Molecular mechanics of the interactions of spermine with DNA: DNA bending as a result of ligand binding

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ABSTRACT

We used energy minimization of a molecular mechanical force field to evaluate spermine interactions with B-form DNA oligomers with either alternating purine/pyrimidine or homopolymeric sequences. Four different positions for spermine docking—within, along, and bridging the minor groove and bridging the major groove—were assessed for each sequence. Interaction at the major groove of alternating purine/pyrimidine sequences appears to be the most favorable of all models assessed, and are associated with significant bending of DNA. Interactions at the major groove of homopolymers were less favorable than those of heteropolymers and showed little or no bending. Interactions with the minor groove were most favorable for spermine positioned near the base of the groove, and became less favorable as spermine was moved toward the top of the groove. Association along the phosphate backbone alone was the least favorable of the interactions.

INTRODUCTION

Polyamines are simple, polycationic ligands present in prokaryotic and eukaryotic cells that are fundamentally important for cellular growth (reviewed in 1,2). The regulation of their synthesis and metabolism is intricate (3); after growth stimulation, the concentrations of intracellular polyamines and the activities of rate-limiting enzymes of polyamine biosynthesis can increase by orders of magnitude. Growth regulation by polyamines may be related in part to interactions with nucleic acids. Early evidence for this interaction emphasized the ability of polyamines to alter DNA conformation by condensation and aggregation (4–7) and to raise the melting temperature (T_m) of DNA (8,9), which implies stabilization of double-stranded over single-stranded forms. It was found recently that polyamines also stabilize both the left-handed Z conformation (10,11) and the right-handed A conformation (12) of nucleic acids. Stabilization of specific DNA

conformations may be important for processes such as nucleosome formation (13), chromatin condensation (14), and gene expression (15). Changes in the conformation of DNA caused by polyamines, polyamine analogues, or other cations depend on both the charge and structure of the cation, which is related to charge distribution along the length of the polyamine (16–18). Furthermore, the ability of a cation to promote DNA condensation does not allow prediction of the ability of the cation to stabilize the Z conformation or to perturb T_m (19,20). Thus, even though polyamines are structurally simple ligands, they probably interact with nucleic acids on several levels. This point is important because available data support both specific and nonspecific interactions between polyamines and DNA (21).

Early model building studies of polyamine/nucleic acid binding were based on electrostatic interactions between the protonated amino groups of polyamines and the negative charges of phosphate groups on the nucleic acid backbone (9,22). Suwalsky *et al.* (23) proposed that DNA condensation was related to the formation of polyamine bridges between DNA molecules. Later, Zhurkin *et al.* (24) rationalized polyamine stabilization of the A form of DNA with a similar model. Their studies support more favorable interactions between polyamine and neighboring interstrand phosphate oxygens in the A than in the B form. Even though this kind of a model provides a specific interaction between the polyamine cation centers and backbone phosphates, it does not directly provide sequence specificity because bases are not involved implicitly in the interaction. Secondary effects (such as bending) of base sequence on DNA conformation have not been addressed in these modeling studies.

In a theoretical study of spermine/DNA interactions, Zakrzewska and Pullman (25) calculated the most favorable spermine positions and conformations in the A and B forms of DNA with several base sequences, and concluded that spermine interactions appear to depend on DNA conformation and base sequence, and that salt may affect comparative binding. Their studies examined spermine localization and placement, but did not allow flexibility in DNA structure.

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Because polyamines are often used to obtain crystals for X-ray diffraction studies, several groups have published crystal structures that contain evidence related to the specificity of nucleic acid/polyamine interactions. In both tRNA^{phe} crystals (26) and B-DNA crystals (27), spermine may be associated with a bend in the major groove. In Z-DNA crystals, spermine is located both at the convex surface and along the phosphate backbone (28). Solution-phase studies of the competitive binding between polyamines and antibodies to specific sites on Z-DNA and Z-RNA also support specific binding of polyamines (29); other experimental results consistent with bending and stiffening of DNA in the presence of spermine have been published recently (16,30,31).

We used energy minimization calculations to model spermine interactions with the major groove of B DNAs (32) and found that spermine/DNA complexes are stabilized by maximizing interactions between proton donors on spermine and proton acceptors on DNA, which was achieved by bending the DNA over spermine, a process that encases the polyamine in a deepened, narrowed, and highly electronegative major groove.

Thus there are considerable data that support specific interactions of polyamines with nucleic acids. More importantly, when polyamines interact with nucleic acids, conformational changes may occur. In order to examine the sequence specificity of spermine binding to DNA and to explore how spermine-induced conformational changes in DNA structure might be related to DNA sequence, we have extended our theoretical studies of spermine/DNA interactions to several different base sequences at four possible sites of binding.

METHODS

Calculation of Spermine Energy Minima. The relative energies of spermine (Fig. 1) were calculated by determining the energies of each conformer. The appropriate C-C or C-N bond was rotated through the three staggered torsion angles of 60°, 180°, and 300° (gauche +, trans, or gauche -) using the appropriate partial charges and constants from Singh and Kollman (33) and Weiner *et al.* (34). Because there are 11 torsion angles in spermine, we sampled a total of 3¹¹ conformations.

Placement of Spermine onto DNA. Spermine, represented by the six lowest energy conformers (Fig. 2), was docked into B DNA decamers of different base sequence using the program MIDAS (35). DNAs were constructed from the coordinates of Arnott and Hukins (36), and the complexes were displayed and manipulated on an Evans and Sutherland Picture System 2. Coordinates used in the modeling will be deposited in the Protein Data Bank at the Brookhaven National Laboratory. We were able to fit four sites on DNA using these methods (Fig. 3): along a single phosphate backbone; bridging the minor groove (the Liquori/Tsuboi model); within the minor groove; and bridging

FIGURE 1. Structure of spermine. Relative energies of spermine were calculated by rotating about angles θ_5 - θ_5' .

the major groove. These positions were selected in part to assess the relative importance of spermine interactions with bases by comparing interaction sites on DNA either including or excluding base functional groups.

Molecular Mechanics. Models were refined with the program AMBER (37) under no constraints. Partial atomic charges and constants were taken from Weiner *et al.* (34) and Singh *et al.* (37), and the partial atomic charges on spermine were calculated using the Gaussian 80-UCSF program (37). Because water was not explicitly included in these calculations, we used a distance-dependent dielectric constant $\epsilon = R_{ij}$. The structures were refined until the root mean square of the energy gradient was less than 0.09 kcal/mol-Å.

Energy minimization methods that model these effects must be used with some caution. First, our assumptions may cause electrostatic effect to be exaggerated. Second, because only one local energy minimum is sampled, possible alternative modes of interaction might be missed. We have sampled multiple sites for interaction in attempts to avoid this pitfall. The local minimum may represent the most stable structure, but this approach ignores the kinetic energy in the real system and the importance of temperature in chemical interaction. We have performed molecular dynamics calculations for the binding of spermine to the major groove of d(GC)₅-d(GC)₅ and d(G)₁₀-d(C)₁₀ (38); solvent water and counterions were included explicitly in the calculations, which support the results reported here.

FIGURE 2. Relative energies of spermine. The numbers labeling each conformation refer to torsion angles at θ_3 , θ_4 , θ_5 , θ_3' , θ_4' , θ_5' . Trans (180°) is coded as 0, gauche - (300°) is coded as 3, and gauche + (60°) is coded as 6. Thus, the lowest energy is all trans (000000).

RESULTS AND DISCUSSION

Relative Energies of Spermine Conformations. We found five conformations of spermine within 1 kcal of the global minimum, the all *trans* conformation (Fig. 2). Two of the five conformations are symmetrical and three are not. The conformations of two published crystal structures for spermine (as either the hydrochloride [39] or the phosphate [40] salts) are among these conformations. In each of the 30 lowest energy conformations,

FIGURE 3. Docking of spermine to B-DNA. DNA is shown schematically by connecting adjacent phosphorous atoms. Spermine was placed in four possible positions for interaction with DNA: within the major groove (top left), within the minor groove (top right), along the phosphate backbone (bottom left), and bridging the minor groove (bottom right).

the three central torsion angles are 180° , which forces the central diaminobutane moiety into a fully extended conformation and produces a single distance (approximately 6.3 Å) between the secondary amino groups (data not shown). The aminopropyl groups attached to the secondary amino groups assume a variety of conformations, however, and there are a variety of distances and positions between the primary and secondary amino groups.

Energetics of Spermine/DNA Complexes. Numerical results for spermine/DNA complexes are summarized in Tables I–IV. Because energies for each DNA sequence will be different, absolute values of energy for each sequence and each complex cannot be compared directly. For each case, both the DNA/spermine complex and the DNA from the complex after energy minimization were compared with DNA energy minimized in the absence of spermine; this allows both the stability of the complex and the stability of DNA within the complex to be determined from a common baseline, and in essence normalizes the two values. The ‘stabilization energy of spermine’ is the difference in potential energy between a complex of spermine and oligomer after energy minimization and an oligomer energy minimized in the absence of spermine; it provides a measure of the amount of stabilization given to the complex by addition of the ligand. ‘DNA destabilization energy’ is related to the conformational changes that DNA undergoes in accommodating spermine and is calculated as the difference between the potential energy of DNA energy minimized in the presence of spermine and the potential energy of DNA energy minimized in its absence. These values are positive because spermine binding perturbs DNA from its minimum energy. We note that control sequences energy minimized in the absence of spermine did undergo some structural change, especially at the ends of the duplex.

Two values given in the tables are not normalized. The ‘intramolecular energy of spermine’ quantitates the stability of spermine in each model. Even though the starting conformations of spermine in each model may be different, the primary structure is unchanged and relative energies can be compared. The ‘interaction energy of spermine’ is the sum of all energies of interaction between each spermine molecule and DNA. It quantitates how well spermine fits the model DNA and should not be confused with the stabilization energy; the intramolecular energies of spermine and DNA are not included in the interaction energy, but are included in the stabilization energy. The

Table I. Energies (kcal/mol) of the Spermine/DNA Interaction in the Major Groove*

	d(AT) ₅ -d(AT) ₅	d(A) ₁₀ -d(T) ₁₀	d(GC) ₅ -d(GC) ₅	d(G) ₁₀ -d(C) ₁₀	d(AC) ₅ -d(GT) ₅
DNA destabilization ⁺	79	67.4	90	74	102
Spermine stabilization of complex [§]	-339	-314	-343	-304	-353
Spermine/DNA interaction [¶]					
Total:	-494	-464	-517	-454	-536
P:	-522	-483	-540	-448	-568
S:	150	146	152	145	158
B:	-113	-114	-117	-137	-117
Intramolecular energy of spermine	76	81	84	77	82

* Results are derived from energy minimization calculations with the program AMBER.

⁺ Quantitates the increase in energy of DNA when energy minimized in the presence and absence of spermine (see text).

[§] Quantitates the decrease in energy of the molecular ensemble when energy minimized in the presence and absence of spermine.

[¶] Quantitates the energy of the spermine/DNA interaction after energy minimization. The total spermine/DNA interaction energy has been divided into the component energies: spermine/phosphate (P), spermine/sugar (S), and spermine/base (B) energies. The sum of P, S, and B do not equal the total interaction energy because the interactions at the ends of the DNA strands are neglected.

Table II. Energies (kcal/mol) of the Spermine/DNA Interaction in the Minor Groove*

	d(AT) ₅ -d(AT) ₅	d(A) ₁₀ -d(T) ₁₀	d(GC) ₅ -d(GC) ₅	d(G) ₁₀ -d(C) ₁₀	d(AC) ₅ -d(GT) ₅
DNA destabilization ⁺	79	70	57	58	77
Spermine stabilization of complex [§]	-308	-297	-299	-250	-316
Spermine/DNA interaction [¶]					
Total:	-460	-455	-430	-396	-443
P:	-426	-451	-438	-407	-419
S:	163	164	149	153	156
B:	-188	-159	-132	-135	-171
Intramolecular energy of spermine	72	88	76	88	77

* Footnotes are the same as given in Table I.

Table III. Energies (kcal/mol) of the Spermine/DNA Interaction Across the Minor Groove*

	d(AT) ₅ -d(AT) ₅	d(A) ₁₀ -d(T) ₁₀	d(GC) ₅ -d(GC) ₅	d(G) ₁₀ -d(C) ₁₀
DNA destabilization ⁺	33	30	31	45
Spermine stabilization of complex [§]	-262	-246	-262	-275
Spermine/DNA interaction [¶]				
Total:	-374	-361	-371	-410
P:	-445	-432	-446	-493
S:	113	115	117	128
B:	-36	-36	-35	-38
Intramolecular energy of spermine	79	85	78	90

* Footnotes are the same as given in Table I.

FIGURE 4. Interaction of spermine with d(AC)₅-d(GT)₅. *Left:* Spermine in the major groove of DNA before energy minimization. The major groove is marked by filled circles and the minor groove by filled triangles. Spermine is in the all trans conformation. *Right:* Spermine in the major groove of DNA after energy minimization. The major groove has enclosed spermine, decreasing its size (note position of solid circles), and the DNA has bent, which increases the size of the minor groove (note position of solid triangles).

interaction energy of spermine is a combination of three interactions, with phosphate, with sugars, and with bases.

There is considerable variation in the energies of interactions

FIGURE 5. View down the helical axis of spermine complexed with d(AC)₅-d(GT)₅ in the major groove after energy minimization. Each amino group on spermine interacts with at least two functional groups in DNA. Also note interaction across the two strands of DNA where the N4 of cytosine interacts with OA of the backbone of the opposite strand.

between spermine and DNA as a function of position of binding and base sequence; the most favorable interactions occur for complexes in which spermine bridges the major groove of heteropolymers. All possible sites of interaction for spermine on DNA have not been modeled in our studies. We chose only sites that seemed favorable based on crystallographic data, calculations of spermine conformational energies, and simple docking maneuvers.

Major Groove Models. Numerical results for the calculation of major groove complexes of spermine with the homopolymers d(G)₁₀-d(C)₁₀ and d(A)₁₀-d(T)₁₀ and the heteropolymers d(GC)₅-d(GC)₅, d(AT)₅-d(AT)₅, and d(AC)₅-d(GT)₅ are listed in Table I. The spermine/homopolymer complexes show relatively less interaction and less stabilization than do the heteropolymer complexes (Tables II and III). Thus, the homopolymers are stabilized by -304 (d[G]₁₀-d[C]₁₀) or -314 (d[A]₁₀-d[T]₁₀) kcal/mol, while the heteropolymers are stabilized by more than -339 kcal/mol. The interaction energies of the homopolymers are -454 (d[G]₁₀-d[C]₁₀) and -464 (d[A]₁₀-d[T]₁₀) kcal/mol, and those of the heteropolymers are all greater than -494 kcal/mol. The homopolymers also tend to be less destabilized by the interaction with spermine (Table I, row 1). Thus the DNA destabilization energy was 67 kcal/mol for d(A)₁₀-d(T)₁₀ and 73 kcal/mol for d(G)₁₀-d(C)₁₀. The d(AC)₅-d(GT)₅ polymer was more destabilized than the other polymers. This may be related to the high interaction energies of these polymers with spermine; better interaction might drive more conformational change in DNA. The intramolecular energy of spermine changed little through each calculation (Table I, row

FIGURE 6. Spermine interaction with d(G)₁₀-d(C)₁₀ in the major groove. The interaction before energy minimization is shown on the left. After energy minimization (right), even though there are some changes in placement of atoms in DNA, there is no evidence of a bend or of gross changes in the dimensions of the major (squares) and minor (circles) grooves.

4). Thus, compared with the homopolymers, the energy of interaction of spermine is more favorable and the perturbations of the intramolecular structure are greater for the heteropolymer. These results suggest the existence of site and sequence specificity for spermine binding in the major groove.

The complex of $d(AC)_5-d(GT)_5$ with spermine in the major groove before (*left*) and after (*right*) energy minimization is shown in Figure 4. After energy minimization (*right*), the helix is bent, the major groove is narrowed (compare the positions of the filled circles *right* and *left*) and the minor groove is broadened (compare the positions of the filled triangles). The magnitude of this bend is 5° – 10° larger than that obtained in our earlier calculations on $d(GC)_5-d(GC)_5$ and $d(AT)_5-d(AT)_5$ (32), but it is formed in a similar manner by interactions between phosphate oxygens and purine N7 groups and spermine primary and secondary amines. A view of the same complex looking down the helical axis of DNA is shown in Figure 5. Spermine interacts with various functional groups in the major groove. Each primary amino group (N2 and N2') interacts with two phosphate oxygens (OA), while the secondary amino groups (N1 and N1') interact with at least one heteroatom on the bases (N7/O4) and a phosphate oxygen. N1' of spermine interacts with both O4 and N7 of different bases stacked on each other. There is also an interaction between a cytosine N4 and a phosphate oxygen (OA) on the opposite strand of DNA. In this region, then, the major groove encloses spermine. In addition, sugar pucker is changed from C2' endo to C3' endo and interphosphate distances decreased considerably (not shown).

The homopolymers $d(A)_{10}-d(T)_{10}$ and $d(G)_{10}-d(C)_{10}$ have less favorable energies of stabilization and interaction than do the heteropolymers. We split the interaction energies of spermine and DNA into three groups: interaction of spermine with phosphates, with sugars, and with bases (Table I). It is apparent that the more favorable (lower) interaction energy of the heteropolymers is a function only of spermine interactions with phosphate; for instance, the spermine/phosphate interaction energy in the homopolymer $d(A)_{10}-d(T)_{10}$ is -483 kcal/mol and in the heteropolymer $d(AT)_5-d(AT)_5$ is -522 kcal/mol. Differences between the energies of the sugar and base interactions are smaller. For the heteropolymer and homopolymer pair $d(GC)_5-d(GC)_5$ and $d(G)_{10}-d(C)_{10}$, there is a greater difference in the phosphate interaction energy (-517 vs. -445 kcal/mol) than found for AT-containing oligomers, but it is partially counterbalanced by a better interaction with the bases in the homopolymer (-117 vs. -137 kcal/mol). Consideration of these results leads to the counterintuitive conclusion that sequence-dependent binding of spermine is not a simple function of interactions with base functional groups per se but depends on the phosphate backbone as well. Reasons for this conclusion are discussed below.

Structures for the complex formed by placing spermine in the major groove of the homopolymer $d(G)_{10}-d(C)_{10}$ before (*left*) and after (*right*) energy minimization are shown in Figure 6. The large bend observed in energy minimized heteropolymer/spermine complexes is not evident, and the 'normal' dimensions of the major groove are essentially retained. (Compare both the solid squares marking the major groove and the solid circles marking the minor groove before [*left*] and after [*right*] energy minimization.) When the complex is viewed along the helical axis (Fig. 7), it can be seen that spermine interacts with phosphates and bases at its N2', N1', and N2 positions. Because the N4 amino groups on cytosine are hydrogen donors, however, there is no obvious position for interaction with the N1 position

of spermine. This inability of spermine to interact fully with the nucleic acid is a possible explanation for the absence of bending in this homopolymer.

Binding of spermine to the homopolymer $d(A)_{10}-d(T)_{10}$ produces a different structure (Fig. 8). Because thymine provides a carbonyl group (O4) in the major groove that may interact with a secondary amino group of spermine (Fig. 9), we expected that the interaction would be more significant than found for $d(G)_{10}-d(C)_{10}$. Energy minimization does produce a significant change in the conformation of the backbone. The major groove (solid circles) decreases in size after energy minimization and the minor groove (solid triangles) increases in size. Neither the change in groove size nor the distinctive bend seen in heteropolymers are as evident in this case, however. Viewed along the helical axis (Fig. 9), it can be seen that three of the four amine nitrogens in spermine (N2, N2', and N1') each interact with at least two functional groups on DNA, while the N1 interacts only with the O4 of thymine. (Although one phosphate oxygen appears to be interacting with the N1 of spermine, the distance of approximately 3.5 Å puts it out of the range of hydrogen bond lengths). Thus, in contrast to $d(G)_{10}-d(C)_{10}$, $d(A)_{10}-d(T)_{10}$ provides sites for interaction at both secondary amino groups of spermine; but even though changes in the conformation are greater than those seen for $d(G)_{10}-d(C)_{10}$, the amount of bending in $d(A)_{10}-d(T)_{10}$ does not approach the amount found for the heteropolymers, and the dimensions of the minor groove remain more within the range found in unperturbed B-DNA.

These results can be rationalized in several ways. One possible explanation is that at least two interactions per spermine nitrogen are necessary to cause a bend in the helix. The energy minimized spermine- $d(A)_{10}-d(T)_{10}$ complex contains a 'nascent' interaction between spermine N1 and phosphate oxygen OA (Fig. 9). The change in nucleic acid conformation necessary to pull this phosphate oxygen closer to N1, however, is not favorable. Moreover, this 'nascent' interaction is qualitatively different from the interactions in the heteropolymers, in which there were at least three phosphate interactions with spermine from each strand

FIGURE 7. View along the helical axis of the energy minimized model shown in Figure 6. Only three of the four amino groups of spermine interact with sites on DNA. The presence of an amino group at cytosine N4 is unfavorable for interaction with N1 of spermine.

of DNA. Instead, this homopolymer favors the interaction of a fourth phosphate on the same strand as three other interacting phosphates; the opposite strand had not bent enough to allow interactions between a third phosphate and spermine. Thus, even the presence of a pyrimidine heteroatom (O4) in position to interact with a secondary amino group on spermine did not provide sufficient driving force to cause a bend. Evidently, heteropolymers in the B conformation as described in AMBER are more easily bent to form a narrow major groove and widened minor groove than are homopolymers, simply because of the different base sequence. It follows that phosphate interactions may influence sequence-dependent binding in the following way: conformational change in DNA may not depend directly on interaction of ligand with base. Base-base interactions may also help to determine the innate flexibility of DNA, which could then regulate the phosphate/spermine interaction.

In a more general sense, the prediction of bending in DNA as a consequence of polyamine binding could have important implications for stabilization of tertiary structure of both DNA and RNA. In tRNA^{Phe}, spermine appears to stabilize a narrowed major groove and to contribute to a 25° bend in the helical axis (26). The Drew-Dickerson crystal structure for B-DNA (41) shows small differences in structures for the nucleic acid per se and with polyamine bound. Gosule and Schellman (7) described a compact form of DNA reversibly induced by polyamines with a regular packing structure (42). Hydrogen/deuterium exchange studies (16) have also been interpreted as evidence for spermine-induced DNA bending. Marquet *et al.* (30,31) have reported

electro-optical measurements consistent with spermine-induced bending for adenine- and thymine-containing DNA polymers but stiffening in guanine- and cytosine-containing polymers. This is consistent with our theoretical data in d(AT)-d(AT) that shows bending in this sequence. The fact that they find stiffening in guanine- and cytosine-containing bases under low salt conditions is consistent with a B-Z transition that takes place in poly (dG-dC)-poly(dG-dC) (10; Feuerstein, B.G., unpublished results).

Changes in DNA structure found in these modeling studies are interesting from another point of view. The decrease in size of the major groove, increase in size of the minor groove, and alterations of sugar-phosphate and intrastrand phosphate distances are characteristic of the A conformation, and stabilization of A-DNA by polyamines has been reported (12). In addition, the left-handed Z conformation contains similar alterations in sugar pucker and intrastrand phosphate distances; polyamines stabilize this conformation as well (11). In the case of Z-DNA, however, the minor groove is narrow and deep, and the major groove appears as a convex surface. Crystals of this molecule with spermine show binding both at the phosphate backbone and major groove (28). In the case of both A- and Z-DNA, spermine thus appears to favor structures with deep grooves and shortened phosphate distances.

Minor Groove Models. The minor groove is a well-known site of interaction between small molecules and DNA and must be considered as a site for spermine binding. We placed spermine within the minor groove of B forms of d(A)₁₀-d(T)₁₀,

FIGURE 8. Spermine interaction with d(A)₁₀-d(T)₁₀. Interaction before energy minimization is on the left, and the structure after energy minimization is on the right. A small bend in the helix can be seen, and the major groove has become somewhat smaller (compare the distance between the circles marking the major groove before and after energy minimization). The minor groove has increased in size (compare triangles).

FIGURE 9. View along the helical axis of spermine/ $d(A)_{10}$ - $d(T)_{10}$ complexed in the major groove after energy minimization. N2, N1', and N2' of spermine each interact with two functional groups on DNA, while N1 interacts only with the O4 of thymine. OA above N1 is greater than 3.5 Å from it (see text).

$d(AT)_5$ - $d(AT)_5$, $d(GC)_5$ - $d(GC)_5$, $d(G)_{10}$ - $d(C)_{10}$, and $d(AC)_5$ - $d(GT)_5$. Results of energy minimization calculations for these complexes are listed in Table II. Comparison between sequences for this position shows that spermine interacts least favorably with the minor groove of $d(G)_{10}$ - $d(C)_{10}$ because spermine stabilizes this complex approximately 50 kcal/mol less than the other sequences examined. Compared with the energy for major groove placement, each complex is stabilized 15–50 kcal/mol less well by placement of spermine into the minor groove. Although the major groove is more favorable than the minor groove for every sequence, the $d(A)_{10}$ - $d(T)_{10}$ sequence shows the least specificity. The stabilization energy for $d(A)_{10}$ - $d(T)_{10}$ differs by only 17 kcal/mol and the spermine-DNA interaction energies differs by only 8 kcal/mol from the major groove position. The lowest DNA destabilization energies were found for $d(GC)_5$ - $d(GC)_5$ and $d(G)_{10}$ - $d(C)_{10}$, which may be related to the higher and therefore less favorable spermine/DNA interaction energies for these complexes.

Differences in the conformation and placement of spermine upon forming minor groove complexes can be seen in Figures 10 and 11. The spermine conformation in the complex with $d(AT)_5$ - $d(AT)_5$ (Fig. 10, *left*) is more extended, while that for the complex with $d(GC)_5$ - $d(GC)_5$ (Fig. 10, *right*) is more bent. Differences in the interaction with DNA are shown in Figure 11, in which spermine is shown with a slice of the water-accessible surface of DNA (43) represented as a series of dots. The minor groove is a pocket in which spermine rests. Spermine approaches the floor of the minor groove in $d(AT)_5$ - $d(AT)_5$ (*left*), but the approach is less favored in $d(GC)_5$ - $d(GC)_5$ (*right*). In the spermine- $d(AT)_5$ - $d(AT)_5$ complex, the primary amino groups of spermine are in contact with O2 of thymine, N3 of adenine, and O1' of appropriate sugar residues at the base of the minor groove (not shown). In the spermine- $d(GC)_5$ - $d(GC)_5$ complex, however, the positively charged guanine 2-amino group

FIGURE 10. Spermine conformations in the minor groove of $d(AT)_5$ - $d(AT)_5$ on the left and $d(GC)_5$ - $d(GC)_5$ on the right after energy minimization. The spermine conformation is more extended when complexed with $d(AT)_5$ - $d(AT)_5$, while the conformation is bent more in the complex with $d(GC)_5$ - $d(GC)_5$.

interferes with interactions between the spermine amino groups and the O2 of cytosine and N3 of guanine. Thus, the secondary amino groups of spermine, which interact primarily with phosphate oxygens in both sequences, are placed more closely to the floor of the groove in $d(AT)_5$ - $d(AT)_5$ but are close to the 'top' of the groove in $d(GC)_5$ - $d(GC)_5$.

These results are related to the electrostatic potentials at the floor of the minor groove. In the presence of spermine, the minor groove floor of $d(AT)_5$ - $d(AT)_5$ is much more negative than that of $d(GC)_5$ - $d(GC)_5$; the minor groove in the former heteropolymer is more favorable for spermine binding (data not shown). Interestingly, the energies for the interactions of spermine with phosphates, sugars, and bases listed in Table II show that base interactions are significantly less in $d(GC)_5$ - $d(GC)_5$ and $d(G)_{10}$ - $d(C)_{10}$ (ca. -130 kcal/mol) compared with sequences containing adenine (-160 to -190 kcal/mol). Because spermine is forced to the 'top' of the minor groove in the sequences containing guanine, it might also be expected that phosphate interactions would be stronger in these cases. This is not generally true, however. The energies of phosphate interactions are comparable in $d(A)_{10}$ - $d(T)_{10}$ (-451 kcal/mol), $d(AT)_5$ - $d(AT)_5$ (-426 kcal/mol), and $d(GC)_5$ - $d(GC)_5$ (-438 kcal/mol), but the least favorable energies of phosphate interaction with spermine were found in $d(G)_{10}$ - $d(C)_{10}$ (-407 kcal/mol). Thus, even when spermine is positioned close to the floor of the minor groove, favorable phosphate/spermine interactions are possible.

Minor Groove Bridge. Energies for docking spermine across the minor groove are listed in Table III. Both spermine stabilization of the complexes and its interaction with DNA are energetically less favorable compared with docking across the major groove. The interaction energy, which is a measure of the fit of spermine with DNA, is 100 to 150 kcal/mol less favorable than found for the major groove docking sites. In general, this position is also less favorable than docking spermine within the minor groove. This is the result of favorable values for base interactions (< -100 kcal/mol) in the major groove and within the minor groove compared to the less favorable (> -35 kcal/mol) base

FIGURE 11. Spermine placement in the minor groove of $d(AT)_5-d(AT)_5$ on the left and $d(GC)_5-d(GC)_5$ on the right, both after energy minimization. DNA is represented by a water-accessible surface. The minor groove is on the top of each DNA molecule. The spermine interaction with $d(GC)_5-d(GC)_5$ (right) is displaced toward the top of the groove, while the spermine interaction with $d(AT)_5-d(AT)_5$ (left) is placed at the bottom of the groove.

FIGURE 12. Spermine bridging the minor groove of $d(GC)_5-d(GC)_5$ before (left) and after (right) energy minimization. DNA is marked by the backbone phosphorus. Spermine is represented by a set of dots defining the van der Waals surface. After energy minimization, spermine has become more extended and the major groove has decreased in breadth.

interaction in the minor groove bridging model. The more favorable sugar-spermine interactions in the minor groove bridging model (113–128 kcal/mol) compared with positions within the minor groove (150–165 kcal/mol) and major groove (145–160 kcal/mol) do not make up this difference. Only the homopolymer $d(G)_{10}-d(C)_{10}$ has similar stabilization and

interaction energies for the two minor groove positions. This is the result of the extremely favorable phosphate-spermine interactions where spermine bridges the minor groove (–493 kcal/mol) compared with the energies for docking within the minor groove (–407 kcal/mol). It is reasonable to expect that the less favorable interaction found in this minor groove model

should destabilize DNA only slightly; indeed, DNA destabilization energies are approximately half the values for both major and minor groove complexes described above.

The Liquori/Tsuboi minor groove model, which was proposed over 20 years ago (9,22) is shown in Figure 12 (*left*). Because spermine bridges over the minor groove (Fig. 13), it cannot interact directly with functional groups on the base pairs, and it would be expected that they would affect energies less directly than in docking modes in which direct interactions between polyamines and base pairs occur (32); the direct spermine

FIGURE 13. Spermine bridging the minor groove before energy minimization. Spermine is displayed as the van der Waal surface. Note the space beneath spermine.

interaction with base pairs (Table III) is more than 100 kcal/mol less favorable than found for the two docking modes described above. The structures of spermine, represented as van der Waals surfaces, docked to d(A)₁₀-d(T)₁₀ before energy minimization are shown in Figures 12 (*left*) and 13, respectively. The *n*-butyl group between the two secondary amines of spermine bridges the minor groove of DNA; there are two distinct angles that allow the propylamine moieties on either side of the 'bridge' to follow the phosphate backbone. A view down the minor groove (Fig. 13) makes it clear that spermine bridges over the minor groove in this configuration. There are notable differences in the structure of the complex after energy minimization (Fig 12, *right*) and these hold true in each complex examined. From the positions of the phosphates before (Fig. 12, *left*) and after (Fig. 12, *right*) energy minimization, it is apparent that the minor groove has decreased in width: the negatively charged phosphates have approached each other in the presence of the positive charge of spermine. The major groove has also increased in size somewhat (not shown) and there is a slight bend in the helical axis. The sharp angles at the secondary amines of spermine have been softened in the energy minimized model, increasing the end-to-end length of the spermine molecule.

Spermine Along the Phosphate Backbone. Results obtained by placing spermine along the phosphate backbone are listed in Table IV. This docking mode creates two groups based on DNA stabilization and interaction energies. The stabilization and interaction energies of the spermine/homopolymer complexes are favored by 80–100 kcal/mol over the spermine/heteropolymer complexes. This is related in part to an increase in DNA destabilization energies (Table IV, row 1) and intramolecular energies for spermine (Table III, row 4) in the homopolymer/spermine complexes. Compared with major groove models, spermine stabilizes the complex 130–150 kcal/mol less well; this docking mode is the least favorable interaction of all models investigated in this study.

The structure of the complex of spermine, represented as its van der Waals surface, docked at the phosphate backbone of d(A)₁₀-d(T)₁₀ before (*left*) and after (*right*) energy minimization is shown in Figure 14. Spermine is shown as the van der Waal surface immediately adjacent to the phosphate backbone. The major groove is above spermine and the minor groove is below. As discussed above, energy minimization produced at least two groups based on stabilization and interaction energies. The structure in Figure 14 (*right*) is the more energetically favorable conformation; the minor groove has decreased in size, similar to the result found in the minor groove bridging model, and spermine has approached the opposite backbone and interacts with an additional nucleic acid phosphate. In the energetically less

Table IV. Energies (kcal/mol) of the Spermine/DNA Interaction along the Phosphate Backbone*

	d(AT) ₅ -d(AT) ₅	d(A) ₁₀ -d(T) ₁₀	d(GC) ₅ -d(GC) ₅	d(G) ₁₀ -d(C) ₁₀
DNA destabilization ⁺	17	31	20	28
Spermine stabilization of complex [§]	-210	-270	-204	-268
Spermine/DNA interaction [¶]				
Total:	-309	-397	-300	-389
P:	-356	-458	-353	-461
S:	93	112	92	116
B:	-33	-36	-32	-34
Intramolecular energy of spermine	77	96	77	94

* Footnotes are the same as given in Table I.

FIGURE 14. Spermine associated with the phosphate backbone. In the model on the left spermine is displayed as the van der Waal surface associated with the phosphate backbone of d(A)₁₀-d(T)₁₀ before energy minimization, and that on the right shows the complex after energy minimization. Spermine decreases the breadth of the minor groove.

favorable conformation there are no significant alterations of the dimensions of the minor groove and no tendency of spermine to bridge across to the opposite backbone.

In the initial models of spermine bound to this position, it was apparent that changes in the dimensions of the minor groove occurred at the ends of the oligomers; because these alterations might reflect these 'end effects,' we repeated the calculations with 20 base pair oligomers and found similar alterations of minor groove dimension and spermine position at some distance from the ends. We also found that distinctions between homopolymer and heteropolymer, changing sequence, or even (in a limited way) placement of spermine did not predict the propensity of the minor groove either to maintain or to alter its dimensions in these ensembles. We believe these results simply imply the existence of multiple local energy minima for this configuration of the DNA-spermine complex. The fact that the stabilization energies for spermine placement along the phosphate backbone are among the least favorable we have found is consistent with lesser specificity of binding in this position.

General Discussion and Conclusions. Our findings of sequence-associated bending may bear upon studies of DNA coiling around a protein core. As DNA winds about a protein, the major and minor grooves will alternately face the protein. It would be expected, therefore, that DNA would bend alternately toward the major and minor grooves as each faces the protein. Analyses of the sequence of DNA complexed to histones in the nucleosome core particle (44,45) has shown a strong preferences for the major groove facing inward for the GpC sequence and for the minor

groove facing inward for the ApA sequence, which implies a propensity for collapse of the major groove for bending GpC sequences and collapse of the minor groove for bending ApA sequences. Gartenberg and Crothers (46) studied the bending of specific sequences complexed with *E. coli* catabolite activator protein (CAP) and came to similar conclusions. Although we find that d(GC)₅-d(GC)₅ bends more toward the major groove than does d(A)₁₀-d(T)₁₀ when complexed with spermine in the major groove, other sequences we investigated do not correlate perfectly with other sequences studied by others, such as ApT in nucleosomes (44,45) and CAP (46). This heteropolymer sequence appears to face the minor groove although we calculate that it is able to bend toward the major groove. Unfortunately, we cannot directly compare the theoretical and experimental results because the bends found in our sequences encompass four or more base pairs while the experimental studies examined only two or three base pairs; moreover, the propensity of DNA to bend probably depends both on specific ligand/DNA interactions and the innate tendency of a sequence to bend. For example, a model of the nucleosome deduced from X-ray diffraction (47) shows sharp bends in specific regions of the DNA helix while other regions apparently are less distorted. In any case, our data do support the concept that an intrinsic propensity of DNA to bend plays an important role in its interaction with ligand and in its tertiary structure. One other result in support of this idea is found in studies of a kinetoplast minicircle DNA segment from *Crithidia fasciculata* (48). This sequence exhibits different amounts of bending when titrated with cationic metals. An organic cation such as spermine may cause similar behavior.

Our results provide a basis for evaluating possible interactions of these physiologically important cations with the genetic material. For interactions in the major groove, bending of the helix may be an important component of the strongest interactions; sequence may influence the intrinsic flexibility of DNA and therefore the strength of ligand binding. Interactions with the minor groove seem to be a continuum in which spermine resides from the floor to the top of the minor groove, at the level of the phosphate residues, and then out into adjacent solvent. The more favorable interactions appear to narrow the minor groove, but these are clearly not as favorable as the most favorable interactions in the major groove.

We have used molecular dynamics to model the major groove interaction of d(GC)₅-d(GC)₅ and d(G)₁₀-d(C)₁₀ including counterion and water (38). The results of these calculations, which show continued association of spermine with the heteropolymer but not with the homopolymer after 40 psec of simulation, support our basic conclusion that spermine interacts most favorably with the major groove of alternating purine/pyrimidine sequences.

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