Influence of Negative Supercoiling and of the Proximity of Left-handed Z-DNA on the Escherichia coli Lactose Repressor-Operator Interaction*

Wang-Ting Hsieh and Robert D. Wells
From the Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, Alabama 35294

The influence of negative supercoiling and of flanking (dC-dG) tracts in either right-handed B- or left-handed Z-structures on the interaction of the Escherichia coli lac repressor was investigated. The operator was embodied within the lac control sequence, which was 95, 59, or 29 base pairs in length. Thus, the (dC-dG) regions (in either B- or Z-conformations) were at different distances from the repressor-binding site. Surprisingly, the presence of the promoter sequence (−59 to −20 relative to the +1 transcription start site) of the lac operator region increases the binding affinity of lactose repressor to the operator at high negative super coil densities. This influence of the promoter region on the binding was abolished when the flanking (dC-dG) tracts were in the left-handed Z-DNA conformation. In contrast, minimal differences in the binding affinities were observed between plasmids containing shorter operator fragments (59 or 29 base pairs), whether the flanking (dC-dG) tracts were in right-handed B- or left-handed Z-forms. The promoter region may be directly involved in the repressor-operator complex in a previously unrecognized manner or may exert structural influence on the operator region. In general, increasing the amount of negative supercoiling increases the binding affinity and decreases the dissociation rate constants for the three operator-containing fragments, both with and without flanking Z-DNA tracts. Thus, the lac operator region possesses a previously unrecognized structural pliability, as influenced by negative supercoiling and neighboring sequences and/or conformations, which modulates its biological properties. The occurrence of a segment of left-handed DNA in a plasmid can have several effects on the rest of the molecule. First, it relaxes the negative superhelical turns of the plasmid, causing long range effects on other segments of DNA by changing the overall torsional stress. Hence, a structural transition in the primary helix at one location can have profound effects on the properties of a distant site, such as a promoter (19) (for review see Refs. 2–5). Second, it may perturb the conformation of a region of neighboring B-DNA. Circular dichroism, 31P NMR, and Raman spectroscopic studies on restriction fragments, as well as nuclease sensitivity studies (such as S1 and BAL31) on recombinant plasmids indicate that the conformational perturbation caused by the presence of a left-handed segment within right-handed DNA can occur over a range of several base pairs (9, 20–23). Restriction and methylation analyses demonstrated that reactions were inhibited when the recognition sites were in the Z-block (9, 12, 17) or at (or near) the B-Z junctions (9), as well as up to 8 base pairs (bp) away from the left-handed blocks (24). Also, physical studies on a 157-bp DNA fragment containing (dC-dG)16 and (dC-dG)14, flanking the 95-bp lac operator sequence indicated that the (dC-dG) blocks were in the Z-conformation in high salt solutions, whereas the 95-bp lac operator region maintained the right-handed conformation, but a substantial portion (∼80%) of the phosphodiester backbone was no longer in a typical B-configuration (23, 25). We were interested in the influence of neighboring regions of left-handed Z-DNA on the binding of a well characterized regulatory protein, the Escherichia coli lactose repressor, to its target site. The repressor binds to the operator region of the lac operon with very high affinity, physically blocking the transcription by RNA polymerase of the genes for metabolic enzymes (26). Upon the addition of inducer, the repressor protein undergoes a conformational change and the binding affinity is decreased. Competition by nonspecific binding from the very large amount of the rest of the genome releases the repressor from the operator region, and transcription begins. Previous work revealed that the interaction between the lactose repressor and operator using Ap lac DNA containing both the primary operator and two pseudo-operators (repressor binding sites at the 3' end of the I-gene and in the Z-gene (27, 28)) was influenced by negative supercoiling (29). Herein, we have constructed two families of plasmids containing the lac operator region on 95-, 59-, and 29-bp segments, both with and without flanking tracts of (dC-dG). Repressor binding determinations as a function of negative supercoil density enabled an evaluation of the presence of neighboring right-handed or left-handed helices as a function

* The abbreviations used are: bp, base pairs; [3H]AdoMet, 3H-labeled S-adenosylmethionine.

A substantial body of information on the structure and properties of left-handed Z-DNA in oligomers, restriction fragments, and recombinant plasmids has been reported (reviewed in Refs. 1–5). The torsional stress of negative supercoiling induces the B to Z transition in (dC-dG) tracts and other suitable sequences in recombinant plasmids under physiological conditions (6–17). The types of sequences that can adopt left-handed structures are currently under evaluation and are, in general, of an alternating purine-pyrimidine (alternating syn-anti) nature, but a strictly alternating purine-pyrimidine is neither necessary nor sufficient (8, 9, 11, 18).
of distance. Surprisingly, we found that the promoter se-
quency region of the lac control region had an influence on 
the binding of the lactose repressor to the operator under the 
influence of negative supercoiling and neighboring left-
handed DNA.

MATERIALS AND METHODS

Chemicals and Enzymes—E. coli DNA polymerase I Klencow frag-
ment, T4 DNA ligase, and all restriction enzymes were purchased from 
Bethesda Research Laboratories, Boehringer Mannheim, or 
New England Biolabs and used as recommended by the supplier. 
HhaI methylase (New England Biolabs) was used as described (12). 
T4 polymerase kinase was obtained from Pharmacia Biotechnol-
ogy, Inc. Wheat germ topoisomerase and calf thymus topoisomerase 
were gifts from R. R. Burgess (University of Wisconsin) and 
J. E. Larson (this laboratory), respectively. Poly(dC-dG) was synthesized 
and characterized as described previously (30). [methyl 3H]aden-
sine, 5'-monophosphate ([H]AdoMet) and 32P-labeled nucleotides were 
from Amersham Corp.

E. coli lac Operator Fragments—The 95-bp E. coli lac operator frag-
ment is an Alul 95-bp fragment containing the complete promoter 
and operator region (−59 to +36) with additional 4-bp EcoRI sticky 
ends (31). The 59-bp lac fragment contains a portion of the promoter 
and lac operator region (+1 to +36) and has 4-bp EcoRI sticky ends. 
The 59-bp operator fragment was generated from the 95-
bp lac operator fragment using the single MspI site in the sequence. 
The 95-bp fragment was ligated to form a polymer which then was 
digested with MspI and filled in with DNA polymerase I Klencow 
fragment plus dATP and dCTP. The filled-in fragment was then 
ligated with pBR322 that had been previously digested with EcoRI 
and filled in with DNA polymerase I Klencow fragment plus dATP 
and dTTP. Recombinant plasmids containing the lac fragment were 
screened according to published procedures (32, 33). Plasmids con-
aining the correct inserts will generate both EcoRI sites on the lac 
fragments. EcoRI digestion of the plasmids containing the lac inserts 
yielded the expected 40- and 59-bp lac operator fragments. The 29-
bp synthetic lac fragment containing the center 22-bp lac operator 
sequences (+1 to +22) with EcoRI linkers (34, 35) was a gift from Dr. 
Kathleen Matthews (Rice University, Houston).

Construction of Plasmids—pRW465, pRW468, and pRW467 are 
recombinant plasmids containing a single copy of the 95-, 59-, 
and 29-bp lac operator fragments in the EcoRI site of pBR322, re-
presently. pRW461 (used below) is a pBR322 derivative whose EcoRI 
site was eliminated by filling in the EcoRI site using the DNA 
polymerase Klencow fragment plus dATP and dTTP.

pRW465, pRW468, and pRW467 were constructed as follows: poly-
dC-dG) was digested with FnuDII to oligomers ranging 
from 20 to 100 bp and were ligated to a pBR322 vector that had been 
digested with EcoRI and BamHI and filled in with DNA polymerase 
plus four dNTPs. The ligation mixture was then transformed  into 
HblOl cells grown in M9 medium supplemented with glucose and 
cysteine and amplics with 150 μg/ml chloramphenicol for 
6–8 h. Two successive transformation reactions were described (36). 
The reaction was carried out at 37 °C for 3 h. The incubation was then 
stopped by phenol extraction, and the plasmid was separated from 
free AdoMet via a Sephadex G-50 or G-75 column. The plasmids 
were labeled to a specific activity of 1.0–1.5 × 10⁶ cpm/μg, which 
enabled the measurement of the Kd down to 10⁻¹⁵ M. No nicking 
or linearization of supercoiled plasmids was observed during the label-
ing. There was no Hha site in the operator DNA sequence; thus, methylation should not influence the binding of repressor to 
the operator.

Previous studies on HhaI methylase have shown that tracts of left-
handed (dC-dG) in recombinant plasmids were not methylated at 
high negative supercoil densities (12, 17). The plasmids containing 
the (dC-dG) tracts were incubated with appropriate amounts of the lactose repressor in the binding buffer at room temperature for 20 min before the addition of AdoMet 
and HhaI methylase. The reaction was further carried out at 37 °C 
for 2 h and stopped by phenol extraction. After ethanol precipitation, 
the plasmids were then digested with PstI and HhaI and analyzed on 
agarose gels. The results showed that the (dC-dG) tracts in the plasmids 
that were methylated at pH 7.4 and analyzed on agarose gels. 
The results showed that the (dC-dG) tracts at high negative supercoil densities (0.085 before the adjustment described under "Results"; 0.057 after the adjustment) were not methylated in the binding buffer. These data 
data concentrations were determined by the fluorescence enhance-
ment of Hecochs dye 33258 (41) with a Perkin-Elmer Model 650-105 
spectrophotometer. pRW465 was used as a standard. 
Nucleic acids that were not supercoiled were observed between 
the binding site of glyce, relaxed, or linear DNA. DNA 
concentrations as low as 3 ng/μl were determined from this method.

Repressor-Operator Binding Assays—DNA binding assays using 
the nitrocellulose filter method were performed as described (42, 43). 
The filtering buffer for both kinetic and equilibrium experiments was 
10 mM Tris-HCl, pH 7.4, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 
150 mM KCl. Bovine serum albumin (nuclease-free, Bethesda Rese-
arch Laboratories) was added to this buffer (50 μg/ml) to stabilize 
the protein and was used as binding buffer. The lactose repressor 
generous gift from Dr. Kathleen Matthews, Rice University, Hous-
ton) was a homogeneous (~99% pure by gel electrophoresis) 
monomer preparation. The repressor was found to be 42% active 
under the stoichiometric conditions. The dissociation constant (Kd) 
was determined from the half-height of the saturation curve (43). 
The dissociation constant (Kd) was monitored from the decrease in 
the labeled filter-bound complex after addition of an excess amount of unlabeled DNA as described previously (44, 45). 
The repressor was added to operator-containing plasmids (2 × 10⁻¹² to 2 × 10⁻¹¹ M) in 
buffer at room temperature for 30 min, a 50–100-fold molar excess of pRW465 was 
added, and 0.5 ml of the incubation mixture was filtered at different 
time intervals. Duplicate or triplicate filters were used for each time 
point. The actual equilibrium or nonspecific DNA binding in the 
presence of inorganic β-thiogalactoside (Bethesda Research Labo-
raries) was determined and subtracted from each point (44, 45). 
The dissociation rate constant was then calculated from the (kd) using 
Kd = 0.693/kd.
Superoiling and Z-DNA on Repressor-Operator Complex

RESULTS

Characteristics of Plasmids—Two series of lac operator-containing plasmids were constructed as described under "Materials and Methods." The first three plasmids, pRW465, pRW468, and pRW467 each contained a single 95-, 59-, or 29-bp lac operator fragment, respectively, in the EcoRI site of pBR322. Right panel, plasmids containing a single operator fragment flanked by (dC-dG)_{13} and (dC-dG)_{13} tracts inserted into the BamHI site of pRW461. Construction of these plasmids is described under "Materials and Methods."

![Plasmids constructed for this study.](image)

FIG. 1. Plasmids constructed for this study. Left panel, plasmids containing a single E. coli lac operator insert in the EcoRI site of pBR322. Right panel, plasmids containing a single operator fragment flanked by (dC-dG)_{13} and (dC-dG)_{13} tracts inserted into the BamHI site of pRW461. Construction of these plasmids is described under "Materials and Methods."

The loss of negative supercoil turns that accompanies the B to Z transition (20) can be conveniently monitored by agarose gel electrophoresis in two dimensions (8, 10, 11, 13-15). Determinations with pRW483, pRW486, and pRW485 revealed that ~12.5 superhelical turns were lost when both the (dC-dG)_{13} and (dC-dG)_{13} blocks underwent B to Z transitions, in excellent agreement with the predicted values (as calculated previously (8, 10, 11, 13-15, 19, 47)). Two separate transitions for the two (dC-dG) blocks for all plasmids, including pRW485, were observed from two-dimensional gel analyses as expected from prior studies (9, 12, 16, 19, 47). This indicated that the lac operator region, including the 29-bp segment in pRW485, did not form a left-handed Z-conformation at the negative supercoil densities used (up to ~0.113 before adjustment; see following paragraph).

Because the lactose repressor-operator interaction is influenced by negative supercoiling per se (29, 48) (irrespective of B to Z transitions), it was necessary to compare the binding of repressor to operator-containing plasmids of similar negative supercoil densities. The negative supercoil densities measured by ethidium bromide fluorescence enhancement (40) were determined, assuming all the sequences were in the B-conformation. The real negative supercoil densities for plasmids containing (dC-dG) inserts after the B to Z transitions will be higher than the actual values according to the relaxation of the superhelical turns. To account for this difference, we adjusted the negative supercoil densities of pRW483, pRW486, and pRW485 after the transitions by adding ~12.5 superhelical turns. Thus, the negative supercoil densities reported (Figs. 2-5) reflect the comparable values between plasmids with or without (dC-dG) inserts.

Effect of Negative Supercoiling and Left-handed Z-DNA on the Dissociation Rate Constants—The influence of negative supercoiling on the ability of the lac repressor to bind to the six plasmids (Fig. 1) was determined; typical data for pRW485 are shown in Fig. 2. The lactose repressor dissociates more slowly from the plasmids as the negative supercoil density.

![Dissociation of lactose repressor from pRW465 with increasing negative supercoil densities.](image)

FIG. 2. Dissociation of lactose repressor from pRW465 with increasing negative supercoil densities. (A), related: \( A, -\gamma = 0.057; B, -\omega = 0.072; C, -\theta = 0.085; D, -\nu = 0.113. \) The dissociation kinetic binding assay was performed as described under "Materials and Methods." [operator] = 0.5-1.0 x 10^{-11} M. [repressor] = 1.0-2.0 x 10^{-11} M.

\[A, -\gamma = 0.057; B, -\omega = 0.072; C, -\theta = 0.085; D, -\nu = 0.113.\]
Supercoiling and Z-DNA on Repressor-Operator Complex

The dissociation rate constants were determined as described under "Materials and Methods." Each point indicates two to six independent measurements of $t_{1/2}$. The error bar indicates the standard deviation.

Panel A, Δ, pRW467; □, pRW468; ●, pRW465; ○, pRW463. Panel B, ■, pRW486; ■, pRW485; □, pRW483. Panel C, △, pRW487; ▲, pRW485. The dissociation rate constants were determined as described under "Materials and Methods" and calculated from $k_d = 0.693/t_{1/2}$. Each point indicates two to six independent measurements of $k_d$ values. The error bar shows the standard deviation.

FIG. 5. Effect of negative supercoiling and (dC-dG) blocks on the dissociation rate constants. Panel A, ○, pRW465; ●, pRW483. Panel B, △, pRW467; ▲, pRW485. The dissociation constant measurements were performed as described under "Materials and Methods." Each point indicates two to six independent measurements of $K_d$ values. The error bar represents the standard deviation.
95-, 59-, or 29-bp lac operator fragments (Fig. 5, relaxed plasmids). The higher \( K_d \) values of repressor binding to pRW468, pRW467, pRW486, and pRW485 (panels B and C) than to pRW465 and pRW483 (panel A) indicates the importance of the promoter region present in the 95-bp (panel A) but not in the 59- and 29-bp sequences (panels B and C, respectively) for the repressor-operator interaction.

Second, determinations as a function of \( \Delta \) revealed that increasing negative supercoil densities decreased the \( K_d \) for all the plasmids measured, except for pRW483 (Fig. 5). The \( K_d \) of pRW465 decreases 5-fold, whereas no major difference in \( K_d \) was observed for pRW483. Minimal differences in \( K_d \) values were found between pRW468 and pRW486 (panel B). These data suggest that the presence of flanking left-handed Z-DNA affects the binding of repressor to the plasmid containing the 95-bp operator but not the 59-bp operator. Thus, the promoter region (−59 to −20) which influences the repressor-operator interaction was further affected by the left-handedness at high negative supercoil densities as revealed above (Fig. 4) for the \( K_d \). The sizes and sequence contexts of the lac operator region have been shown to affect the \( K_d \) values (45, 49−61). A small difference in \( K_d \) values was observed between relaxed pRW468 and pRW467 (Fig. 5, panels B and C, relaxed plasmids; also Table I). For some reason, which may be due to a DNA structural change, at high negative supercoil densities (\( \Delta \) = −0.085), pRW467, which contains the 29-bp lac operator, was a better operator than the 59-bp lac operator-containing plasmid, pRW468 (Table I). The extent of decrease of \( K_d \) for pRW485 (−2-fold) as the negative supercoil density increased from 0 to 0.085 was less than that for pRW467 (−4.5-fold). Because we did not observe any difference in \( K_d \) values, the difference in \( K_d \) (0.98 × 10^{−12} M for pRW467 versus 3.3 × 10^{−12} M for pRW485) at high negative supercoil densities indicated that the influence of the neighboring left-handed Z-DNA blocks must be in the \( K_d \) values (also discussed below). The nonspecific binding affinity for repressor to right-handed poly(dG-dC) (dG-dC) is lower than to other DNA polymers (52). Furthermore, the binding of repressor to left-handed poly(dG-dC) (dG-dC) (generated in a solution containing 4 mM MnCl{\textsubscript{2}} and heated at 65 °C for 20 min; the presence of a Z-DNA structure was proven by both CD and ultraviolet spectroscopy) is ~10-fold lower than to the right-handed form (data not shown). Hence, if the lactose repressor has contacts with the DNA sequence outside the 29-bp operator region, the transition of the (dG-dC) blocks from B-form to Z-form may also increase the \( K_d \) of pRW485.

**Effect of Negative Supercoiling and Left-handed Z-DNA on the Association Constants**—It is important to determine how the association of repressor to operator is influenced by negative supercoiling and left-handedness. Because we are able to measure the \( k_s \) values directly, the association rate constants were calculated from the equation \( K_d = k_s/k_{\text{diss}} \). The results are shown in Table I. Only the \( k_s \) values of relaxed and supercoiled (−\( \Delta \) = 0.085) plasmids are presented. The influence of negative supercoiling on the \( K_d \) values was very different from the effects on \( k_s \) and \( K_d \) values. Although both \( K_d \) and \( k_s \) values of plasmids containing the lac operator alone, pRW465, pRW468, and pRW467 were decreased under the influence of negative supercoiling, minimal changes in \( k_s \) values were observed. Also, minimal differences of \( k_s \) values for pRW483 were observed between relaxed and supercoiled DNAs. However, a small decrease in \( k_s \) (2-fold) of pRW483 was observed for plasmids at high negative supercoil densities. This suggested that the promoter region, which was influenced by the flanking left-handed Z-DNA, also affected the rate of association of repressor to the operator in pRW483. If the 29-bp lac operator sequence was altered by the flanking left-handed (dC-dG) blocks, the association of repressor to this operator region would be slower. The \( k_s \) for the pRW485 at high negative supercoil density was, in fact, lower than the \( k_s \) for the plasmid without the left-handed blocks, pRW467.

**DISCUSSION**

The dissociation rate constants and dissociation constants were measured for plasmids containing different lengths of lac operator regions as a function of negative supercoil density. Increasing negative supercoiling increased the binding affinity for all the plasmids investigated. Enhancement of binding affinity of the lactose repressor to the operator region has been attributed to unwinding of DNA sequences by the protein (29). Studies of the lactose repressor-operator complex using covalently closed \( \lambda \)lac5 phage showed a maximum of ~14-fold increase of binding affinity with increasing negative supercoil densities (29). We observed only a 5-fold increase of the binding affinity for repressor using plasmids containing the 95-bp or shorter operator sequences, which suggested that the unwinding angle of the operator by the repressor is smaller than the 90 °C determined using the \( \lambda \)lac5. The difference may be attributed to the differences of the assay conditions or the size of DNAs used (50,000 versus 5,000 bp, respectively).

Surprisingly, we observed an influence of the promoter region (−59 to −20) on the repressor-operator interaction under the influence of negative supercoiling. Determination of the repressor contact regions on the operator sequence in the linear DNA fragment by either dimethyl sulfate methylation, DNase I footprinting, or cross-linking did not reveal contacts in this region (53, 54). However, some contacts upstream of the lac operator upon binding of lactose repressor were observed using photofootprinting techniques (55). The sequence from −8 to −25, which has weak homology to the primary operator, was proposed to bind the repressor. If this sequence is involved in the formation of the repressor-operator complex, it may explain the higher binding affinity we found for pRW465 than pRW468, because pRW468 contains only half of this proposed pseudo-operator. However, similar contact regions between the supercoiled plasmid and linear DNA were observed by primer extension rapid footprinting methods (56). In that study, the presence of the pseudo-operator in the I-gene may mask the influence of the promoter. Whether this promoter region is directly involved in the formation of repressor-operator complex under torsional stress remains a question. A strong S\textsuperscript{S} nucleosome-sensitive region has been mapped at the lac promoter-operator sequence in the supercoiled plasmid, which suggested that there were unusual secondary structures caused by the influence of torsional stress (57). Also, specific S\textsuperscript{S} and mung bean nuclease

### Table I

**Comparison of binding parameters for repressor-operator complex**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>( \Delta )</th>
<th>( K_d \times 10^4 )</th>
<th>( k_s \times 10^{12} )</th>
<th>( k_{\text{diss}} \times 10^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRW465</td>
<td>0</td>
<td>2.1 ± 0.8</td>
<td>3.2 ± 0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>pRW468</td>
<td>0.085</td>
<td>0.43 ± 0.2</td>
<td>0.51 ± 0.05</td>
<td>1.2</td>
</tr>
<tr>
<td>pRW467</td>
<td>0.085</td>
<td>6.8 ± 0.6</td>
<td>3.0 ± 0.3</td>
<td>0.44</td>
</tr>
<tr>
<td>pRW476</td>
<td>0.085</td>
<td>2.6 ± 0.9</td>
<td>1.3 ± 0.2</td>
<td>0.55</td>
</tr>
<tr>
<td>pRW483</td>
<td>0.085</td>
<td>0.38 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>pRW484</td>
<td>0.085</td>
<td>2.3 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td>1.1</td>
</tr>
<tr>
<td>pRW486</td>
<td>0.085</td>
<td>3.1 ± 0.8</td>
<td>1.3 ± 0.1</td>
<td>0.53</td>
</tr>
<tr>
<td>pRW487</td>
<td>0.085</td>
<td>6.1 ± 1.5</td>
<td>3.0 ± 0.1</td>
<td>0.49</td>
</tr>
<tr>
<td>pRW488</td>
<td>0.085</td>
<td>1.8 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.67</td>
</tr>
<tr>
<td>pRW489</td>
<td>0.085</td>
<td>6.2 ± 0.4</td>
<td>8.9 ± 0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>pRW490</td>
<td>0.085</td>
<td>3.3 ± 1.4</td>
<td>1.5 ± 0.4</td>
<td>0.45</td>
</tr>
</tbody>
</table>
cleavage of intact Aplac phage DNA decreased the binding of repressor to the operator (49, 50). It is possible that when the unorthodox secondary structure is formed in the promoter region, it influences the neighboring operator structure, thus affecting the binding affinity. It may be noteworthy that in the L-arabinose operon of E. coli, two of the mutations causing repression-defective phenotypes were found in the RNA polymerase-binding site of P<sub>lac</sub> (58).

The presence of left-handed Z-blocks perturbs the structure of neighboring B-DNA. The physical studies (reviewed in the Introduction) demonstrate this perturbation clearly. Furthermore, Z-DNA inhibits the digestion or modification by the restriction endonucleases BamHI, BssHII, EcoRI, and H<sub>H</sub>al, and methylases H<sub>H</sub>al and EcoRI, when their recognition sequences were in or close to left-handed Z-DNA (see Refs. 8, 35, and 54, and Footnote 2). Transcription by RNA polymerase is terminated by left-handed (dC-dG) tracts (13). Lesions in left-handed (dC-dG) polymers were not repaired by the repair enzymes (59, 60). All of these studies suggested that these proteins did not recognize or utilize Z-DNA or sequences altered by Z-DNA as a substrate. The binding affinity of the lactose repressor to the (dC-dG) polymer (B-helix) is the lowest among the synthetic polymers measured (52). Binding of repressor to left-handed poly(dC-dG) is even less tight than to the right-handed form. This indicated that the lactose repressor also prefers binding to B-DNA than Z-DNA. However, we observed minimal effects of left-handed Z-DNA on the dissociation rate constants for plasmids containing either the 95- or 29-bp operator fragment. Because the binding of the lactose repressor to the operator has a very high affinity (K<sub>d</sub> = 10<sup>-13</sup> M) and unwinds the operator sequence when the repressor reaches the operator (29), it may change the conformation of the altered operator region to the conformation it prefers. Therefore, the binding affinity was maintained. These results indicate that it is possible for a strong regulatory site to coexist next to left-handed blocks without losing the efficiency of regulation. In this context, it will be interesting to learn whether the inhibition of the restriction endonucleases and methylases is due to the loss of binding to their recognition sequences or to an inability to execute the actual catalytic reaction.

The influence of the promoter region on the binding of repressor to the operator at high negative supercoil densities was diminished by the presence of the left-handed Z-DNA. We propose that when the flanking (dC-dG) tracts undergo the B to Z transition, the left-handed Z-DNA perturbs the secondary structure of this promoter region. If there is direct contact of the repressor to this region, it may be diminished. If the influence of the binding is due to structural effects on the operator region, the influence is blocked. Therefore, the binding affinity of repressor to the 95-bp operator sequence flanked by the left-handed Z-DNA is decreased.

All of these results indicate that the interaction of the lac repressor with its binding site is increased by the presence of the neighboring RNA polymerase-binding site; also, the torsional strain on the DNA and the presence of flanking unorthodox conformations profoundly influence this important regulatory process. Hence, this segment of DNA seems remarkably pliable from a structural standpoint as found previously (9) for other systems. Thus, DNA structure as well as the DNA sequence are important for protein-DNA interactions and may be involved in gene regulation.

Also, a simple method is described to label supercoiled plasmids without affecting the negative supercoiling using the H<sub>H</sub>al methylase and [H]<sub>1</sub>AdoMet. Because the labeling was carried out after the plasmids were purified, the need for using thymine-requiring strains and isolating DNA in the presence of [H]<sub>1</sub>thymine was eliminated. No nicking or linearization of the supercoiled plasmids was observed after methylation.

Because only small portions of the DNA sequences were methylated (31 H<sub>H</sub>al sites in pBR322), this should not interfere with the experiments unless the methylated sites were important for the interaction.

REFERENCES

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