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Published on Web 04/03/2002

Bending of DNA by Asymmetric Charge Neutralization: All-Atom Energy Simulations†

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Received November 12, 2001

Abstract: DNA dodecamers of the alternating d(CG)·d(CG) sequence with six phosphate groups either charge-neutralized or substituted by neutral methylphosphonates across the major or minor groove have been subjected to energy minimization to determine the conformational effect of the asymmetric elimination of phosphate charge. We report bending angles, directions of bending, and detailed structural characteristics such as groove widths and local base-pair parameters. Our principal results are that charge neutralization on one face of the DNA induces significant bending toward the neutralized face, in agreement with theoretical predictions on a simplified model and experimental data on a similar base-pair sequence, and that the DNA conformation averaged over all stereospecific methylphosphonate substitutions is nearly the same as the conformation produced by charge neutralization of the phosphates. Individual isomers, however, cover a wide range of structures, with the magnitude and direction of overall bending sensitive to the precise stereochemical pattern of neutralization. Our simulation does not explicitly contain counterions, and the results therefore suggest that counterions can influence DNA structure by neutralizing the phosphate charge. These data provide new hints into the molecular mechanisms which underlie the deformations of DNA structure induced by the binding of positively charged proteins and other tightly associated cationic species.

1. Introduction

The current interest in "what's in charge" of DNA structure has focused attention on the influence of the ionic charge of the phosphate groups and counterions and the mediating dielectric behavior of the solvent (solvent polarization).[1−3] The sequence-specific coordination of monovalent and divalent cations in the grooves of a growing number of ultrahigh-resolution crystal structures,[4] along with the observed[5−7] and simulated[8,9] asymmetric build-up of counterions around DNA in solution, point to the critical role of electrostatic interactions in sequence-dependent bending. These studies confirm early theoretical predictions of (i) the effects of base sequence on the electrostatic potential and distribution of counterions around double helical DNA[10−13] and (ii) the role of localized charge neutralization on DNA collapse.[14,15] The nonuniformity of counterions at individual base-pair steps is expected to bend the double helix along the same lines as site-specific phosphate neutralization, whereby DNA collapses into chemically modified sites[16] or closes patches of helix in contact with cationic amino acids of protein.

DNA bending by proteins is important in many cellular processes. In addition to the histones which wrap DNA in nucleosomes, numerous eukaryotic transcription factors show the ability to increase the local curvature of DNA molecules. The "activated" DNA regions are believed to lead to initiation of transcription.[17] For example, strong (∼90°) bending of the DNA recognition site is induced by the *Escherichia coli* catabolite activator protein (CAP),[18] which functions primarily as an activator of transcription by binding to specific DNA sites

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† This work was taken in part from the dissertation of Konstantin M. Kosikov written in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Rutgers University, 1998.

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10.1021/ja0125218 CCC: $22.00

located at or near promoters. The recognition elements of CAP induce DNA wrapping toward and around the sides of the dimeric protein, with the 22 base-pair (bp), 2-fold symmetric consensus sequence forming a nearly planar bend.[19−21] Another interesting example of DNA strongly bent by protein occurs at the "TATA-box" of various organisms.[22−28] A TA-rich, 8 bp segment of promoter DNA binds to the TATA-box binding protein (TBP) by bending ∼80° away from the protein into the major groove and out of plane, producing a wide and shallow minor groove and strong underwinding.

While both TBP and CAP induce significant DNA deformation, the mechanisms of bending are quite different, typifying two kinds of DNA bending proteins.[29] In the TBP-DNA complex, bending is induced primarily by intercalation of hydrophobic protein residues, leading to kinking at the insertion sites. As a result, the DNA is bent away from the protein, and the contacts are at the convex surface of the bent DNA. In contrast, the CAP-DNA complex prominently features cationic protein residues in the contact interface, suggesting involvement of electrostatic interactions with DNA phosphate groups. The DNA is bent toward and around the protein, so that it is the concave side of the bent DNA that is present in the interface.

Electrostatic interactions are also thought to play a major role in DNA bending by the histone proteins in nucleosomes. This idea, originated by Mirzabekov and Rich,[14] was theoretically developed by Manning et al.[15] There is no protein in the theory. Instead, the action of cationic protein groups is reduced to mathematical neutralization of a patch of phosphate charges on one side of the DNA. The electrostatic stretching that contributes to maintenance of a straight DNA structure consequently vanishes on that side. The remaining interatomic forces that had balanced the stretch in the original straight equilibrium DNA structure become unbalanced, since the stretch has vanished. The unbalanced forces collapse the DNA to a new equilibrium structure that must be bent toward the neutralized side. The theory does not address the problem of determining how the unbalanced forces are apportioned among the many atomic-level interactions within the DNA molecule. The only question asked is whether the unbalancing of forces by charge neutralization is large enough to influence DNA structure. The calculations of Manning et al., based on polyelectrolyte theory, predict a significant amount of bending.

To check the effect of asymmetric charge neutralization on DNA bending in a protein-free environment, Strauss and Maher designed and constructed a series of DNA molecules in which selected phosphates were replaced by neutral methylphosphonate analogues.[16] Six phosphate groups were modified on one side of the double helix across the minor groove and the neutralization sites were phased with respect to A-tracts with known global bending features. The direction and magnitude of bending caused by phosphate neutralization were then followed using comparative electrophoresis measurements. Neutralization across the minor groove of a 21 bp GC-rich segment was found to induce bending of ∼20° toward the neutralized surface. Repeated experiments in the presence of multivalent cations showed reduced bending, in accordance with the predictions of counterion condensation theory.[15,30] These results point to the electrostatic nature of the bending. Similar studies of synthetic DNAs with tethered ammonium ions as a source of phosphate group neutralization[31] support the data obtained with methylphosphonate substitutions. Electrostatic phasing showed that modifications across the minor groove produce a bend of ∼4° toward the neutralized face for a hexyl tether and ∼8° for a shorter propyl tether. Neutral acetylated derivatives of the tethered amines produced no bending. Methylphosphonate substitutions were further utilized to model the bending caused by positively charged protein residues in DNA complexes with the PU.1 transcription factor.[32] PU.1, a member of the ETS family of DNA binding proteins, uniformly bends DNA into the major groove by ∼8° as it curves around the protein.[33] Selective neutralization of phosphate groups by methylphosphonate substitutions at observed sites of electrostatic contacts between DNA and protein bends the DNA (in the absence of protein) by ∼28° toward the neutralized face, although the direction of curvature is slightly different from that in the X-ray structure of the protein-DNA complex.

In principle, methylphosphonate substitutions of DNA phosphate groups can be made in two positions, creating either R or S isomers. Most of the experiments of Strauss and Maher were done with a racemic mixture of R and S methylphosphonate substitutions. It has been shown that incorporation of the two pure isomers into the B-DNA double helix has different effects on local structure. Kan et al.[34] found using NMR, UV, and CD spectroscopic measurements that the S isomer exhibits slightly greater base overlap than the R isomer in d(ApA) dimers. Also, thermodynamic studies by Reynolds et al.[35] have shown that incorporation of *S*-methylphosphonate isomers produces a sequence-dependent reduction in thermal stability, while no such effect is found for R isomers. Other work by Vyazovkina et al.[36] has shown the decrease in thermal stability caused by S isomers and, in addition, has found that incorporation of R isomers increases thermal stability compared to that of normal, unmodified DNA. Furthermore, gel mobility profiles of DNAs with selective substitutions in R positions show about 30% less bending than the racemic mixture.[37] The difference is

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attributed to additional bending by S isomers (present in the racemic mixture) by local nonelectrostatic effects.

The theory devised by Manning et al.[15] that predicts the bending of DNA subjected to asymmetric phosphate charge neutralization is semimacroscopic. It applies the classical theory of elastic rods to a microscopic but highly idealized polyelectrolyte model. Important aspects of bending cannot be analyzed, such as the actual molecular-level distortions underlying the bending, the influence of the detailed spatial location and the redistribution of partial atomic charge of the neutralized phosphates, and a possible dependence on the mode of neutralization. In this paper we deepen that analysis by optimizing the energy of an all-atom DNA model subjected to selective phosphate neutralization and/or chemical substitutions of charged phosphates by neutral methylphosphonates.

2. Computational Perspectives

The present DNA simulations in the absence of protein combine features of previous computational studies. We follow Sanghani et al.[38] in neutralizing patches of phosphates on one side of the DNA double helix, and like Swarnalatha and Yathindra,[39−41] we introduce site-specific neutralizing chemical substitutions of phosphate oxygens. These earlier calculations provide useful reference points for our work. The all-atom minimization studies of Sanghani et al.[38] predict considerable bending of poly(dA)·poly(dT), poly(dG)·poly(dC), and poly(dTA)·poly(dTA) repeating polymers which are asymmetrically neutralized by addition of positive charge to the anionic phosphate oxygens on one side of the double helix, with concomitant closure of both major and minor grooves at the modified sites. The computed direction of bending depends on the size of the neutralization patch, and the computed degree of bending varies to some extent with sequence, being slightly lower in partially neutralized poly(dA)·poly(dT) than in comparably modified derivatives of the other two sequences. In studies of polymers with 20−50% neutralization, i.e., 4−10 uncharged phosphates per double helical turn, the computed magnitude of bending is greatest at 30−40% neutralization (6−8 modified phosphates in each complete turn of the 10-fold duplex). The predicted degree of neutralization is roughly twice that found by Strauss and Maher (2 phosphates per 21 bp repeat or 14% neutralization)[16] to bend DNA at comparable levels but less than the predicted neutralization of the nucleosome core particle.[14] Analysis of the published crystal structures of the nucleosome core particle,[42−44] however, shows that only 15−20% of the phosphate side groups are in close contact with positively charged amino acids (Y. Li and W. K. Olson, unpublished data).

In subsequent energy minimization studies of protein-free DNA, Gurlie et al.[45,46] looked at the effect of neutralizing those phosphates in the DNA recognition sequence which are directly contacted by the protein in the 2.5 Å CAP-DNA crystal complex.[20] The result was an increase by ∼13° without change of direction of an already substantial intrinsic curvature of the DNA sequence. The full neutralized set of phosphates included, but was more extensive than, those directly contacted by cationic protein side chains (some of the contacts in the complex are by neutral side chains and backbone protein atoms). A minimal set of three neutralized phosphates that are contacted across the minor groove by cationic side chains in the crystal structure was found to enhance a secondary bend into the minor groove at the end of an A-tract in the recognition sequence. The net bending of the partially neutralized DNA differed only slightly in magnitude and direction from the bend toward the protein in the actual complex.

The molecular mechanics studies by Swarnalatha et al.[41] of B- and Z-type $(dCG)_n$·$(dCG)_n$ fragments of lengths 4−12 bp with all phosphates substituted by neutral *R*- or *S*-methylphosphonate groups do not show any bending of the double helix. This behavior is consistent with the expectation[47] that the compressive force unmasked by phosphate neutralization over the entire DNA surface (as distinguished from neutralization on only one side of the DNA) may be sufficient to buckle the double helical axis of persistence-length segments of DNA, but not shorter segments. The S isomer distorts the canonical B-DNA duplex slightly, deforming the structure along a short portion of the classic B → A conformational pathway, i.e., the sugar ring repuckers to a form with a lower pseudorotation phase angle in concert with reorientation of the base about the glycosyl linkage ($\Delta\chi < 0$), and adjustment ($\Delta\zeta > 0$) of the rotation about the P−O3′ bond in the sugar−phosphate backbone.[48] The repuckering is more pronounced for the cytosine furanose rings than the guanine sugars, in accordance with well-established patterns of DNA sequence-dependent deformability.[49] At the same time, the R-substituted oligomers undergo no significant local structural changes. The uniform positioning of methyl groups into the major groove in the case of the S isomer (see discussion below regarding side-group orientation) effectively reduces the major groove width while leaving the minor groove width essentially unaltered. The external orientation of methyl groups in the R form produces quite the opposite effect, increasing the width of the major groove and decreasing the minor groove width.

The recent molecular dynamics refinement of the NMR structure of a 10 bp DNA duplex with a single chiral alkyl phosphonate substitution (an *R* or *S*-*n*-octyl-phosphonate) at the central base-pair step of one strand, d(CCACC*p*GGAAC)·d(GTTCCGGTGG) with *p* the site of modification,[50] reveals a partial B → A transition coupled to moderate bending at the modified dimer. The simulations, carried out with state-of-the-art methods in the presence of explicit solvent and counterions, further indicate that the distortions of structure are more pronounced for the S than the R diastereoisomer, i.e., the same

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conclusion reached by Swarnalatha et al.[41] with rapid, but more primitive computations based on the implicit treatment of solvent through a distance-dependent dielectric function. Calculations of the latter sort clearly provide a useful starting point for more elaborate investigations of the structure and dynamics of partially neutralized DNA helices.

3. Computational Approach

Here we report our analyses of 12 bp B-like fragments of alternating poly(CG)·poly(CG), a sequence very close to the modified DNA constructed in the laboratory by Strauss et al.[16] All-atom models were generated for arbitrary placement of the constituent bases using the chain generating scheme and potential functions outlined in the recent computer studies of DNA compression and extension by Kosikov et al.[51] Energy optimization was performed at the local base-pair level, using as independent variables (1) the six rigid-body parameters at each successive base-pair step [three angles (Tilt, Roll, Twist) and three variables (Shift, Slide, Rise) with dimensions of length] and (2) the two parameters, Propeller and Buckle, known to distort complementary base pairs significantly in high-resolution structures.[52] Interatomic interactions were assessed with the Poltev potential, a force field developed specifically to reproduce the known distances of separation[53] and enthalpies[54] of nucleic acid base stacking. The magnitudes of partial charges on base and phosphate atoms are somewhat smaller than those employed in the AMBER[55] and CHARMM[56] force fields, consequently weakening electrostatic interactions of complementary base pairs and phosphate groups[57] compared to effects reported by others[1−3,8,9] in related studies of DNA partially neutralized by the asymmetric distribution of mobile cations.

To minimize end effects, periodic boundary conditions have been introduced, with the eleventh base-pair step set equal to the first step so that base pairs 11 and 12 always mimic the changes of the first and second base pairs. Such a representation of the double helix makes it possible to extend the conformational repeating fragment to longer chain lengths and thereby determine the global bending. We define the global bending angle β, following Zhurkin et al.,[58] as the angle between the planes of base pairs i and $i + 10$ at the centers of consecutively repeated bending/neutralization sites (corresponding in Figure 1, where $i = 5$, to base pairs 5 and 15). The direction of bending α is measured, also using the approach of Zhurkin et al.[58] with respect to the long axis of the 10 bp repeating unit. In the case of bending between base pairs 5 and 15 (Figure 1), α is the angle between the average long axis of the two base pairs (i.e., the *y*-axis of the so-called "middle" base-pair frame[59]) and the axis of intersection of the two base-pair planes (i.e., the rotation axis that brings the two planes into coincidence[60]). Bending into the major groove, the global counterpart of positive Roll coupled with zero Tilt at a single base-pair step, corresponds to $\alpha \approx 0°$ (see Figure 1). Bending into the minor groove, analogous to negative Roll in an isolated dimer, corresponds to $\alpha \approx 180°$. Deformations of the double helix to accordion-like structures with one DNA strand over-

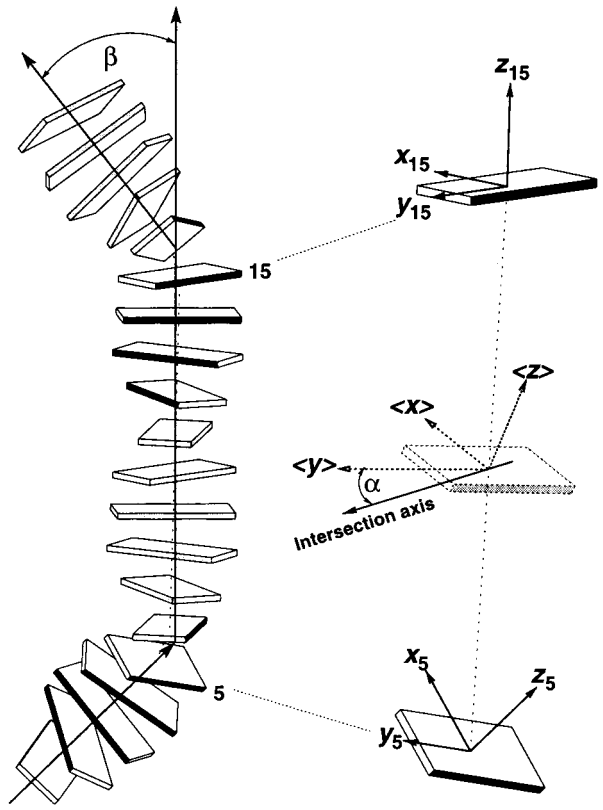

Figure 1. Schematic illustration obtained with 3DNA[67] of the magnitude β and direction α of global bending of a 20 bp DNA chain with two identical repeating fragments centered at steps 5 and 15. Minor groove edges of individual base-pair planes are designated by heavy shading and opposing (major-groove) faces by light shading. Reference frames of individual base pairs are constructed in accordance with standard guidelines.[68] Mean frames (dotted axes) of base pairs at consecutive sites of duplex distortion are obtained by a sequence of symmetrized Euler rotations.[58,59] The intersection axis (solid arrow in middle right image) is obtained from the vector product of base-pair normals.[60]

stretched at the dimer level and its complement correspondingly compressed are characterized by α near $\pm 90°$.

The major and minor groove widths (unless otherwise noted) are defined as the distances between P atoms in different strands separated by 3−4 base pairs, i.e., P′$(i - 2)$···P$(i + 2)$ across the minor groove with three intervening base pairs and P$(i - 2)$···P′$(i + 2)$ across the major groove with four intervening base pairs, corresponding to the P···P distances between labeled phosphate groups across the type 1 phosphate modification sites of the ideal B-DNA duplexes in Figure 2 (see below for further details of modification patterns). Here P(i) and P′(i) are the 5′-phosphates of the complementary nucleotides that comprise base pair i, with the prime used to denote the nonsequence (complementary) strand. For example, the computed minor (m1) and major (M1) groove widths at base-pair step 5 in Figure 3 correspond respectively to the distances between P′(3)···P(7) and P(3)···P′(7).

The simulations were performed under the same "low salt" conditions used in recent simulations of DNA stretching by Kosikov et al.[51] In these conditions, all phosphate groups initially bear a full electronic charge (−1). Counterions are not included in the simulations. We use a sigmoidal, distance-dependent dielectric constant of the form,

$$\epsilon(r_{ij}) = \epsilon_\infty - (\epsilon_\infty - 1)\left[\frac{1}{2}(sr_{ij})^2 + sr_{ij} + 1\right]\exp(-sr_{ij}) \qquad (1)$$

where $\epsilon_\infty = 78.3$, the dielectric constant of bulk water at room temperature. In "low salt" conditions, corresponding to a version of this function with relatively gradual slope defined by the controlling parameter $s = 0.356$, the variation of $\epsilon(r_{ij})$ has the effect of enhancing

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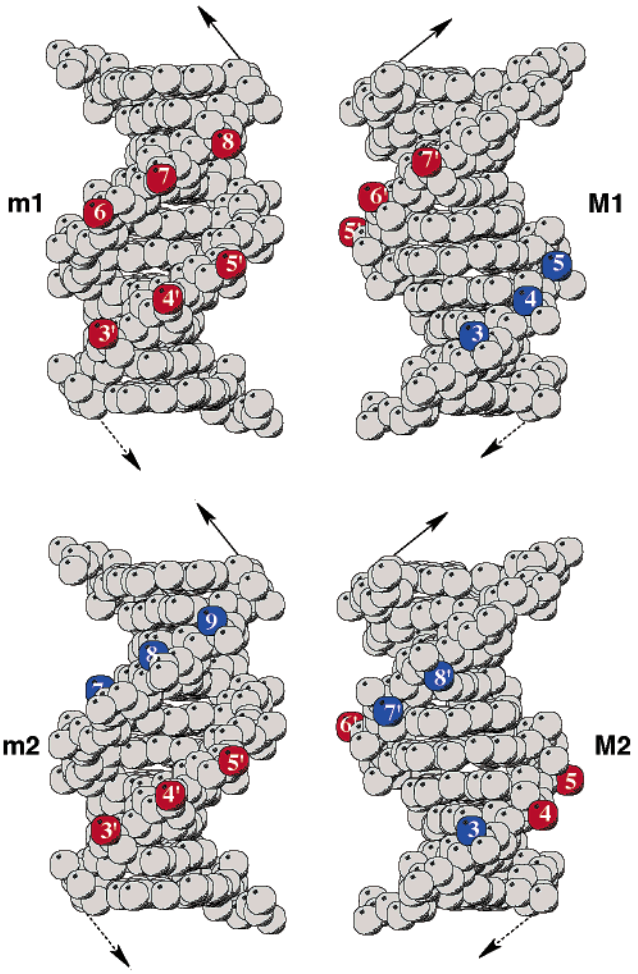

Figure 2. Space-filling representations of ideal 10-base pair B-form d(CG)·d(CG) duplexes[69] illustrating stereoselective chemical neutralization of six phosphate groups at four different modification sites. Three consecutively modified phosphate groups on complementary strands are separated by one (m1, M1) or two (m2, M2) base pairs and placed across the minor (m1, m2) or major (M1, M2) groove, on one side of the helix. Red: "R" oxygens directed outward in equatorial positions. Blue: "S" oxygens in axial positions pointing into the major groove. (Designations in quotations correspond to the absolute configurations of the methyl phosphonates that would be produced by replacement of these atoms by a methyl group.) Solid and dashed arrows show the respective 5′ → 3′ directions of sequence and nonsequence (complementary) strands. Numbers designate the 5′-phosphates of selected base pairs, with primes added to atoms on the complementary strand. Molecular images depict specific patterns of stereoselective substitution: m1, RRR−RRR; M1, SSS−RRR; m2, RRR−SSS; M2, SRR−RSS.

local electrostatic interactions (ϵ ranges from 2.0 to 4.7 as r_{ij}, the nonbonded distance between atoms i and j, increases from 3 to 5 Å).

Uniform partial neutralization of a given phosphate group was obtained by adding +0.42 esu to both side-group phosphate oxygens. The added charge mimics the increase in the computed charge of the phosphate group produced by the replacement of an oxygen by a methyl group in ab initio quantum mechanical studies of model compounds.[41] Substitutions were also performed stereoselectively, with a methyl group replacing one of the oxygens, creating an R or an S isomer with the same increase in net charge, i.e., 0.84 esu, concentrated on the methyl group. The two isomers are defined, following IUPAC guidelines,[61,62] such that the P → C_M bond vector forms an acute angle with the positive normal to the plane containing the O3′−P−O5′ linkage in the S form and an obtuse angle in the R-form, with the normal to the plane given by the vector product of phosphodiester bond vectors, (O3′ → P) × (P → O5′). (According to this definition, the methyl group in the S configuration is rotated in a positive right-handed (∼ +120°) sense and that in the R configuration in a left-handed (∼ −120°) sense with respect to O5′, when viewed along the O3′−P bond.[39,40]) In B-DNA the R-substituted methyl group adopts a pseudoequatorial position with respect to the chain backbone whereas the S isomer assumes a pseudoaxial position. The equatorial oxygens (highlighted in red in Figure 2) point outward from the major groove while the axial oxygens (in blue) point into the major groove.

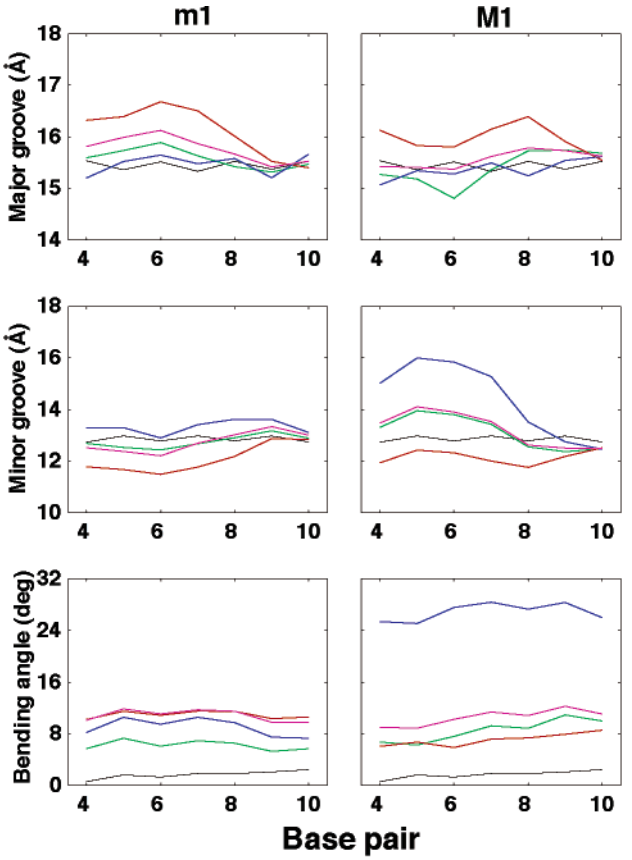

Figure 3. Major and minor groove widths and global bending angles β of energy-optimized 12-bp B-DNA duplexes subjected to various forms of phosphate neutralization at sites m1 (left) and M1 (right); values of β are defined with respect to the designated base-pair planes. Chemically unmodified B-DNA (black); duplexes with uniform phosphate neutralization (green), all-R (red), and all-S (blue) methylphosphonate substitutions; average structure (magenta) based on the mean rigid-body parameters of base pairs and dimer steps in 20 duplexes with 3R and 3S substitutions.

Phosphate groups were selectively neutralized on one side of the helix to induce bending toward that side. The simulations were performed for four arrangements of neutralized sites. The first one, termed site m1, places six modified phosphate groups, on either side of a given base pair (base pair 5 in Figure 2), along the edges of the minor groove in accordance with the experiments of Strauss et al.,[16] while the second arrangement with neutralization on the opposite side of the helix (site M1) places the modified phosphate groups across the major groove. In the other two arrangements (m2 and M2) there is a 2 bp gap between modified phosphate groups on opposite strands (base pairs 5 and 6, the lower pair of images in Figure 2). In all simulations reported here, the neutralization sites are centered on base pair 5 for m1 and M1, and on base pairs 5 and 6 for m2 and M2. Not shown are available data generated by sliding the neutralization sites up and down the duplex, since these results have substantially similar features.

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(62) Joint Comission on Biochemical Nomenclature (JCBN), I. *Eur. J. Biochem.* **1984**, *138*, 5−7.

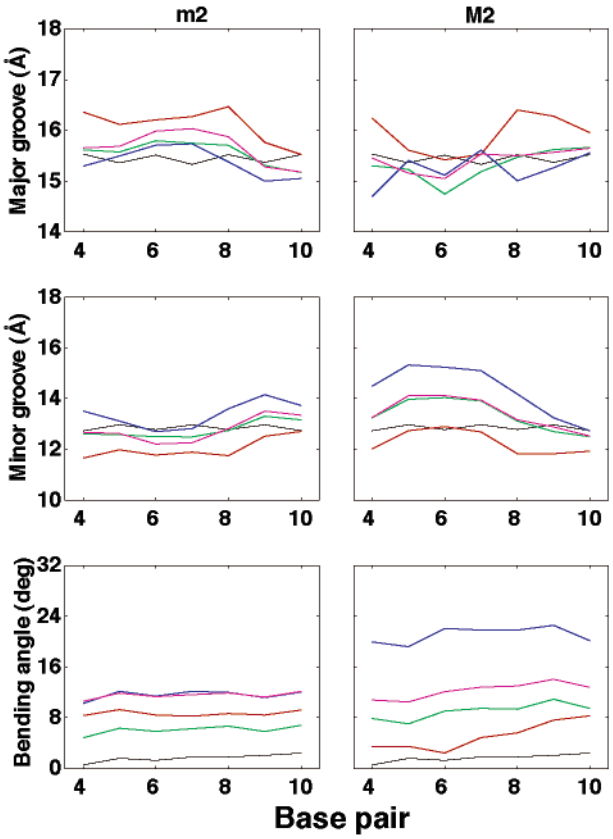

Figure 4. Major and minor groove widths and global bending angles β of energy-optimized 12-bp B-DNA duplexes subjected to various forms of phosphate neutralization at sites m2 (left) and M2 (right). See legend to Figure 3.

4. Results

4.1. R vs S-Modified Neutralization Patches. Our simulations show the conformational effects of all possible R and S substitutions on DNA helices with chemically modified patches of neutralization. Substitutions of all six phosphates by R methylphosphonates at either site m1 or site M1 increase the major groove width and decrease the minor groove width (red curves in Figure 3) along the same lines found by Swarnalatha et al.[41] in simulations of uniform R-substituted B-DNA chain fragments. (The unexpected narrowing of the minor groove by stereospecific neutralization of phosphate groups lining the major groove is discussed in further detail below.) Corresponding substitutions with six S isomers widen the minor groove (significantly in case of the M1 phosphates), but have almost no effect on the major groove (blue curves in Figure 3)—an effect that is missed in computer studies of model compounds with all S-modified phosphates.[41] The bending in the two extreme cases in Figure 3 (all-R or all-S) differs significantly from that found for uniform neutralization of phosphate oxygen charges (green curves) at the same sites. It is quite clear that local steric and electrostatic factors add to the effects of charge neutralization for methyl substitutions in both R and S configurations. Note particularly the enhanced magnitudes of major groove bending of all-R substituted methylphosphonates (Figure 3, red curves in the top two panels) regardless of the base pair i used to define groove width ($i = 5$ corresponds to the modifications in Figure 2). Similar results for neutralization sites m2 and M2 are shown in Figure 4.

Table 1. Magnitude and Direction of Global Bending of DNA Duplexes Neutralized Stereospecifically at Four Different Modification Sites[a]

	magnitude, β (deg)				direction, α (deg)			
pattern[b]	m1	m2	M1	M2	m1	m2	M1	M2
uniform	7.3	6.3	10.9	10.9	175.2	164.8	−18.7	30.3
RRR−RRR	11.5	9.3	7.9	7.6	161.1	150.3	156.0	137.2
SSS−SSS	10.5	12.2	28.1	22.5	−170.7	−157.0	−15.5	6.6
RRR−SSS	15.7	16.1	13.5	18.4	−153.9	−139.5	−64.5	−21.8
RSS−RRS	10.3	13.1	8.9	11.0	−170.0	−163.4	37.6	37.9
SRS−RRS	11.8	13.6	10.2	12.1	−176.4	−154.9	−100.5	−39.3
SSR−RRS	17.3	16.9	10.3	8.2	−164.9	−163.9	−65.0	−58.9
SSR−RSR	13.8	14.7	13.6	11.6	179.9	−160.1	−42.7	−39.7
SRS−RSR	10.7	10.4	14.5	14.2	−176.7	−166.5	−39.7	−21.1
RSS−RSR	8.2	10.1	16.6	12.1	−173.6	−173.3	16.8	32.7
RRS−RSS	11.4	11.1	13.4	10.1	159.9	155.5	56.3	69.3
RSR−RSS	15.0	14.4	8.8	7.4	169.5	166.6	55.0	72.9
SRR−RSS	19.3	16.9	8.8	5.7	172.1	−179.4	−136.1	170.0
SRR−SRS	12.1	12.9	10.3	8.0	172.3	167.4	−106.2	85.4
RSR−SRS	11.1	10.4	7.9	10.9	164.8	155.1	41.5	54.1
RRS−SRS	8.1	9.6	11.2	14.6	140.7	148.0	36.8	48.9
RSS−SRR	7.0	6.7	16.8	21.7	175.9	−171.1	−9.0	19.4
SRS−SRR	8.2	7.9	17.3	20.2	−173.1	−165.2	−50.7	−14.0
SSR−SRR	13.6	10.4	13.7	17.9	−173.3	−161.9	−62.1	−17.1
RRS−SSR	8.5	7.3	13.2	18.5	145.9	148.1	27.4	39.6
RSR−SSR	9.0	10.8	15.4	14.7	153.0	168.6	4.9	45.4
SRR−SSR	14.7	12.6	12.8	11.7	164.3	177.1	−67.7	57.8
SSS−RRR	15.8	15.8	8.1	10.8	137.0	144.3	87.0	85.7
average	12.0	12.0	12.2	13.0	172.5	180.0	−10.7	29.6

[a] Neutralization and substitutions of phosphate groups centered at base pair 5. [b] Pattern of stereospecific phosphate neutralization found at phosphates $i-2$, $i-1$, i in the sequence strand and i', $i'+1$, and $i'+2$ in the complementary strand. Also see Figure 2. [c] Positioning of modified phosphates (p in the sequence strand and q in the complementary strand) at different sites along the 10 bp chain repeat is as follows:

minor groove sites (m1, m2)

major groove sites (M1, M2)

The neutralization of individual phosphates by stereoselective methylphosphonate substitution is asymmetric in the sense that there is a small net positive charge on the methyl group and a residual negative charge of greater magnitude on the remaining (double-bonded) side-group oxygen. The repulsive interactions between the side-group oxygens at different phosphonate sites apparently compete with the neutralization of charge centered on the methyl group, giving rise to the variation in groove widths and bending associated with specific chemical configurations (Figures 3 and 4).

4.2. Bending of Racemic Mixtures vs Uniformly Neutralized Phosphates. To minimize steric effects we attempted to model the racemic mixture of R and S isomers, used in most experiments. We compared the average bending of the set of substitution structures with that of uniform phosphate neutralization. In a racemic mix of six substituted phosphates, the dominant population will have 3 R and 3 S substitutions. These substitutions are randomly arranged among the six phosphate positions, giving 20 possible combinations (see Table 1 and discussion below for further details). We simulated all 20 of these steric combinations in the four neutralization sites shown in Figure 2. The bending diagrams in Figures 5 and 6 illustrate that, even though the magnitudes and directions of bending are

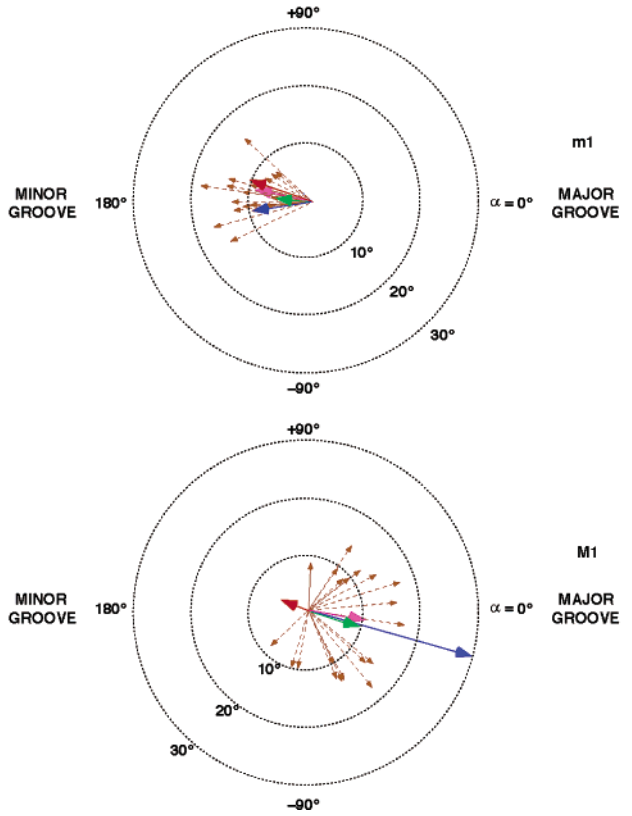

Figure 5. Circular plots of the magnitudes β (radial displacements) and directions α (arrows) of global bending centered at base-pair 5 in energy-minimized 12 bp B-DNA duplexes neutralized uniformly and through site-specific methylphosphonate substitutions across the minor groove at site m1 (top) and the major groove at site M1 (bottom). Duplex with uniform phosphate neutralization (green), all-R (red), and all-S (blue) methylphosphonate substitutions; average structure (magenta) based on the mean rigid-body parameters of base pairs and dimer steps in 20 duplexes with 3R and 3S substitutions.

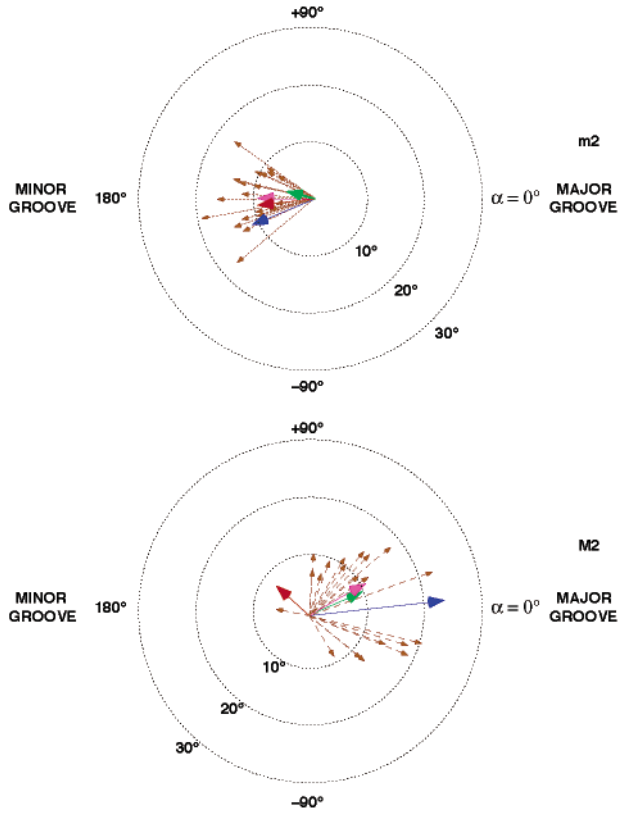

Figure 6. Circular plots of the magnitudes β (radial displacements) and directions α (arrows) of global bending centered at base-pair 5 in energy-minimized 12 bp B-DNA duplexes neutralized uniformly and through site-specific methylphosphonate substitutions across the minor groove at site m2 (top) and the major groove at site M2 (bottom). See legend to Figure 5.

spread over a wide range of values for the 20 different stereospecific substitutions (thin dashed brown vectors), the average (racemic) bending vector (thick magenta arrow) remains close to that found for phosphate neutralization (thick green arrow). Overall, these diagrams show that neutralization of phosphates on one side of the helix bends the DNA toward the neutralized side. This is true for neutralization across both the major and the minor grooves. A notable exception is the all-R substitution across the major groove, which generates a slight bend into the minor groove (thick red arrows in Figures 5 and 6 with magnitudes given by the value of β at base pair 5 in the lower right panels of Figures 3 and 4). The magnitude of bending is also generally greater for bending into the major groove than into the minor groove (compare the lengths of vectors in lower vs upper circles in the Figures 5 and 6).

4.3. Modes of Global Bending. Figure 7 illustrates the different modes of duplex bending brought about by site-specific versus uniform and racemic neutralization of phosphate groups in poly(CG)·poly(CG) oligomers. The energy optimized configurations of the 12 bp repeating fragments of B-form structures with selected chemical modifications across the major groove (site M1) are superimposed at their 5′-terminus and represented in stereo. The different magnitudes and directions of bending of the all-R (red) and all-S (blue) substituted helices are immediately apparent from the upper pair of images in Figure 7. These extreme examples bracket the predicted bending of the same chain subjected to uniform phosphate neutralization at site M1 and the structure obtained by averaging over all 20 3R/3S substitutions. The similar global bending of the latter structures reveals itself in the lower images in Figure 7, where the chain with neutralized phosphates (green) is superimposed on the (magenta) configuration generated from the mean values of both the rigid-body parameters relating complementary base pairs and the step parameters between successive base pairs.

4.4. Stereoselective Control. As evident from Figures 5 and 6, the computed direction of DNA bending is sensitive to both the site and stereochemistry of neutralization. Although the double helix generally bends in the direction of the groove that is outlined by uncharged phosphates, there are exceptions to the rule (see leftward pointing arrows in the lower circles of Figures 5 and 6 that depict the bending into the minor groove of double helices with selected patterns of neutralization across the major groove). Table 1 summarizes the computed magnitude and direction of bending for these unusual cases and for other stereoselective substitutions of phosphates across the major and minor grooves. The entries in the first column detail the specific chemical modifications introduced in the sequence and complementary strands at different neutralization sites. For example, the designation RSS−SRR points to the presence of an R-substituted 5′-phosphate followed by two *S*-methylphosphonates in successive residues ($i-2$, $i-1$, i) along the sequence strand, in combination with an *S*-methylphosphonate followed by two R-substituted 5′-phosphates in the hydrogen-bonding partners of base pairs (i', $i'+1$, and $i'+2$) in the

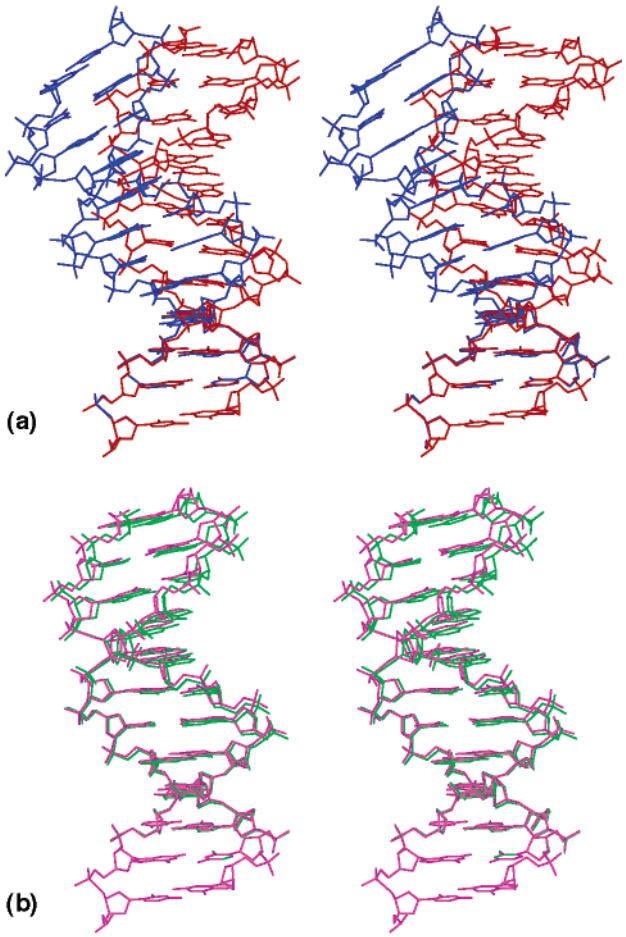

Figure 7. Color-coded stereoimages of the simulated bending of 12 bp B-DNA duplexes modified across the major groove at site M1 via (a) all-S (blue) vs all-R (red) phosphate substitutions; (b) uniformly neutralized (green) vs average over 20 duplexes with 3R and 3S substitutions (magenta). Configurations of lowest energy structures are generated with 3DNA[67] from the rigid-body parameters relating complementary base pairs and successive dimer steps. Chains are aligned in a common frame in the first base pair and oriented so that the minor groove is always shown at the lower end of each duplex.

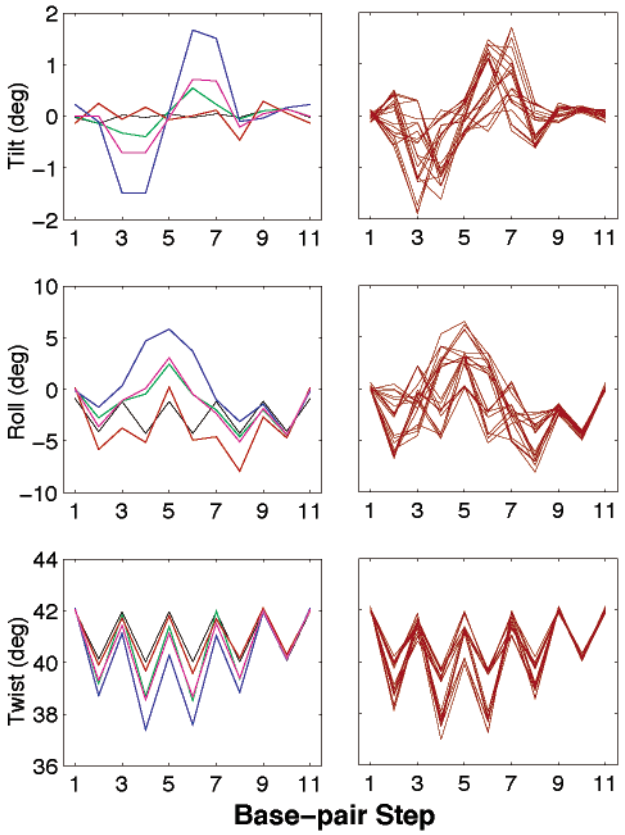

Figure 8. Local angular step parameters (Tilt, Roll, Twist) vs base-pair position in energy-optimized 12 bp B-DNA duplexes neutralized at site M2 and centered on base pairs 5 and 6. Odd numbered steps correspond to CG dimers and even numbered steps to GC dimers. Colors on the left designate the mode of neutralization: (red) all-R; (blue) all-S; (green) uniformly neutralized; (magenta) average over 20 duplexes with 3R and 3S substitutions; (black) chemically unmodified DNA. Parameters characterizing the 20 3R/3S substitutions are shown in brown on the right.

complementary strand. For neutralization across the minor groove, the modifications along the complementary strand precede those of phosphates in the leading strand in terms of base-pair order, i.e., $i' \leq i - 1$ (see preceding definitions of groove widths and footnote to Table 1). In the case of substitutions across the major groove, the modifications of the complementary strand occur further along the base-pair sequence, i.e., $i' \geq i$.

As evident from Table 1, the computed effects of neutralization are more sensitive to the stereochemical pattern of neutralization than to the precise sites of groove modification. Global bending is roughly comparable for chains with phosphate modifications at sites m1 and m2 on the edges of the minor groove, but is distinct from that found for helices with phosphate substitutions at sites M1 and M2 across the major groove. By contrast, small changes in the absolute stereochemistry of methylphosphonate substitutions perturb both the magnitude and direction of bending. For example, the shift of an *R*-methylphosphonate from the center to one end of a neutralization site, e.g., RRS−RSS to SRR−RSS, enhances and reorients chain bending into the minor groove at sites m1 and m2. The corresponding modifications of phosphates along the major groove edges have even more pronounced effects on overall structure. Whereas the SRR−RSS combination bends the duplex into the minor groove, the RRS−RSS modification introduces a deflection of greater magnitude in the major groove direction. Examination of the data in Table 1 reveals stereochemical signals that may underlie the computed structural deformations. For instance, the terminal *S*-methylphosphonates and penultimate R substitution common to some of the 3R/3S arrangements give rise to the unexpected bending of the duplex into the minor groove at the M1 modification site (see SRX−XXS motifs in Table 1 where X is either configurational center and $|\alpha| \geq 90°$). Electrostatic repulsions between closely spaced, side-group oxygens at these configurational centers seemingly contend with the tendency of the neutralized duplex patch to collapse into the major groove.

4.5. Deformations of Local DNA Structure. We have made detailed comparisons of the evolution of the six local base-pair step parameters along the optimized decamer repeats of DNAs neutralized stereospecifically and uniformly at sites m1, m2, M1, and M2. As an example, we show the results for chains with various phosphate substitutions at the M2 site in Figures 8 and 9. The data on the right half of these figures correspond to all 20 R/S combinations (thin brown lines). The similarity in the left half of the figures between the average step parameters of the 20 different optimized structures (magenta line) and the parameters of the structure determined with charge-

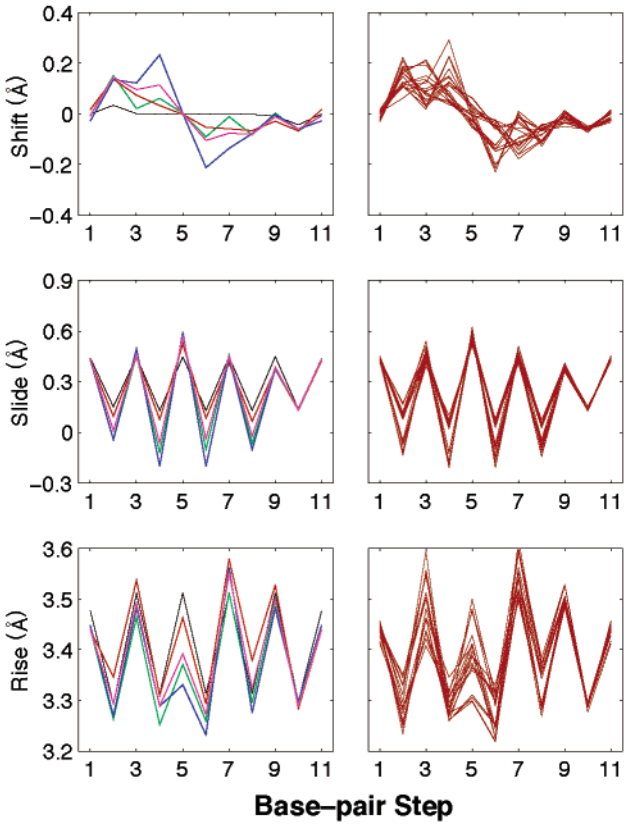

Figure 9. Local spatial translations (Shift, Slide, Rise) vs base-pair position in energy-optimized 12 bp B-DNA duplexes neutralized at site M2 and centered on base pairs 5 and 6. See the legend to Figure 8.

neutralized phosphates (green) is remarkable. Other examples of such striking structural similarities may be seen for the groove widths in Figures 3 and 4 averaged over the 20 R/S isomers and those of the neutralized conformers. These results, and those discussed above, suggest that the chemical substitutions of one side-group oxygen per phosphate group by a methyl, when averaged over all possible combinations of R and S isomers (which is equivalent to a racemic mixture of R/S isomers), produces nearly the same structural effect as uniform neutralization.

Importantly, the patterns in Figures 8 and 9 mimic observed conformational trends in DNA, adding to the reliability of the present computations. The predicted changes in global structure reflect the known tendency of DNA to deform preferentially via Twist, Roll, and Slide.[63,64] The computed changes in these parameters are also coupled in the correct sense, with a decrease of Twist leading to a reduction in Slide. Furthermore, the Twist of successive base-pair steps alternates between the over- and underwound states typical of CG and GC dimer steps found in high-resolution crystal structures,[63] although the predicted magnitudes of Twist are greater than observed mean values. The computed anticorrelation of Tilt and Shift, which is easily seen for the all-S substituted molecules (blue plots on the left in Figures 8 and 9), similarly follows known conformational trends. The pronounced bending of DNA into the major groove brought about by incorporation of all-S phosphate modifications at site M2 arises from a build-up of large positive values of Roll consistent with the strong bending deformations observed in known crystal structures, e.g., CAP,[18−21] and the bending into the minor groove associated with R-substitutions at the same site stems from an accumulation of slightly negative Roll over several base-pair steps, reminiscent of the conformational pattern found in the negatively curved, high-resolution NMR structure of the Dickerson dodecamer in the aqueous dilute liquid crystalline phase.[65]

5. Conclusions

Energy optimization of B-like poly(CG)·poly(CG) dodecamers shows that electrostatic neutralization of six phosphate groups on one side of the double helix bends the helix toward the neutralized face, in general agreement with the theoretical model of DNA bending by asymmetric phosphate neutralization suggested by Mirzabekov and Rich[14] and subsequently extended by Manning et al.[15] The magnitude of bending is moderate, being on the average greater when the neutralized phosphates are placed across the major groove than when they lie across the minor groove (∼11° vs 7°). This result is not unexpected, since the major groove is wider than the minor groove in B-DNA and allows more room for bending without steric clashes. The spacing of neutralized phosphates on complementary strands, i.e., separation of three modified residues by one or two base pairs, has almost no effect on the magnitude and direction of bending. Our simulation does not explicitly contain counterions, and the results therefore suggest that counterions can influence DNA structure by neutralizing the phosphate charge.

Strauss et al. synthesized a racemic mixture of R and S isomers to eliminate the ionic charge on six phosphate groups across the minor groove of a 21 bp GC-rich DNA fragment and observed an apparent bend of ∼20° toward the neutralized face in gel electrophoresis tests. They interpreted the bend as resulting from the elimination of charge. In support of their interpretation, we find that the same effect produced in our simulations by uniform electrostatic neutralization of six phosphate groups on one side of the helix can be achieved by a racemic mixture of R and S methylphosphonate isomers, although the calculated bending angles are somewhat smaller than the experimentally observed ones. Quantitative agreement with experiment, however, is not expected for the simplified solvent/counterion model used in this study. On the other hand, our calculations show that all-R or all-S methylphosphonate substitutions of the phosphate oxygens produce different magnitudes and directions of bending from uniform electrostatic neutralization of phosphate charges, especially for substitutions across the major groove. These differences are easily understood in terms of the stereochemistry of these substitutions. The S-configuration, in which the methyl group points into the major groove and contacts the preceding sugar, is expected to have stronger local structural and electrostatic effects than the R-form, in which the methyl group points away from the B-DNA helix. Strauss-Soukup et al.[37] have also observed different effects of pure R substitutions compared to racemic methylphosphonates on DNA bending. Duplexes neutralized by incorporation of pure R methylphosphonate isomers appear to be bent ∼30% less than

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duplexes neutralized by racemic methylphosphonates. In accordance with these experimental results, we find that racemic mixtures of R- and S-substituted phosphates bend DNA more strongly than all-R substitutions for all four sites studied. The difference is higher for bending across the major groove (71% for M2 and 54% for M1) compared to the minor groove bending (29% for m2 and only 4% for m1). We further predict that patches of phosphate groups neutralized by *S*-methylphosphonate substitutions along the major groove will bend double helical DNA more strongly than the corresponding racemic mixture.

Gel electrophoresis measurements are not necessarily expected to detect the different directions of intrinsic bending of stereoselectively neutralized helices reported in this work. In the absence of a quantitative theory, the interpretation of DNA gel mobilities is restricted to the qualitative notion that a naturally straight DNA molecule can "snake" more easily through the pores of a gel than a chain with intrinsic curvature.[66] The observed displacement is a function of the length, ionic atmosphere, natural equilibrium structure, and deformability of the chain sequence. Furthermore, the minimum energy configurations of differently substituted oligonucleotide duplexes are not representative of the ensemble of chain structures associated with the movement of polymeric DNA through a gel. The comparable magnitudes of intrinsic bending of model helices with stereospecific (3R and 3S) methylphosphonate substitutions are, nevertheless, suggestive of similar relative mobilities and consistent with the narrow bands of material observed in gel migration studies of a racemic mixture of modified oligonucleotides.[16]

Finally, the stereospecific effects of phosphate modification on the bending of model duplexes suggest how positively charged proteins and other cationic ligands may selectively control DNA deformations through preferential interactions with side-group oxygens. The shortest distance across the major groove between "S" side-group oxygens of the canonical B-DNA duplex is approximately 5 Å less than that between "R" oxygens (the designations in quotations correspond to the stereochemistry of the methyl phosphonates that would be produced by substitution of these atoms by a methyl group rather than the standard IUPAC convention whereby the *pro-S* oxygen of DNA becomes an *R*-methylphosphonate and vice versa[61,62]). Not surprisingly, stereoselective neutralization of the phosphate oxygens on the edges of the major groove has a marked effect on the predicted direction of duplex bending. The greater (3−4 Å) separation of "S" versus "R" oxygens across the minor groove correspondingly influences the magnitude and direction of bending into the minor groove. It is thus possible to envision how a protein might take advantage of these stereochemical features to trigger the bending of DNA required for close association and/or biological activity.

Acknowledgment. The authors are grateful to Drs. Gary L. Olson and Narayanarao Yathindra for helpful discussions. Support for this study has been provided by the U.S. Public Health Service (NIH grants GM20861 to W.K.O. and GM36284 to G.S.M.) and the Program in Mathematics and Molecular Biology based at Florida State University (fellowship support to K.M.K.). Computations were carried out at the Rutgers University Center for Computational Chemistry.

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