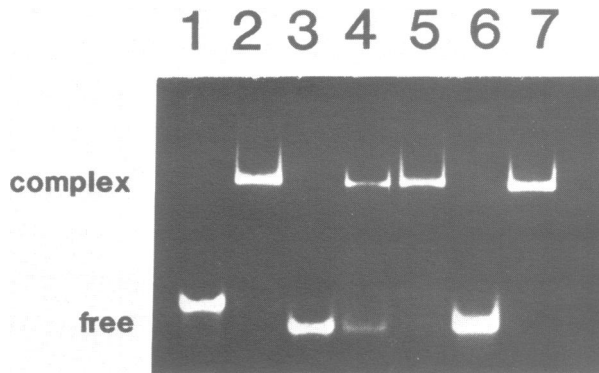


FIG. 4: Gal repressor binding to operator fragments in CD samples. 20  $\mu$ l of the Gal repressor-DNA solutions used in the CD experiments were electrophoresed on a 7% polyacrylamide gel and stained with ethidium bromide as described in "Experimental Procedures". DNA concentrations were 0.8  $\mu$ M. Lanes 1 and 2 show  $O_I$  DNA with 0.0 and 1.6  $\mu$ M repressor; lanes 3 to 5 show  $O_{E}M1$  DNA with 0.0, 0.8  $\mu$ M, and 1.6  $\mu$ M repressor; lanes 6 and 7 show  $O_E$  DNA with 0.0 and 1.6  $\mu$ M repressor.

assumed to occur by a simple one to one complex for which the association constant is  $K = [C]/[D][P]$ , [C], [D] and [P] being the concentrations of the complex, the free DNA and the free protein dimer respectively. The free

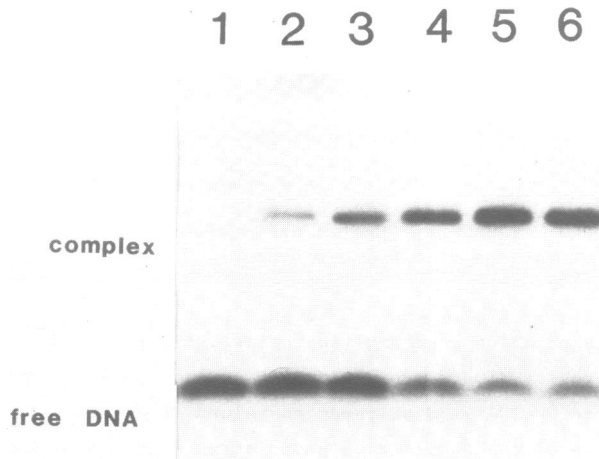


FIG. 5: Gal repressor binding to  $O_E$  fragment. Autoradiograph of polyacrylamide gel displaying  $^{32}P$  labeled free and complexed  $O_E$  DNA. DNA concentrations were 20 nM in each lane. Concentration of Gal repressor in lanes 1 through 6 were in nM; 0.0, 20., 40., 80., 120., and 160.



protein concentration is determined from the relation  $[P] = \alpha[P_T] - [C]$ , where  $\alpha$  is the percentage of the total protein active for site specific DNA, and  $[P_T]$  is the total Gal repressor added. By rearranging the equation for the equilibrium constant and taking the log of both sides (26), one obtains the equation  $\log([C]/[P]) = \log[D] + \log K$ . The data were plotted in accordance with this relationship to determine K. We find that  $K \approx 10^8 \text{ M}^{-1}$  for Gal repressor binding to the  $O_E$  DNA site. Preliminary experiments indicates that the equilibrium constant of the  $O_I$  DNA site is similar to the  $O_E$  DNA, while the binding constant of Gal repressor for the  $O_{EM1}$  DNA is decreased by one to two orders of magnitude.

#### DISCUSSION

The binding of Gal repressor to  $O_E$  and  $O_I$  DNAs alter their circular dichroism spectra. It is interesting to note that the CD change described here is similar to the changes induced by the Lac repressor and Tet repressor on their respective operator sites (14,15). In both cases the intensity of the positive band is increased and shifted slightly to a higher wavelength. Site specific complexes between the cyclic AMP receptor protein, CRP, and its DNA binding site (16), or the Cro repressor and its binding site (17) show qualitatively different CD changes. The spectral similarity between the Gal repressor-DNA complex and the Lac and Tet repressor-DNA complexes suggests that the structures in these three systems are alike. This idea gains support particularly for the Gal and Lac repressors where there are strong similarities in the operator sites and the proteins. Both operators are of similar length and have sequences with hyphenated dyad symmetry. There is a strong similarity in the amino acid sequence of these two proteins (25). Structural domains of  $\alpha$ helix-turn- $\alpha$ helix commonly found in subunits of many DNA binding proteins have been proposed to interact with bases in DNA major grooves (27,28). The binding domain of the Lac repressor has an 8 amino acid long  $\alpha$ -helix, followed by a 3 amino acid turn, and then a 9 amino acid  $\alpha$ -helix from residues 6 to 25 (28,29). The Gal repressor segment from amino acid 4 to 23 has a primary structure which is also consistent with an 8 amino acid  $\alpha$ -helix, 3 amino acid turn, and 9 amino acid  $\alpha$ -helix (18). Moreover, 4 out of 8 amino acids in the first helix of both proteins are identical, the sequence of the turns are identical, and 6 out of 9 amino acids of the second helix are identical.

The absence of a CD change for the Gal repressor  $O_{EM1}$  DNA complex suggests that one or both of the G-C pairs at positions 8 and 8' are necessary to induce the structural change in the  $O_E$  site. This is not the

case for the G-C pairs at positions 1 and 1' at the ends of the  $O_E$  site. The magnitude of CD change of the Gal repressor  $O_{EM2}$  DNA complex was similar to the spectra of the Gal repressor  $O_E$  fragment. Since dimethyl sulfate protection has indicated contacts for both sets of G-C pairs (12), the CD changes observed with  $O_E$ ,  $O_{EM2}$  and  $O_I$  DNA appear to reflect an alteration in DNA conformation rather than just G-C contacts. If we assume a B conformation DNA, the two major grooves exposing the N7 atoms of guanines at positions 1 and 1' of  $O_E$  lie on the same face. A third major groove showing the N7 atoms of guanines at positions 8 and 8' is almost located on the opposite face (12).

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