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A Positively Charged Residue Bound in the Minor Groove Does Not Alter the Bending of a DNA Duplex

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The purine repressor (PurR) from *Escherichia coli* belongs to a growing class of regulatory proteins that bend DNA by making specific contacts in the minor groove of a target DNA sequence.[1,2] The DNA bending, a single 45° kink, is directed away from the protein and toward the major groove and results primarily from the interdigitation of dyad-related leucine side chains of the hinge helices into the central CpG base pair step of the operator site.[1] As a consequence, the minor groove broadens and the DNA helix is unwound locally. In addition to this minor groove binding element, PurR possesses a helix-turn-helix (HTH) motif that recognizes the major groove. Recently, an electrostatics-based model has been proposed[3] and explored computationally,[4] to explain the origin of minor groove bending toward the major groove. We decided to test what the effect of a positive charge in the minor groove would be in the context of a DNA-binding protein that makes additional contacts in the major groove. Crystallographic studies on wild type PurR and a site-directed mutant bound to DNA reveal that the presence of a positively charged group in the minor groove does not significantly alter DNA conformation.

Lys55 makes specific base contacts in the minor groove of the operator.[1] To examine the influence of Lys55 on DNA minor groove binding and on promoting the sharply bent and underwound conformation of the operator, this residue was substituted with an alanine (Lys55Ala).[5] DNA-binding studies on the wild type (WT) and Lys55Ala proteins were carried out by fluorescence anisotropy using a 24 base pair fluoresceinated oligonucleotide duplex encompassing the *purF* operator site.[6] WT protein binds this sequence with a K_d of 2.5 nM, while Lys55Ala binds approximately 360-fold more weakly with a K_d of 900 nM. The lower affinity of the Lys55Ala mutation is clearly in line with the role of the wild type residue as an anchoring residue, as described by Werner *et al.*,[2] and represents an increase in $\Delta\Delta G_{binding}$ of 1.7 kcal/mol per monomer to operator half site.

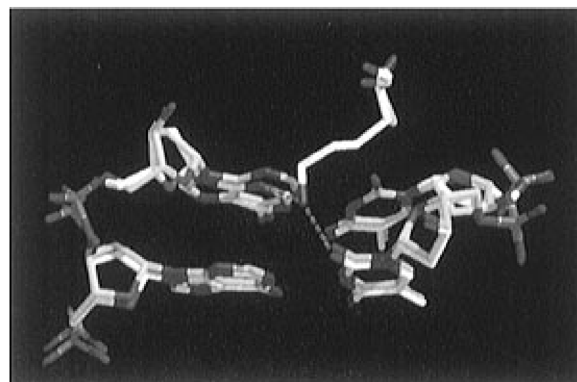

Figure 1. Model of the WT−palF complex (white carbons) overlaid with the Lys55Ala−palF complex (yellow carbons) by minimizing the rms distances between the C_α and P atoms of the two models. Residue 55 and base pairs 7:7′ and 8:8′ are shown for each model. The image was prepared with *MidasPlus* software.[9]

To investigate the structural basis for this loss in binding affinity, X-ray structures of both WT and Lys55Ala repressors bound to a palindromic DNA sequence (palF) based on the *purF* site[7] were solved by Fourier difference methods.[8] The root mean square (rms) deviation[9] between the C_α carbon atoms of the DNA-binding domains of the WT and Lys55Ala proteins is 0.27 Å and demonstrates directly that the loss of affinity between Lys55Ala and the *purF* target sequence is not related to conformational changes in the protein. In the WT structure, the C_ϵ of Lys55 makes a van der Waals contact to C2 of adenine 8, and N_ζ appears positioned to form a hydrogen bond with either N3 of A8 or O2 of T7′ of the adjacent base pair (Figure 1). The high *B*-factors for the last three atoms of the lysine side chain (C_δ, 81; C_ϵ, 94; N_ζ, >100) indicate sufficient flexibility, that any of these contacts might predominate for a particular conformer, and would combine to give an averaged contribution to the free energy of binding. Removal of the lysine side chain from position 55 results in loss of these contacts and opens a solvent accessible pocket in the structure of the complex. However, no density was observed for any static solvent molecules in that region.

As expected, the substitution of Lys55 with an alanine has a significant impact on the electrostatic potential of the PurR DNA-binding element that interacts with the minor groove (Figure 2).[10] While the local parameters for the A8:T8′ base pair do not show substantial distortion from those of the DNA in the WT complex, the substitution of alanine for lysine might be expected to result in a relatively broader minor groove and a more sharply bent DNA structure, since the loss of positive charge in the minor groove could enhance the interstrand electrostatic repulsion between phosphates.[4] However, the local

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(6) The 5′-fluoresceinated duplex was prepared from two complementary 24mers, one of which contained the fluorescent label, by the method of Ebright (Ebright, R. *Meth. Enzymol.* **1996**. In press). The sequence of the labeled oligonucleotide is 5′-F-GAAAAAGAAAACGTTTGCGTAGGG. The anisotropy measurements were carried out at room temperature in 1 mL of binding buffer (250 mM potassium glutamate, 150 mM NaCl, 10 mM magnesium acetate, 1 mM EDTA, 5% w/v glycerol, 100 mM HEPES, pH 7.5) containing 1 μg of poly dI·dC, 100 μM hypoxanthine, and 2 nM of the labeled duplex. The repressor was titrated into this mixture, and readings were taken after each addition using a Beacon Fluorescence Polarization instrument.

(7) The sequence of the duplex containing the palindromic sequence is 5′-TACGCAAACGTTTGCGT-3′. The numbering of the sequence is 1-2-3-4-5-6-7-8-9-9′-8′-7′-6′-5′-4′-3′-2′, respectively.
(8) Crystals were grown as described by Schumacher *et al.* (Schumacher, M. A.; Choi, K. Y.; Zalkin, H.; Brennan, R. G. *J. Mol. Biol.* **1994**, *242*, 302−305.) Refinement was performed using the TNT package (Tronrud, D. E.; Ten Eyck, L. J.; Matthews, B. W. *Acta Crystallogr.* **1987**, *A43*, 489−501.) The WT structure was solved to 3.0 Å resolution with an *R*-factor of 15.7% (bond lengths showed 0.013 Å rms deviation from ideality, bond angles 1.63°). The Lys55Ala structure was solved to 2.7 Å resolution with an *R*-factor of 16.8% (bond lengths showed 0.017 Å rms deviation from ideality, bond angles 1.90°). Coordinates for the structures have been deposited in the Brookhaven Protein Data Bank and have PDB ID codes 1BDH and 1BDI for the Lys55Ala−hypoxanthine−palF and WT−hypoxanthine−palF complexes, respectively.
(9) Calculated using *MidasPlus*. Ferrin, T. E.; Huang, C. C.; Jarvis, L. E.; Langridge, R. *J. Mol. Graphics* **1988**, *6*, 13−37.
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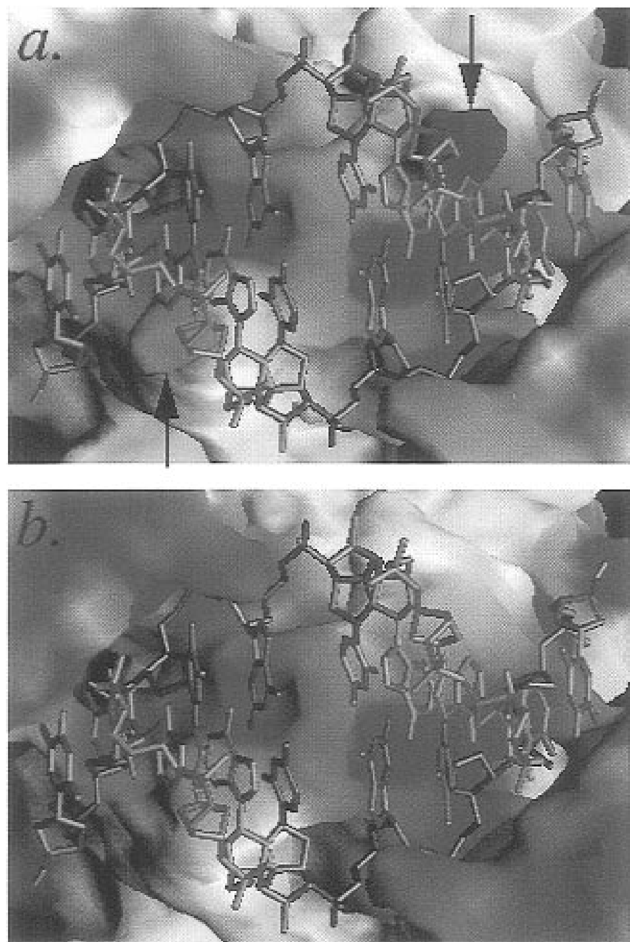

Figure 2. Electrostatic surface potential maps (negative, red; positive, blue) calculated with the program *GRASP*.[10] The position of the palF duplex is shown in green. (a) WT PurR with the position of Lys55 indicated by arrows. (b) The Lys55Ala mutant. The dyad-related Leu54 residues of the PurR dimer are located in the center of each image.

and global conformation of the DNA duplex is nearly identical in the two structures (Table 1),[11] and the small differences that do exist are within the uncertainty associated with the atomic positions of the models. From these data it is clear that the presence of a positively charged residue in the minor groove plays at best a small role in determining the conformation of the DNA.

The analysis of the binding activities and structures of the WT and Lys55Ala repressors indicates that the major role of Lys55 is to enhance the affinity of the repressor for the *purF* operator sequence. Does this role suit other instances in which charged residues bind in the minor groove of DNA? In two structures of homeodomains, those of engrailed and Matα2, complexed with their target DNA sequences, arginine residues in a flexible peptide N-terminal to the helix-turn-helix motif make minor groove contacts.[12,13] In neither of those structures do the helical twist angles for the DNA duplex appear to correlate with the presence of charged atom groups in the minor groove. Thus, DNA-binding elements other than Lys55 appear to be critical in determining the bent conformation of DNA bound to PurR. This is further supported by the observation that the lactose repressor−$lacO_{\mathrm{symm}}$ complex possesses bent DNA similar to that found in the PurR−*purF* complex, despite its lack of a positively charged residue positioned to interact with the minor groove.[14] While it is likely that the local environment around phosphate groups has a role in determining the conformation of bound DNA sequences,[4,15] the significance of this role should not be overemphasized in comparison to the those of the intrinsic conformational flexibility of specific DNA sequences[16] and of the specific contacts along the entire protein−DNA interface that demand a particular DNA conformation.[1,2] In particular, the "wedge"[2] formed by the dyad-related Leu54 residues of PurR requires a sharp bend in the operator to accommodate the van der Waals interactions they make when intercalated in the central CpG step.[1] Furthermore, major groove binding interactions made by PurR to base pairs further removed from the central CpG step are likely to help enforce the bent conformation. The placement of Lys55 in the minor groove takes advantage of the conformation of the operator in order to enhance binding affinity, but Lys55 does not itself appear to play a significant role in generating that conformation.

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Table 1. Roll and Twist Parameters Calculated for the palF Duplex When Complexed to Wild Type and Lys55Ala PurR Using the Program *Curves*[11]

	palF with WT		palF with Lys55Ala	
step	twist (deg)	roll	twist (deg)	roll
C3/G4	36.2	−8.7	37.8	−7.4
G4/C5	35.5	4.3	30.9	3.3
C5/A6	34.2	−1.7	37.9	2.4
A6/A7	34.0	3.7	34.9	3.0
A7/A8	36.5	3.6	33.6	4.2
A8/C9	28.8	2.2	29.6	1.7
C9/G9′	19.0	43.7	17.7	46.4
global curvature (deg)	54		49	
minor groove width (Å)[a]	8.69		8.32	

[a] At central GpG step.

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