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An amino-terminal fragment of *lac* repressor binds specifically to *lac* operator

(dimethyl sulfate methylation/tryptic peptide/bromouracil DNA/protein domains)

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ABSTRACT **We have examined the effect of an amino-terminal peptide from the *lac* repressor (residues 1–59) on the methylation of purines in the *lac* operator with dimethyl sulfate. The peptide perturbs the methylation of the operator, and the peptide-induced pattern of inhibition and enhancement of methylation, across the operator, closely resembles the pattern induced by intact repressor. This demonstrates that this small amino-terminal peptide binds specifically to the *lac* operator and that the mechanism of recognition and binding is basically the same as that of intact repressor.**

How does the *lac* repressor bind to the *lac* operator? Does it hold the operator in a groove on its surface or does it reach out with "arms" to grasp the operator? Six years ago, Adler *et al.* (1) proposed that the first 50 amino acids at the amino terminus of the *lac* repressor bind directly to the *lac* operator. The small size of this region suggested to them that the operator-binding structure is a protrusion that reaches out to the operator. They based these ideas on their observation that most mutations that damage the repressor's ability to bind operator but not its ability to form tetramers—i^{-d} mutations—map within the first 20% of the *i* gene (1).

Additional support for these ideas came from the work of Weber, Miller, and their colleagues (2–5). Studies on translational reinitiation within the *lac* repressor demonstrated that repressor molecules lacking 23–61 amino-terminal amino acids have no operator-binding capacity but show a normal affinity for inducer and have a normal tetrameric structure (4, 5). Furthermore, the amino terminus is highly susceptible to protease digestion: when repressor is treated with trypsin under nondenaturing conditions, only the amino terminus up to amino acid 59 is attacked (2, 3), and the trypsin-resistant core, the remainder of the molecule, forms a tight, compact structure that maintains the tetrameric structure and binds inducer.

Recently, Geisler and Weber (6) developed a method for assessing directly the role of the amino terminus in operator binding. They found that, in 1.0 M Tris·HCl, pH 7.5/30% (vol/vol) glycerol, tryptic cleavage is limited to only two sites in the *lac* repressor—arginine-51 and lysine-59. Cleavage under these conditions yielded the tetrameric trypsin-resistant core and approximately equal amounts of two amino terminal "headpieces": one spanning amino acids 1–59 (long headpiece) and the other amino acids, 1–51 (short headpiece) (as well as an octapeptide accounting for amino acids 52–59). Geisler and Weber reasoned that, if the binding structure were to be a protrusion, its structure should be relatively unaffected by the rest of the molecule and one or both of these headpieces might retain its native structure and bind specifically to the *lac* operator. Using the nitrocellulose filter binding assay (7), they demonstrated that the headpieces have a substantial affinity for DNA. However, they could not detect any specific preference of the headpieces for *lac* operator. Müller-Hill *et al.* (8), also using the filter binding assay, obtained an analogous result with repressor grafted to β-galactosidase: fusion of the first 60–80 residues of *lac* repressor to β-galactosidase yielded a chimeric protein that exhibited a strong but non-operator-specific affinity for DNA.

Here, we have examined the interaction between the long repressor headpiece and *lac* operator by using methylation with dimethyl sulfate as a probe for protein–DNA contacts (9). The lack of operator specificity found by Geisler and Weber might have been due to the inability of the filter binding method to discriminate between specific and nonspecific binding. Because nonspecific binding sites, such as the phosphates of DNA, in general outnumber specific sites by several orders of magnitude (by almost 50,000 in the studies by Geisler and Weber), filter binding reveals specific interactions only if they are strong enough to compensate for the difference in numbers. In contrast, the methylation method responds only to specific protein–DNA interactions. Nonspecific binding is either not detected at all or appears as a uniform effect along the entire length of a DNA molecule.

Isolation of repressor headpieces

Trypsin cleaves the repressor first at residue 59 and then at residue 51. A 45-min digestion produces an 80% pure sample of long headpiece; digestion for 3–4 hr produces virtually pure short headpiece. Fig. 1 shows a time course and the purity of our isolated headpieces.

Methylation method

Dimethyl sulfate methylates the purines in DNA at the N-7 of guanines and at the N-3 of adenines (12). The methylation method (9) exploits three characteristics of this reaction. First, the reaction takes place under very mild, nondenaturing conditions—conditions that do not interfere with normal protein–DNA binding reactions. Second, methylation produces a weak link in the DNA backbone. We identify the site of methylation simply by breaking the DNA at the methylated base with heat and alkali and measuring the distance between the break and the end of the DNA strand by sizing an end-labeled fragment on a polyacrylamide gel. Third, proteins bound to DNA perturb the methylation reaction at the protein-binding site. Thus, we measure the extent of methylation at each purine in a DNA sequence in the absence and presence of a DNA-binding protein. Differences in the level of methylation outline the site of interaction on the DNA and point out specific sites of purine–protein contact. A DNA-binding protein can both decrease (protection) and increase (enhancement) the level of methylation of purines in the contact region. Protection is most likely caused by steric hindrance. Although the mechanism of enhancement is unknown, it appears likely to represent a close approach of the protein to DNA in such a way as to

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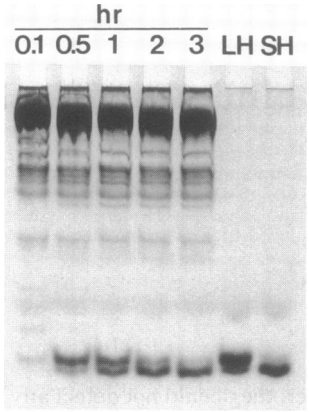

FIG. 1. Slab gel (12.5% acrylamide, 1.25% bisacrylamide, 8 M urea, 0.1% sodium dodecyl sulfate, 0.1 M PO_4/Tris at pH 6.8) (10) showing the time course of the tryptic digestion of the *lac* repressor and the purified products from 45-min (LH) and 3-hr (SH) digestions. Conditions were as described by Geisler and Weber (6). The gel was run at 6 V/cm for 18 hr, stained (11) for several hours at 37°C, and destained in 5% methanol/7.5% acetic acid. The light smear migrating just behind the headpieces is Congo red.

produce a hydrophobic pocket that increases the local concentration of the reagent. Because the N-7 position lies in the major groove of DNA and N-3 lies in the minor groove, a perturbation of the methylation reaction at a guanine or adenine suggests a protein contact in the major or minor groove, respectively.

In the present experiments, we took a 55-base-pair long DNA fragment containing the *lac* operator and labeled it at one or the other 5′ terminus with ^{32}P (9, 13). (The sequence of this fragment is displayed at the top of Fig. 4.) We treated this end-labeled fragment with dimethyl sulfate such that, on the average, fewer than one purine per DNA strand was methylated, cleaved the strands at the methylated bases with heat and alkali, separated the products by electrophoresis on a polyacrylamide gel, and visualized the ^{32}P-labeled fragments by autoradiography. Because of the low level of methylation, all purines were methylated randomly, and fragments corresponding to methylation at each purine in the sequence were generated. These fragments appear in order of size as bands in the autoradiogram. The intensity of each band is proportional to the level of methylation of the corresponding purine. Thus, repressor- or headpiece-induced perturbations of the methylation reaction are detected as changes in the intensities of bands in the autoradiogram.

Long headpiece binds specifically to the *lac* operator

Fig. 2 shows the methylation-induced cleavage products from the top strand (in Fig. 4) of the 55-base-pair fragment. Methylation was carried out in the absence of headpiece and in the presence of four concentrations of headpiece, ranging from 12 to 80 μg/ml (1.8–12 μM). We chose this range of headpiece concentrations by reasoning as follows. Two repressor subunits are adequate for almost normal repressor-operator binding (16, 17). If the headpiece contains most of the operator-binding components of the repressor and if it is capable, by itself, of binding to the operator, then we might expect that the number of headpiece–operator contacts is about one-half the number of repressor–operator contacts. This would give a headpiece–operator binding energy of about one-half the repressor–operator binding energy and a headpiece-operator dissociation constant roughly equal to the square root of the repressor–operator dissociation constant. Under low-salt conditions, the repressor–operator dissociation constant is 10^{-13} M and this

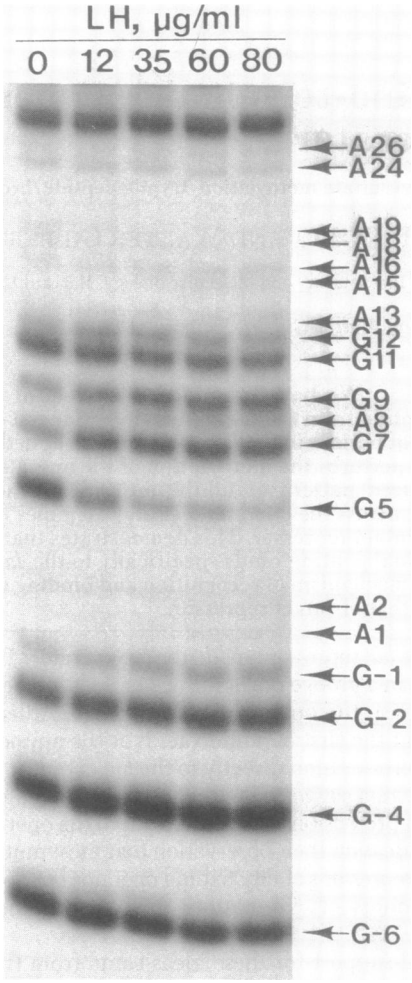

FIG. 2. Autoradiogram of a 20% polyacrylamide gel (prepared as described in ref. 14) showing the effect of long headpiece (LH) on methylation of the 55-base-pair-long operator-containing fragment. The sequence of this fragment is given in Fig. 4. Here, the 5′ end at the *Hpa* II cleavage site is labeled with ^{32}P, and methylation-induced cleavages at purines in the top strand of the fragment appear as bands in the autoradiogram. Each band represents cleavage at the purine listed to the right of the band. The fragment was isolated and labeled as described (13). Headpiece concentrations were determined spectrophotometrically (15). The reaction mixture consisted of 1 pmol of labeled fragment in 100 μl of 50 mM cacodylate, pH 8/10 mM $MgCl_2$/0.1 mM EDTA containing sonicated calf thymus DNA (10 μg/ml) and bovine serum albumin (200 μg/ml). Long headpiece and 1 μl of dimethyl sulfate were added and the mixture was incubated at room temperature for 5 min. The sample was then precipitated and prepared as described in ref. 14 for "strong guanine/weak adenine cleavage" except that heating at 90°C was for 10 min at pH 7.0 and for 5 min in 0.1 M NaOH. The 55-base-pair-long fragment used in these studies was about 50% substituted with 5-bromodeoxyuridine (see text). Bromodeoxyuridine substitution causes the relatively diffuse quality of the bands; this is minimized by heating for shorter periods.

increases to about 10^{-10} M in the presence of inducer (18, 19). Therefore, we guessed that the headpiece–operator dissociation constant would fall in the range 10^{-5}–10^{-6} M.

The data in Fig. 2 demonstrate that the headpiece strongly affects the methylation of specific purines in the top strand of the operator; the other strand is affected similarly. We regard this clear headpiece-induced effect on methylation exclusively at the operator as proof that the headpiece binds specifically to the operator sequence. This conclusion is independent of whether or not the individual purines affected would have been similarly affected by intact repressor. However, the inhibition

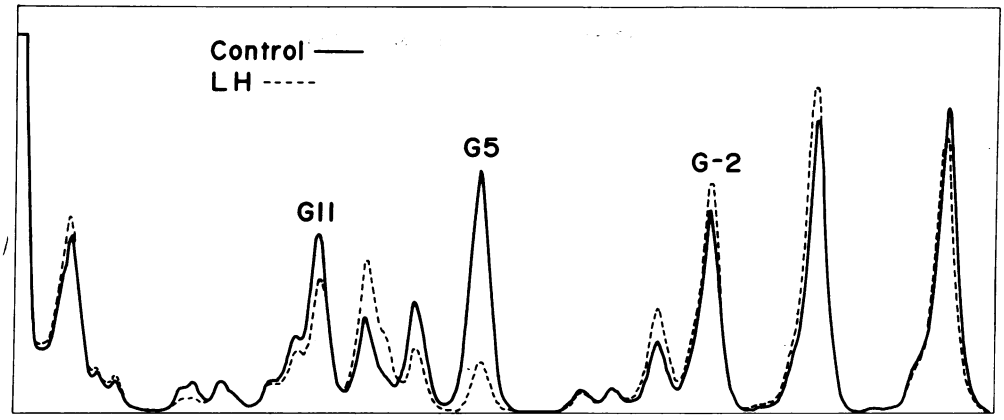

FIG. 3. Superimposed densitometer (Ortec model 4310) tracings of autoradiograms similar to those shown in Fig. 2. The tracings describe the results of methylation in the absence and presence (70 μg/ml) of long headpiece (LH). These tracings were used to measure the intensity of each band and to generate the graphs shown in Fig. 4.

and enhancement of methylation at individual purines in the operator are remarkably similar for intact repressor and for the long headpiece.

Using a densitometer, we measured the intensity of each band in the autoradiograms. Fig. 3 shows such a quantitation of the effect of the long headpiece on purine cleavage bands. The effects of repressor and long headpiece on methylation of purines in and proximal to the operator are summarized in Fig. 4. These data show clearly the striking resemblance between the methylation protection-enhancement patterns of intact repressor and long headpiece.

The results obtained with long headpiece are not caused by contamination with intact repressor. In these experiments the effect of the headpiece on the methylation was saturated at approximately 10 μM in the presence of 10 nM ^{32}P-labeled operator (Fig. 2). If this effect were to be caused by contaminating repressor, the repressor concentration would have to be about 10 nM because binding is essentially complete at these

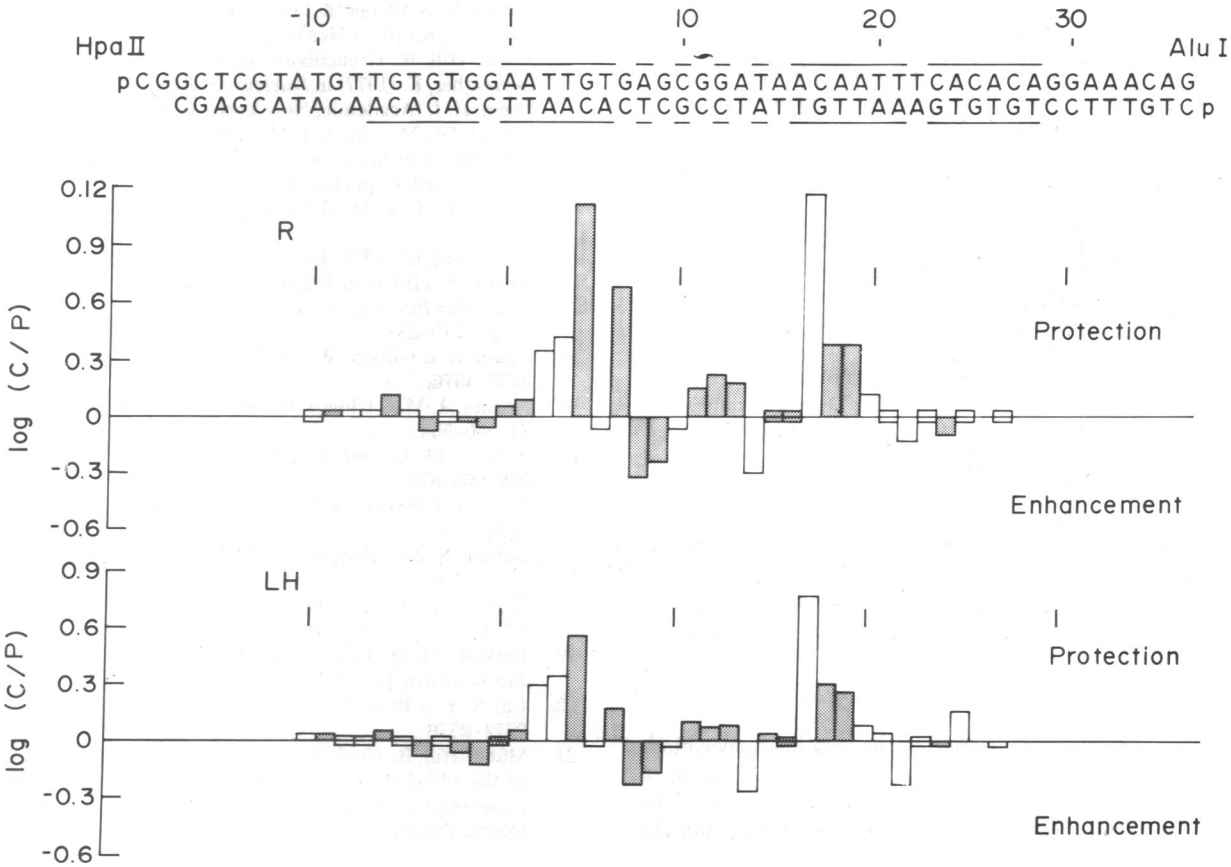

FIG. 4. The effect of repressor (R) and long headpiece (LH) on methylation of the 55-base-pair-long fragment. The sequence of the 55-base-pair-long fragment containing the operator is given at the top. Each bar in the figure is aligned with a purine in the sequence and gives the effect of added protein on methylation at that purine. Shaded bars refer to purines in the top strand and clear bars, to purines in the bottom strand. The effect on methylation is given as the logarithm of the ratio C/P, in which C is the level of methylation in the absence of added protein and P is the level of methylation in the presence of repressor or long headpiece. Methylation is enhanced when log (C/P) < 0, inhibited when log (C/P) > 0, and unaffected when log (C/P) = 0. These data represent the average of four separate experiments; C/P values were reproducible to within 10–15%.

concentrations. Adding excess unlabeled operator to the reaction should displace the repressor molecules from the labeled operator, and methylation should give the pattern observed in the absence of headpiece. However, the pattern induced by headpiece under these conditions was not affected by the addition of 125 nM unlabeled operator in a control experiment.

The operator-containing DNA fragments were substituted about 50% with 5-bromodeoxyuridine (BrdUrd) (13). The BrdUrd–operator gives a stronger pattern of protection and enhancement with the headpiece than does the normal (dThd-containing) operator. Qualitatively, the patterns are the same for both the BrdUrd and dThd–operators. The quantitative differences are probably due to a tighter binding to the BrdUrd operator. The *lac* repressor itself binds to BrdUrd–operator about 10-fold more strongly than to dThd–operator (20).

The pattern of protection and enhancement exhibited by intact repressor in Fig. 4 differs slightly from that described earlier (9). Prominent differences occur at A-18 (from no effect in ref. 9 to strong protection here) in the top strand and G-10 (enhancement to very weak enhancement) and A-14 (no effect to strong enhancement) in the bottom strand. Smaller discrepencies are at G-11 (no effect to weak protection) and A-13 (no effect to weak protection) and at A-22 (no effect to weak enhancement). Although the previous study used the tight-binding mutant repressor X-86, none of the differences can be attributed to differences in the repressor molecules; X-86 and wild-type repressor make the same contacts to the *lac* operator. The effects at A-18, G-11, A-13, and A-22 were simply overlooked earlier; changes at the latter three purines were considered to be marginally small and the protection at A-18 was obscured by a contaminating band. The weak enhancement at G-10 measured here is peculiar to BrdUrd-substituted DNA: enhancement at G-10 is consistently stronger with dThd–operator. And the strong enhancement at A-14 is related to BrdUrd substitution and temperature: A-14 is enhanced at room temperature (Fig. 4) and at 0°C with BrdUrd–operator and at room temperature with dThd–operator but not affected at 0°C with dThd–operator (9). An interesting feature of the present protection-enhancement patterns is the symmetry they share with the operator sequence.

That the amino-terminal region of the control protein contains the information to make the specific DNA contacts is not unique to the *lac* repressor. R. Sauer and C. Pabo (personal communication) have found that a papain fragment spanning the amino-terminal half of the *lambda* repressor makes all the relevant *lambda*–operator contacts.

The small differences in the protection-enhancement patterns doubtless reflect small differences in the structures of the long headpiece and the amino-terminal region of the *lac* repressor. Thus, the methylation method can be used as a structural probe.

Summary

The present results demonstrate that the long headpiece binds specifically to the *lac* operator and that the mechanism of binding is essentially the same as with intact repressor. The relationship between the affinities of the headpiece and the whole repressor for the operator and the strong dyadic symmetry of the methylation pattern across the operator suggest that two headpieces make all the DNA contacts. We conclude that (*i*) the amino-terminal region of intact repressor directly touches the *lac* operator and constitutes most, if not all, of the repressor's operator binding site, (*ii*) the tertiary structure of the long headpiece is very similar to the tertiary structure of the corresponding region of intact repressor, and (*iii*) the structure of the amino-terminal region of the repressor forms a distinct structural element (probably a protrusion) whose conformation is largely independent of the rest of the protein. Müller-Hill and his colleagues (21) also reached this last conclusion based on their work with repressor–galactosidase fusion proteins.

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