Molecular dynamics of spermine–DNA interactions: sequence specificity and DNA bending for a simple ligand

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ABSTRACT

We used molecular dynamics to model interactions between the physiologically important polyamine spermine and two B-DNA oligomers, the homopolymer $(dG)_{10}$-$(dC)_{10}$ and the heteropolymer $(dGdC)_5$-$(dGdC)_5$. Water and counterions were included in the simulation. Starting coordinates for the spermine-DNA complexes were structures obtained by molecular mechanics modeling of spermine with the two oligomers; in these models, spermine binding induced a bend in the heteropolymer but not in the homopolymer. During approximately 40 psec of molecular dynamics simulation, spermine moves away from the floor of the major groove and interacts nonspecifically with $d(G)_{10}$-$d(C)_{10}$. In contrast, a spermine-induced bend in the helix of $(dGdC)_5$-$(dGdC)_5$ is maintained throughout the simulation and spermine remains closely associated with the major groove. These results provide further evidence that the binding of spermine to nucleic acids can be sequence specific and that bending of alternating purine-pyrimidine sequences may be a physiologically important result of spermine binding.

INTRODUCTION

Polyamines are structurally simple linear aliphatic compounds found in all cells that fundamentally affect the regulation of cellular growth and other processes (1–3), possibly by binding to and altering the conformation of DNA (4–9). Depletion of polyamines in tumor cell lines affects the viscoelastic properties of DNA and the cytotoxicity of DNA crosslinking drugs (10,11), phenomena that may be related to alterations in DNA conformation (12). Several recently synthesized polyamine analogs that have growth inhibitory properties decrease the levels of physiologic polyamines and have quantitatively different effects on DNA conformation than do the parent compounds in vitro (13). The mechanisms of interaction of polyamines with DNA may be crucial for polyamine regulation of normal growth; if these mechanisms were known in some detail, it might be possible to manipulate cell growth with polyamine analogs.

Early models for the interaction of polyamines and DNA assumed that binding was the result of electrostatic attraction between the positively charged polyamines and the negatively charged phosphates (9,14,15). It was later suggested that binding was the result of nonspecific interactions between polyamines and nucleic acids (16). Recent structural evidence of spermine bound to a B-DNA dodecamer (17,18), to $tRNA^{phe}$ (19), and to Z-DNA and Z-RNA (20,21), and studies of the effect of polyamines on the H-D exchange kinetics of nucleic acids (22) as well as the binding and effects on DNA conformation of polyamine analogs (23–26) suggest that the interaction of polyamines with nucleic acids is at least in part specific.

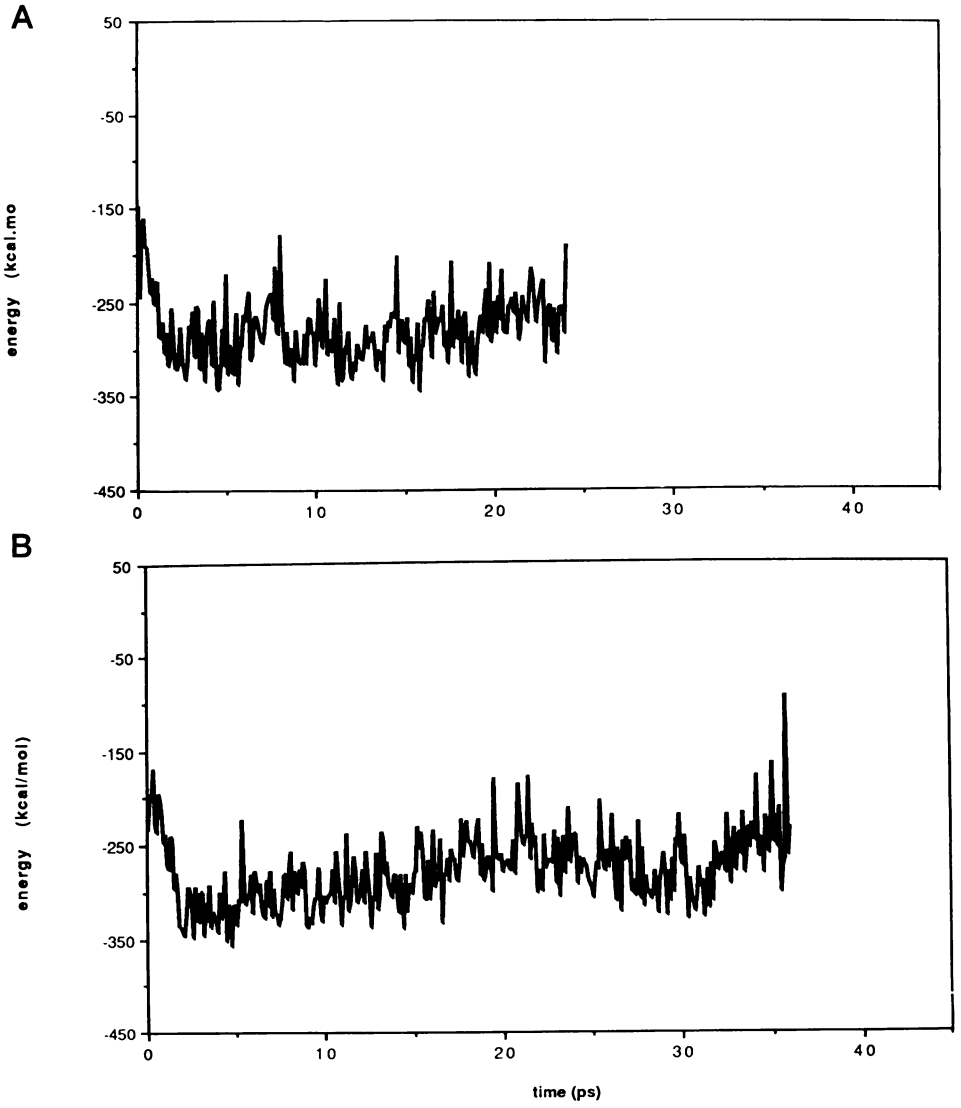

FIGURE 1. Intramolecular energy of $d(G)_{10}$-$d(C)_{10}$ plotted against time of simulation; values are plotted at 0.1 psec intervals. (A) Control simulation in the absence of spermine. (B) Simulation in the presence of spermine. Note that the values for the energies are similar; average values are -272 ± 35 kcal/mol in (A) and -282 kcal/mol in (B) after 4 psec are allowed for equilibration.

Using energy minimization methods, we modeled several possible sites for specific interactions of spermine with B-DNA oligomers and found that the energetically most favorable sites of binding are the major groove of oligomers with alternating purine/pyrimidine sequences (27,28). Binding of spermine induces a bend in the oligomer that decreases the size of the minor groove. The position of binding and the presence of

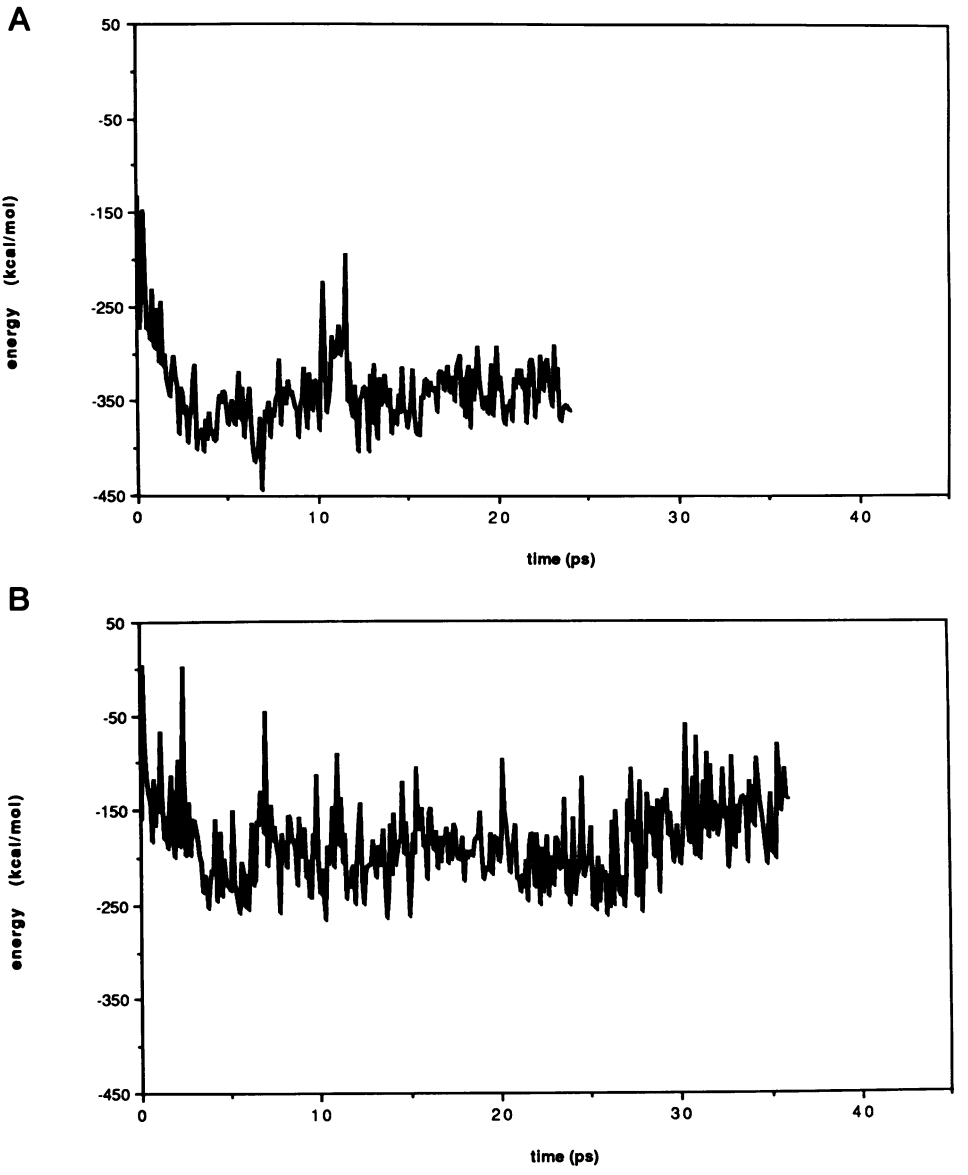

FIGURE 2. Intramolecular energy of $d(GC)_5$-$d(GC)_5$ plotted against the time of simulation. (A) Control simulation in the absence of spermine. (B) Simulation in the presence of spermine. Note the average value of energy in DNA complexed with spermine is higher than control DNA. Average values are -344 ± 31 kcal/mol in (A) and -187 ± 40 kcal/mol in (B). Note also that the heteropolymer alone is more stable than the homopolymer (Fig. 1).

the bend are supported by crystal structures for a spermine/dodecamer complex (17,18), in which spermine is bound to an alternating purine-pyrimidine sequence at the major groove, and to $tRNA^{phe}$ (19), in which the major groove size decreases from 12 Å to 8.6

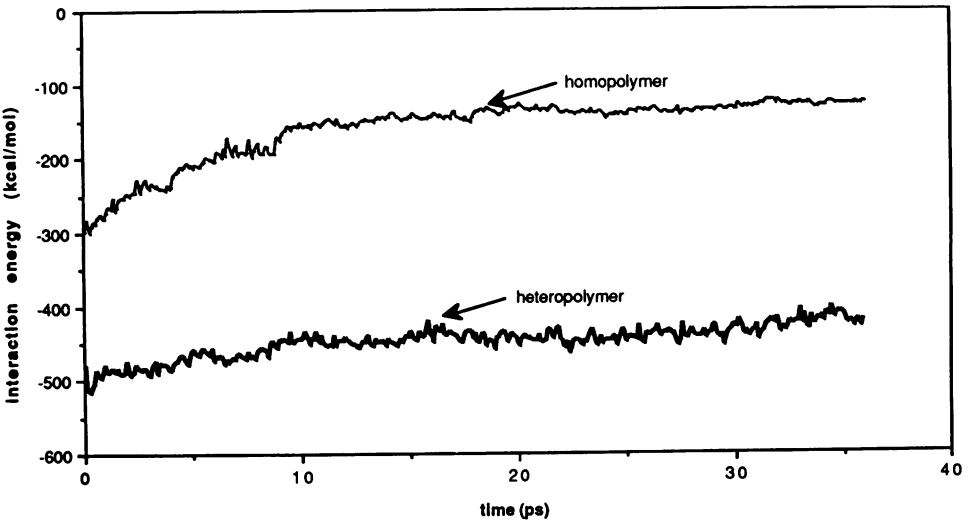

FIGURE 3. Interaction energies between $d(G)_{10}$-$d(C)_{10}$, $d(GC)_5$-$d(GC)_5$, and spermine vs. simulation time. The energy of the interaction of the homopolymer with spermine increases steadily and then plateaus at approximately 10 psec. The interaction energy between the heteropolymer and spermine, however, increases slowly to a plateau at approximately 10 psec. The lower interaction energy for the heteropolymer with spermine indicates a significantly lower interaction in the complex.

Å and where spermine binding appears to stabilize a 26° kink between anticodon and D stems. Other recently reported results for spermine-DNA interactions in solution support the idea of a spermine-induced bend in DNA (22,29).

Based on the most stable structures identified in our molecular mechanics studies (27), we have now used molecular dynamics to simulate the binding of spermine to the major groove of the heteropolymer $d(GC)_5$-$d(GC)_5$ and the homopolymer $d(G)_{10}$-$d(C)_{10}$ in the presence of individual water molecules and counterions. As controls, we simulated the dynamics of the same polymers in the absence of spermine.

METHODS

Spermine was docked into the B-DNA decamers using program MIDAS (30,31). DNAs were constructed using the coordinates of Arnott and Hukins (32) and complexes were displayed and manipulated on an Evans and Sutherland Picture System 2. Sodium counterions were placed initially on the phosphate bisector 3Å from the phosphorus atom to assure electrical neutrality. For models of DNA-spermine complexes, four counterions closest to spermine were removed to maintain neutrality.

The models were refined using energy minimization with the program AMBER (33). Constants and partial atomic charges were taken from Weiner *et al.* (34) and Singh and Kollman (35), and the partial atomic charges on spermine were calculated using the Gaussian 80-UCSF program (35). The assembly was placed into a bath of repeating cubes of TIPS3P water molecules obtained from a Monte Carlo simulation of liquid water (36). Water was then energy minimized in the presence of the DNA/cation ensemble with a dielectric constant of 1.

Molecular dynamics were run on the assemblies obtained by energy minimization at 300° K under periodic boundary conditions and a dielectric of 1. All solute-solute nonbonded interactions were calculated; other interactions were subject to an 8 Å residue-based cutoff. Calculations were performed on the Cray XMP at the San Diego Supercomputer Center. Control DNA was built and treated similarly, except that spermine was never introduced into the simulation. Four extra counterions were placed to assure electrical neutrality. The control assemblies ran 24 psec and the experimental assemblies ran 36 psec.

RESULTS AND DISCUSSION

The intramolecular energies of the DNA as a function of time in the control simulations of $d(G)_{10}$-$d(C)_{10}$ and $d(GC)_5$-$d(GC)_5$ in the presence of water and counterions only are shown in Figures 1a and 2a, respectively. (This energy is simply a measure of the stability of DNA in the assembly of polymer, water, and ions.) High frequency changes in energy, probably related to thermal activity, oscillate about a baseline; changes in the baseline may represent alternative conformations of the oligomers, although this was not analyzed extensively. In the first 4 psec of simulation, the homopolymer and the heteropolymer systems equilibrated. After equilibration, the average energy of the heteropolymer is -344 ± 31 kcal/mol and that of the homopolymer is -272 ± 35 kcal/mol. Because the number and type of atoms are the same in each system, the heteropolymer is more stable than the homopolymer, perhaps because of stacking interactions (37,38).

The intramolecular energy of the homopolymer $d(G)_{10}$-$d(C)_{10}$ complexed with spermine as a function of simulation time is shown in Figure 1b. After equilibration is achieved (near 2.5−3.0 psec), there is an apparent stabilization of the energy until 31 psec, when there appears to be a slight increase in the baseline intramolecular energy. Energy plots for the homopolymer with water and monovalent cation only (Fig. 1a) and with spermine bound (Fig. 1b) are similar, as shown by the mean energy and standard deviations (-272 ± 35 kcal and -282 ± 31 kcal, respectively).

The intramolecular energy as a function of time for a simulation of the spermine-$d(GC)_5$-$d(GC)_5$ complex, based on the bent DNA structure identified in the energy minimization studies (27), is shown in Figure 2b. Unlike the other three simulations in which the initial decrease in the energy of DNA occurs over the first 2.5−3.0 psec, there is an initial 'metastable' state near −150 kcal/mol that appears to go through two high-energy spikes (near 0 kcal/mol) at approximately 0 and 2 psec. This is followed by a slight decrease in energy, after which the energy plateaus until approximately 30 psec, where a slight increase in energy occurs. The mean intramolecular energy after 4 psec is 187 ± 40 kcal/mol, which is approximately 150 kcal/mol higher than the control and is consistent with a large and continuing effect of spermine on DNA that holds it in a high energy state. This simulation is characterized by multiple swings in intramolecular energy, often as great as 100 kcal/mole, that may be related to the presence of spermine. The standard deviations of the intramolecular energies for all simulations are similar (between ± 31 and ± 40 kcal/mol), however, which suggests that variations in DNA intramolecular energy are not related to the presence of spermine. Because the average intramolecular energy of $d(GC)_5$-$d(GC)_5$ complexed with spermine is so much higher than that of the other oligomers, however, different and higher energy conformations of DNA are more accessible to the spermine-complexed heteropolymer.

Interaction energies as a function of time for complexes of spermine and $d(G)_{10}$-$d(C)_{10}$ and $d(GC)_5$-$d(GC)_5$ are shown in Figure 3. These data are a measure of the strength of

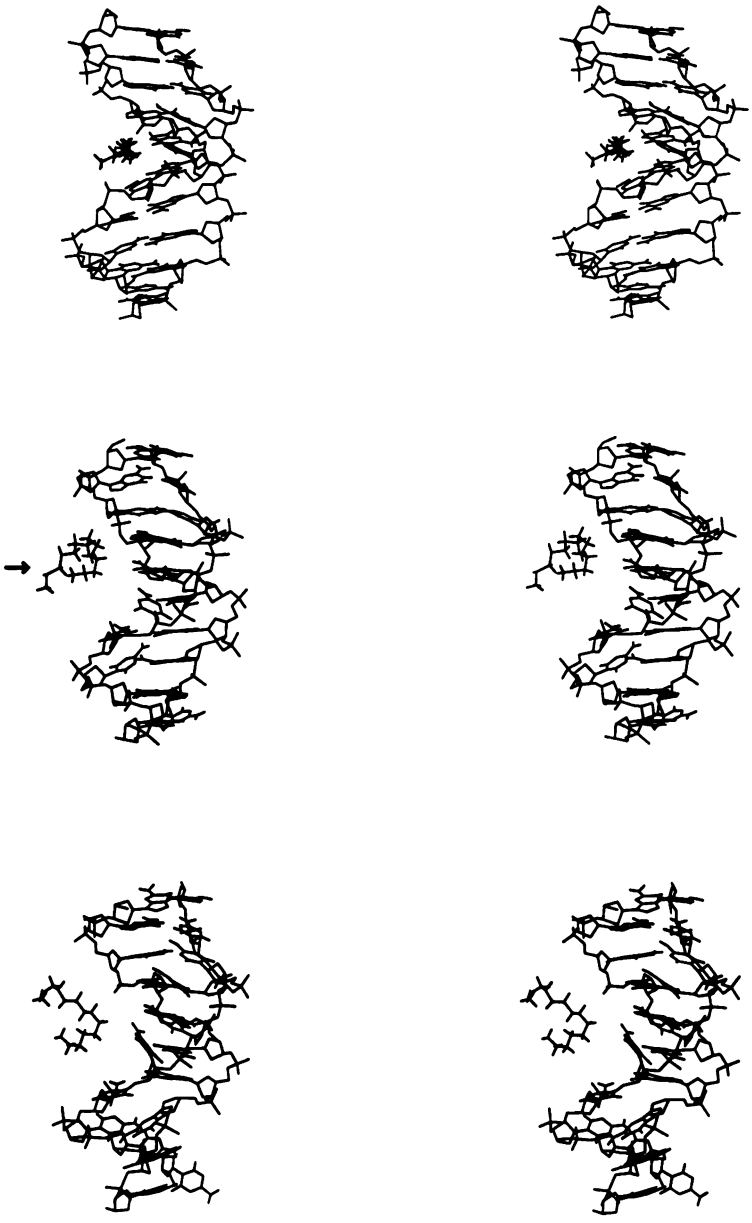

FIGURE 4. Stereoviews of the structures of the $d(G)_{10}$-$d(C)_{10}$/spermine complex before (*top*), during (*middle*), and after (*bottom*) molecular dynamics simulation. Spermine has moved away from the DNA bases. Spermine is marked by the labeled primary amino group (N2).

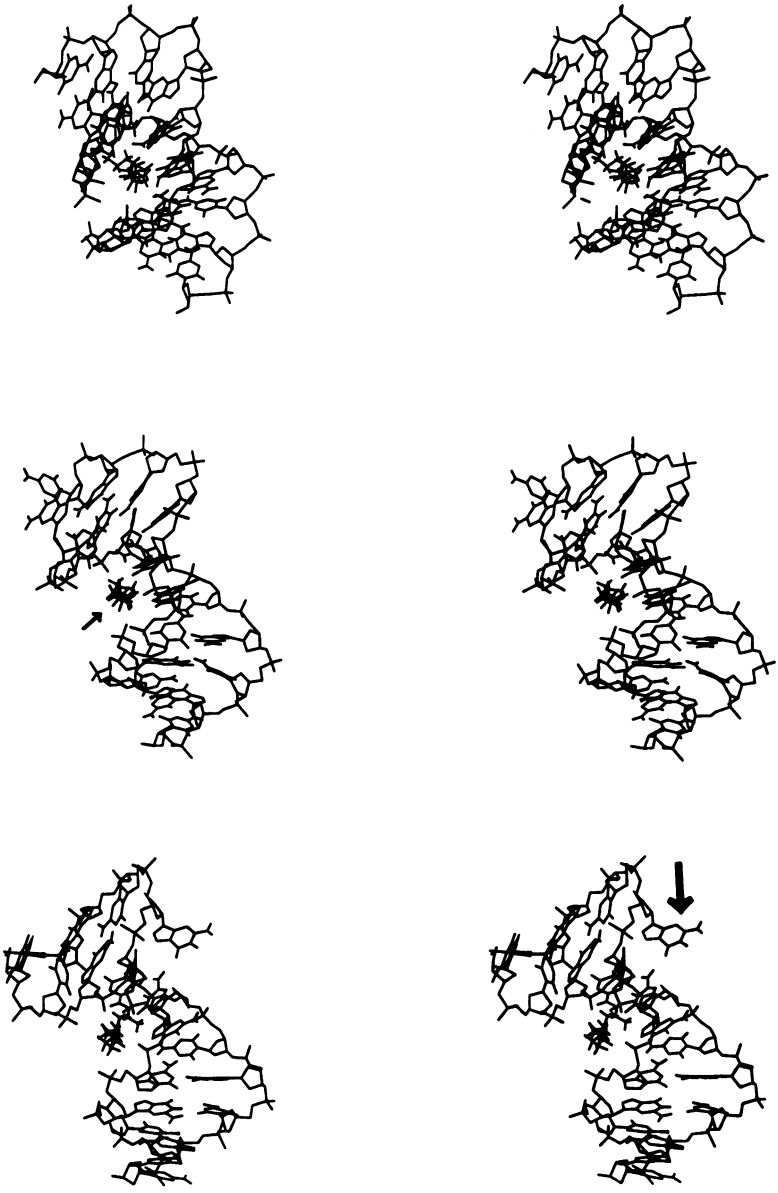

FIGURE 5. Stereoviews of the structures of the $d(GC)_5$-$d(GC)_5$/ spermine complex before (*top*), during (*middle*), and after (*bottom*) molecular dynamics simulation. Spermine remains associated with the major groove of the heteropolymer, and the bend in the helical axis is maintained. One dG (*arrow*) has rotated out of the polymer base stacking arrangement.

binding between spermine and DNA, and are not simply the stability of a single molecule; they should not be confused with the intramolecular energies of DNA described above. The initial interaction energy for spermine and $d(G)_{10}$-$d(C)_{10}$ of approximately -300 kcal/mol increases, with two short interruptions, to a plateau region that begins at approximately 10 psec. The average energy is -140 ± 8 kcal/mol, which is a weaker interaction than predicted by energy minimization (27,28) and is consistent with the negligible effects of spermine on the intramolecular energy, and thus on the conformation, of $d(G)_{10}$-$d(C)_{10}$. The interaction energy of spermine with $d(GC)_5$-$d(GC)_5$ begins with a much lower value of -500 kcal/mol that slowly increases to a plateau at approximately 10 psec, after which the mean interaction energy is -439 ± 11 kcal/mol. The large differences in the energies for spermine binding to the heteropolymer and the homopolymer suggest that the modes of binding for spermine to these oligomers are quite different.

Structures of spermine bound to $d(G)_{10}$-$d(C)_{10}$ and $d(GC)_5$-$d(GC)_5$ before (*top*), during (*middle*), and after (*bottom*) each molecular dynamics run are shown as stereoviews in Figures 4 and 5, respectively. The initial structures are the result of energy minimization studies reported elsewhere (27). At the start of the dynamics run, spermine is within the major groove of the homopolymer, but by 36 psec of simulation it has moved away from the floor of the major groove. At this distance, the surface of DNA is well solvated and any interactions with spermine are nonspecific: spermine is 'sliding' along the major groove. In the initial structure for $d(GC)_5$-$d(GC)_5$, however, the major groove envelops spermine and causes a prominent bend in the DNA. After 36 psec of simulation, the DNA is still bent, the major groove surrounds spermine, and spermine is interacting strongly with the N7 and O6 positions of the appropriate dGs. The interaction of spermine with nucleic acid is strong enough to destabilize base pairing and base stacking; in the latter instance, one dG is rotated out of the normal base stacking configuration (arrow, Fig. 5). This behavior is especially interesting because of the ability of spermine to stabilize Z-conformations of nucleic acids (13,23–26) in which purines change from anti to syn conformations by rotation about the glycosyl bond. Even though base stacking in the region of spermine binding has been disrupted somewhat, hydrogen bonding continues to hold the strands together. In addition, a phosphate/primary amino group hydrogen bond of spermine has been disrupted by a Na^+ (all not shown). It is reasonable to expect initial differences in energies and in the behavior of the complexes in these molecular dynamics calculations because simulations were run on energy-minimized models of the spermine/nucleic acid systems, and because the results of the energy minimizations were different for the homopolymer and the heteropolymer. The finding that spermine continues to be intimately associated with the heteropolymer and that the energy of the heteropolymer remains high throughout the simulation in solution strongly suggests that there are basic differences in the interactions of spermine with the two sequences. The intramolecular energy of the homopolymer shows no change attributable to the presence of spermine. The interaction energy between spermine and the homopolymer clearly increases from a stable position defined by molecular mechanics to a less stable plateau value. These quantitative measures describe the motions of the spermine and DNA quite well; spermine moves away from a homopolymeric DNA sequence not specifically receptive to binding, and interacts in a nonspecific manner. For the heteropolymer, however, the large increase in intramolecular energy (150 kcal/mol) in the presence of spermine, compared to the energy in the absence of spermine, is maintained. At the same time, an interaction energy between heteropolymer and spermine that is significantly stronger than that between the homopolymer

and spermine changes little during the simulation, which strongly suggests that there is a more favorable interaction between spermine and the heteropolymer. These differences in interaction also suggest that physiologic processes controlled in part by polyamine binding to DNA may depend both on base sequence and conformational changes in DNA.

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