Non-contacted Bases Affect the Affinity of Synthetic P22 Operators for P22 Repressor*

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The affinity of synthetic P22 operators for P22 repressor varies with the base sequence at the operator's center. At 100 mM KCl, the affinity of these operators for P22 repressor varies over a 10-fold range. Dimethylsulfate protection experiments indicate that the central bases of the P22 operator are not contacted by the repressor. The $K_D$ for the complex of P22 repressor with an operator bearing central T-A bases ($9T$) increases less than 2-fold between 50 and 200 mM KCl, whereas the $K_D$ for the complex of repressor with an operator bearing central C-G bases ($9C$) increases 10-fold in the same salt range. The DNase I cleavage patterns of both bound and unbound P22 operators also vary with central base sequence. The DNase I pattern of the repressor-9C operator complex change markedly with salt concentration, whereas that of the 9T operator-repressor complex does not. These changes in nuclease digestion pattern thereby mirror the salt-dependent changes in the P22 operator's affinity for repressor. P22 repressor protects the central base pair of the 9T operator from cleavage by the intercalative cleavage reagent Cu(II)-phenanthroline, while repressor does not protect the central bases of the 9C operator. Together these data indicate that central base pairs affect P22 operator strength by altering the structure of the unbound operator and the repressor-operator complex.

The $c_2$ gene of the lambdoid bacteriophage P22 codes for a DNA-binding protein called the repressor (Sauer et al., 1982). This protein displays extensive structural and functional homology with other lambdoid bacteriophage repressors (Hochchild et al., 1983; Poteete et al., 1980; Poteete and Ptashne, 1982; Ptashne, 1986; Sauer et al., 1982). Despite these homologies, the various phage repressor proteins each bind only the specific operator sequences present in its own phage (Ptashne, 1986). The repressor is required by its cognate phage for the establishment and maintenance of the lysogenic state. Each phage contains two operator regions, $O_R$ and $O_L$, consisting of three closely spaced binding sites (Poteete et al., 1980). In all these phages, the repressors bind as dimers of identical subunits to these six binding sites. Efficient functioning of the genetic switch controlling these phages' decision between lysis and lysogeny requires that the phage's repressor have different affinities for these operators (Ptashne, 1986). The ability of the repressors to discriminate between these operators is critical for the growth life cycle choices of the bacteriophage. Despite their highly homologous structures and functions, the two well studied repressors of the bacteriophages 434 and $\lambda$ employ different structural mechanisms to discriminate between each of their six operators. The purpose of this investigation is to begin to determine the structural basis for P22 repressor-operator discrimination.

The results of NMR investigations show that, like the other lambdoid phage repressors, the repressor protein of bacteriophage P22 belongs to the class of proteins containing a helix-turn-helix structural unit (Senn et al., 1987). This unit has been shown to be responsible for DNA binding specificity (Wharton and Ptashne, 1986). In complexes of $\lambda$ repressor and 434 repressor with their cognate operators, one dimer of the protein is bound to a partially 2-fold rotationally symmetric operator sequence on B-form DNA (Aggarwal et al., 1988; Anderson et al., 1987; Jordan and Pabo, 1988). The 2-fold related "recognition" $\alpha$-helices, one from each protein monomer, lie in successive major grooves on one face of the DNA. Each recognition helix is positioned in the major groove so that its side chains can make base-specific contacts with the outermost base pairs of the operator (Aggarwal et al., 1988; Anderson et al., 1987; Jordan and Pabo, 1988). Mutagenesis experiments show that this view of sequence-specific protein-DNA contacts is true for P22 repressor (Wharton and Ptashne, 1986). In this "specificity swap" experiment, replacing the solvent exposed residues of 434 repressor's recognition helix with those present at homologous positions in P22 repressor's putative recognition helix results in a hybrid protein that binds specifically to P22 operators. Moreover, this hybrid protein has the same order of preference for the binding sites in P22 $O_R$ as does wild-type P22 repressor.

While the importance of base-specific protein-DNA contacts in P22 repressor-operator complexes to binding specificity is clear from the above description, this may nonetheless be an incomplete portrait of the determinants of its DNA binding specificity. The idea that the "recognition helix" is solely responsible for determining DNA sequence specificity cannot account for the changes in operator affinity for protein that occur when non-contacted base pairs at the center of a binding site are mutated (Koudelka et al., 1987). For example, in the case of 434 repressor, biochemical and x-ray crystallographic studies show that neither the helix-turn-unit nor any other part of the proteins can contact the four base pairs at the center of the 434 operator (Aggarwal et al., 1988; Koudelka et al., 1987, 1988). Nevertheless, 434 operator's affinity for 434 repressor is determined, in part, by the base composition at the center of the operator (Koudelka et al., 1987, 1988).

Several lines of evidence suggest that like 434 repressor, the ability of P22 repressor to discriminate between various P22 operators is modulated, at least in part, by the base composition at the center of the operator. First, analysis of the sequences of the six 18-base-pair operator sites reveals a

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providing pattern: the symmetrically arrayed outermost base pairs of the P22 repressor are conserved, while the sequences in the center of the operator are highly divergent (Fig. 1) (Poteete and Ptashne, 1982). Despite the view that these outermost conserved bases are the only sites for specific protein-DNA contacts (Wharton and Ptashne, 1985, 1986), the affinity of P22 repressor for these operators varies over a 50-fold range1 (DeAnda et al., 1983). Second, the specificity swap experiment shows that when P22 amino acid sequences are grafted onto a protein that is sensitive to the sequences at the center of the operator, the 434 repressor, the resulting hybrid protein has the same order of preference for the binding sites in P22 O₁ as does wild-type P22 repressor (Wharton and Ptashne, 1985). This suggests that P22 repressor, like 434 repressor, also uses a central operator sequence to aid in distinguishing between various operators.

To examine more directly whether the central bases of the P22 operator play a role in determining its affinity for P22 repressor, a series of P22 operators, mutant in their central base pairs, were synthesized and their affinity for P22 repressor determined (Fig. 1). The evidence presented here shows that although the central bases are not contacted by P22 repressor, the sequence of these base pairs has a significant role in determining the affinity of operator for repressor. The order of preference of the P22 repressor for central base sequences differs from that seen with 434 repressor, suggesting that P22 repressor uses a different mechanism in recognizing these base pairs. To further characterize the mechanism by which the central bases modulate P22 operator strength, we determined the salt dependence of these interactions. We show that the affinity of one operator for P22 repressor is salt insensitive, while that of another is markedly salt sensitive. These data suggest that the structure of the unbound P22 operator and/or P22 repressor-operator complexes varies with central sequence. The results of nuclease digestion studies support this conclusion.

**Experimental Procedures**

**Binding Sites, Plasmids, DNA Fragments, and End-labeling—DNA manipulations were performed as described (Maniatis et al., 1982). The binding sites oligonucleotides were synthesized in an Applied Biosystems model 380A DNA synthesizer, annealed, and ligated into the unique Sall site in pUC18 (Messing, 1983). The sequences of the binding sites used are given in Fig. 1. The sequences of the resulting plasmids were confirmed by dideoxy methods. These DNA molecules were cleaved at the EcoRI site and 3'-end-labeled by repairing the recessed ends with Klenow and [γ-32P]ATP or 5'-end-labeled with [γ-32P]ATP and polynucleotide kinase. These ~2700-bp pair linear DNAs were used directly in filter binding studies. For all the nuclease digestion and dimethylsulfate protection experiments, the labeled DNA was cut with HindIII and the resulting ~800-base pair operator-containing DNA fragment was gel-purified. Control experiments show that the affinity of repressor for DNA and the structure of the repressor-DNA complexes are independent of DNA length.

**Protein Preparation**—P22 repressor was isolated from the Escherichia coli strain X90 bearing the plasmid pTP125 which causes overproduction of the P22 repressor (DeAnda et al., 1983). This plasmid was a gift from A. Poteete (University of Massachusetts Medical Center, Worcester, MA). The P22 repressor was purified as described by DeAnda et al. (1983).

**DNA Binding Assays—**DNA binding assays were performed essentially as described by Johnson et al. (1979). Briefly, binding reactions were performed in 10 mM Tris-HCl, pH 7.8, 1 mM MgCl₂, 1 mM dithiothreitol, 2.5 µg/ml of sonicated chicken blood DNA, 100 µg ml⁻¹ of bovine serum albumin, ~1 nM operator DNA, either 50 or 150 mM KCl, and an appropriate amount of protein. After a 10-min incubation at 25 °C, sufficient DNase I was added to give, on average, one cleavage/DNA molecule in 5 min of further incubation. Cu(I)-phenanthroline protection assays were performed essentially as described by Spassky and Sigman (1985). The reaction conditions for these experiments were identical to those for the DNase I protection experiments except that 1 mM mercaptoethanol was used in place of dithiothreitol. Dimethylsulfate protection assays were performed as described by Wharton et al. (1986). In this experiment, 50 mM sodium cacodylate was used as the buffer ion, and all other components were identical to the those used in DNase I experiments. Guanine-specific cleavage was affected by heating the DMS-modified DNA in piperidine at 90 °C. For G = A cleavages, the DMS-modified DNAs were incubated in phosphate buffer, pH 7.0, at 0 °C for 30 min prior to piperidine cleavage. Following reaction of the DNA with the modifying agent, the ethanol-precipitated samples were resuspended in 90% formamide dixy mix and denatured by heating at 90 °C. The samples were electrophoresed on 25 × 30-cm denaturing 7.5% polyacrylamide gels containing 7 M urea, 89 mM Tris-HCl, 89 mM sodium borate, and 1 mM EDTA. Autoradiography was performed by exposing the gel to preflashed Kodak XAR-5 film with or without an intensifying screen at ~80 °C. Relative band intensities of all DMS and footprinting results were quantified by densitometric scanning of the autoradiographs. The numbers quoted in the text represent averages obtained from scans of at least two separate autoradiographs resulting from separate experiments. No significant differences in either the quantitative or qualitative appearance of the footprinting results were detected between the replicate experiments. All footprinting and DMS protection results were obtained at saturating concentrations of binding protein; results of experiments performed with a 3-fold higher protein concentration were not qualitatively or quantitatively different from the results reported here.

**Nucleotide Filter Binding Experiments**—Nitrocellulose filter binding experiments were performed as described (Wharton and Ptashne, 1985). The reaction buffer for this assay contained 10 mM Tris-HCl, pH 7.8, and 50 mM, 100 mM, 150 or 200 mM KCl. The counts retained on the filter as a function of P22 repressor concentration were converted to fractional saturation values (Lin and Riggs, 1972). Fractional saturation values were determined in duplicate. Three to five duplicate measurements were averaged and dissociation constants determined from non-linear least squares fits to the data. The maximum variation in the Kd determined from each duplicate measured was less than 2-fold. The standard deviation of the reported dissociation constants was less than 5% of the value. Protein concentrations cited are correct for protein activity (~85% of protein is active in DNA binding).

**Results**

**Affinity Order of P22 Repressor for Synthetic Operators—**Using filter binding, we have determined the affinity of P22 repressor for four synthetic operators at 100 mM KCl (Fig. 1). These operators are identical in sequence except for the central two bases of the dyad symmetric operator site. Table 1 shows that changing the two central bases, positions 9 and 10, together from T·A·A·T to any other base composition exam- ined decreases the affinity of the synthetic P22 operator for P22 repressor. At 100 mM KCl, the affinity of these operators for P22 repressor varies over 10-fold. Under these conditions, P22 repressor displays a hierarchy of preference.

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1. L. Wu and G. B. Koudelka, unpublished data.

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2. The abbreviations used are: DMS, dimethylsulfate; CuOp, bis-(1,10-phenanthroline)copper (I).

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**Fig. 1. P22 operator sequences.** Sequences of the naturally occurring and synthetic P22 operators along one strand. The boxed regions of the naturally occurring operators highlight the regions of high sequence conservation. The bases in the synthetic operators that were varied in these experiments are circled.
TABLE I

Affinity of P22 synthetic operators for P22 repressor

For the nomenclature used for the operators, see Fig. 1. Binding affinities are expressed as the concentration of repressor monomers needed to give a fractional saturation of 0.5 for each operator in a filter binding experiment. These values have been normalized to the dissociation constant for the repressor-9T operator complex. Under the conditions of these experiments, unity corresponds to 1.6 × 10⁻⁸ M. The standard deviations of the individual dissociation constants are less than 5% of their value.

| Operator | Relative Kd |
|----------|-------------|
| 9T       | 1           |
| 9G       | 5.7         |
| 9A       | 6.8         |
| 9C       | 9.3         |

Dependence of P22 Repressor-Operator Complex Stability on Salt Concentration—In an attempt to gain insight into the mechanism by which the non-contacted bases affect the affinity of P22 operator for P22 repressor, the salt dependence of the stabilities of the P22 repressor-synthetic operator complexes was determined. The Kd for the repressor-9T operator complex increases less than 2-fold between 50 and 200 mM KCl, whereas the Kd for the P22 repressor-9C operator complex increases 10-fold in this same salt range. These results are displayed graphically in Fig. 3. Analysis of these data by the method of Record et al. (1977) indicates that when P22 repressor binds, different numbers of cations are released from each of the two operators. This analysis suggests that 0.5 cations are displaced by each monomer of repressor binding to the 9T operator whereas 1.3 cations are displaced by each monomer when repressor binds to the 9C operator. These results imply that the structures of the repressor complexes with the 9T operator and 9C operators are different.

In terms of the number of cations released by P22 repressor binding, the results obtained in this study are similar to those reported by DeAnda et al. (1983). Using a DNA fragment containing all six naturally occurring operator sites, they interpreted the salt dependence of dissociation constants as corresponding to the release of 1.5–2 cations/operator half-site. It should be noted that both our results and those of DeAnda et al. (1983) predict a smaller number of cations released than the number of protein-phosphate backbone contacts identified in ethylnitrosourea interference experiments (Potete et al., 1980). This is consistent with the idea that salt dependences of affinity identify only interactions of charged protein groups with the DNA backbone.

DNase I Digestion Studies of P22 Operators and Repressor-Operator Complexes—DNase I can be used to study the backbone structure of DNA. Its rate of cleavage of the DNA phosphate backbone is influenced by the width of the minor groove near the site of cleavage, the flexibility of the DNA in this region, or the presence of bound protein (Drew and Travers, 1985; Johnson et al., 1979; Lomonosoff et al., 1981; Satchwell et al., 1986). The rate of cleavage of the DNA backbone is proportional to the observed band intensity. To determine whether the structure of the unbound operators and/or the P22 repressor-operator complex varies with central

Fig. 2. Dimethylsulfate protection analysis of complexes formed between P22 repressor and synthetic 9T (A) and 9G (B) operators. Experiments were performed as described under "Experimental Procedures." For each unbound operator and repressor-operator complex, the DMS-modified DNAs were treated to favor cleavage after guanines (G) or guanine and adenine (G = A). This allows analysis of DMS protection events that occur in the major groove or major and minor grooves, respectively. The sequence of the operator is shown at the center. The numbers denote operator positions as described in Fig. 1.

for base compositions at the central operator positions. At positions 9 and 10 of the operator, P22 repressor prefers T-A-A-T > G-C-C-G > A-T-T-A > C-G-G-C. The results of dimethylsulfate protection experiments show that P22 repressor does not protect any purines in either the central or outer positions in any of the synthetic operators from methylation. Fig. 2 shows a representative experiment using the 9T and 9G operators. Inspection of the DMS results in Fig. 2 and other results (not shown) shows that P22 repressor does not protect the central base pairs in any operator in either the major (G-reaction) or minor (G = A reaction) groove. Parallel DNase I protection experiments, performed under conditions identical to those used in the DMS experiments, showed that the synthetic operators were fully saturated with P22 repressor (data not shown). The DMS results therefore indicate that the central base pairs of the P22 operator are not contacted by the P22 repressor. Together, the affinity measurements and the DMS protection results indicate that the central base composition of the P22 operator affects its affinity for repressor although these bases are not contacted by the protein.

Fig. 3. Salt dependence of P22 repressor-operator dissociation constants. Plotted is the -log Kd versus -log [KCl] for the 9C (triangle) and 9T (circles) operators. Error bars on the measured Kd are smaller than symbol size used to represent the value. The lines represent linear least squares fits to these data.
base sequence, the conformation of the DNA-phosphate backbone of the 9T and 9C operators were probed with DNase I in the absence and presence of protein at different concentrations of KCl. Fig. 4 shows that the DNase I cleavage patterns of both the unbound and bound 9T operator are different from those of the unbound and bound 9C operator. Some of these changes in DNase I pattern occur far from the site of base substitution, while others occur at or near the site of base substitution.

Probing the unbound 9T and 9C operators at 50 mM KCl, shows that the relative intensity of the band resulting from cleavage at position 15 is 2-fold greater in the 9C operator than in the 9T operator (Fig. 4A). These differences appear to represent a “long distance” effect of central base substitution on synthetic P22 operator structure. There are also significant differences in the relative intensity of the bands corresponding to cleavage at positions 10 and 12 in the two operators. Cleavage of the DNA backbone of the 9C operator at position 12 is about 1.5-fold greater relative to the 9T operator, but at position 10, cleavage in the 9C operator is 2-fold lower. These are “local” effects and relate more directly to the sequence changes at the operator’s center (positions 9 and 10). Both the long distance and local effects of central base substitution on operator structure can be detected by DNase I at 150 mM KCl (Fig. 4B). These data suggest that the structure of the unbound P22 synthetic operators varies with central base sequence.

At both 50 and 150 mM KCl, adding protein to the 9T and 9C operators alters the observed DNase I cleavage pattern, relative to that seen with the unbound operators (Fig. 4). Consistent with the idea that the structure of the P22 repressor-operator complex varies with central base sequence, the magnitudes and types of changes in DNase I cleavage pattern differ between the bound 9T and 9C operators. At 50 mM KCl, the presence of bound repressor does not affect the accessibility of DNase I to the DNA at position 16 in the 9T operator. In contrast, bound repressor increases the accessibility of DNase I to the DNA at this position by 1.7-fold in the 9C operator. At 150 mM KCl, bound repressor affords very little protection of the DNA backbone at operator positions 15 and 16 of the 9T operator. Similarly, bound repressor also affords little protection to the base at position 15 of the 9C operator. By contrast with the 9T operator however, repressor binding increases by 2-fold DNase I cleavage at position 16 of the 9C operator. Another difference in the repressor effects on cleavage of the two repressor-bound operators is seen at position 11. Repressor slightly enhances cleavage at this position in the 9T operator but has no effect in the 9C operator. Taken together these data show that the structures of the bound 9T and 9C operators are different. Moreover, these DNase I-detected alterations in structure are seen both at or near the site of base substitution and at a distance.

The effects of salt on the DNase I cleavage pattern of the 9T and 9C operators both free in solution and in complex with repressor appear to mirror the salt effects on affinity (Fig. 3). Consistent with the relative salt insensitivity of the 9T-repressor complex, as the salt is changed from 50 mM to 150 mM KCl, the DNase I cleavage patterns of the unbound and repressor-bound 9T operator change only slightly. Only two effects of salt are seen: 1) in the unbound 9T operator, the intensity of the band at position 16 increases 2-fold at high salt, and 2) repressor protects the DNA backbone at position 15 by 3-fold at low salt, whereas repressor protects this position by 1.5-fold at higher salt. By contrast, multiple effects of varying salt concentration are apparent for both the unbound and repressor-bound 9C operator. Relative to the results at low salt, the intensity of cleavage of the unbound 9C operator at positions 12 and 15 decreases at high salt by 3- and 1.5-fold, respectively. Similar to the results with the bound 9T operator at low salt, repressor nearly completely protects operator position 15 in the 9C operator from cleavage. Unlike the result with the 9T operator at high salt repressor fails to protect position 15 from cleavage. Additionally, at low salt repressor protects position 10 of 9C from cleavage but completely fails to do so at low salt. More subtle changes (1.3-1.5-fold) resulting from varying salt concentration are seen in relative intensities of cleavages and extent of repressor protection of positions 6 and 15 of the 9C operator.

**Cu(I)-Phenanthroline Digestion Studies of P22 Operators and Repressor-Operator Complexes—Cu(I)-phenanthroline (CuOP) and its derivatives are chemical nucleases that cleave DNA in a two-step reaction, minor groove binding followed by strand scission (Chen and Sigman, 1990). The binding of these reagents is guided, at least in part, by intercalation (Veal and Rill, 1991). As a result of this binding, these reagents can also be used as a probe of DNA conformation (Spaßky and Sigman, 1985). Alterations in the base structure which affect the ability of the reagent to bind and/or intercalate should modify the rate of CuOP cleavage. To further characterize the apparent dependence of the structures of the unbound and bound P22 operator on central base sequence, CuOP digestions of the unliganded and repressor-liganded 9T and 9C operators were performed. The CuOP cleavage patterns of the unbound 9T and 9C operators, at both 50 and 150 mM KCl, differ only at position 7 (Fig. 5). At this position, CuOP cleavage of the 9C operator is about 3-fold stronger than that in the corresponding position of the 9T operator. Compared to the unliganded operators, adding protein to these reactions markedly alters the pattern of CuOP cleavage of both the 9T and 9C operators (Fig. 5). P22 repressor similarly protects positions 8 and 9 of both the 9T and 9C operator from CuOP cleavage. A striking difference in the ability of P22 repressor to protect DNA from CuOP modification is seen at operator position 10 (Fig. 5). In the 9T operator, P22 repressor protects the base at position 10 from CuOP attack, whereas the presence of protein enhances the
Effect of Non-contacted Bases on Phenanthroline Cleavage at Position 10 in the 9C Operator

Experiments were performed as described under "Experimental Procedures." Shown is an autoradiograph of the gel. The sequence of the operator is shown in the center. The numbers denote operator positions as described in Fig. 1. Bands resulting from Cu(I)-phenanthroline footprinting experiments of the 9T and 9C operator complexes at 50 mM (A) and 150 mM KCl (B). Cu(I)-phenanthroline footprinting experiments were performed as described under "Experimental Procedures." Shown is an autoradiograph of the gel. The sequence of the operator is shown in the center. The numbers denote operator positions as described in Fig. 1. Bands resulting from Cu(I)-phenanthroline footprinting experiments of the 9T and 9C operator complexes at 50 mM (A) and 150 mM KCl (B).

Cleavage at this position in the 9C operator by 1.5-fold. This phenanthroline cleavage at position 10 in the 9C operator shows that the reagent can access this base in the presence of P22 repressor. Protection of the base at position 10 by repressor in the 9T operator indicates that bound repressor either physically occludes access of the reagent to these bases or induces an altered conformation of the bases preventing the intercalation of the reagent. In any case, these data show that the structures of the complexes of P22 repressor with the 9T and 9C operators are different.

**DISCUSSION**

The affinity of P22 operator for P22 repressor varies with base composition at the center of the operator (Table I). P22 repressor does not protect these bases from dimethylsulfate modification (Fig. 2). These data indicate that the central base pairs of the P22 operator affect its affinity for operator by influencing DNA conformation and not by interacting directly with the protein.

Two lines of evidence suggest that the central base pairs in the P22 operator alter the structure of the repressor-operator complexes. First, the $K_d$ for the repressor-9T operator complex is relatively independent of KCl concentration, whereas that for 9C operator-repressor complex is more salt-sensitive (Fig. 3). This difference in salt dependence is probably caused by differences in the number, or more likely, the strength of charged amino acid side chain interactions with the DNA-phosphate backbone in the two repressor-operator complexes. Second, the DNase I protection patterns of the repressor-bound 9T and 9C operators are different (Fig. 4). The rate of DNase I strand scission is very sensitive to sugar-phosphate backbone conformation (Drew and Travers, 1985; Lomonosoff et al., 1981; Satchwell et al., 1986). Thus, these differences in cutting pattern must be reflecting central sequence-dependent adjustments in the DNA structure in the two repressor-operator complexes.

A consideration of the salt dependence of operator strength together with the changes in DNase I cleavage pattern suggests that the central base pairs affect operator affinity for repressor, in part, by altering the structure of the repressor-operator complex. At low salt, where the affinities of the two operators for repressor are comparable, the DNase I pattern of the two complexes are highly similar, whereas at high salt, where the two operator strengths differ by 10-fold, the patterns are obviously different. The most significant changes in DNase I cleavage pattern with salt are seen in the 9C operator-repressor complex. Interestingly, the position of salt and/or central sequence-dependent DNase I cleavages in both the 9T and 9C operators corresponds to the positions of phosphates whose ethylation interferes with P22 repressor binding (Poteete et al., 1980). This observation provides a further justification for assuming that the salt and central sequence effects on binding affinity are related to the changes in DNase I cleavage patterns. That the structures of the repressor-operator complexes do vary with central base sequence is confirmed by the central base composition-dependent differences in the ability of repressor to protect the central base pair from cleavage by DNA by Cu(I) phenanthroline.

How do the central bases influence P22 operator affinity for P22 repressor? Although we do not yet know the structure of the P22 repressor-operator complex, the experiments of Wharton and Ptashne (1985) and the structural homology between 434 and P22 repressor suggest that it is very similar to the 434 repressor-operator complex. In that complex, the DNA at the center of the operator is configured to bring each half-site of the operator into proper juxtaposition with at least one monomer of the protein (Aggarwal et al., 1988) This allows each monomer of the bound dimer to make optimal contacts within each operator half-site. The differences in DNase I cleavage patterns between the bound and 9T and 9C P22 operators indicate that, like 434 repressor, P22 repressor also deforms the operator when it binds.

Realizing that the structures of both the unbound and bound P22 operators vary with central base sequence, we suggest that the central sequence could affect operator affinity for P22 repressor by either one or both of the following mechanisms. In one mechanism, the central base sequence could affect affinity by limiting the degree to which the operator can be deformed in the protein-DNA complex. As such, the central sequence changes would globally alter the number and/or geometry of protein-DNA contacts. This would change the stability of the complex. This idea is consistent with the observed differences in the salt dependence of the dissociation constants for the repressor-9T and 9C operator complexes. Further support for this view comes from a more extensive analysis of the salt dependence data (de-Haseth et al., 1977). By extrapolating the salt dependence of $K_d$ data to 1 M salt concentration and assuming that: 1) the repressor itself does not bind or release cations in the binding reaction, and that 2) the proton equilibrium of the operator or repressor are not shifted by complex formation, the portion of the binding free energy that is due to non-charged interactions between the protein and DNA can be calculated. Using these assumptions, the non-charged interactions contribute 2 kcal/mol less to the overall binding energy of the 9C operator-repressor complex than they do to the 9T operator-repressor complex.

In the additional or alternative mechanism, central sequence-dependent changes in the equilibrium structure of the unbound operator could alter the degree to which the operator must be deformed in order to form a complex with repressor. Since the amount of energy needed to deform DNA increases with the square of the amount of deformation (Barkley and Zimm, 1979), operators whose structure most closely resemble that which they assume in the repressor-operator complex should bind repressor with a higher affinity than those operators whose structure differs markedly from that found in the repressor-operator complex. These data in this paper cannot distinguish the relative contributions of these two mechanisms in determining the affinity of operator for...
P22 repressor, although they do firmly establish these possibilities. The sequence of the non-contacted bases in both 434 and P22 operators influence their affinity for the cognate binding protein. Despite this analogy, these data presented here suggest that the underlying structural basis for the effects of non-contacted bases in the two protein-DNA interactions are very different. In contrast to the P22 repressor-operator interaction, the DNA backbone conformation in the 434 repressor-operator complexes does not vary with central base sequence. By contrast with the hierarchy of affinities that operators bearing different central base sequences have for P22 repressor, 434 operators having either T·A or A·T at their central two positions bind repressor equally well, whereas those having G·C or C·G bases at these positions bind repressor equally poorly (Koudelka et al., 1987, 1988). Together, these observations indicate that the mechanism by which the central base sequence affects P22 repressor-operator interaction is distinct from that seen the 434 repressor-operator complex. Moreover, in the 434 repressor-operator interaction, the effects of central base pair composition are local, modulating operator strength by influencing both the equilibrium structure and structural flexibility of the central segment of the operator (Aggarwal et al., 1988; Anderson et al., 1987; Koudelka et al., 1987; Carlson and Koudelka, 1992). The effect of central base sequence on unbound and bound P22 operator cleavage patterns indicates that the sequences of these bases have both local and longer distance effects on operator structure. We cannot determine whether the central base-dependent changes in operator strength result from influences on either equilibrium structure of unbound and bound operator, its ease of distorting the operator conformation, or both. These data presented here do, however, suggest that the central sequence effect on structure is distributed over the entire protein-DNA interface and not localized to the center of the operator.

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