A molecular mechanics study of spermine complexation to DNA: a new model for spermine–poly(dG-dC) binding

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SUMMARY

Molecular mechanics calculations of the binding of spermine to a number of solvated DNA helices have led to the development of a new model for spermine complexation. The structural details of the complexes formed with d(CGCGCGCGCGC)\textsubscript{2} and d(ATATATATAT)\textsubscript{2} decamers allowed a rationalization of the observed experimental differences for binding to these two helices. For d(ATATATATAT)\textsubscript{2} it was concluded that spermine remains in a cross-major groove binding site. Conversely, for d(CGCGCGCGCGC)\textsubscript{2} spermine reorientation via specific ligand–base-pair hydrogen-bond formation allows complexation along the major groove. The solvent plays an important role in differentiating the two binding modes. A mechanism of spermine complexation to natural DNA is postulated from these results. Past experimental data are also considered in the context of the new model.

1. INTRODUCTION

Polyamines are increasingly being recognized as having an important role in many cellular processes, including cell growth and replication (Heby & Persson 1990; Goldemberg & Algranati 1989). The detailed mechanisms by which the polyamines work are far from clear, but it is known that they bind to B-DNA and produce a conformational change (Marquet et al. 1985). One member of the class, spermine (NH\textsubscript{2}(CH\textsubscript{2})\textsubscript{3}NH\textsubscript{2}(CH\textsubscript{2})\textsubscript{4}NH\textsubscript{2}(CH\textsubscript{2})\textsubscript{3}NH\textsubscript{2} at physiological pH) induces helical bending and ultimately condensation of DNA into toroidal particles (Marquet et al. 1985). Polyamines also induce the B–Z transition and stabilize Z-DNA (Chen et al. 1984; Thomas & Messner 1986, 1988; Vertino et al. 1987). A crystal structure of spermine bound to the Z-DNA hexamer d(CGCGCG)\textsubscript{2} shows two distinct modes of spermine complexation: one of these interacting with the phosphate backbone and the other depending on spermine–base-pair interactions (Gessner et al. 1989). Another X-ray structure, of the A-DNA octamer d(GTGTACAC)\textsubscript{2}, has spermine bound deep in the major groove of the helix (Jain et al. 1989). The in vivo implications of the specific conformational stability induced by spermine complexation are as yet unclear, but it is possible that such behaviour may be a key element in fundamental processes such as gene expression (Rich et al. 1984).

The structural simplicity of polyamines is deceptive and it is likely that a myriad of specific and non-specific interactions may be involved in their complexation with DNA. Crystal data is only available for a limited number of B-DNA sequences and appears not to account for all experimental data in the literature, in particular solution data for homopolymers. In solution spermine binds more strongly to an alternating GC helix (henceforth referred to as poly(dG-dC)) than to the equivalent AT helix (poly(dA-dT)) (Stewart 1988). Marquet & Houssier (1988) provide evidence for different binding modes to the two helices from electric dichroism mean relaxation times and from ultraviolet (uv) spectra of the two complexes. They interpret their data in terms of spermine inducing a bend in the poly(dA-dT) helix, resulting in condensation of the helix at higher spermine concentration. This behaviour is similar to that observed for natural DNA (Marquet et al. 1985). In contrast, spermine complexation to poly(dG-dC) results in a stiffening of the helix (Marquet & Houssier 1988). This behaviour is, in both cases, consistent with observed changes in the hydrogen–deuterium exchange rates of the helix amino and imino protons upon spermine complexation (Basu et al. 1987). The decrease in exchange rate observed for the adenine amino protons is indicative of major groove spermine binding, whereas the increase in rate for the imino proton suggests increased solvent accessibility to this atom, consistent with a bent helix and a widened minor groove. The H–D exchange rate of the major groove (cytosine) amino protons decreases on spermine binding to poly(dG-dC), again consistent with major-groove complexation. There is, however, a negligible change in the minor groove (guanine) amino proton exchange rate, and this may be because spermine complexation does not induce a helical bend. It is, however, also possible that the wider minor groove in the free poly(dG-dC) helix already permits solvent access to these protons. Thus any opening of the
1. METHODS

(a) Introduction

The main theoretical tool used in this work is molecular mechanics, in which all the atom-atom interactions in the system are parameterized in terms of bond stretches, bond angle bends, bond torsions and non-bonded (including van der Waals, electrostatic and H-bonding) atom-atom interactions. The limited range of bonding types shown by the main group atoms enables a reasonably accurate parameterization, and hence energy determination, even for a system as large as the spermine-DNA-water complex studied in this work. An important feature of the calculations of the present work (in contrast to previous studies) is the inclusion of specific solvent molecules.

In performing these calculations we proceeded as follows. (i) From consideration of all available crystal data (in this case spermine binding to B-, A- and Z-DNAs) many possible types of binding site were postulated. (ii) We then attempted to build spermine-DNA complexes of each type with reasonable atom-atom bonded and non-bonded distances. (iii) Preliminary energy considerations were used to eliminate some structures. (iv) The geometries remaining at the end of
step (iii) were then refined using an energy minimization routine. In these calculations each atom (typically 1000 atoms) were allowed to move. The result is a structure that is lower in energy than the starting one, but usually resembles it. (v) Further calculations with slightly different starting structures were then done. Convergence of minimized structures and energies is indicative of a true minimum (local or global) having been located on the energy surface. (vi) The energies and structures resulting from energy minimizations for each possible complex were then compared to deduce a global minimum.

(b) Model building

In any molecular mechanics approach, a key element in obtaining a realistic answer is a sensible starting point for the energy refinement. In many studies this has been achieved by using X-ray coordinates. There have been several crystal structures of DNA duplexes reported with spermine present. The d(CGGCGATTCCGCG) B-DNA duplex has the spermine bound cross-groove between G2 and C3 (Drew & Dickerson 1981). In contrast, with the A-DNA octamer d(GTGTACAC) spermine binds in a down-groove manner (Jain et al. 1989). Two recent studies of the B-DNA d(CGATCG) hexamer have both spermine and drug molecules present (Frederick et al. 1990; Williams et al. 1990). The spermine adopts a variety of geometries, all of which involve binding in and across the major groove. The binding sites are clearly influenced by the presence and identity of the drug molecules. These structures are, in each case, of mixed helices, and it is unclear to what extent the data can be transferred directly to a study of homopolymers. As such they do not provide a natural starting point for energy minimization of spermine binding to poly(dG-dC) and poly(dA-dT). Despite this, however, the X-ray structures provide invaluable information regarding the general binding position of the spermine. In addition, specific spermine–helix interactions observed in the crystals provide a framework within which models for the spermine–poly(dG-dC) complex can be constructed.

All the spermine–B-DNA X-ray structures have the polyamine bound in or across the major groove. In addition, in one such structure, that of the d(CGGCGATTCCGCG)2 dodecamer (Drew & Dickerson 1981), a direct hydrogen bond occurs between a spermine NH$_2$ and a guanine base. Changes in the poly(dG-dC) (but not in the poly(dA-dT)) UV spectrum upon spermine complexation further support the existence of an interaction between spermine and the DNA base pairs for this polynucleotide in solution (Marquet & Houssier 1988). A direct hydrogen bond is less likely for poly(dA-dT) because of the centrally situated amino group in the major groove. Therefore, in constructing new models for the poly(dG-dC)–spermine complex that differ from those possible for poly(dA-dT), a guiding principle was to maximize the hydrogen bonding between the spermine and the base pairs. On the basis of the crystallographic evidence and the H–D exchange rates cited above model building was restricted to major groove complexes only.

Because the experimentally observed helical bending in the spermine–poly(dA-dT) complex is apparently due to spermine–phosphate electrostatic interactions and can be explained by the Feuerstein cross-groove complex, it seems likely that a different orientation of the spermine is required to explain the opposite effect of helix stiffening observed in the poly(dG-dC) complex. This suggests a down-groove complexation site might be appropriate, which would be consistent with spermine–base-pair hydrogen bonding. Such an orientation has not been observed with a B-DNA duplex, but this may be because none of the literature structures contains a sufficiently long run of GC base pairs. Down-groove binding is observed in spermine complexes with A-DNA (Jain et al. 1989) and Z-DNA (Gessner et al. 1989), but the different helical conformations in these cases makes it unclear as to the relevance of these observations to the present study. The difficulties associated with interpreting clearly the X-ray information for solution modelling is exemplified by the recent structure determination of Clark et al. (Clark et al. 1990). This helix crystallizes in the A conformation, but nuclear magnetic resonance (NMR) studies show it to be in the B conformation in solution. Thus the presence of a spermine bound to phosphates close to the minor groove may be a consequence of the crystallization process, rather than an indication of where it is found in solution. This is the only example we are aware of in which spermine binds closer to the minor groove than to the major groove.

Several models for the spermine–poly(dG-dC) complex were constructed within the framework described above. In building the models, the conformation of the spermine was varied, but the allowed region of torsional space was restricted to approximately trans or gauche conformations about each of the backbone bonds. The inherent flexibility of the spermine structure is such that conformations having several gauche bonds are only a few kcal mol$^{-1}$ higher in energy than the global minimum all-trans conformer (Feuerstein et al. 1986). Rotational barriers between conformers will be sufficiently high to prevent rotational isomerization during the minimization. However, by starting energy refinements using different spermine conformations, the associated multiple minima problem is circumvented to a large extent.

A model in which the central NH$_2$ units hydrogen-bond successively to guanine bases on the same DNA strand (i.e. a 1,3 complex with respect to the base pairs) was rejected because the central (CH$_2$)$_4$ unit cannot adequately span this distance. On this basis, it seems that a down-groove binding orientation with poly(dG-dC) would have to be of a general type where the spermine hydrogen-bonds to successive base pairs (i.e. successive guanines on opposite strands). This could occur in a number of ways, involving either both central NH$_2$ group or one NH$_2$ group and an NH$_3^+$ group. Several models were constructed on this basis. The sterically most promising complex was one in which the spermine binds approximately parallel to the helical axis (figure 1b), with its central NH$_2$ units making hydrogen bonds with N7 and O6 atoms on successive guanines, thus forming a seven-membered cyclic arrangement in each case (figure 2). For a single bound molecule the terminal groups are then free to interact with phosphates or more remote base pairs. This model and a Feuerstein-like cross-groove model (figure 1a) for the spermine–poly(dG-dC) complex were then compared using the procedure outlined above (steps (iv)–(vi)).

(c) Solvent

The most important difference between this work and previous theoretical studies of spermine–DNA complexation is the inclusion of solvent molecules. This is important to obtain a more realistic description than that offered by a gas-phase model. Because it seemed essential to allow the water to be free to move in the minimization routines, the amount of solvent we were able to include was limited. There is a serious problem when using fully solvated helices in energy refinement routines, as the shallowness of the overall energy surface can result in the failure of the minimization. Although we accept that the overall helix solvation will exert an indirect influence on the binding, as we required the relative stabilities of different DNA–spermine structures, solvent in
Figure 2. The proposed model of spermine binding to the poly(dG-dC) major groove. The main feature of this interaction is the efficient hydrogen bonding between spermine NH⁺ groups and guanine N7 and O6 atoms.

...the region of the binding site is the most important. After comparing several approaches, we concluded that a 4 Å radius sphere of solvation around each phosphate was adequate. This treatment places some water molecules in the major groove, but does not extend to a full representation of the DNA solvent shell (Subramanian & Beveridge 1989). About one hundred specific water molecules were included in the calculations. For this reason we view the solvent as a restraint in the minimization procedure and we do not consider water-solute interactions to be quantitatively significant. We are currently using molecular dynamics simulations to obtain a more quantitative treatment of the solvation effects. In the results reported below the exact solvent energy contribution is omitted but, as will be shown, the presence of solvent significantly influences the relative energy of the spermine-DNA complexes.

The treatment of water in this work is intermediate between full helix solvation with individual water molecules and a gas phase representation. There is some difficulty in deciding upon the type, value and even meaning of the dielectric constant. In gas-phase calculations (i.e. no actual solvent present) a simple distance-dependent dielectric function is used to evaluate groove and phosphate interactions. This is already an over-simplification, as the dielectric constant will vary differently in the two regions of the helix. Inside the grooves the conditions might be reasonably represented by a constant dielectric function. Outside the helix grooves, representation of bulk water as a continuum requires a distance-dependent dielectric. This however, still allows the unrealistically close approach of formally charged species. Water is necessary to prevent this. In our calculations, we have included water molecules that primarily shield the phosphates. Phosphate-spermine interactions can therefore be evaluated using a constant dielectric.

In the groove, use of a similar dielectric will not introduce significant errors in the spermine-DNA binding energies as noted above. The question remains as to what constant value should be adopted for the dielectric. In our calculations we used a unit value for the dielectric constant, realizing that computed binding energies will be unrealistically high. However, our interest is in relative values and we have not picked an arbitrary dielectric to scale our numbers.

(d) Computational details

The QUANTA 2.1 package was used for model building. Gas-phase calculations were done in the presence and absence of counterions using the AMBER 3.1 force field (Weiner & Kollman 1981; Weiner et al. 1984, 1986). When sodium counterions were included these were given parameters which mimic a hydration shell. The sodium parameters used (van der Waals radius = 5.0 Å, well depth = 0.1 kcal mol⁻¹ for the non-bonded parameters and charge = +1) were in accord with previous practice (Singh et al. 1985). As detailed below, it became apparent that a gas-phase representation of the complexation was inadequate and inclusion of explicit solvent molecules was necessary to mediate the spermine-helix electrostatic interaction. Helices constructed with QUANTA were therefore solvated using AMBER by adding 4 Å radius spheres of solvation around each phosphate group. Energy refinement for both gas phase and solvated helices was done by using full conjugate gradient minimization. A root mean square (r.m.s.) error in the gradient of 0.1 kcal mol⁻¹ Å⁻¹ was used as the convergence criterion. For gas-phase calculations a distance-dependent dielectric constant (of the form r⁻²) was included in evaluation of the electrostatic interaction to simulate solvent screening. A dielectric constant of unity was used for the solvent-mediated calculations. Standard AMBER charges and potentials were used for the helices. Point charges for the...
Table 1. Computed gas-phase binding energies (kcal mol\(^{-1}\)) for spermine–DNA complexes

<table>
<thead>
<tr>
<th>complex</th>
<th>(\Delta E_{\text{DNA}})</th>
<th>(\Delta E_{\text{sper}})</th>
<th>(E_{\text{sper–DNA}})</th>
<th>(E_{\text{bind}})</th>
<th>(E_{\text{sper–DNA/Na}^+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT (cg)</td>
<td>34.4</td>
<td>14.2</td>
<td>-354.1</td>
<td>-305.5</td>
<td>-305.9</td>
</tr>
<tr>
<td>AT (dg)</td>
<td>18.4</td>
<td>17.4</td>
<td>-294.7</td>
<td>-258.9</td>
<td>-227.6</td>
</tr>
<tr>
<td>GC (cg)</td>
<td>32.5</td>
<td>9.9</td>
<td>-392.6</td>
<td>-350.2</td>
<td>-330.4</td>
</tr>
<tr>
<td>GC (dg)</td>
<td>31.8</td>
<td>12.8</td>
<td>-361.4</td>
<td>-316.8</td>
<td>-294.7</td>
</tr>
</tbody>
</table>

\(a\) (cg) refers to the cross-groove model, (dg) to the down-groove model.
\(b\) The conformational energy change of the helix upon complexation.
\(c\) The conformational energy change of spermine upon complexation.
\(d\) The spermine–helix interaction energy.
\(e\) The binding energy, a sum of \(\Delta E_{\text{DNA}}\), \(\Delta E_{\text{sper}}\) and \(E_{\text{sper–DNA}}\).
\(f\) The spermine–helix interaction energy in the presence of sodium counterions (see text).

Spermine tetracations were evaluated by fitting to the electrostatic potential of the AM1-derived wave function (Ferencycz et al. 1990). This gave charges (in milli-electrons) of 40 (N1 = N14), 258 (H1), -99 (C2), 132 (H2), -121 (C3), 96 (H3), -130 (C4), 142 (H4), 77 (N5 = N10), 255 (H5), -158 (G6), 144 (H6), -102 (G7), 91 (H7), where N1 is a protonated primary amine.

Full calculations were done on two major groove binding modes, a cross-groove complex and a down-groove complex, as illustrated in figure 1a, b respectively. For each model several different starting points were used in an attempt to locate a locally global minimum. Clearly these calculations are beset by the multiple minima problem but we have some confidence that, by considering a series of starting spermine positions, a reasonable global minimum is located for each complex. All reported results are the lowest energy complexes found in each case. For cross-groove complexes, the spermine–helix starting separation was varied, but no specific hydrogen bonds were included prior to minimization. For down-groove complexes an attempt was made to maximize the spermine–base-pair hydrogen bonding in the starting structure. Additional calculations were done for a more general down-groove complexation in which no specific hydrogen bonds were included before the energy refinement.

The helices used were all decamers in the B-DNA conformation (as constructed by using the qanta package). Spermine was positioned in a central binding site for both models. Thus for the spermine–d(GCGCGCGCGC)\(_2\) complex (helix nomenclature 5'G1C2...G9C10, 5'G1C12...G19C20), the cross-groove model had a spermine molecule spanning the major groove between the G5+C6 and C6+G15 base pairs. Similarly for the down-groove model, hydrogen bonds were formed between the ligand's protonated secondary amine groups and the G5 and G15 bases (figure 2). Complexation of spermine to three mixed helices was also considered; d(GCGGCGCGCG), d(GCGGCGAAC), d(GC ATGGCGGC), d(GCGGCGATGC) and d(GCGTGTGC GC), d(GCGCGACGCG). These are referred to in the following discussion as GC(4T), GC(3A4T) and GC(4T6T) respectively, reflecting the positions of substitution in the first strand.

3. RESULTS

Molecular mechanics binding energies (\(E_{\text{bind}}\)) for spermine complexes with d(GCGCGCGCGC)\(_2\) and d(ATATATATAT)\(_2\) (henceforth referred to as GC and AT respectively) are given in table 1. The numbers refer to gas-phase calculations and comprise three terms. \(\Delta E_{\text{sper}}\) gives the conformational energy change of the spermine upon complexation, as determined by the difference between the internal energy of complexed spermine and that evaluated for a free spermine molecule. The lowest energy conformation of the free spermine tetracation is all-trans, as expected on steric grounds. \(\Delta E_{\text{DNA}}\) is the conformational energy change of the helix upon complexation. \(E_{\text{sper–DNA}}\) gives the interaction energy of the spermine with the helix (in the absence of counterions). Gas-phase calculations were also done in the presence of counterions and the final column of table 1 shows the interaction energy term (\(E_{\text{sper–DNA/Na}^+}\)) including counterion effects. The general screening effect exerted by the counterions, first noted by Zakrzewska & Pullman (1986), is shown by the less negative interaction energies obtained for all the complexes. This effect seems to be similar for each complex, i.e the counterions do not appear to influence the spermine complexation such that one binding mode is significantly favoured. We agree with Zakrzewska & Pullman that counterion effects may have a role to play in spermine binding specificity but, again in accord with their conclusions, we do not feel that current representations of counterions probe such behaviour subtly enough to allow further comment. All further discussion refers to systems in which counterions are excluded, as generally they exert a similar effect for each binding site.
The binding of spermine to DNA

Table 2. Computed solvent-mediated binding energies (kcal mol\(^{-1}\)) for spermine-DNA complexes

(The definition of energy terms as for table 1. Details of the helix nomenclature are given in the text.)

<table>
<thead>
<tr>
<th>complex</th>
<th>(\Delta E_{\text{DNA}})</th>
<th>(\Delta E_{\text{spec}})</th>
<th>(E_{\text{spec}}-\Delta E_{\text{DNA}})</th>
<th>(E_{\text{bind}})</th>
<th>(\Delta E_{\text{DNA-water}}^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT (cg)</td>
<td>-63.2</td>
<td>1.1</td>
<td>-149.8</td>
<td>-211.9</td>
<td>111.6</td>
</tr>
<tr>
<td>AT (dg)</td>
<td>-59.0</td>
<td>7.3</td>
<td>-221.4</td>
<td>-273.1</td>
<td>68.1</td>
</tr>
<tr>
<td>GC (cg)</td>
<td>-24.1</td>
<td>7.5</td>
<td>-321.4</td>
<td>-351.1</td>
<td>186.9</td>
</tr>
<tr>
<td>GC (dg)</td>
<td>-2.0</td>
<td>15.9</td>
<td>-365.4</td>
<td>-351.1</td>
<td>186.9</td>
</tr>
<tr>
<td>GC (dg-a)(^b)</td>
<td>-12.7</td>
<td>13.6</td>
<td>-283.6</td>
<td>-282.7</td>
<td>—</td>
</tr>
<tr>
<td>GC(4T) (dg)</td>
<td>36.6</td>
<td>17.1</td>
<td>-288.9</td>
<td>-235.2</td>
<td>—</td>
</tr>
<tr>
<td>GC(3A4T) (dg)</td>
<td>2.3</td>
<td>16.9</td>
<td>-244.5</td>
<td>-225.3</td>
<td>—</td>
</tr>
<tr>
<td>GC(4T6T) (dg)</td>
<td>15.9</td>
<td>11.5</td>
<td>-246.1</td>
<td>-218.7</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\) The behaviour of the AT and GC helices in the solvent-mediated calculations is of interest. The negative \(\Delta E_{\text{DNA}}\) component suggests that the internal energy of the helix has been lowered (relative to the energy of the free helix) by the spermine complexation. As detailed in the text, we do not consider the solvent representation to be adequate enough to quote quantitative solvent–solvent interactions. We do include, however, the change in solvation energy for the AT and GC helices upon spermine complexation (the final column). These numbers can only be considered qualitatively, but it is interesting that the spermine complexation appears to decrease the helix-solvent interaction energy (i.e. this term is less negative for the complexed helix, hence the positive value of \(\Delta E_{\text{DNA-water}}\)).

\(^b\) The GC down-groove complex initially constructed without inclusion of specific hydrogen bonds (see text).

will in practice be significantly lower than that predicted by gas-phase calculations, owing to solvent screening. The absolute magnitude of the computed binding energies is much higher than typical experimental values. As discussed above, this is related to the choice of dielectric constant, which in this work has been set at unity. Because for all complexes the electrostatic component dominates the interaction energy, a simple scaling by the inverse of the dielectric constant is all that is necessary to obtain more realistic values.

The fact that the electrostatic component of the interaction energy is dominant for both cross-groove and down-groove binding disguises the significant differences between these two complexation modes. This is primarily because in amber most of the energy derived from hydrogen bonding is included in the electrostatic term. A more revealing comparison is that between the ligand interaction energies with the base pairs and the backbone. For the cross-groove model interaction with the base pairs provides only a small amount of the total, about one sixth, whereas for the down-groove model with GC it provides one third of the total.

The cross-groove model is favoured for both AT and GC which, as discussed earlier, does not seem to satisfactorily account for the experimentally observed differences. However, the presence of water molecules will affect the relative binding energies. We have attempted to simulate such behaviour by including specific water molecules in the calculation (as 4 Å radius spheres of solvation centred on each phosphate group). Computed binding energies for GC and AT spermine complexes using solvated helices are given in table 2. The component energy terms are similar to those in table 1 and were determined in the same way. Free helix energies for calculation of \(\Delta E_{\text{DNA}}\) were obtained using minimized, solvated helices. The immediate conclusion to be drawn from the binding energies is that, for GC, the down-groove binding site is now considerably favoured over the cross-groove site. To a lesser extent, however, this is also true for the AT helix, which is apparently not in agreement with the experimental data discussed above. There are two possible explanations for this. First, the AT down-groove complex may be intrinsically more stable than the cross-groove complex, as predicted, but the down-groove complexation is kinetically unfavourable; an argument we return to below. A second possibility is that the balance of solvent screening in the simulation may be weighted so that this effect is over- emphasised for the spermine-phosphate interaction, relative to the spermine–base-pair interaction. Hence the magnitude of the interaction energy for the spermine–phosphate coulombic interaction, which is overestimated by gas-phase studies, may have been underestimated relative to the spermine–base-pair coulombic interaction in the solvated study. Further screening of this latter interaction would reduce the binding strength for the down-groove complex and could make this binding mode unfavourable for AT. For a similar additional screening of the spermine–base-pair interaction in the GC complex, the down-groove model would still be favourable (as the current data suggests the cross-groove/down-groove energy difference to be much larger for GC than for AT).

4. DISCUSSION

(a) A possible mechanism for spermine complexation

The data in table 2 suggest that it is energetically and stereochemically possible for the spermine in the down-groove geometry to bridge two guanine bases in opposite strands of poly(dG-dC). This complex is considerably more stable than the cross-groove complex and provides a possible explanation for the helix stiffening observed experimentally. In themselves, however, the energies do not provide a picture of the spermine complexation mechanism, nor explain why
there are differences between poly(dA-dT) and poly(dG-dC). Examination of structural details of the complexes is more revealing and allows us to suggest a possible cooperation mechanism that accounts for the poly(dA-dT) versus poly(dG-dC) differences. This in turn also provides a possible explanation of some experimentally observed behaviour of spermine with natural DNA.

We begin by examining the cross-groove spermine complexes of AT and GC. The minimized solvent-mediated complexes have significant structural differences that are similar to those in the gas-phase complexes of Feuerstein et al. (1986). The spermine spans the AT groove in a broadly symmetrical manner, remaining close to the all trans conformation (cf. the small value for $\Delta E_{\text{sper}}$ obtained for this complex). Spermine–helix interaction is almost exclusively via solvent-mediated spermine–phosphate hydrogen bonds (with water bridges) with the magnitude of the spermine–phosphate interaction being similar for both strands of the helix. No spermine–base-pair hydrogen bonds are established. In contrast, in the GC complex the groove-spanning spermine molecule has a significant kink at the right-hand NH$_2$ group, as shown in figure 1a. This arises because the NH$_2$ function forms a strong hydrogen bond with G15(N7) and spermine binding becomes a balance between ligand–base-pair and ligand–phosphate interactions. The asymmetry in the solvated GC cross-groove complex also occurs in the Feuerstein gas-phase model, in which there is a clear imbalance in the spermine–phosphate interactions calculated for the two phosphate backbones.

The secondary amine–guanine N7 hydrogen bond is present in the Feuerstein model, but is formed by both the secondary amine groups; in one case to (using our nomenclature) G5(N7) and in the other to G15(N7). In the Feuerstein AT complex there is also significant spermine–base-pair interaction (dispersed over the neighbouring base pairs and possibly including hydrophobic interactions). Inclusion of solvent before energy refinement forces the spermine to adopt a position more distant from the helix and reduces the spermine–base-pair interaction. One hydrogen bond still forms in the cross-groove complex, however, (it should be emphasized that the starting points for the minimizations did not in any way impose this hydrogen bond upon the complex) and this is an essential feature in the following discussion. It should be noted that the significant helical bend induced by the spermine in the gas-phase calculations (Feuerstein et al. 1986) does not occur to any great extent in the solvent-mediated minimizations. The spermine–helix intermolecular separation must be influential in this behaviour and we suggest that large helical bending requires the cumulative effect of several cross-groove bound spermine molecules.

It seems reasonable to assume that, whatever the final structure of the complex, the initial spermine–helix interaction (for any base-pair sequence) must be due to the coulombic attraction between the spermine tetracation and the phosphate backbone. Furthermore, it is likely that this results in a complex that is satisfactorily represented by the cross-groove model. For GC this can occur with formation of a spermine–base-pair hydrogen bond whereas for AT such an interaction does not occur. We carried out energy refinement on a general GC down-groove complex (denoted by (dg-a) in table 2) in which spermine was positioned in the major groove with no attempt to form hydrogen bonds prior to minimization. Interestingly, the hydrogen bond (NH$_2$-G15(N7)) found in the GC cross-groove complex also forms in this down-groove model. It is possible that these static structures illustrate a dynamic series of events in which the spermine initially binds cross-groove to the GC helix electrostatically and then forms a hydrogen bond with (G15(N7)) about which a pivoting motion allows it to move into the major groove. At this point further rearrangement (permissible because of the flexibility of the ligand) allows formation of a more stable, groove-bound complex represented by the postulated down-groove model. Such behaviour cannot occur for AT because the initial hydrogen bond is not formed, as although the adenine N7 atom is available for hydrogen bond formation the proximity of the 6-NH$_2$ blocks a close approach of the spermine via electrostatic repulsion. The guanine 6-carbonyl oxygen will have the opposite effect. Thus, even if the AT down-groove complex is more stable than the cross-groove complex, as predicted by the computed binding energies, it will be kinetically unlikely to form.

The complexation sequence accounts for the experimentally observed behaviour of natural DNA. Initial addition of spermine to calf thymus DNA causes helix stiffening (Marquet et al. 1985), but increasing the spermine concentration induces helical bending with, at high concentration, toroidal particle formation. The stronger binding for the GC cross-groove complex relative to the equivalent AT complex suggests that a proportionally higher number of spermine molecules will bind to GC-rich regions of DNA at low spermine:base-pair ratio ($r_{\text{sh}}$). Calculations on mixed helices (table 2) suggest what might be expected for natural DNA. The down-groove complexes for mixed helices have significantly less negative $E_{\text{sper}}$–DNA interaction energy terms than the equivalent term for the GC down-groove complex. (It is probably more instructive to compare interaction energies, rather than binding energies, as there are difficulties associated with the internal helical energy component $\Delta E_{\text{dna}}$ – see footnote to table 2.) The data in table 2 suggest that an AT base pair next to the central G5-C16,C6-G15 binding site (as in GC(4T), which has a T4-A17 pair) might tolerate a down-groove complexation but further G-C to A-T substitutions probably preclude down-groove binding. Thus, assuming the cross-groove model to be transient relative to the down-groove complexation, it seems likely that at low $r_{\text{sh}}$ there will be a tendency for the majority of bound spermine molecules to occupy down-groove binding sites, resulting in a stiffening of the helix. As the spermine concentration increases the cross-groove complexation will predominate (because there are more potential sites) and the AT helix-type behaviour, i.e. helical bending, will occur. The postulated model has interesting implications for the in vivo role of spermine.
spermine, as the degree of helical stiffening and bending could be subtly controlled by small changes in the spermine concentration (or in the concentration of other ions because this significantly changes the association constant for the spermine complexation). This behaviour would also be dependent on the GC:AT ratio and specific base sequence of the cellular DNA.

(b) Re-interpretation of experimental data for the spermine-poly(dG-dC) complex

We finally consider some previously published experimental data and show how some of this may be re-interpreted in the light of the new model for the spermine-GC binding. The data available are from dichroism experiments and UV spectra (Marquet & Houssier 1988) and equilibrium binding data in the form of a Scatchard plot (Winkle & Crooks 1988). We consider each of these in turn.

The main result from the dichroism (bipolar pulse) experiments is that the poly(dG-dC)-spermine complex has no net permanent dipole perpendicular to the helical axis. This was previously assumed to imply a symmetric arrangement of asymmetric binding sites (Marquet & Houssier 1988) but may also be reasonably explained (Fredericq & Houssier 1973) by spermine binding parallel to the helical axis, as in the down-groove complex. Such a ligand geometry would have no permanent dipole perpendicular to the helical axis and so would not be detected by the dichroism experiment. UV studies (Marquet & Houssier 1988) of spermine binding to poly(dG-dC) as a function of binding ratio show two points at which the wavelength maximum and peak amplitudes change abruptly: at approximate rsb of 0.2 and 0.4. This is in contrast to spermine-poly(dA-dT) binding, for which no significant changes are observed. Because the down-groove model requires a run of about five base pairs per spermine molecule (rsb = 0.2), this suggests that this binding mode prevails until all available sites are occupied. At this point a second binding interaction, possibly resembling the cross-groove model, commences and is characterized by a change in the UV spectrum. In contrast to spermine bound externally (cross-groove) to poly(dA-dT), these externally bound molecules cannot cause a bending of the helix since the internal (down-groove) spermines prevent it, thus accounting for the different helix flexibility observed for the two polynucleotides in the presence of spermine. The explanation of spermine-induced GC and AT helical flexibility changes is analogous to the behaviour of spermine with natural DNA, as discussed above. The main difference is in the number of potential groove-bound (internal) complexation sites. For natural DNA only a small proportion of the helix can accept a groove-bound spermine and hence the cross-groove complexation overwhelms the effect of the internally bound molecules and helical bending is induced. For poly(dG-dC) the internal to external complexation ratio favours the internal binding (as every site in the major groove is a potential down-groove binding site) and the resultant behaviour is helical stiffening. For poly(dA-dT) little or no down-groove binding occurs and helix bending is induced almost immediately spermine is added.

There is further evidence to support the two distinct binding modes of spermine to poly(dG-dC) in the Scatchard plot of Winkle & Crooks (1988). The original interpretation of the results is a quadratic function (broken line, figure 3) fitted to the data and it was concluded that this implied some form of cooperative binding. The plot, however, is not in accord with the form expected for a single cooperative binding mode (McGhee & von Hippel 1974, 1976). It is, however, precisely what would be expected for an initially positively cooperative complexation (i.e. binding to a site neighbouring a complexed molecule is favoured over binding to an isolated site) followed by an approximately non-cooperative binding mode. Thus the solid line fit (figure 3) is a re-interpretation of the data in which the plot is viewed as two distinct Scatchard plots representing internal down-groove complexation (rsb < 0.02) and a second, externally bound mode that commences at rsb = 0.2, in accord with the UV data. For the internal binding, using the Winkle & Crooks binding constant of K_int = 2200, a computational fit, based on the modification of McGhee & von Hippel, gives values for the internal binding site length, n_int, of four to five base pairs and the cooperativity factor, α, of 50–60. The curve shown has parameters K_int = 2200, n_int = 5 and α = 60, the ω value indicating positive cooperativity in the spermine-poly(dG-dC) internal binding mode. The second binding mode gives an approximately linear plot from which values of K_ext and 1/n_ext may be obtained by extrapolation from the intercepts on the line rsb = 0.2 (as this is approximately the ratio at which the second
binding mode commences) and the $r_{sb}/L$ axis respectively. $K_{ext}$ thus has a value of about 27000 and (since the external binding mode commences at $r_{sb} = 0.2$) $1/n_{ext} = 0.7–0.2 = 0.5$, giving $n_{ext} = 2$.

However, precipitation of poly(dG-dC) begins at about $r_{sb} = 0.4$ (Marquet & Houssier 1988), so the theoretical maximum binding ratio for externally bound spermine molecules (one per two base pairs) is not actually attained. Assuming a saturation level of internal binding at $r_{sb} = 0.2$, we estimate one externally bound spermine for every four to five base pairs at the point of precipitation.

The exact nature of the second (external) binding mode with poly(dG-dC) cannot be predicted from the data, but it seems likely that the spermine is electrostatically bound to the phosphate backbone. The geometry may resemble the cross-groove complex, but calculations designed to probe this have thus far proved inconclusive. If, however, this is the case, there is a striking resemblance between the overall model for the B-DNA spermine–poly(dG-dC) complex with the X-ray structure reported for the same complex having the helix in the Z-conformation (Gessner et al. 1989). Detailed discussion of this point does not seem appropriate here, but it is interesting to note that spermine has a significant effect on the ability of GC helices to undergo the B–Z transition (Thomas & Messner 1988).

5. CONCLUSION

In this study we have examined the differences between spermine complexation to poly(dG-dC) and poly(dA-dT). These differences are clearly observed experimentally and it seems likely that they are the result of different complexation geometries with the two helices. The Feuerstein cross-groove model is a good representation of spermine–phosphate complexation and, similarly, we believe the down-groove model developed in this work to be representative of this type of complexation. The flexibility of the spermine tetracation allows it to occupy a wide variety of (related) binding sites and all a theoretical model can do is to show the main features of such complexation.

For spermine–poly(dG-dC) these are the formation of direct spermine–base-pair hydrogen bonds and binding that occurs deep in the major groove.

Several interesting questions arise from the calculations and data re-interpretation. The origin of the cooperativity in the spermine–poly(dG-dC) complexation is unclear. The specific behaviour of spermine with GC-rich helices may have important in vivo implications and this requires further study. Finally, counterion and solvation effects have only been dealt with approximately in this study, but their effect cannot be completely ignored. Inclusion of solvent proved to be critical in obtaining a good description of the complexation, owing to mediation of the spermine–helix electrostatic interaction. Molecular dynamics simulations currently in progress probe these effects in a more quantitative manner and should give a more complete picture of the spermine binding.

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