Molecular Mechanics Simulations on Covalent Complexes between Anthramycin and B DNA

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We present molecular mechanics simulation of the covalent interactions of the potent antitumor antibiotic belonging to the pyrrolo[1,4]benzodiazepine class, anthramycin, with six deoxydecanucleotides, d(GCGCGCGCGC)$_2$, d(G$_{10}$)·d(C$_{10}$), d(GCGCGTGCGC)·d(GCGCACGCGC), d(GCGCGAGCGC)·d(GCGCTCGCGC), d(GGGGGAGGGG)·d(CCCCTCCCCC), and d(GGGGGTGGGG)·d(CCCCACCCCC), in their minor grooves. The complexes are characterized by both a network of hydrogen bonds between the drug and the polynucleotide and good packing interactions. The DNA double helix in these complexes shows very minimal distortion, and interactions of the drug with the decanucleotides seem to be not very sensitive to the sequence variation around the site of complex formation. The conformational features in the complexes obtained are generally consistent with the experimentally derived conclusions by recent NMR and 2-D NOE studies.

Anthramycin is a potent antitumor antibiotic and belongs to the pyrrolo[1,4]benzodiazepine class derived from *Streptomyces refuineus*.[1] The biological effectiveness of this drug has been proposed to result from its inhibition of nucleic acid synthesis through its covalent attachment to DNA.[2-6] Several investigations have demonstrated the formation of a well-defined thermally labile covalent adduct with DNA.[7-9] The drug is known to bind in the minor groove of the polynucleotides covering a 3-base-pair region,[5,6,10,11] reacting specifically with DNA containing guanine.[2-5] It does not react with mononucleotides, polynucleotides, and RNA not containing guanines. The complex has unusually high stability and survives conditions that disassociate the DNA complexes of most other antibiotics.[12] Anthramycin-modified DNA is inactivated as a template for RNA and DNA polymerase reactions and as a substrate for nuclease reactions.[13,14]

Earlier hydrolysis studies on the reaction of DNA with anthramycin had suggested C-11 on the drug to be a possible site of binding to DNA.[12] Later studies by Ostrander et al.[9] have confirmed the points of covalent attachment to be N2 of guanine and C-11 of anthramycin. On the basis of these observations, a CPK model of anthramycin–DNA adduct was proposed[8] and in such a model the drug was shown to lie completely within the minor groove of the polynucleotide. Recently, several structural features of the adduct have been studied by ^{1}H and ^{13}C NMR spectroscopies.[15] The assignments of carbon and proton resonances have been shown to be consistent with the above-mentioned points of covalent attachment on the drug and the DNA. These studies also predicted loss of helical symmetry upon the covalent binding. The biological consequences of the damage due to anthramycin–DNA interactions on repair-proficient and -deficient xeroderma pigmentosum cells have also been recently examined.[16,17] In such studies, the drug was shown to produce persistent excision-dependent single- and double-strand breaks. These observations were shown to be consistent with the earlier proposed CPK models[8] for the DNA–anthramycin interactions.

The structure of anthramycin has been elucidated by crystallographic analysis, and the drug is shown to have a right-handed twist relative to its long axis.[18] To date, however, no crystallographic analysis of an adduct between anthramycin and DNA fragment has been reported. Though model-building studies on the DNA–anthramycin adducts have been carried out, the details of energetics of interactions between the drug and the polynucleotide have not been reported in the literature. In the present investigations, we endeavor to do so by using molecular mechanical methods on models built by the methods of computer graphics.

While this study was in progress, independent investigations on the modeling of anthramycin–DNA interactions were carried out by Remers et al. (personal communication). In that study, binding of anthramycin and related pyrrolo[1,4]benzodiazepine antibiotics to a double-helical deoxyhexanucleotide, d(ATGCAT)$_2$, has been examined, utilizing the united atom force field parameters[19] for the drug moieties. It may be noted that in the present study we have treated the drug on an all atom level.

Anthramycin is known to readily interconvert with 11-epianthramycin,[12] and therefore we have considered both the configurations in our analysis of the complexes. Further, we also seek to examine the sensitivity of DNA–anthramycin interactions to sequence variation around the

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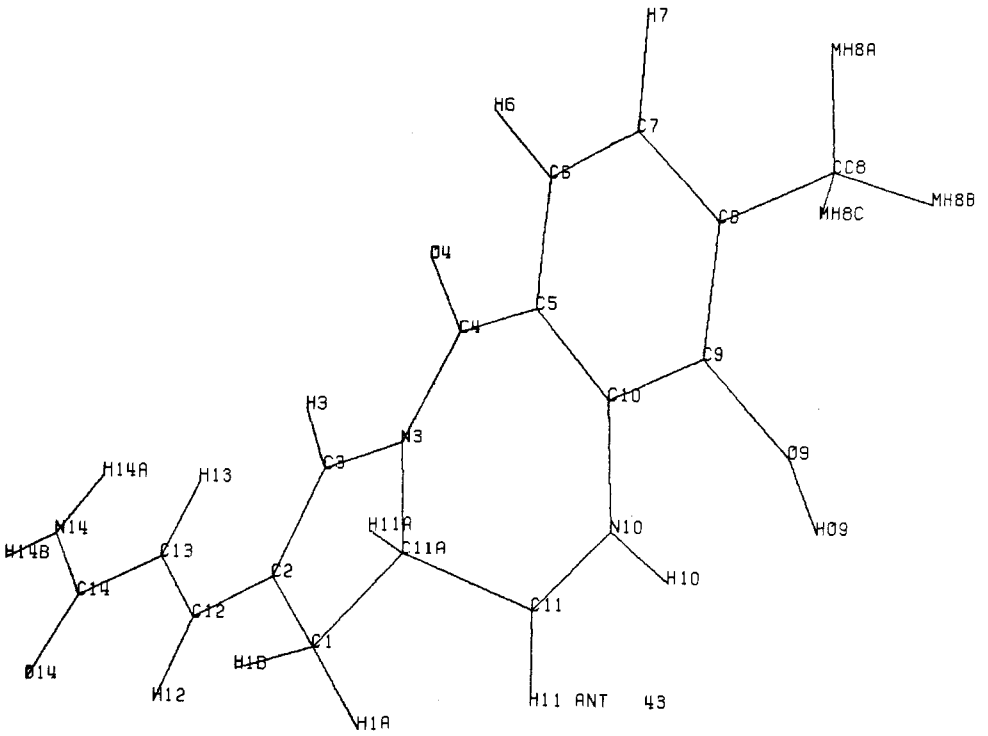

Figure 1. Schematic representation of anthramycin used in the covalent complexes with the decanucleotides. The dashed line indicates the points of covalent attachment on the drug.

site of covalent complex formation. This is particularly relevant in view of the fact that the reactivity of this drug is sensitive to the sequence-specific microheterogeneity in DNA.[8,20,21] We point out that while our investigations were in progress, Hurley and co-workers reported footprinting analysis of DNA binding sites for anthramycin and related drugs.[22] These studies have suggested sensitivity of the drug–DNA interactions to the nature of the bases on either side of the covalently alkylated guanine.

We have studied models of complexes between anthramycin (also referred to as AM) and two deoxydecanucleotides [d(GCGCGCGCGC)$_2$ and d(G$_{10}$)·d(C$_{10}$)]. In view of the interactions between the drug and the decamers (as will be discussed below), the base at the 3′-end of guanine, which is covalently linked to the drug, was changed to adenine and thymine with corresponding Watson–Crick partner on the complimentary strand in both the decamers. Thus, four additional decanucleotides, d(GCGCGTGCGC)·d(GCGCACGCGC), d(GCGCGAGCGC)·d(GCGCTCGCGC), d(GGGGGAGGGG)·d(CCCCTCCCCC), and d(GGGGGTGGGG)·d(CCCCACCCCC), were complexed with anthramycin. In order to understand the long-range effects of the complexation, we have bound the drugs in the central portion of the sequence, as has been described below. As also will be discussed in a later section, the salient conformational features of our models are consistent with some of the recently obtained 2-D NMR data on anthramycin complexes with the hexanucleotide mentioned above.

Methods

The conformational analyses in the present investigations were carried out by the methods of molecular mechanics, wherein energy calculations were performed with the program AMBER-UCSF (assisted model building with energy refinement).[23,24] We have employed the force field parameters presented by Weiner et al.[19] United atom force field parameters were used for the polynucleotide part of the drug–DNA complexes, while the all-atom force field parameters were used for the drug. The molecular mechanical energies were evaluated by eq 1 in ref 19, and the structures were refined until the rms gradient was less than 0.1 kcal/mol A. In all the calculations, we have used a distance-dependent dielectric constant $\epsilon = R_{ij}$. The charges on anthramycin were obtained from quantum chemically derived electrostatic potentials[25] and are listed in Appendix 1 (supplementary material).

We have supplemented the force field parameters[19] with appropriate bond length, bond angle, and dihedral parameters corresponding to the additional atom types defined in anthramycin. Also, we have defined new atom types for some of the atoms of the guanine bases that are involved in covalent linkages with the drugs and have supplemented the corresponding force field parameters. These are listed in Appendix 2 (supplementary material).

For the sake of convenience, the six oligonucleotides referred to above have been referred to as G10C10, GC10, GCA, GCT, G10C10A, and G10C10T, respectively. The following nomenclature has been used in designating residues of these oligonucleotides. In the oligomers GC10, GCA, and GCT, residues have been counted from the 5′-end to the 3′-end of each strand as GUA1, CYT2, GUA3, CYT4, etc., with suitable changes to accommodate adenine and thymine residues. Thus, the last residue at the 3′-end of the second strand would be referred to as CYT20. In the other three oligomers, the residues on the G strand have been counted as GUA1, GUA2, GUA3, etc., from the 5′-end to the 3′-end, while those on the C strand have been

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counted as CYT11, CYT12, CYT13, etc. The phosphate groups have been referred to as P_{n-m}, where n and m are the serial numbers of the bases at respectively, 5′- and 3′-ends. For example, the phosphate groups intervening GUA3 and CYT4 in GC10 and GUA3 and GUA4 in G10C10 are designated as P_{3-4}. We have followed the nomenclature of atom numbering in anthramycin (Figure 1) used by Hurley and co-workers.[26]

Independent investigations on the complexes between anthramycin and a nucleic acid fragment (Remers et al., personal communication) have demonstrated that noncovalent models are characterized by both hydrogen-bonding and van der Waals interactions between the drug and the hexanucleotide. These models indicate that the drug binds very tightly to the polynucleotide due to its verysnug fit into the minor groove. In such models, the distance between C11 atom of the drug and the site of covalent binding on the polynucleotide, the N2 of guanine, is appropriate for the facilitation of bond formation between these atoms.

In the present investigations, the DNA–anthramycin covalent complexes were model built by docking the drug in the minor grooves of the above polynucleotides by the computer graphics program CHEM[27] on an Evans and Sutherlands Picture System 2. The geometry used for the nucleic acid fragments was the standard B DNA proposed by Arnott and co-workers.[28] This was followed by the creation of a covalent bond between the C11 on drug and N2 of GUA5. The force field parameters for this bond were chosen to be the same as for the analogous C–N bond involved in glycosidic linkages between bases and sugars in nucleic acids. The covalent complexes corresponding to the oligonucleotides G10C10, GC10, GCA, GCT, G10C10A, and G10C10T are denoted by the suffix AM, for example G10C10-AM, GC10-AM.

It may be noted that, in view of the possibility of epimerization at the C11 site in anthramycin, the covalent bond between the guanine and the drug could have two possible orientations relative to the seven-membered ring of the latter. We denote these two configurations as R and S. The corresponding complexes are denoted with subscripts r and s, respectively. Only in the case of G10C10-AM and GC10-AM were both the configurations investigated; in the other four cases, only configuration S was studied (for reasons to be discussed below). Calculations were also carried out by reversing the orientation of the drug in the minor groove relative to that in all the complexes mentioned above. The corresponding energy-minimized models were obtained only for configuration S in the decanucleotides G10C10 and GC10 and are referred to as G10C10-AM_{rev} and GC10-AM_{rev}.

Results

In all of the DNA–anthramycin complexes investigated in the present study, the polynucleotide backbone and glycosidic conformations after energy refinement are very similar to those found in a B DNA structure.[27] The latter has gauche$^+$, trans, trans, gauche$^-$, gauche$^-$, and anti conformations about the C4′–C5′, C3′–O3′, C5′–O5′, P–O3′, P–O5′, and glycosidic bonds with C2′ endo sugars. Therefore, our discussions on the conformational aspects of the polynucleotide part in the complexes have been restricted to only those cases where the deviations are significant from those in the standard B DNA.[28] Also the energy refinement of the oligonucleotides not covalently bound to the drug yielded structures whose conformational features were very close to those of the B DNA[28] and are hence not discussed in this paper.

Table I. Hydrogen Bond Parameters (Involving the Drug–Polynucleotide Interactions) in the Covalent Complexes between Anthramycin and the Decanucleotides Listed in Text[a]

complex	X	Z	length H--Z	angle X–H–Z
G10C10-AM_r	N14 (AM)	OA (P_{5-6})	1.69	166.2
	N11 (AM)	O2 (CYT16)	1.93	125.8
G10C10-AM_s	N2 (GUA6)	O9 (AM)	1.76	171.8
	N14 (AM)	OA (P_{18-19})	1.68	157.0
GC10-AM_r	N14 (AM)	OB (P_{5-6})	1.70	167.5
	O9 (AM)	O2 (CYT16)	1.82	159.0
	N11 (AM)	O2 (CYT16)	1.89	131.9
GC10-AM_s	N14 (AM)	OB (P_{5-6})	1.68	163.2
	O9 (AM)	O2 (CYT16)	1.79	174.7
G10C10-AM_{rev}	N2 (GUA4)	O9 (AM)	1.84	150.1
GC10-AM_{rev}	N2 (GUA17)	O9 (AM)	1.86	137.5
GCA-AM	N14 (AM)	OB (P_{5-6})	1.68	162.9
	O9 (AM)	O2 (CYT16)	1.80	165.0
GCT-AM	N14 (AM)	OB (P_{5-6})	1.68	163.5
	O9 (AM)	O2 (CYT16)	1.78	173.4
G10C10A-AM	N14 (AM)	OA (P_{18-19})	1.68	156.0
G10C10T-AM	N14 (AM)	OA (P_{18-19})	1.68	155.1

[a] In a hydrogen bond X--H···Z, X and Z are, respectively, donor and acceptor atoms, with H being the hydrogen atom. The hydrogen bond lengths and angles are in angstroms and degrees, respectively. The hydrogen bond length corresponds to the distance between H and Z, while the angle is X–H–Z.

As in the case of our investigations on DNA–mitomycin complexes,[29,30] we find that DNA–anthramycin complexes are characterized by stabilization due to hydrogen bonds between the drug and the polynucleotide. Table I lists the parameters such as donor and acceptor atoms, lengths (distances between the hydrogens and the acceptors), and angles (formed at the hydrogen atoms), for these hydrogen bonds in all the complexes. For the sake of convenience of description of sugar puckers, we have divided the phase (W) space into three broad regions. In accordance with this classification, W values from 0 to 72°, 72 to 144°, and 144 to 180° correspond to sugar puckers in the C3′ endo, O1′ endo–C1′ exo, and C2′ endo regions. In the case of intermediate sugar puckers (O1′ endo–C1′ exo) the phases of individual sugar moieties are explicitly mentioned.

As was done in our earlier investigations on drug–DNA interactions,[29,30] we have carried out component analysis of the total energies of various complexes in order to understand their relative stabilities. We wish to emphasize that comparison of the total energies of the complexes is often not meaningful since different sequences of nucleotides are represented. However, the energies of the complexes with different configurations of the covalent bond between the drug and the oligonucleotide for a given sequence can be compared. In these analyses, sugars, bases, and phosphates are treated as individual groups. The phosphate group includes both O3′ and O5′ atoms. In addition, the drug in each complex is treated as a separate group. The energies of interactions between various groups have been indicated by appropriate arrows connecting the groups. In addition, we have evaluated the drug–helix interaction energy and the helix destabilization energy

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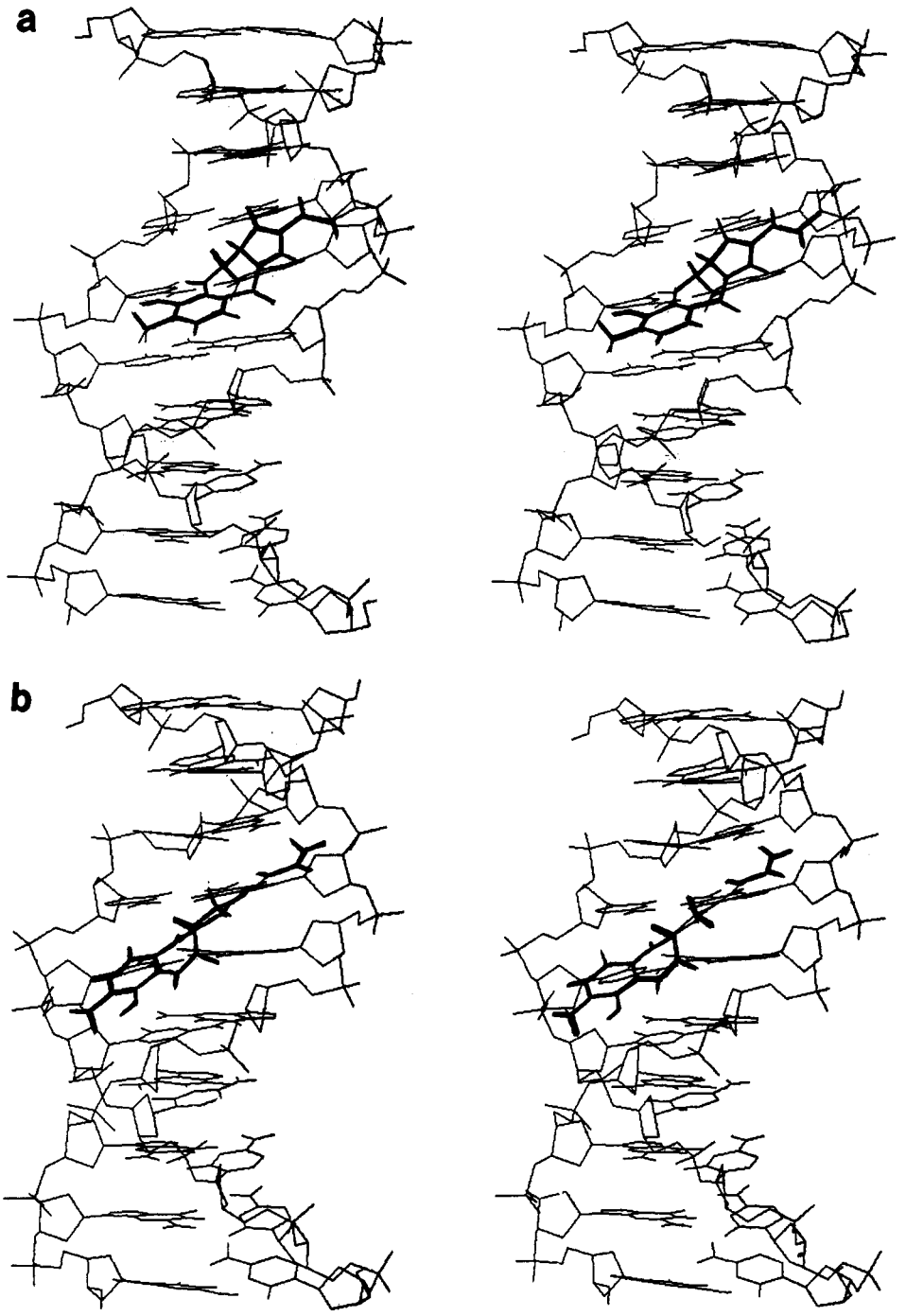

Figure 2. Stereopairs of the monolinked covalent complexes between anthramycin and $d(G_{10})\cdot d(C_{10})$ with (a) the drug covalently attached with configuration *R* and (b) the drug covalently attached with configuration *S*.

(also called the polynucleotide strain energy) in order to understand the effects of covalent complexation. The latter is defined as the difference between the total energy of the refined structure of the oligonucleotide and that of the polynucleotide part of the drug–DNA complexes. These energy components together with the total energies of the complexes have been listed in Table II.

We find that, in all the complexes investigated, the covalent linkage of anthramycin to the oligonucleotides produces no significant deviations in the conformations of the backbone and the bases relative to the standard B DNA. The only significant changes are confined to sugar puckers and P–O3′ torsions. The glycosidic orientations of all the bases are in the anti region as in the B DNA.

(1) Covalent Complexes between G10C10 and Anthramycin. In G10C10-AM$_r$ and G10C10-AM$_s$, the p–O3′ bonds at the 3′-end of CYT17 have trans configurations. In G10C10-AM$_r$, the sugars in GUA3, GUA4, GUA6, and CYT12 have O1′ endo–C1′ exo geometries while the rest of the sugars are predominantly C2′ endo. In G10C10-AM$_s$, the sugars in GUA3, GUA4, and CYT12 change pucker to the intermediate O1′ endo–C1′ exo. In this complex, the sugar pucker in GUA6 is C2′ endo (W = 168°). The stereopairs of these two complexes (Figure 2a,b) reveal that, in G10C10-AM$_r$, one of the three hydrogen bonds (N2···O2) in the Watson–Crick pair with cytosine on the complementary strand is broken, while in the other covalent complex none of the three hydrogen bonds are broken.

The complexes G10C10-AM$_r$ and G10C10-AM$_s$ are stabilized by two hydrogen bonds between anthramycin and the phosphates and bases of the oligonucleotides. As seen from Table I and Figure 2a, the amino group in the amide fragment of anthramycin in G10C10-AM$_r$ is involved in

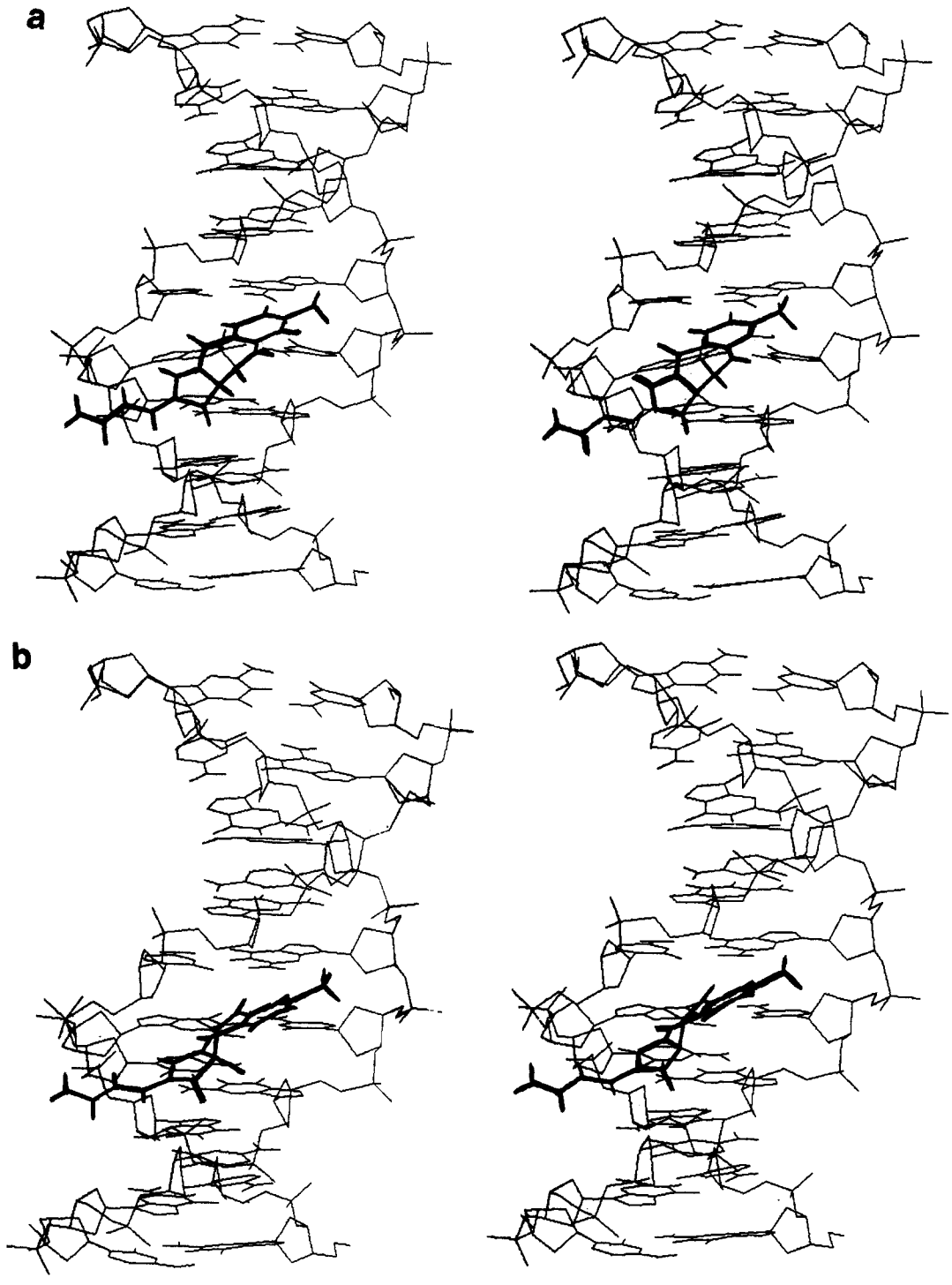

Figure 3. Stereopairs of the monolinked covalent complexes between anthramycin and d(GCGCGCGCGC)$_2$ with (a) the drug covalently attached with configuration *R* and (b) the drug covalently attached with configuration *S*.

hydrogen-bonding interactions with one of the pendant oxygens in P_{5-6}. Further, the phenolic hydroxyl (O9–HO9) and O5′ of CYT16 are involved in favorable electrostatic interactions. In G10C10-AM$_s$, the amino group in the amide fragment of the drug is oriented away from P_{5-6} and instead forms a hydrogen bond with one of the pendant oxygens on P_{18-19}. This change is accompanied by the reorientation of the phenolic hydroxyl, with the O9 getting directed toward the amino group on C2 of guanine at the 3′-end of GUA5, thus leading to an N–H···O hydrogen bond. Further, the N10–HN10 bond also points toward the same amino group on GUA6, leading to favorable electrostatic interactions between HN10 (anthramycin) and N2 (GUA6). It may be noted that the bond in the drug was oriented toward the carbonyl of CYT16, in G10C10-AM$_r$.

(2) Covalent Complexes between Anthramycin and GC10. In GC10-AM$_r$ (Fig. 3a), the sugars corresponding to CYT2 and CYT12 have intermediate puckers while in GC10-AM$_s$ (Figure 3b) in addition to these sugars those in GUA3, GUA13, and CYT14 also have O1′ endo–C1′ exo puckers. These variations in sugar geometries are similar to those obtained in DNA–mitomycin covalent complexes, which were investigated earlier in our laboratory.[28,29] The P–O3′ conformation is trans at the 3′-ends of CYT4, CYT6, CYT8, GUA15, and GUA17. As in the case of the covalent complexes between G10C10 and anthramycin, the hydrogen bond N2···O2 between GUA5 and CYT16 is disrupted

Table II. Total Energies and Drug, Drug–Helix, Helix, and Polynucleotide Destabilization Energies (kcal/mol) of the Covalent Complexes between Mitomycin and Deoxydecanucleotides

complex	total	drug	helix[a]	helix destabilization[b]	drug–helix[c]
G10C10-AM$_r$	-856.3	7.0	-796.6	37.2	-66.7
G10C10-AM$_s$	-873.7	9.6	-807.9	25.9	-75.3
GC10-AM$_r$	-875.0	13.3	-820.4	30.0	-67.8
GC10-AM$_s$	-893.8	13.3	-830.9	19.5	-76.1
G10C10-AM$_{rev}$	-871.0	9.4	-799.7	34.1	-71.3
GC10-AM$_{rev}$	-889.2	15.1	-832.6	17.8	-71.7
GCA-AM	-908.3	13.3	-845.5	23.5	-76.1
GCT-AM	-910.4	13.8	-847.8	22.0	-76.3
G10C10A-AM	-890.8	9.3	-828.0	27.9	-72.1
G10C10T-AM	-892.9	9.7	-829.5	25.8	-73.1

[a] Helix energy is the energy of the polynucleotide part of the drug–DNA complexes. [b] Helix destabilization energy is the difference in energies of the decanucleotides refined in the absence of the drug and the polynucleotide part of the complexes. The total energies (kcal/mol) of the decanucleotides energy refined without anthramycin covalently bound to them are as follows: GC10, -850.4; G10C10, -833.8; GCA, -869.0; GCT -869.8; G10C10A, -855.9; G10C10T, -855.3. [c] Drug–helix energy is the energy of interaction between the atoms of the drug and those of the polynucleotide.

in GC10-AM$_r$ and not in GC10-AM$_s$. Unlike in the case of G10C10-AM complexes, in GC10-AM complexes the change of configuration of covalent bond formation between the oligonucleotide and anthramycin does not lead to changes in the hydrogen-bonding partner of the amino group in the amide fragment of the drug. Thus, as revealed by the stereopairs in Figure 3a,b, this amino group is hydrogen bonded to one of the pendant oxygens on P_{5-6} in both the complexes. In both GC10-AM$_r$ and GC10-AM$_s$, the phenolic hydroxyl (O9–HO9) is oriented toward the carbonyl of CYT16, forming hydrogen-bonding interactions. However, the N10–HN10 bond is oriented differently in the two structures. In the former, it is also in the vicinity of the carbonyl in CYT16, leading to favorable electrostatic interactions, while in the latter it is directed toward the exocyclic amino group on C2 of GUA15. We find that, in both the sets of complexes involving G10C10 and GC10, the carbonyl group in the amide fragment of anthramycin is not involved in any hydrogen-bonding interactions with any of the groups on the oligonucleotides.

In the four complexes analyzed thus far, we note that the network of hydrogen-bonding and electrostatic interactions (Coulombic) is dependent on the nature of the bases at the 3′-end of GUA5 and 5′-end of CYT16. We also note that no features of such interactions are sensitive to the type of base at the 5′-end of GUA5 and 3′-end of CYT16. Further, base pairs that are further apart along the polynucleotide also do not influence the interactions with anthramycin. In view of this, we carried out additional model-building exercises on DNA–anthramycin complexes by replacing CYT6 in GC10 and GUA6 in G10C10 by adenine and thymine and making corresponding changes on the complementary strand to maintain Watson–Crick base-pairing schemes. In both the additional models, configuration *S* of the covalent bond C11–N2 was considered, as it corresponded to energetically favorable structures with the G- and C-containing oligonucleotides.

Figure 4a,b (supplementary material) shows stereopairs of the covalent complexes between anthramycin and GCA and GCT, respectively. Figure 5a,b (supplementary material) shows the corresponding structures for the complexes with oligonucleotides G10C10A and G10C10T. Not surprisingly, the conformations of the backbone and the bases in these structures did not differ significantly from those in the complexes without the A and T bases. No major changes in the anthramycin orientation in the minor groove are noticed upon the incorporation of an A-T or T-A base pair instead of a G-C base pair in either of the two oligonucleotides. Comparing Figures 3b and 4b (supplementary material), it is found that the weak hydrogen-bonding interactions involving the phenyl hydroxyl and the amino group in GUA16 of the former are absent in the latter since the base in ADE16 lacks the amino group at C2. However, these two complexes do retain the weak hydrogen-bonding interactions involving the N10–HN10 of anthramycin and the C2 carbonyl of the pyrimidine at the 3′-end of GUA5. In Figure 4b (supplementary material), N10–HN10 is oriented toward the N3 of ADE6, while the O9–HO9 bond is oriented toward the C2 carbonyl in CYT16. In the case of adenine replacing guanine in residue 6 in the homopolymer, the loss of amino group at C2 deprives the phenyl hydroxyl oxygen of the hydrogen-bonding interactions (Figure 5a) (supplementary material). On the other hand, replacement by thymine leads to stabilization of the drug–DNA interactions through the hydrogen-bonding interactions between the carbonyl oxygen of the pyrimidine and N10–HN10 bond of the drug.

(3) Covalent Complexes between Anthramycin and Decanucleotides (Drug Orientation Reversed). G10C10-AM$_{rev}$. Figure 2c (supplementary material) shows the stereopair of the covalent complex between anthramycin and G10C10 with the amide tail of the drug in the vicinity of the nucleotides GUA7 and GUA8 instead of GUA3 and GUA4 in G10C10-AM$_s$. Also, in contrast to the latter complex, the amino group in this fragment of the drug is involved in hydrogen-bonding interactions with P_{7-8} in G10C10-AM$_{rev}$. The proton HN10 is directed toward the amino nitrogen in GUA4, and the hydroxyl group O9–HO9 is involved in electrostatic interactions with the O1′ of GUA5. It may be noted such interactions involving sugar ring atoms are not present in other models of anthramycin–DNA interactions discussed thus far. As in G10C10-AM$_s$, the polynucleotide part of the complex does not undergo any helical distortions, with the base pairs remaining intact.

GC10-AM$_{rev}$. As in the case of G10C10-AM$_{rev}$, the conformational characteristics of the polynucleotide part of this drug–DNA complex (Figure 3c) (supplementary material) are very similar to those in GC10-AM$_s$. The drug–DNA interactions are, as to be expected, characterized by a different set of hydrogen bonds than in GC10-AM$_s$. The amino group in the amide fragment of anthramycin is involved in hydrogen-bonding interactions with one of the pendant oxygens on P_{8-9}, instead of P_{5-6} in GC10-AM$_s$. The hydroxyl group O9–HO9 is oriented toward N3 of GUA5. The oxygen atom in this group is also involved in favorable electrostatic interactions with the amino group of GUA17. In contrast to GC10-AM$_s$, no fragment of the drug is close to CYT16 in GC10-AM$_{rev}$. The section of the drug containing the aromatic and the flexible seven-membered ring is nicely accommodated in the minor groove, while the amide tail is drawn toward the chain containing GUA5 in order to form hydrogen-bonding interactions with the phosphate group.

Energetics

The conformational features of the models discussed above are reflected in their component analysis diagrams. From Table II it is clear that configuration *R* models are energetically destabilized relative to those with configuration *S*. This difference is contributed to by both the

helix destabilization and drug–helix interaction terms. In both G10C10-AM$_s$ and GC10-AM$_s$, the helix destabilization energy is less than that in G10C10-AM$_r$ and GC10-AM$_r$, respectively, by about 10 kcal/mol. This is easily understood in view of the fact that, in the latter complexes, one of the hydrogen bonds in GUA5-CYT16 base pair is disrupted (Figure 6; supplementary material), while such is not the case in the other two structures. This is also reflected in the diagrams presented in Figures 7 and 8 (supplementary material), which, respectively, represent the G10C10-AM and GC10-AM complexes. In both these diagrams, the energy values without parentheses correspond to configuration *S* and those within parentheses correspond to configuration *R*. As is clearly demonstrated, for example in Figure 7 (supplementary material), the interaction energy between the bases CYT16 and GUA5 is around −20.0 kcal/mol in G10C10-AM$_s$ and −12.8 kcal/mol in G10C10-AM$_r$. Configuration B also leads to more favorable drug–helix interactions (by about 8 kcal/mol) in both sets of structures.

The energies of interactions between anthramycin and various groups on the decamers show qualitative consistency with the conformational features of interactions between drug and DNA discussed earlier. In Figure 7 (supplementary material), the drug–P_{5-6} interactions are favored by more than 15 kcal/mol in G10C10-AM$_r$ compared to G10C10-AM$_s$, while those with P_{18-19} are favored in the latter by about 17 kcal/mol. Drug–GUA6 interactions are favored in the latter complex by about 6 kcal/mol, while interactions with CYT16 are favored in the former to a smaller extent (2.8 kcal/mol). As is to be expected from the earlier discussions on conformational features, in Figure 7 (supplementary material) the anthramycin–P^{5-6} interactions are different in G10C10-AM$_r$ and G10C10-AM$_s$ by only about 1 kcal/mol. The differences in interactions with CYT6 and CYT16 favor GC10-AM$_s$ and GC10-AM$_r$, respectively, by about 3.5 and 2.3 kcal/mol.

A combined look at the component analysis diagrams in Figures 7 and 8 (supplementary material) reveals the following points. In both sets of structures, covalent complexation produces configuration-sensitive changes in base–base stacking interactions on the strand containing GUA5. The stacking interactions between GUA5 and the base at the 3′-end in the sequence are more favorable in G10C10-AM$_r$ and GC10-AM$_r$ than in the corresponding complexes with configuration *S* by about 3–5 kcal/mol. This is due to the fact that, in the former complexes, covalent complexation rotates the N2–H2 bond in GUA5 so as to enhance favorable electrostatic interactions with the negatively charged carbonyl oxygen (in case of pyrimidine) and nitrogen N3 (in case of purine) in the base at the 3′-end of GUA5. In the latter complexes, on the other hand, since no hydrogen-bonding base-pairing interactions are affected, the stacking energies are quite similar to those found in normal B DNA.

We note some sensitivity of interactions between anthramycin with P_{16-17} to the oligonucleotide sequence. In Figure 8 (supplementary material) these interactions have similar energy values in both GC10-AM$_r$ and GC10-AM$_s$, whereas in G10C10-AM$_r$ (Figure 7) (supplementary material) they are favored by more than 5 kcal/mol compared to G10C10-AM$_s$. This difference in G10C10-AM complexes could be attributed to the fact that configuration *R* leads to more favorable electrostatic interactions between the hydroxyl group O9–HO9 and the said phosphate group than does configuration *S*. In the structure with the latter configuration of covalent binding, this hydroxyl group is oriented toward the exocyclic amino group of GUA6, away from P_{16-17}. As mentioned earlier in the paper, this hydroxyl group is involved in interactions with groups on the neighboring bases in GC10-AM$_r$ and GC10-AM$_s$ and are located too far away from P_{16-17} to have any significant interactions.

Figures 7 and 8 (supplementary material) also show the effects of the presence of A-T and T-A base pairs instead of G-C base pair at the 3′-end of GUA5 (and at the 5′-end of CYT16) on the interactions between anthramycin and various groups in the decamers G10C10 and GC10. It is found that, in the case of GCA and GCT, these sequence variations do not bring about any drastic changes on the interactions between the drug and the nucleotides. In fact, the drug–helix interaction energies in GCA-AM and GCT-AM are very similar to that in GC10-AM$_s$, as seen from Table II, supporting the above observation. None of the individual interactions differ by more than 1.0 kcal/mol in either GCA-AM or GCT-AM from those in GC10-AM$_s$.

However, in the case of G10C10A and G10C10T complexes, the interaction energies between anthramycin and ADE6 (in G10C10-AM) and THY6 (in G10C10T-AM) are higher by nearly 3 kcal/mol than in the corresponding interactions with GUA6 in G10C10-AM$_s$. The interactions between the drug and the corresponding complementary bases are not significantly altered. Also, the insertion of the A-T and T-A base pairs does not in these cases seem to bring about any changes in the interactions between the drug and other nucleic acid fragments. Consistent with this is the fact that the drug–helix interactions in these two complexes are higher by about 2–3 kcal/mol than in G10C10-AM$_s$.

The effects of reversing the orientation of the drug in the minor groove on the energetics of anthramycin–DNA interactions are shown in the component analysis diagrams in Figure 9 (supplementary material). Here, the energy components in G10C10-AM$_{rev}$ and GC10-AM$_{rev}$ are represented within and without parentheses, respectively. The conformational features of these two complexes are reflected by the interaction energies of −16.1 and −8.5 kcal/mol between the drug and P_{7-8} and GUA5, respectively. The latter is about 3.5 kcal/mol lower than that in either G10C10-AM$_s$ or GC10-AM$_s$. Also, the interactions between the drug and GUA17 is favored in GC10-AM$_{rev}$ by about 3 kcal/mol over that in GC10-AM$_b$, while in the latter the interactions between the drug and CYT16 are favored by around 9 kcal/mol.

In the case of G10C10-AM$_{rev}$, the interactions between anthramycin and the bases in CYT17 and CYT16 are energetically similar to those in G10C10-AM$_s$, despite the fact that in the two complexes different fragments of the drug are in the vicinity of these two bases. In comparison to the latter complex, G10C10-AM$_{rev}$ is characterized by favorable interactions between the drug and the base at the 5′-end of GUA5 and the sugar in GUA5. The interactions between the drug and P_{7-8} are around 16 kcal/mol as in GC10-AM$_{rev}$. However, unlike the latter, the interactions of the drug with the base in GUA5 are higher in energy by about 2.3 kcal/mol compared to the corresponding interactions in G10C10-AM$_s$.

Discussion

DNA–anthramycin covalent complexes have been model built and energy refined. In these complexes, we find several points of resemblance with the conformational features of the mitomycin C–DNA covalent complexes when the drug was anchored in the minor groove. The most prominent of these features is the distortion suffered by the polynucleotide part of the complexes upon the

covalent binding of the drug. In the cases of both the complexes, the distortions are minimal in the backbone and glycosidic conformational parameters. The DNA base pairs are all intact in both mitomycin–DNA and energetically most favored anthramycin–DNA complexes. In both cases, the drug–nucleic acid interactions are stabilized by an extensive hydrogen-bonding network.

We emphasize that counterions and solvent effects have not been explicitly represented in our potential functions, and thus, the quantitative features of these investigations cannot be directly correlated to the experimentally observed activity of anthramycin. However, the high-resolution models that have been arrived at provide useful qualitative insight into the various conformational features of the anthramycin–DNA complexes. The results could provide impetus for further experimental studies on these complexes. Recently, a complex between anthramycin and $d(ATGCAT)_2$ has been analyzed by ^1H and ^{13}C NMR,[15] 1- and 2-D NOE techniques.[31] The results obtained from these studies are in general consistent with the qualitative features obtained from the present investigations.

The NMR studies have found the site of covalent attachment to the amino N2 of guanine and have ruled out N7 in the major groove as a possible site. Also, the DNA duplex was stabilized by the formation of the covalent complex. Our models find that the energetically most favorable structures do have the drug snugly packed in the minor groove, with its tail providing additional stabilizing interactions with the backbone of the polynucleotide. 2-D NOE experiments[31] suggest that the formation of the covalent adduct does not disrupt any of the base pairs in the oligonucleotide and hence does not significantly alter the stacking interactions. The low-energy models obtained in our investigations, with configuration *S* of covalent attachment, are consistent with such a picture. Further, this study also "predicted" (ref 32) the orientation of the drug in the minor groove and the configuration of covalent attachment at the C11a of anthramycin. In our models with the amide fragment of the drug toward the 5′-side of the guanine base to which it is linked, the drug–DNA interaction energies are favored over those in models with reverse orientation of the drug by about 5 kcal/mol. The low-energy models are also consistent with the observation of an NOE between the methyl protons in the aromatic ring of the drug and the H2 of purine two bases removed from the guanine covalently attached at the 3′-end.

The low-energy models obtained in our investigations have features such as nondistortion of the polynucleotide helix that were suggested in experimental studies on S_1 nuclease and BND cellulose chromatography.[33] These features have been made use of in the interpretation of the experimental results on the biological consequences of DNA damage by anthramycin.[17] For example, the formation of excision-dependent single-strand and double-strand breaks in normal and XP cell lines has been attributed to the fact that the covalent linkage is inside the minor groove, which makes the repair enzyme complex unable to discern which strand of DNA is modified by anthramycin. The lack of distortion in the helical structure of DNA might result in the excision repair on the wrong strand, leading to single-stranded regions still containing the drug. Thus, our models are consistent with the suggested role of anthramycin in biological systems.

In conclusion, molecular mechanical simulations of the DNA–anthramycin complexes have yielded models that have several points of consistency with available experimental data. The binding energies of the drug in various complexes as well as the distortion energies in the oligonucleotide fragment of the complexes are consistent with those obtained in the independent investigations on interactions between $d(ATGCAT)_2$, and it is interesting to note the two sets of model-building exercises have led to slightly different roles in interactions for the tail of the anthramycin molecule.

While our study suggests interactions with the phosphate group in the vicinity of this group, independent investigations by Remers et al. (personal communication) find this fragment to lie entirely in the minor groove of the polynucleotide. Given that the calculations do not contain counterions or salt, we do not consider this difference significant; such a difference is likely to be due to slight differences in the model building. Our experiences in netropsin–DNA interactions also suggest that the importance of phosphate–drug interactions will be exaggerated in models such as used here.[34]

Our studies suggest that variation of the sequence of the polynucleotide around the site of complex formation does not seem to have a marked effect on the binding energies of anthramycin with DNA. Recently, it has been demonstrated using MPE·Fe(II) footprinting technique that there is a marked preference for covalent binding of pyrrolo[1,4]benzodiazines to guanines flanked by purines rather than pyrimidines.[22] Such preferences could be due to environmental factors such as solvent and counterions and the resultant dynamic effects, and these have not been taken into explicit consideration in the present study. The lack of explicit representation of such effects could also be attributed to the absence of any major conformational changes in the proximity (of around three to four base pairs) of the drug-binding site, as predicted by these experimental studies.[22] Molecular dynamical studies incorporating counterions and solvent effects on the covalent complexes between anthramycin and oligonucleotides are in order and are likely to highlight the significance of sequence specificity of such interactions.

It may be noted that the present studies have been carried out only with the drug bound in the minor groove of the B form of DNA. Several studies on the conformations and structures of oligo- and polynucleotides have demonstrated sensitivity of polymorphism to nucleotide sequences.[35–38] A different conformational environment for anthramycin in polymorphic forms other than B is likely to influence its interactions with polynucleotides. It is therefore likely that sequence variations together with the corresponding polymorphism could influence the nature of anthramycin–DNA interactions.

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Supplementary Material Available: Tables of charges on anthramycin in its covalently bonded form (Appendix 1), additional bond lengths, bond angles and dihedral parameters for anthramycin (Appendix 2), stereodiagrams of structures of the complexes G10C10-AM$_{rev}$ (Figure 2c), GC10-AM$_{rev}$ (Figure 3c), GCA, GCT, G10C10A, and G10C10T (Figures 4a,b and 5a,b, respectively), and energy component analysis diagrams (Figures 7-9) (12 pages). Ordering information is given on any current masthead page.

Conformations of Complexes between Pyrrolo[1,4]benzodiazepines and DNA Segments[1]

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The molecular mechanics program AMBER, assisted by CHEMLAB II, was used to model the covalent and noncovalent binding of anthramycin, tomaymycin, and neothramycin A to the hexanucleotide duplex d(GCATGC)$_2$ in the B-form conformation. Structures covalently bonded at N2 of guanine gave excellent fits when placed in either direction in the minor groove. However, energy analysis showed a preference for the direction wherein the side chain points toward the 5′ end of the covalently bound strand. This preference agrees with published NMR studies. Noncovalent binding of anthramycin in the minor groove near guanine gave good fits with almost no distortion in the helix, and the reactive center of the ligand was close enough to N2 for subsequent covalent bond formation. Anthramycin also gave a good noncovalent complex near adenine in the minor groove, but binding in the major groove had decreased dispersion attractions. Binding of tomaymycin was similar to that of anthramycin, although the smaller size of tomaymycin resulted in less binding energy. Neothramycin noncovalent binding was characterized by strong electrostatic interactions, partly involving the 3-OH group, and by part of the molecule lying outside the minor groove. AMBER was used for the exploratory design of an anthramycin analogue that theoretically would bind as well as anthramycin but not cause cardiotoxicity. A related study involving anthramycin, tomaymycin, and the pentanucleotide duplex d(AAGAA/TTCTT) was undertaken to evaluate further the ability of AMBER to predict sequence specificity. It indicated a preferred direction of binding toward 5′ in the minor groove of the duplex, but rather weak interaction with the noncovalently bound strand. This prediction agreed with experiments on tomaymycin that showed separation of the duplex and alignment of the drug toward the 5′ end of the covalently bound strand.

This paper and the preceding paper in this issue[1] represent independent investigations on modeling the binding of anthramycin and related pyrrolo[1,4]benzodiazepine antibiotics to specific segments of double helical B DNA. The other paper utilizes all atom force field parameters for the drug, emphasizes the appropriate stereochemistry for covalent bond formation at C11 of anthramycin, and explores the effects of sequence specificity when the covalent bond is made with the 2-amino group of guanine. The present paper utilizes united atom force field parameters to investigate both covalent and noncovalent binding at guanine and other locations on DNA. It includes anthramycin, tomaymycin. and neothramycin A. It also explores the use of molecular mechanics in the design of anthramycin analogues.

The pyrrolo[1,4]benzodiazepine antitumor antibiotics are potent agents produced by actinomycetes.[4-7] Included in this family are anthramycin, tomaymycin, sibiromycin, and neothramycins A and B. Their structures and numbering are shown in Figure 1. All of them have the same structural nucleus but differ in the benzene ring substituents and in the degree of saturation and substituents of the pyrrole ring.[8,9] Anthramycin and tomaymycin are observed by X-ray diffraction to possess a right-handed twist conformation along the length of the molecule, controlled mainly by the C11a hydrogen.[10,11] The neothramycins by structural analogy should have a similar twist, but unsaturation in the pyrrole ring of sibiromycin limits twisting. A carbinolamine group or its equivalent at N10–C11 are required for covalent binding to DNA.[9] Although the structure of tomaymycin often is written with a methyl ether group at C11, this is an artifact of crystallization from methanol. The natural product probably has an OH group like that of the anthramycin.[12] The neothramycins are N10–C11 imines, but they are known to undergo hydration in aqueous solution.[12]

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