ABSTRACT

Previous electrophoretic experiments suggest that the AP-1 site in duplex DNA bends in response to the pattern of amino acid charges distal to the basic region in bound bZIP proteins. The extent and direction of apparent DNA bending are consistent with the prediction that DNA will collapse locally upon asymmetric phosphate charge neutralization. To prove that asymmetric phosphate neutralization could produce the observed degree of DNA bending, the present experiments partially substitute anionic phosphate diesters in the AP-1 site with various numbers of neutral methylphosphonate linkages. DNA bending is induced toward the neutralized face of DNA. The degree of DNA bending induced by methylphosphonate substitution (∼3.5° per neutralized phosphate) is comparable to that induced by GCN4 variants carrying increasing numbers of additional basic amino acids. It is plausible, therefore, that asymmetric phosphate neutralization is the cause of DNA bending in such complexes.

INTRODUCTION

Naked duplex DNA behaves as a stiff, worm-like coil. DNA stiffness can be described in terms of persistence length, the distance over which a segment of the molecule tends to remain in a linear trajectory (1). This parameter is estimated to be ∼150 bp for duplex DNA (2). Because of its inherent stiffness, the biologically-relevant bending and folding of DNA into compact nucleoprotein structures such as nucleosomes (3), recombination complexes (4) and transcription complexes (2,5,6) requires energy. This energy is provided by favorable protein–DNA interactions. Can DNA bending by proteins be understood in terms of simple principles? Besides their fundamental significance, appreciation of such principles might allow the design of artificial DNA bending agents.

High resolution structural information suggests that at least two motifs exist in nucleoprotein complexes involving bent DNA. These two motifs suggest different underlying mechanisms. An important class of DNA bending proteins (here termed Class 1) contact bent DNA on its convex surface, such that the double helix curves away from the bound protein. Molecules in this class include TBP (7,8), HMG box proteins such as SRY and LEF-1 (9,10), and others that are often classified as ‘architectural’ binding proteins (10–12). One mechanism of DNA bending by Class 1 DNA bending proteins involves intercalation of hydrophobic amino acids between base pairs in the minor groove of DNA, dramatically enlarging this groove, and thereby altering the helix axis (10,12). It has been suggested that the relatively low dielectric character of the intercalated protein enhances interphosphate repulsions within the double helix, contributing to DNA deformation in these cases (13).

A second class of DNA bending proteins (here termed Class 2) is exemplified by the histone octamer, responsible for the remarkable wrapping of ∼150 bp of DNA by almost 720° in the nucleosome (3). Many other Class 2 DNA bending proteins have been described (14–17). Class 2 proteins contact bent DNA on its concave surface, curving the double helix toward the bound protein. The engaged surfaces of Class 2 proteins typically contain cationic amino acids, suggesting an important role for electrostatic interactions.

We are interested in learning how electrostatic effects contribute to DNA bending by Class 2 proteins. One interpretation is that bending occurs to maximize the protein–DNA interface, driven by the free energy release associated with favorable Coulombic interactions. We have been exploring a second interpretation, originally suggested by Mirzabekov and Rich (18), and subsequently addressed mathematically by Manning and co-workers (19). According to this model, the asymmetric neutralization of partial DNA phosphate charges by the cationic surface of a Class 2 protein alters the balance of electrostatic forces present within the DNA double helix. In particular, cancellation of phosphate charges on one helical face of DNA should promote ‘collapse’ of the DNA toward that surface, under the ‘weight’ of the unbalanced interphosphate repulsions remaining on the opposite face of the helix.

We have undertaken a series of experiments to test this intriguing (if less intuitive) model for DNA bending by Class 2 proteins. Our strategy has been to simulate the electrostatic consequences of protein binding by chemical synthesis of DNA duplexes in which the charge distribution is altered by partial substitution with either neutral phosphate analogs (20–22), or
tethered cations (23,24). DNA shape is then indirectly monitored by electrophoresis experiments. In support of the model in question, these studies have consistently shown that asymmetric phosphate neutralization causes moderate DNA bending in the predicted direction. DNA bending would not be anticipated in these model systems if the mechanism of Class 2 proteins involved only maximization of favorable charge–charge interactions between protein and DNA, because no proteins are present in the experiments.

The present work was motivated by our desire to apply the predictions of asymmetric phosphate neutralization to specific DNA sequences thought to be bent when complexed with proteins. The case of dimeric basic-zipper (bZIP) DNA binding proteins including Fos/Jun (25), Jun/Jun, CREB (26) and GCN4 (27) is particularly intriguing. Electrophoretic phasing experiments suggest that different members of this family have different effects on DNA shape (28–31). The intrinsic shapes of the binding sites for these proteins in DNA have also been shown to be significant (26,32). Amino acids adjacent to the basic region of each bZIP monomer lie near the DNA double helix. It was noted that the pattern of charged amino acid residues in this region correlates with apparent DNA bending in the resulting complex (Fig. 1A; 26,30). The significance of this relationship has been directly demonstrated by showing that changing the charges of these residues induces apparent changes in the bend angle of bound DNA (33–36). The character (anionic or cationic) and net charge of the protein surface have been shown to correlate with apparent DNA bend direction. If cationic amino acids are present, DNA bending is induced away from the leucine zipper of the bound protein so that, from the perspective of its N-termini, the DNA bending is induced away from the leucine zipper of the bound DNA (33–36). The character (anionic or cationic) and net charge of the protein surface have been shown to correlate with apparent DNA bend direction. If cationic amino acids are present, DNA bending is induced away from the leucine zipper of the bound protein so that, from the perspective of its N-termini, the protein dimer behaves as a Class 2 bending protein (Fig. 1B). In studies of both modified Fos/Jun (33), and GCN4 proteins (34–36), the degree of DNA bending within the AP-1 recognition sequence responds to the total charge of the bZIP protein in this region. Interestingly, the presence of anionic amino acids in these positions was observed to produce proportional DNA bending in the opposite direction, consistent with a role for charge repulsion in determining DNA shape (36). Thus, from the perspective of the N-termini of GCN4 mutant proteins, conversion of cationic amino acids to anionic amino acids effectively converts the dimers from Class 2 to Class 1 bending proteins.

The observation that cationic amino acids positioned on one DNA face induce apparent DNA collapse toward that face suggests that such bZIP proteins act as Class 2 DNA bending molecules. However, the electrostatic mechanism of DNA bending is not revealed in such experiments. Both direct Coulombic attraction (33) and asymmetric phosphate neutralization (34,35) have been proposed to explain these data. To directly test the hypothesis that asymmetric phosphate neutralization is responsible for DNA bending by cationic domains of these bZIP proteins, we have now applied the ‘phantom protein’ strategy to measure the effect of partial phosphate neutralization on the shape of the AP-1 site in duplex DNA. Our approach is shown in Figure 1B and C. DNA duplexes containing AP-1 sites have been synthesized such that certain phosphates are chemically neutralized (Fig. 1C). These neutralizations are intended to mimic the effects of various numbers of cationic amino acids positioned along the DNA minor groove, opposite to the leucine zipper of a bound bZIP protein (Fig. 1B). DNA bending is predicted according to the asymmetric phosphate neutralization hypothesis, but not if the bending force involves ‘attraction’ of the DNA to the protein.

Figure 1. Experimental design. (A) Certain bZIP proteins (e.g. GCN4 homodimers) are thought to bind the AP-1 site without introducing major distortions into the DNA. The amino acids just N-terminal to the basic region in such proteins (black) tend to be neutral. (B) Other bZIP proteins and GCN4 derivatives with cationic amino acids just N-terminal to the basic region (indicated by +) position these residues on one face of the DNA and appear in electrophoretic phasing assays to induce DNA bending toward the minor groove. The bending force presumably involves either attraction of the anionic double helix toward the cationic amino acids or collapse of the double helix due to laterally asymmetric phosphate neutralization. (C) If DNA bending arises from asymmetric phosphate neutralization, an AP-1 site containing neutralized phosphates should spontaneously bend to mimic the effect of bZIP protein binding. Such bending is not predicted according to an ‘attraction’ model. The structure of the neutral methylphosphonate internucleoside linkage is shown below. Racemic mixtures of methylphosphonate stereoisomers were used in this study. (B and C) DNA bending is exaggerated for clarity.

The results of our experiments show that partial phosphate neutralization within the AP-1 sequence induces DNA bending. Induced bending is both qualitatively and quantitatively similar to that induced by GCN4 variants thought to position cationic amino acids on one face of the DNA helix.

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides were prepared as previously described (37). All oligomers were purified by denaturing polyacrylamide gel electrophoresis, eluted from gel slices, and desalted using C18 reverse phase cartridges. Oligonucleotide concentrations were determined at 260 nm using molar extinction coefficients (M−1 cm−1) of 15 400 (A), 11 700 (G), 7300 (C), 8800 (T) assuming no hypochromicity. Compositions of oligonucleotides containing methylphosphonate substitutions were verified by mass spectrometry.

Gel electrophoresis and data analysis

Analysis of DNA shape was performed by comparative gel electrophoresis of ligated DNA duplexes through native polyacrylamide gels as has been described (20,38). Relative curvature values were determined as in previous studies (20,38,39). Briefly, the distance migrated by duplex DNA standards of known length were measured and fit by a least-squares method to an exponential function. The apparent length of DNA in each gel band was then
estimated using the derived function and the distance migrated. An equation of the form:

\[ R_l - 1 = (pL^2 - q)(\text{relative curvature})^2 \]

was fit by a least-squares method to data for a standard DNA duplex (duplex 21 in Fig. 2) containing one intrinsically-curved A<sub>6</sub> tract per 21 bp (relative curvature = 0.5 A<sub>6</sub> tract equivalents per helical turn) and no methylphosphonate substitutions (40). \( R_l \) is the ratio of the apparent DNA length to the actual DNA length (L) for each band in the experimental ligation ladder. Best fits for the values of the constants, \( p \) and \( q \), were then determined for each gel using mobility data for this standard DNA duplex for the length range 120 < length < 190 (39). The resulting equation was then used to obtain estimates for unknown relative curvature values (A<sub>5</sub>–6 tract equivalents per helical turn of DNA) for duplexes containing A<sub>5</sub> tracts and either unmodified or partially neutralized AP-1 sequences. The intrinsic DNA curvature due to the A<sub>5</sub>–6 tract is considered to be a vector of magnitude \( \alpha \), directed toward the minor groove in a reference frame shifted 0.5 bp 3' from the center of the A<sub>5</sub>–6 tract (38). Estimates for the value of \( \alpha \) in test duplexes were obtained by comparison of the electrophoretic behavior of their ligation products to those containing duplex 21 (Fig. 2). To estimate the degree of bending induced by charge neutralization, \( b \), the direction of induced bending was assumed to be toward the center of the neutralized DNA surface, at radial angle \( \theta \) from the intrinsic A<sub>5</sub>–6 tract bend. This assumption is consistent with qualitative experimental results. Satisfactory fits of the bending data were obtained by considering the degree of induced bending toward the neutralized surface to be:

\[ b = kn \]

where \( n \) is the number of neutralized phosphates, and \( k \) gives the induced relative curvature per neutralized phosphate. The single best value of \( k \) was estimated by individual fits to bending data for each phasing, where the measured (net) relative curvature, \( c \), is related to the intrinsic A<sub>5</sub>–6 tract curvature \( (a) \) and the induced bend \( (b) \) according to:

\[ c = a \cos\theta - 2abcos(180-\theta) \]

The appropriate apparent value of \( a \) (in relative curvature units) was used for each phasing to account for the presence of a small additional intrinsic curvature within the AP-1 site. Determination of \( b \) from equation 3 thus deconvolutes the apparent net DNA curvature \( (c) \) into components due to the intrinsic curvature of the A<sub>5</sub> tract \( (a) \) and the unknown bending due to charge neutralization \( (b) \). Multiplication by a factor of 36 converts the units of \( a \) and \( c \) to degrees of DNA bending, and converts the units of \( k \) to degrees of DNA bending per neutralized phosphate.

**RESULTS AND DISCUSSION**

**Experimental design**

Comparative electrophoresis experiments allow estimation of the magnitude and direction of DNA bends induced by sequence, structure and/or chemical modifications (20, 38, 40). In this approach, DNA duplexes comprising two turns of the double helix are chemically synthesized with cohesive molecular termini for unidirectional ligation in the presence of T4 DNA ligase. Each DNA duplex contains an A<sub>5</sub>–6 tract that is intrinsically curved by \( \sim 18^\circ \) in an orientation that has been established (38). Because the A<sub>5</sub>–6 tracts in the resulting ligated multimers are aligned in a plane, multimers are increasingly curved, and show length-dependent mobility retardation in native polyacrylamide gels. Comparative electrophoresis is very sensitive to DNA shape because the electrophoretic anomaly is related to both the square of multimer length and the square of the relative curvature present in each of the ligated DNA duplexes (see equation 1 in Materials and Methods; 38).

In order to study the effects of phosphate neutralization within the AP-1 site, 21 DNA duplexes were synthesized (Fig. 2). Duplexes 1–3 and 7–9 are unmodified, containing AP-1 sites and A<sub>5</sub> tracts in various positions. Duplex 21 is a well-studied mobility standard containing a single A<sub>5</sub> tract in a GC-rich sequence context (40). The remaining duplexes contain two to
four methylphosphonate substitutions within the AP-1 site, in positions simulating DNA sites thought to be in close contact with cationic amino acid side chains in Jun/Fos or GCN4 mutants that bend DNA toward the minor groove (26,33–35,41).

The comparative electrophoresis technique measures overall DNA curvature as a function of the phasing between a site of uncharacterized DNA deformation (the partially-neutralized AP-1 site) and a reference bend provided by an A₅-6 tract. When favorably phased, the two helix deformations will add constructively to enhance the overall curvature of the duplex and decrease electrophoretic mobility. In unfavorable phasings, the two elements of curvature will tend to cancel and the electrophoretic mobility will increase. Estimates of the direction and extent of DNA bending due to charge neutralization can be deduced from these experiments (20).

**Helical repeat of DNA duplexes**

Application of comparative electrophoresis requires determination of the length of DNA that best approximates an integral number of helical turns. This parameter was confirmed experimentally using DNA duplexes 1–6 (Fig. 2), containing the AP-1 site in unmodified form (duplexes 1–3) or bearing two methylphosphonate substitutions (duplexes 4–6). Duplexes 1 and 4 are 20 bp in length, duplexes 2 and 5 are 21 bp in length and duplexes 3 and 6 are 22 bp in length. When ligated into multimers, the two duplexes should appear equally curved. However, as shown in Figure 4 (lanes 4, 7 and 9), and in Figure 5A and F, these three duplexes did not comigrate. Figure 5F shows that duplexes 7 and 9 (cis and trans phasings) were equally curved, while duplex 8 (the ortho phasing) was somewhat less curved. In addition, although each of the three duplexes contained one A₅ tract, apparent curvature was less than for the unmodified standard duplex 21 lacking an AP-1 sequence.

These data suggest the presence of intrinsic curvature within the AP-1 site that explains the phase-dependent differences in net DNA curvature for unmodified duplexes 7–9. The intrinsic AP-1 site curvature is most completely opposed to the A₅ tract in the ortho phasing. The symmetry of the curvature data further suggests that the intrinsic AP-1 site curvature counteracts the A₅ tract bends of cis and trans duplexes equally, and to a lesser extent than in the ortho case. A simple quantitative model was devised to account for these data. In this model, the A₅ tracts in duplexes 7–9 each induce an identical curvature of ~16° (within 10% of the conventional value; 42) in the expected direction. In addition, the model includes a second intrinsic curvature of ~4° within the AP-1 site, directed toward the major groove of DNA in a reference frame ~1 bp 5′ of the center of the AP-1 site (see summary depiction in Fig. 7). The different phasings of these two elements of curvature accounts for the different mobilities of duplexes 7–9.

A small AP-1 site curvature of approximately this magnitude (3–8°) and direction has previously been detected in several kinds of electrophoretic phasing analyses (26,33,35,43). The curvature of the AP-1 site toward the major groove near the center of the site would tend to slightly narrow the major groove in the direction of the leucine zipper of an incoming GCN4 homodimer (e.g. see Fig. 1A). It remains possible that the locus of slight intrinsic curvature lies just outside the AP-1 site.
DNA bending by phosphate neutralization within the AP-1 site

Having obtained estimates for the intrinsic shapes of unmodified phasing probes bearing the AP-1 site, we constructed four sets of partially-neutralized duplexes bearing methylphosphonate substitutions at various patterns of phosphates within the AP-1 site (duplexes 5–20 in Fig. 2). Duplexes 5, 10 and 11 represent three phasings of a partially-neutralized AP-1 site bearing one neutralized phosphate on each strand (1 + 1 = 2 case) with neutralizations placed in the sugar–phosphate backbones flanking the minor groove opposite the leucine zipper in an approaching GCN4 dimer (Fig. 1C). This placement is intended to mimic the positions of potential salt bridges between cationic amino acid side chains of GCN4 mutants bound to the AP-1 site (35).

An example of electrophoretic data is shown in Figure 4 for the 1 + 1 = 2 case. Partial neutralization of the AP-1 site alters the overall shape of the modified duplexes (Fig. 4, compare lanes 12, 14 and 17). Neutralization on approximately the same DNA face as the intrinsic A5 tract curvature enhances overall bending (Fig. 4, compare lanes 12 and 14 with lanes 4 and 7). As the neutralized face is phased away from the A5 tract bend, overall bending is reduced (Fig. 4, lane 17).

Similar bending data were collected for the 0 + 3 = 3, 1 + 2 = 3 and 1 + 3 = 4 cases. The entire data set is depicted graphically in Figure 5. Figure 5B–E shows the electrophoretic anomaly of increasingly neutralized AP-1 sites as a function of the length of the ligated species. Figure 5F–J shows the data replotted as RL – 1 versus the square of the DNA length. Also shown in Figure 5F–J are fits of equation 1 (Materials and Methods), used to estimate the net curvature of DNA duplexes in which multiple elements of curvature are interacting. Deduced net curvatures are presented in Table 1. The data demonstrate that AP-1 site bending induced by partial neutralization is directed toward the neutralized face, as expected, and increases with increasing phosphate neutralization (compare Fig. 5F–J). Importantly, data for DNA duplexes with both 0 + 3 = 3 and 1 + 2 = 3 arrangements of three neutralizations are comparable, differing by only 6, 9 and 20% in cis, ortho and trans cases, respectively (Fig. 5H and I; Table 1). This result suggests that the precise pattern of neutralizations is not a major determinant of DNA shape.

Complete analysis of the DNA bending data requires consideration of (i) the A5 tract curvature, (ii) the intrinsic curvature present in the AP-1 site and (iii) the DNA bend induced by partial phosphate neutralization. The measured net curvature for each phasing was taken to be the independent combination of these bends. Analysis was performed by considering the vector sum of projections of each DNA segment on a plane normal to the initial DNA path. For small bend angles, these projections are roughly proportional to the angles themselves. The phasing data were analyzed using equations 2 and 3 (Materials and Methods).
Table 1. Relative curvature values for AP-1 duplexes

<table>
<thead>
<tr>
<th>Neutralization pattern</th>
<th>Phasing$^b$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>cis</td>
</tr>
<tr>
<td>0 + 0 = 0</td>
<td>0.41</td>
</tr>
<tr>
<td>1 + 1 = 2</td>
<td>0.60</td>
</tr>
<tr>
<td>0 + 3 = 3</td>
<td>0.67</td>
</tr>
<tr>
<td>1 + 2 = 3</td>
<td>0.71</td>
</tr>
<tr>
<td>1 + 3 = 4</td>
<td>0.79</td>
</tr>
</tbody>
</table>

$^a$The total number of neutralized phosphates within the AP-1 site of each 21 bp duplex is shown as the sum of neutralizations on the top strand and bottom strand, respectively (see Fig. 2).

$^b$Data are shown as the relative curvature (A 5–6 tract equivalents per helical turn), deduced from Figure SF-1. The data measure overall DNA curvature due to both intrinsic and induced bends, and reflect the mean value derived from two experiments. Standard deviations are <5% of reported values.

Using this procedure, a model was obtained wherein the extent of DNA bending induced by electrostatic effects is a linear function of the number of neutralized phosphates. In this model, each neutralized phosphate contributes $\sim$3.5° of DNA bending toward the neutralized surface. As shown in Figure 6, this model accounts well for the electrophoretic data. The manner in which net curvatures are decomposed into individual components is shown graphically in Figure 7.

It is interesting to note that the estimate of $\sim$3.5° of induced DNA bending per neutralized phosphate is also in excellent agreement with earlier studies of an unrelated DNA sequence, wherein six phosphate neutralizations flanking the minor groove induced $\sim$21° of bending (20). These data also compare favorably with the observed effect of neutralizing two phosphates flanking the minor groove at the center of the CRE sequence in duplex DNA, where the modifications at least partially reversed an intrinsic curvature of $\sim$11° toward the major groove (34).

Results of another study comparing AT-rich versus GC-rich sequence contexts tend to support this unexpected notion that bending by phosphate neutralization is relatively independent of DNA sequence (44).

Alternative explanations

The application of a DNA analog to mimic electrostatic effects raises the concern that the analog itself induces changes in DNA structure through non-electrostatic effects (45,46). We have previously performed four kinds of experiments to address these concerns. First, DNA bending by methylphosphonate-substituted DNA was performed in the presence of different oligovalent cations (20). Non-electrostatic effects on DNA shape would not be expected to depend on counterion valence. DNA bending due to asymmetric phosphate neutralization should be highly sensitive to the residual charge per phosphate, a parameter influenced by counterion valence (47). The observation that DNA bending relaxes in the presence of oligocations is evidence for an inherent electrostatic effect in these studies. Second, we have shown that multiple methylphosphonate substitutions do not alter the helical repeat parameter for duplex DNA (20). Third, we have simulated asymmetric phosphate neutralization using unrelated DNA analogs in which the phosphate backbone is not altered, but additional cations were tethered to bases on one DNA face (23,24).

The qualitative agreement of DNA bending in these studies supports an electrostatic effect. Fourth, in an attempt to limit non-electrostatic contributions to DNA shape, we have compared DNA bending after partially substituting only the favorable Rp methylphosphonate stereoisomer rather than racemic mixtures (21). Similar DNA bending results were obtained, suggesting that effects of methylphosphonate chirality impact bending estimates by <30%. Although it is impossible to eliminate the possibility that some portion of the DNA deformation in these studies is due to non-electrostatic consequences of methylphosphonate substitution, the results summarized above support an electrostatic interpretation of the present data.

Comparing bZIP and ‘phantom’ proteins

Studies of bZIP proteins such as Jun homodimers, Fos/Jun heterodimers (28,29,31), CRE-BP1 homodimers (26), GCN4 homodimers (26,35,48), mutants of these proteins (26,33–26), and other bZIP family members (30) suggest that they modify the shape of the AP-1 site in different ways. It must be noted that studies involving DNA bending by Fos/Jun remain controversial because of debate over interpretation of electrophoretic phasing experiments (49–51).

Recent studies with recombinant variants of Fos/Jun (33) and GCN4 (35,36) containing different numbers of charged amino acids near the basic region support the hypothesis that the shape of the AP-1 site responds to electrostatic features of the protein (Fig. 1). It remains possible, however, that the electrophoretic effects observed for these differently-charged protein variants arise from changes in DNA rigidity or some other property of the protein–DNA complex (49). The results of the present study support an interpretation involving DNA bending.

It is interesting to compare apparent bending of the AP-1 site induced by the binding of a series of increasingly cationic GCN4 variants (36) with DNA bending observed in the present study as a result of partial phosphate charge neutralization. This comparison is presented in Figure 8. Considering both the crude manner in which methylphosphonate substitution mimics interactions between cationic amino acid side chains of GCN4 and DNA phosphates, and differences between the two kinds of electrophoretic phasing experiments, the agreement between the two bending studies is
notable. As shown in Figure 8, both studies suggest a similar shape for the unbound AP-1 site. Bend magnitudes and directions upon increasing asymmetric phosphate neutralization are also comparable. The slope of a linear fit through the previous DNA bending data for GCN4 complexes is the same (3.5° per neutralized phosphate) as predicted by the bending model derived from the current studies with methylphosphonates (Fig. 8). We interpret this striking quantitative agreement between experiments involving ‘real’ and ‘phantom’ proteins as evidence that DNA bending explains the electrophoretic phasing data obtained with GCN4 mutants (35,36), and that asymmetric phosphate charge neutralization causes DNA bending in these complexes.

These bending data can also be compared with previous studies of DNA bending upon binding of PU.1 protein (17,52). Partial neutralization of the PU.1 DNA binding site by methylphosphonate substitution was used to mimic salt bridges observed on one face of the DNA helix within the PU.1–DNA complex (22). In that study, asymmetric phosphate neutralization resulted in DNA bending (28°) that was more than sufficient to account for the DNA curvature observed in the co-crystal (8°).

CONCLUSION

The AP-1 site in duplex DNA appears to bend in response to the pattern of amino acid charges distal to the basic region in bound bZIP proteins. The extent and direction of apparent DNA bending are consistent with the prediction that DNA will collapse locally upon asymmetric phosphate charge neutralization. The present experiments partially substitute AP-1 phosphates with various numbers of neutral methylphosphonate linkages. The degree of DNA bending induced by methylphosphonate substitution (−3.5° per neutralized phosphate) is comparable to that induced by GCN4 variants carrying increasing numbers of additional basic amino acids. It is therefore plausible that asymmetric phosphate charge neutralization is the basis for DNA bending in such complexes.

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