*Articles*

# Effects of Neutralization Pattern and Stereochemistry on DNA Bending by Methylphosphonate Substitutions†

J. K. Strauss-Soukup,‡,§ M. M. Vaghefi,||,⊥ R. I. Hogrefe,||,⊥ and L. J. Maher, III*,§

*Department of Biochemistry and Molecular Biology and Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska 68198, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, Minnesota 55905, and Genta Inc., 3550 General Atomics Court, San Diego, California 92121*

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ABSTRACT: Asymmetric phosphate neutralization has been hypothesized to play a role in DNA bending by proteins. Neutralization is thought to involve salt bridges between the negatively charged phosphate backbone of duplex DNA and the cationic amino acids of an approaching protein. According to this model, the resulting unbalanced charge distribution along the duplex DNA induces the double helix to collapse toward the neutralized surface. Previous work has confirmed that DNA bending is induced by the asymmetric incorporation of racemic methylphosphonate linkages creating a neutral region on one face of duplex DNA. Neutralization was accomplished by substitution of three consecutive phosphodiesters on each strand, arranged across one minor groove of the DNA (a total of six neutralized phosphates). We now measure DNA bending induced by a more diffuse patch of neutralization (alternating neutralized and anionic phosphates) and explore the effect of methylphosphonate stereochemistry. DNA duplexes with patches of alternating methylphosphonate and phosphodiester linkages are less bent than DNAs wherein consecutive phosphates are neutralized. Furthermore, duplexes neutralized by incorporation of pure ($R_P$)-methylphosphonate isomers are bent ∼30% less than duplexes neutralized by racemic methylphosphonates.

Neutralization of the phosphate backbone of duplex DNA by cationic amino acids has been suggested to be a driving force in DNA bending by some proteins (Manning et al., 1989; Mirzabekov & Rich, 1979; Strauss & Maher, 1994). According to this view, phosphate neutralization on one face of the double helix induces an unbalanced charge distribution along the DNA. The double helix is then envisioned to collapse toward the neutralized surface in response to asymmetric electrostatic repulsions. Experimental evidence tends to support this hypothesis. For example, asymmetric incorporation of six racemic methylphosphonate linkages creates a neutralized region on one face of duplex DNA that induces ∼20° of bending (Figure 1; Strauss & Maher, 1994). In these previous experiments, neutralization involved modification of three consecutive phosphodiesters on each strand, arranged across one minor groove of DNA (six neutralized phosphates per two turns of the double helix). Subsequent experiments have shown that asymmetric tethering of cations to DNA yields qualitatively similar results (Strauss et al., 1996a,b).

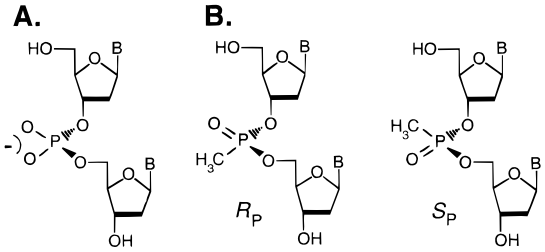


FIGURE 1: Structures of DNA analogs: (A) anionic phosphodiester linkage; (B) neutral methylphosphonate linkages (synthesized either as racemic mixtures of $R_P$ and $S_P$ isomers or as the pure $R_P$ isomer).

Two questions are addressed in the present work. First, to what extent does a "diffuse" patch of neutralization (i.e., alternating neutralized and anionic phosphates) bend DNA? Second, what is the role of methylphosphonate stereochemistry in DNA bending? An alternating pattern of anionic and neutral phosphates results in partial neutralization of the DNA double helix, with the neutralization distributed over more of the minor groove than in the case previously studied. We hypothesized that this more diffuse neutralization would result in less DNA bending than had been measured for a patch of consecutive neutralized phosphates. It has also been of interest to determine if features of the methylphosphonate linkage (other than the absence of charge) deform the shape of duplex DNA. We have previously examined the helical repeat parameter of duplexes containing up to ten racemic methylphosphonate substitutions and have found the helical repeat to be unchanged (10.4 bp[1] per helical turn versus 10.5

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* Author to whom correspondence should be addressed.
‡ University of Nebraska Medical Center.
§ Mayo Foundation.
|| Genta Inc.
⊥ Current address: TriLink BioTechnologies, Inc., 11585 Sorrento Valley Road, Suite 106, San Diego, CA 92121.

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[1] Abbreviations: bp, base pair(s); NMR, nuclear magnetic resonance.

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for the same unmodified sequence; Rice & Crothers, 1989; Strauss & Maher, 1994). However, further examination of this question is now made possible by the availability of chiral dinucleoside phosphoramidites containing pure ($R_P$)-methylphosphonate isomers.

The present studies demonstrate that patches of alternating anionic and neutral phosphates induce less DNA bending than patches of consecutive neutralized phosphates. In addition, duplexes containing chiral ($R_P$)-methylphosphonate substitutions are shown to be somewhat less bent than duplexes with racemic methylphosphonate substitutions. The significance of these results is discussed in the context of understanding electrostatic effects in DNA bending.

## MATERIALS AND METHODS

*Oligonucleotides.* Unmodified oligonucleotides were prepared using an ABI model 394 DNA synthesizer according to standard procedures. Oligonucleotides were cleaved and deprotected in hot ammonia. Oligonucleotides containing site-specific racemic methylphosphonate substitutions were synthesized at a 1 $\mu$mol scale using methylphosphonamidite monomers obtained from Glen Research (Sterling, VA). Isobutyryl derivatives of cytosine were used to facilitate cleavage from the solid support and deprotection as previously described (Hogrefe et al., 1993). All oligomers were purified by denaturing polyacrylamide gel electrophoresis, eluted from the gel, and desalted using $C_{18}$ 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), and 8800 (T) assuming no hypochromicity.

The synthesis of dinucleoside methylphosphonate synthons of defined chirality was as previously described (Reynolds et al., 1996). Briefly, dimethoxytritylated, base-protected diastereomeric dinucleoside methylphosphonates were synthesized in solution and separated by silica gel flash chromatography. Purification was based on the earlier chromatographic elution of the $R_P$ diastereomer. Purified 5′-*O*-dimethoxytrityl base-protected methylphosphonate dinucleosides of defined chirality were converted to the corresponding 3′ $\beta$-cyanoethyl phosphoramidites by phosphitylation using standard procedures (Reynolds et al., 1996). Purities were confirmed by $^1$H and $^{31}$P NMR. Oligonucleotides containing alternating methylphosphonate ($R_P$) and anionic phosphate diester internucleoside linkages were then synthesized using standard procedures (Hogrefe et al., 1993; Reynolds et al., 1996).

*Detection of DNA Shape by Gel Electrophoresis.* Analysis of DNA shape was performed by comparative gel electrophoresis of ligated DNA duplexes as previously described (Crothers & Drak, 1992; Strauss & Maher, 1994). Relative curvature values and estimates of electrostatic bend angles were determined as previously described (Crothers & Drak, 1992; Koo & Crothers, 1988).

## RESULTS AND DISCUSSION

Native gel electrophoresis has been used extensively to study intrinsic and induced DNA bending (Crothers & Drak, 1992; Koo & Crothers, 1988; Strauss & Maher, 1994). Mobilities of DNA fragments through polyacrylamide gels exhibit remarkable shape dependence. Thus, two molecules of the same contour length but with different average conformations will migrate differently. In the present studies, DNA duplexes forming two turns of the double helix are ligated into polymers. DNA shape is then analyzed using the phasing method (Crothers & Drak, 1992), wherein anuncharacterized site of helix deformation (the neutral surface) is placed at different positions relative to an internal reference deformation (an $A_6$ tract whose magnitude and direction are well characterized; Crothers & Drak, 1992). Molecules wherein the reference deformation is enhanced by proper phasing with an electrostatic bend exhibit reduced electrophoretic mobility relative to other phasings. Because all molecules are composed of 21 bp duplexes of identical molecular weight and charge, mobility differences can be attributed entirely to molecular shape.

*Duplexes with Alternating Racemic Methylphosphonate/Diester Linkages.* Previous studies of DNA bending by asymmetric substitution of methylphosphonate linkages showed that ~20° of bending results when three phosphates on each side of one minor groove are neutralized. Realistic patterns of phosphate neutralization in protein/DNA complexes may be more diffuse. We wished to determine if such a diffuse patch of neutralization (i.e., alternating neutralized and anionic phosphates) changes the extent of DNA bending relative to bending by consecutive neutralized phosphates. Synthetic duplexes were therefore created to measure DNA bending by alternating neutral methylphosphonate residues (racemic) and anionic phosphate diesters in a patch on one face of duplex DNA (Figure 2). This pattern will be henceforth termed alternating racemic methylphosphonate/diester linkages.

The electrophoretic phasing method was used to measure bending of these modified double helices. Phasing analysis requires determination of the helical repeat parameter (number of bp per helical turn of DNA) for the test sequence. The unmodified sequence used in these studies has a previously established helical repeat of 10.4 ± 0.1 bp per helical turn (Rice & Crothers, 1989). To measure the helical repeat of DNAs modified with alternating racemic methylphosphonate/diester linkages, we monitored gel mobility as a function of duplex length for duplexes having the $A_6$ tract and methylphosphonate substitutions on the same face (cis configuration). Duplex length was varied from 20 to 22 bp. Synthetic duplexes **1**−**3** were synthesized for this purpose (Figure 2A). The helical repeat was determined by finding the duplex length that gave rise to the greatest electrophoretic anomaly, corresponding to the arrangement wherein elements of curvature are phased most exactly with each other. Analysis of the dependence of gel mobility anomaly ($R_L$) on duplex length shows that the helical repeat of duplexes containing alternating racemic methylphosphonate/diester linkages is 10.4 bp per helical turn, near the canonical value for B-DNA and similar to that of the unmodified duplex (Figure 3). Subsequent experiments to study the behavior of two helical turns of modified DNA therefore employed 21 bp duplexes.

We then monitored DNA bending induced by site-specific neutralization of duplex DNA with alternating racemic methylphosphonate/diester linkages arrayed across the minor groove on one face of the double helix (Figure 2B). Duplex **2** contains the cis phasing arrangement (the center of curvature due to the reference $A_6$ tract lies on the same helical face as the center of the patch of alternating racemic

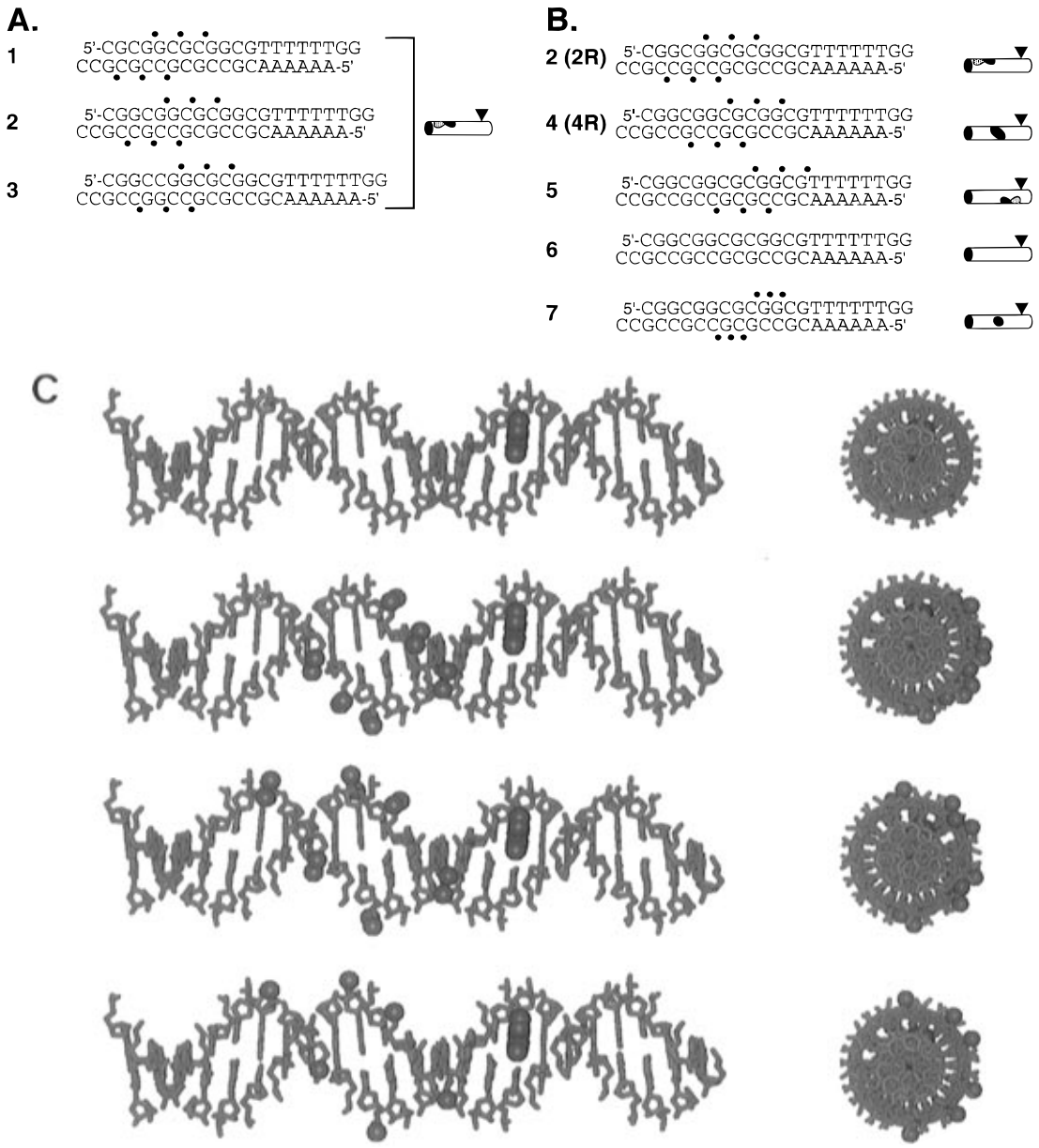


FIGURE 2: Synthetic oligonucleotides for phasing experiments. (A) Oligonucleotides for determining helical repeat parameters. Duplexes **1**−**3** (20, 21, and 22 bp in length, respectively) were used to establish the helical repeat of DNA containing alternating neutral and anionic linkages. Sites of racemic methylphosphonate substitution are identified (●). Cylinders at right depict elements of curvature. The direction of intrinsic curvature due to an $A_6$ tract results in the right end of the double helix curving upward at the filled arrowhead. The filled oval depicts the helical face partially neutralized by methylphosphonate substitutions. (B) Oligonucleotides for bending assays. Duplexes contain the neutralized sequence (alternating neutral and anionic linkages) in different phasings relative to the stationary $A_6$ tract. In duplexes **2** and **2R**, the minor groove at the center of the neutralized sequence is on the same helical face as the bend due to the $A_6$ tract (elements separated by ∼15°; cis configuration). In duplexes **4** and **4R** these elements are separated by ∼80° (orthogonal configuration). In duplex **5**, the separation is ∼150° (trans configuration). Duplex **6** is an unmodified standard that contains an $A_6$ tract. Duplex **7** has previously been studied and contains racemic methylphosphonate substitutions that form a patch of consecutive phosphate neutralizations (Strauss & Maher, 1994). Methylphosphonate substitutions in duplexes **2**, **4**, and **5** are racemic, while duplexes **2R** and **4R** contain only $R_P$ stereoisomers. (C) Molecular models of duplexes **6**, **7**, **4**, and **4R** (top to bottom). End views are shown at right. Atoms comprising the 3′ adenine in each $A_6$ tract are rendered as red spheres. Oxygen atoms substituted by methyl groups in methylphosphonate linkages are shown in magenta. DNA molecules are depicted with a helical repeat parameter of 10.4 bp/turn using the Insight II molecular modeling system.

methylphosphonate/diester linkages). Duplex **4** contains the orthogonal phasing arrangement (centers of curvature of these elements are positioned ∼80° away from each other), and duplex **5** contains the trans phasing arrangement (centers of curvature are separated by ∼150°). Duplex **6** is an unmodified standard that contains a single $A_6$ tract. The curvature of the $A_6$ tract in duplex **6** is taken to be 18° toward the minor groove in these experiments (Crothers & Drak, 1992).

Electrophoretic data were obtained to characterize induced curvature due to the patch of alternating racemic methylphosphonate/diester linkages (Figure 4, lanes 6−12). The mobilities of the respective 9-mer ligated products (189 bp actual length) are indicated by dots in Figure 4. DNA shape depends on helical phasing, as is evident from the differences in migration of the 9-mer species in cis, orthogonal, and trans arrangements (Figure 4, compare lanes 7, 9, and 12). The cis arrangement of the $A_6$ tract and neutralized patch results in the slowest migration (Figure 4, lane 7), suggesting that the curvature due to the patch of alternating racemic methylphosphonate/diester linkages tends to enhance the $A_6$

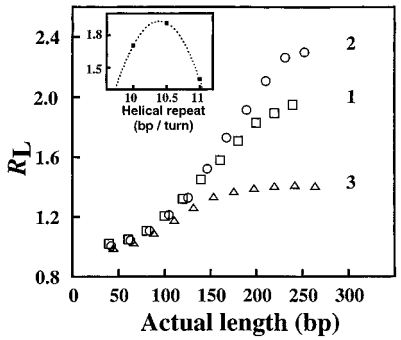


FIGURE 3: Experimental measurement of DNA helical repeat. Mobilities are shown for ligated duplexes **1** (□), **2** (○), and **3** (△) containing the cis configuration of alternating racemic methylphosphonate/diester linkages relative to an $A_6$ tract. The inset depicts $R_L$ values for the 189 bp species (average from two experiments) as a function of helical repeat. The overall helical repeat parameter was estimated as the value of the *x*-axis at the maximum of a parabolic function fit to the data (inset: dotted curve). Standard deviations of the $R_L$ estimates were less than 0.01.

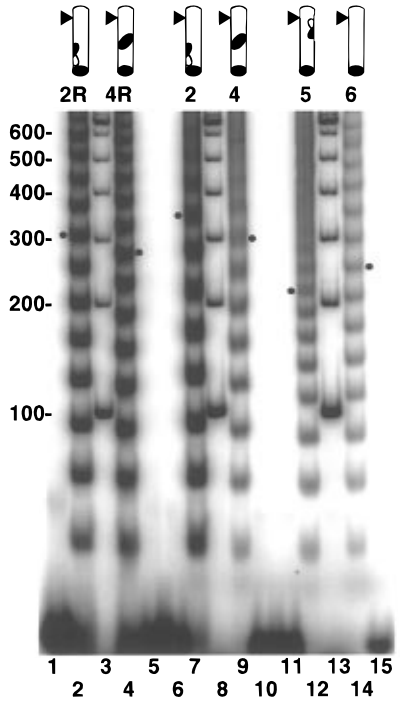


FIGURE 4: Electrophoretic assay of DNA shape. Labeled DNA duplexes were ligated and analyzed by electrophoresis through nondenaturing polyacrylamide gels as described in Materials and Methods. Samples without DNA ligase (lanes 1, 5, 6, 10, 11, and 15) contain only 21 bp species. The 100 bp duplex DNA ladders (lengths indicated at left) are included for reference (lanes 3, 8, and 13). The 189 bp species is indicated (●) for each ligated sample.

tract curvature when the elements are on the same DNA face. This result is in accord with previous studies wherein bending toward the minor groove is induced by neutralization along the minor groove (Strauss & Maher, 1994). The ratio of apparent length to actual length ($R_L$) is plotted versus the actual length of the duplexes in Figure 5A. The presence of phased elements of static curvature is manifested by increasingly positive deviations from $R_L = 1.0$ for increasing molecular lengths. Overall, the data in Figure 5A confirm that the patch of alternating racemic methylphosphonate/diester linkages induces bending toward the minor groove, enhancing the $A_6$ tract bend. Lowest overall curvature (greatest mobility) is observed for the trans phasing.

The mobility data for duplexes containing a patch of alternating racemic methylphosphonate/diester linkages were transformed to allow fitting to linear functions relating gel anomaly to the relative curvature for each phasing (Figure 5B). Resulting estimates for relative curvature ($A_6$ tract equivalents per helical turn) were determined for duplexes **2**, **4**, and **5** (Table 1), as described in Materials and Methods. Data from all three phasing arrangements were then combined to generate quantitative estimates for induced DNA bending (Strauss & Maher, 1994). Relative curvature estimates are plotted as a function of the radial angle between the center of the $A_6$ tract and the center of the patch of alternating racemic methylphosphonate/diester linkages (Figure 6). Data fitting to the phasing equation indicates that the magnitude of the bend angle due to the patch of alternating racemic methylphosphonate/diester linkages is ∼13° toward the minor groove (Table 1). This result is in qualitative agreement with the previous observation that a patch of consecutive racemic methylphosphonate linkages induces an ∼20° DNA bend toward the minor groove (Strauss & Maher, 1994).

The reduced bending by alternating racemic methylphosphonate/diester linkages relative to consecutive linkages (∼13° vs ∼20°, respectively) is statistically significant (Student's *t*-test; $0.05 < P < 0.10$; Bhattacharyya & Johnson, 1977). An explanation for this reduced bending is suggested by consideration of the individual bending force vectors attributable to each neutralized phosphate when the DNA is viewed end-on (Figure 7). For example, the orthogonal configurations of duplexes **4** and **7** are shown in Figure 2 (panels B and C). Duplex **7** contains a patch of consecutive methylphosphonates [as previously studied by Strauss and Maher (1994)]. The end views of duplexes **4** and **7** illustrate that the neutralized patch is more diffuse in the alternating case (duplex **4**). Force vectors due to each neutralized phosphate are more divergent in this case (Figure 7). The resultant vector sum is expected to be larger for a patch of consecutive neutralized linkages, consistent with greater bending for that case. In any case, the theory of Manning et al. (1989) predicts less bending with a decreased fractional neutralization.

*Duplexes with Alternating ($R_P$)-Methylphosphonate/Diester Linkages.* Using the bending data obtained for alternating racemic methylphosphonate/diester linkages, synthetic duplexes were created to test whether the stereochemistry of methylphosphonate linkages affects DNA bending (Figures 1B and 2B). Duplex **2R** contains the cis phasing arrangement in which the center of curvature due to the reference $A_6$ tract lies on the same helical face as the center of the patch of alternating ($R_P$)-methylphosphonate/diester linkages. Duplex **4R** contains the orthogonal phasing arrangement (centers of curvature of these elements are positioned ∼80° away from each other). Syntheses of duplexes with pure ($R_P$)-methylphosphonate stereoisomers were limited to the cis and orthogonal configurations due to availability of dimer synthons.

Electrophoretic data for duplexes **2R** and **4R** are shown in Figure 4 (lanes 1−5). The effect of phasing on DNA bending was qualitatively similar to that observed for duplexes with a patch of alternating racemic methylphosphonate/diester linkages. The cis configuration again results in the lowest mobility (Figure 4, compare lanes 1−5 with lanes 6−12), suggesting that the curvature due to the

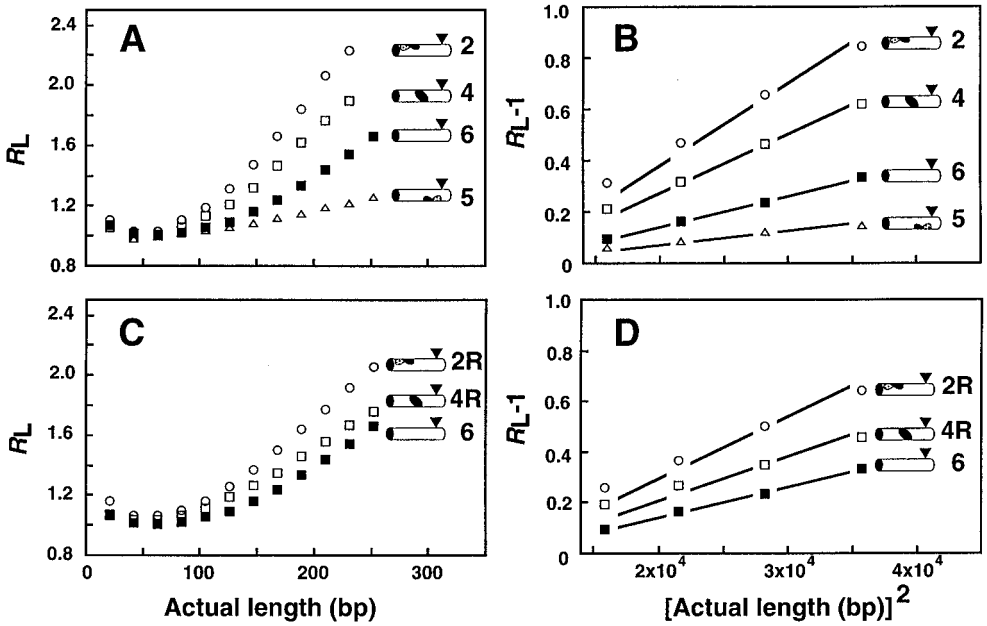


FIGURE 5: (A) Graphical depiction of DNA bending induced by incorporation of six racemic methylphosphonate linkages alternating with anionic phosphates (duplexes **2**, **4**, and **5**). Apparent lengths of ligated DNA duplexes were calculated relative to a standard curve on the basis of mobilities of the 100 bp duplex DNA ladder. The ratio of apparent length to actual length ($R_L$) is plotted versus actual length for each duplex. (B) Estimation of relative DNA curvature (number of $A_6$ tract equivalents per helical turn). Relative DNA curvature estimates were obtained by fitting data from panel A as described in Materials and Methods, using the behavior of duplex **6** (relative curvature set at 0.5 $A_6$ tract equivalents per helical turn) as a reference. (C) Graphical depiction of DNA bending induced by incorporation of six ($R_P$)-methylphosphonate linkages alternating with anionic phosphates (duplexes **2R** and **4R**). Apparent lengths of ligated DNA duplexes were calculated as described above. (D) Estimation of relative DNA curvature. Relative DNA curvature estimates were obtained by fitting data from panel C as described above. All relative curvature estimates reflect data from at least two experiments.

Table 1: DNA Bending Induced by Six Neutralized Phosphates

| 21 bp duplex (six neutralizations) | relative curvature[b] | | DNA bend angle[c] (deg) |
|---|---|---|---|
| consecutive[a] (racemic) | cis | 0.93 + 0.01 | |
| | ortho | 0.61 + 0.02 | 20.7 + 4.0 |
| | trans | 0.26 + 0.02 | |
| alternating (racemic) | cis | 0.84 + 0.03 | |
| | ortho | 0.71 + 0.03 | 13.0 + 1.2 |
| | trans | 0.37 + 0.02 | |
| alternating ($R_P$) | cis | 0.69 + 0.03 | 9.3 + 0.35 |
| | ortho | 0.61 ± 0.01 | |

[a] Strauss & Maher, 1994. [b] Average ± standard deviation based on at least two experiments, where 0.5 is the relative curvature due to the A6 tract present in each 21 bp duplex. [c] Based on best fits to phasing equations. In all cases the indicated bending is toward the minor groove. The average value is given ± standard deviation based on at least two experiments.

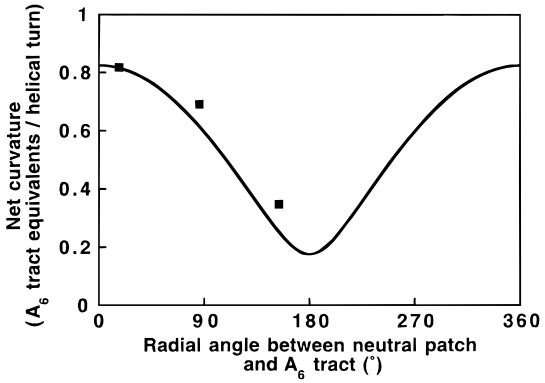


FIGURE 6: Interpretation of phasing data for duplexes with alternating racemic methylphosphonates and anionic phosphate diesters. The plot depicts estimates of the magnitude of net DNA curvature (■) as a function of the radial angle between the $A_6$ tract and the locus of bending under study. The curve indicates least squares fitting of the phasing data to an empirical cosine function that deconvolutes the net curvature into components due to the $A_6$ tract and the bending locus under study (see Materials and Methods).

neutralized surface enhances the $A_6$ tract curvature when the elements are on the same DNA face. Gel mobility data are plotted in Figure 5C,D. Quantitative relative curvature and bend angle estimates are summarized in Table 1. The patch of alternating ($R_P$)-methylphosphonate/diester linkages induced ~9° of DNA bending.

It is notable that DNA bending by pure ($R_P$)-methylphosphonate isomers is somewhat reduced relative to racemic mixtures of methylphosphonates (~9° vs ~13°; Table 1), though the predominant electrostatic effect is still clearly detectable. The difference in DNA bending for pure ($R_P$)- vs racemic methylphosphonates is statistically significant (Student's *t*-test; $0.01 < P < 0.05$; Bhattacharyya & Johnson, 1977).

Why do DNA duplexes with racemic methylphosphonate linkages bend more than duplexes with pure ($R_P$)-methylphosphonate linkages? Previous studies have suggested that, although similar in local geometry, ($R_P$)- and ($S_P$)-methylphosphonate isomers differ in their accommodation into duplex DNA (Bower et al., 1987; Kan et al., 1980;Vyazovkina et al., 1994). For example, DNA duplexes are modestly destabilized by incorporation of ($S_P$)-methylphosphonate isomers (Reynolds et al., 1996). The reduction in thermal stability appears to be sequence-dependent. A possible explanation for this effect is the disposition of the methyl group on the surface of DNA. ($R_P$)-Methylphosphonate isomers position the methyl group away from the major groove and directly toward the solvent, while ($S_P$)-methylphosphonate isomers direct the methyl group toward the major groove where sequence-dependent unfavorable con-

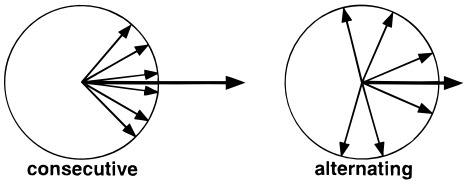


FIGURE 7: Possible interpretation of reduced DNA bending by alternating methylphosphonates and anionic phosphate diesters. The figure depicts end views of DNA helices with three consecutive phosphates neutralized on each DNA strand (left) or three alternating phosphates neutralized on each strand (right). Vectors represent compression forces arising from unbalanced electrostatic forces along the DNA. Compression force vectors direct DNA bending away from the helix axis toward each neutralized phosphate (thin arrows). The resultant bending force vector (thick arrow) has a greater magnitude in the case of consecutive phosphate neutralizations (left).

tacts can occur.

One interpretation of our data is that both ($R_P$)- and ($S_P$)-methylphosphonate isomers contribute to DNA bending byelectrostatic mechanisms. In addition, however, $S_P$ isomers may perturb DNA structure in a subtle manner through nonelectrostatic effects such as unfavorable steric contacts in the major groove. The latter class of contacts may then induce structural changes that tend to modestly exaggerate the electrostatic contribution to DNA bending. Based on this reasoning, previous estimates of the contribution of asymmetric charge neutralization to DNA bending may have been as much as 30% too large.

If the electrostatic behavior of DNA duplexes with racemic mixtures of methylphosphonate isomers is in some sense the weighted average of the many combinations of stereoisomers present, our results predict that duplexes with pure ($S_P$)-methylphosphonate isomers should appear more bent than the racemic mixture. Unfortunately, the limited availability of synthetic materials has prevented direct experimental confirmation of this prediction.

A puzzling aspect of our data involves the absence of electrophoretic band broadening in populations of DNA duplexes with racemic methylphosphonate substitutions versus what is observed for oligonucleotides containing methylphosphonates of pure chirality (e.g., Figure 4, compare lanes 6−12 and lanes 1−5). If the magnitude of DNA bending by methylphosphonate substitution is stereoisomer-dependent, populations of DNA duplexes with racemic methylphosphonate linkages must contain more diverse molecular shapes than samples with pure methylphosphonate chirality. Our expectation has been that such shape variability would be detected as band broadening or smearing upon electrophoresis of samples containing racemic methylphosphonate linkages. The routine detection of sharply defined electrophoretic bands for both kinds of molecular populations suggests that electrophoresis somehow displays the time-average behavior of each molecular population rather than an envelope of all molecular mobilities.

*Perspective.* The present experimental results have been interpreted in terms of an electrostatic model, wherein DNA bending is induced by an unbalanced distribution of anionic charges along the helix axis. In the present case, this unbalanced charge distribution is created by incorporation of neutral phosphate diester analogs in the DNA backbone. While we favor a purely electrostatic explanation for DNA bending, the analogs employed could cause other kinds of perturbations in DNA structure. In particular, it is unknown from theory or experiment how appended methyl groups alter the hydration of the DNA and/or the ion distribution surrounding the double helix. The present DNA analogs are extremely simple models designed to isolate electrostatic effects that might be present in protein/DNA complexes where many other forces undoubtedly contribute to modifying the shape of the double helix.

Interestingly, little structural data are available for helical structures involving methylphosphonate analogs. A recent report describes the NMR structure of a DNA oligonucleotide with alternating ($R_P$)-methylphosphonate/diester linkages, hybridized to a complementary RNA molecule (Mujeeb et al., 1997). The double-helical structure differs from A- or B-forms, and sugar puckers in each strand appear distinct. No structural anomalies are suggested for the DNA strand containing methylphosphonates. In particular, the helix is not bent, consistent with the uniform radial distribution of neutral phosphate analogs about the helix axis. Interestingly, these authors report that NMR experiments with a similar duplex in which racemic methylphosphonate analogs were present gave broad spectral peaks (Mujeeb et al., 1997). This result suggests the presence of many slightly different structures in the racemic mixture.

For proteins that bend DNA, some of the favorable free energy of interaction must be "spent" reconfiguring the shape of the double helix. This reasoning predicts that the same proteins will bind with higher apparent affinity to DNA molecules that are structurally preconfigured in a shape like that observed in the protein/DNA complex. Could DNA duplexes bent by methylphosphonate substitution be tested in this manner? Though initially an attractive strategy, we have not pursued these experiments because of an important limitation. While incorporation of methylphosphonate analogs may prebend the DNA to better accommodate protein binding, the modified phosphates are, by definition, sites where important stabilizing electrostatic interactions are thought to occur with the protein. The presence of appended methyl groups at these phosphates will undoubtedly sterically hamper complex formation, much as ethylation interference experiments monitor important phosphate contacts by introducing steric obstacles to protein binding.

## CONCLUSIONS

Data have been obtained identifying both the distribution pattern and stereochemistry of methylphosphonate linkages as factors affecting DNA bending. In all cases the data support a measurable role of asymmetric phosphate neutralization in DNA bending. When the patch of neutralization is more diffuse, less DNA bending is observed. This result is attributed to the reduced coherence of force vectors due to each neutralized phosphate within the diffuse patch and agrees with theoretical predictions (Manning et al., 1989). DNA duplexes containing pure ($R_P$)-methylphosphonate substitutions are ~30% less bent than duplexes containing racemic methylphosphonates. This effect may arise from nonelectrostatic perturbations of DNA structure by ($S_P$)-methylphosphonate isomers present in racemic mixtures. Even after taking this nonelectrostatic effect into account, however, DNA bending by asymmetric charge neutralization is clearly evident in these experiments.

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