Ternary interactions of spermine with DNA: 4′-epiadriamycin and other DNA: anthracycline complexes

Loren Dean Williams, Christine A.Frederick, Giovanni Ughetto[1] and Alexander Rich*
Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA and
[1]Istituto di Structturistica Chimica, CNR, Area della Ricerca di Roma-Montelibretti, Italy

Received April 16, 1990; Revised and Accepted July 2, 1990

ABSTRACT

The recently developed anthracycline 4′-epiadriamycin, an anti-cancer drug with improved activity, differs from adriamycin by inversion of the stereochemistry at the 4′-position. We have cocrystallized 4′-epiadriamycin with the DNA hexamer d(CGATCG) and solved the structure to 1.5 Å resolution using x-ray crystallography. One drug molecule binds at each d(CG) step of the hexamer duplex. The anthracycline sugar binds in the minor groove. A feature of this complex which distinguishes it from the earlier DNA:adriamycin complex is a direct hydrogen bond from the 4′-hydroxyl group of the anthracycline sugar to the adenine N3 on the floor of the DNA minor groove. This hydrogen bond results directly from inversion of the stereochemistry at the 4′-position. Spermine molecules bind in the major groove of this complex. In anthracycline complexes with d(CGATCG) a spermine molecule binds to a continuous hydrophobic zone formed by the 5-methyl and C6 of a thymidine, C5 and C6 of a cytidine and the chromophore of the anthracycline. This report discusses three anthracycline complexes with d(CGATCG) in which the spermine molecules have different conformations yet form extensive van der Waals contacts with the same hydrophobic zone. Our results suggest that these hydrophobic interactions of spermine are DNA sequence specific and provide insight into the question of whether DNA:spermine complexes are delocalized and dynamic or site-specific and static.

INTRODUCTION

The biological polyamines, putrescine, spermidine and spermine, are found in millimolar concentrations within cells and are essential for growth and proliferation (for reviews, see 1−3)). These aliphatic polycations interact with anionic cellular components such as membranes (4,5) and nucleic acids. Polyamines stabilize duplex DNA (6−9), condense DNA (10−13) and chromatin (14,15) and promote the B-DNA to Z-DNA transition (16,17).

Molecular aspects of nucleic acid:polyamine interactions are generally obscure. Polycations may bind to DNA as delocalized condensates with the association driven by release of bound ions (8,9,18,19) as predicted by the theory of Manning (20). If release of bound ions is the major driving force, the association would be highly dynamic and would lack significant structural and sequence specificity. Alternatively, it has been suggested that polycations may bind to DNA by more direct molecular interactions such as hydrogen bonds (21−25). If so, structural and chemical considerations would contribute to the stability of nucleic acid:spermine complexes. The association should then be relatively static and would display DNA structural and sequence specificity. X-ray crystallographic solution of several nucleic acid:spermine complexes (26−29) appears to support formation of specific complexes although additional, delocalized complexes cannot be excluded by this technique.

Many biological properties of polyamines involve ternary interactions of DNA:spermine complexes with other DNA binding factors. For example, polyamines increase the rate of movement of DNA replication forks (30), increase the fidelity of restriction endonucleases (31), interfere with DNA binding of antibiotics such as actinomycin-D (32), and recognize discrete binding sites on nucleosome core particles (33). This report describes the three dimensional structure of another ternary complex, spermine bound to a DNA:anthracycline complex.

Anthracyclines are DNA intercalators which constitute a widely used family of chemotherapeutic agents. The biological properties of anthracyclines, like those of polyamines, appear to involve ternary interactions of DNA:drug complexes with other factors (34,35). The activities of anthracyclines depend strongly on minor modifications in chemical structure (36,37). Daunomycin is effective for treating acute leukemia while adriamycin, differing only by the addition of a hydroxyl group, is more effective for treating solid tumors. A recently developed analogue, 4′-epiadriamycin (Figure 1), differing from adriamycin solely by an inversion of the stereochemistry at the 4′-position, is better tolerated and is now in clinical use in Europe (36). We have cocrystallized 4′-epiadriamycin with the DNA hexamer d(CGATCG) (the complex is referred to here as epia-AT) and solved the three-dimensional structure to 1.5 Å resolution with x-ray crystallography. As expected, two 4′-epiadriamycin

* To whom correspondence should be addressed

molecules intercalate at the d(CG) steps of each hexamer duplex. As in daunomycin and adriamycin complexes with the same DNA hexamer (29), (complexes referred to here daun-AT and adri-AT) two symmetry-related spermine molecules bind in the major groove of each complex. Surprisingly, the conformation of the bound spermine varies from complex to complex even though the conformation of the DNA is nearly the same. The only covalent differences between these three complexes are in the hydroxyl groups of the anthracyclines. Other anthracycline complexes with a different DNA sequence, 11-deoxydaunomycin bound to the phosphorothioated d(CGTsACG) (38) (referred to here as 11dd-TsA) and daunomycin bound to d(CGTACG) (39) (referred to here as daun-TA) do not contain observable, bound spermine. In the previous report of daun-AT and adri-AT (29), the spermine molecules were described in a preliminary fashion, omitting analysis of van der Waals contacts and hydrophobic interactions. The spermine interactions of all three complexes (daun-AT, adri-AT and epia-AT) are described in detail here.

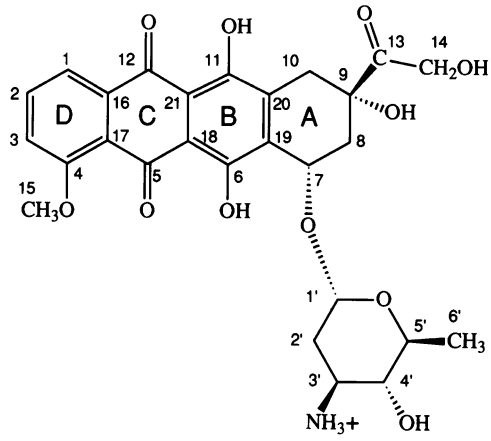

Fig. 1. 4'-Epiadriamycin.

MATERIALS AND METHODS

The self-complementary DNA hexamer d(CGATCG) was synthesized by the phosphotriester method and purified with SEP-PAK C18 cartridges (Waters). Anthracyclines were kindly provided by Farmitalia Carlo Erba, Milan, Italy. Epia-AT crystals were grown in sitting drops at room temperature using the vapor diffusion technique. The crystallization mother liquor contained 1.2mM DNA (single-strand concentration), 20mM sodium cacodylate buffer, pH 6.0, 2.5mM spermine, 1.2mM $MgCl_2$ and 8%, 2,4-methyl-pentane-diol. The sitting drops were equilibrated against a reservoir of 30% 2,4-methyl-pentane-diol. Tetragonal crystals appeared within a day and grew to a size of about 0.4×0.4×0.2mm. The space group was $P4_12_12$ with cell dimensions $a=b=28.04$ Å and $c=53.15$ Å.

A crystal was sealed in a glass capillary with a small volume of mother liquor and data were collected at room temperature on a Rigaku AFC5 rotating anode diffractometer in the 19 scan mode. Intensities were corrected for Lorentz, polarization and absorption effects. The total number of unique reflections greater than 1.5 σ(F) from 1.5 to 10.0 Å was 1714, with 235 reflections between 1.5 and 1.7 Å. The 4'-epiadriamycin:d(CGATCG) complex is nearly isomorphous with the adriamycin:d(CGATCG) complex (29) and those coordinates, minus water molecules and the sugar moiety at the 7-position of the anthracycline, were used as a starting model.

The structure was refined with the Konnert-Hendrickson constrained least squares refinement procedure (40) as modified for nucleic acids (26). The complex is positioned on a crystallographic twofold axis with only one strand of the DNA in the asymmetric unit. The complex was refined at lower symmetry using both strands of the duplex with frequent averaging of symmetry related atoms. Fourier electron density ($2F_o$-F_c) and difference (F_o-F_c) maps were displayed on an Evans and Sutherland PS390 graphics terminal and manual manipulation of the models was performed with the program FRODO (41). Solvent molecules were added slowly throughout the refinement. Until the latter stages of refinement, the spermine molecule was refined as a string of water molecules. At 1.5 Å resolution, first shell water molecules usually refine as tight spheres of Fourier electron density. However, the continuous, elongated shape of the solvent electron density in the major groove clearly suggested that certain water molecules should be replaced with a spermine and this was done in the final refinement. With the exception of the Watson-Crick hydrogen bonds of the base pairs, hydrogen bonds and van der Waals contacts were not constrained during refinement. The final refined structure of the epia-AT complex contains one strand of DNA, one 4'-epiadriamycin molecule, one unique spermine and 38 unique water molecules and had an rms deviation in bond lengths from ideal of 0.019 Å. The final R-factor was 19.6%. The atomic coordinates will be deposited in the Brookhaven Data Bank.

RESULTS

In crystallized complexes of anthracyclines with d(CGATCG) or d(CGTACG), two anthracycline molecules bind to reach hexamer duplex (29,38,39,42,43). The chromophore intercalates at the d(CG) steps and the sugar moiety at the 7-position binds in the minor groove. The relative orientation, conformation and interactions with DNA of the sugar moiety vary from complex to complex (29,38) consistent with the suggestion that the sugar is mobile in solution (38,43). A schematic compilation of the hydrogen-bonding interactions in five related daunomycin-type complexes (daun-TA, daun-AT, adri-AT, 11dd-TsA and epia-AT) is shown in Figure 3. These hydrogen-bonding interactions are observed in complexes representing three modifications of daunomycin, one modification of the DNA backbone, two DNA sequences and two crystal systems. The conformation and interactions in the complex formed by 4'-epiadriamycin with d(CGATCG) (Figure 2) are consistent with the previously reported complexes. The hydrogen-bonding contacts between DNA and 4'-epiadriamycin are listed in Table I. Hydrogen bonds and van der Waals contacts are not directly observed at the resolution obtained from these crystals but are inferred from distance and geometry relationships. The criteria we have used for a hydrogen bond are that an inter-atomic contact be 3.4 Å or less and satisfy reasonable hydrogen bond geometry and donor/acceptor relationships. The criteria for van der Waals contact are that an inter-atomic distance be equal to or less than the sum of van der Waals radii (the sums are approximately 3.4 Å) and not satisfy hydrogen bond geometry and donor/acceptor relationships. The criterion for hydrophobic interaction is that inter-molecular proximity exclude water molecules from contact with hydrophobic functional groups.

With the exception of the structurally distinct nogalamycin

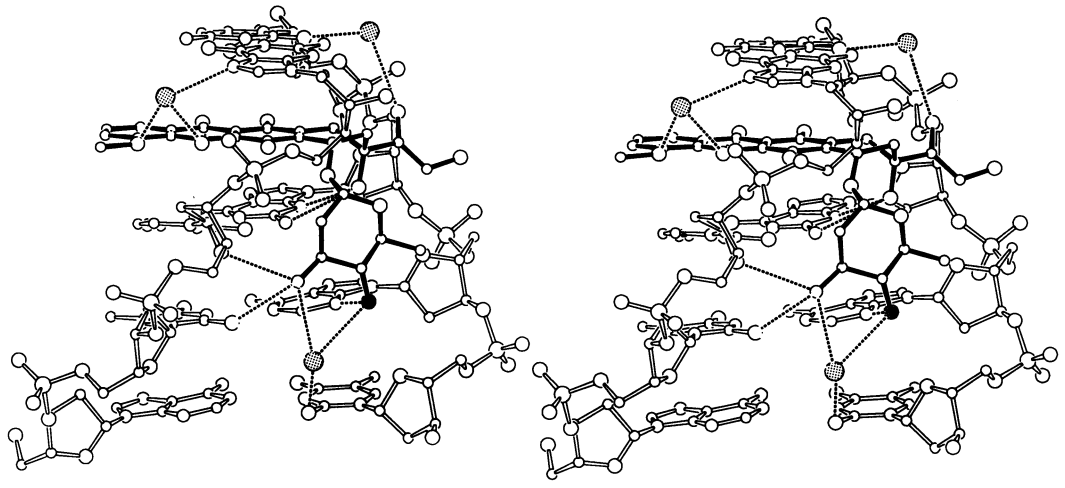

Fig. 2. Stereoview ORTEP (50) representation of the epia-AT complex looking into the minor groove. The DNA is drawn with hollow bonds, 4′-epiadriamycin is drawn with solid bonds and the hydrogen bonds linking the drug and the DNA are drawn with dashed lines. Water molecules, marked with stippling, are the largest spheres. The 4′-oxygen of 4′-epiadriamycin is marked by darker stippling.

complex (43), which is not included in the compilation of Figure 3, throughout the series of crystallized DNA:anthracycline complexes (29,38,39,42), neither the DNA nor the bound anthracycline are conformationally stressed, suggesting that B-DNA can easily accommodate simple intercalators. The orientation of the intercalated chromophore within the DNA is essentially invariant, with the long axis of the chromophore slightly skewed from the perpendicular of the long axis of the base pairs.

One feature of the epia-AT complex which distinguishes it from other DNA:anthracycline complexes is a direct hydrogen bond from the 4′-hydroxyl group of the anthracycline sugar to the N3 of A(3) (Table I and Figures 2 and 3). This hydrogen bond is not observed in other DNA:anthracycline complexes (29,38,39,42) and results directly from inversion of the stereochemistry at the 4′-position of 4′-epiadriamycin. The 9-hydroxyl of 4′-epiadriamycin (and other daunomycin-type anthracyclines) engages in what may be a common mode of hydroxyl group recognition of guanine from within the minor groove. The 9-hydroxyl group simultaneously accepts a proton in a hydrogen bond to N2 of G(2) and donates a proton in a hydrogen bond to N3 of the same base. The angle between these two bonds is acute (42°). A water molecule binds *via* the same pattern of hydrogen bonds with another guanine (the hydrogen-bonding contacts between the DNA and water molecules are listed in Table II). This water molecule (30WO5) forms two hydrogen bonds in the minor groove with G(12) with an angle of 40° between the bonds.

There are two instances where a water molecule simultaneously forms two hydrogen bonds to 4′-epiadriamycin and a third hydrogen bond to the DNA (the hydrogen-bonding contacts between 4′-epiadriamycin and first shell water molecules are listed in Table III). A water molecule (22W02) binds to O4 and O5 of the chromophore and also to N7 of residue G(12). Due to possible tautomerization of the chromophore (38) the direction of proton donation in this hydrogen bond is ambiguous and was omitted from Figure 3. A second water molecule (21W09) binds simultaneously to the N3′ and O4′ of the anthracycline sugar and to O2 of residue T(4) (Figure 2 and 3). In an interaction that is observed in each complex, a water mediated hydrogen bond (*via* 17WO4 in this complex) is observed from the O13 of the A ring to the O2 of C(1).

Table I. Hydrogen-bonding contacts between DNA and 4′-epiadriamycin

drug atom	DNA atom	distance (Å)	angle (degrees)	to third atom
O9[1]	G(2)N2	3.15	118	drug C9
	G(2)N3	2.59	120	
07	G(2)N3	3.35	103	drug C7
N3′	T(10)O2	3.37	119	drug C3′
	C(11)O2	3.27	75	
	C(11)O1′	3.31	120	
O4′	A(3)N3	3.06	111	drug C4′

1) The N2-O9-N3 angle equals 42°.

Anthracycline complexes with the DNA hexamer of sequence d(CGATCG) differ from those with d(CGTACG) in two ways. The first difference is in the minor groove where the anthracycline sugar interacts more intimately with d(CGATCG) than with d(CGTACG) forming direct hydrogen bonds to d(CGATCG) but not to d(CGTACG). The epia-AT complex is consistent with this pattern and the positively charged N3′ forms hydrogen bonds to O1′ of residue C(11) and O2 of residue T(10) (Table I). The geometric relationship between O2 of C(11) and the amine bond of 4′-epiadriamycin makes a hydrogen bond to N3′ improbable. As described above however, the 4′-hydroxyl group of the 4′-epiadriamycin sugar forms a hydrogen bond to N3 of A(3).

Spermine interactions

The second difference between the two DNA sequences is in the major groove where spermine molecules bind to d(CGATCG) but are not observed with d(CGTACG). The spermine molecules bound to the epia-AT complex can be seen in Figure 4. The two spermine molecules per hexamer duplex are related by a two-fold axis and the interactions and conformations of the pair are thus identical. In each complex with d(CGATCG) the methylene and amino groups of spermine form van der Waals contacts with both the DNA and the drug. In the major groove a continuous hydrophobic zone is formed by the 5-methyl and C6 of residue (T(10), C5 and C6 of residue C(11) and C1, C2 and C3 of the

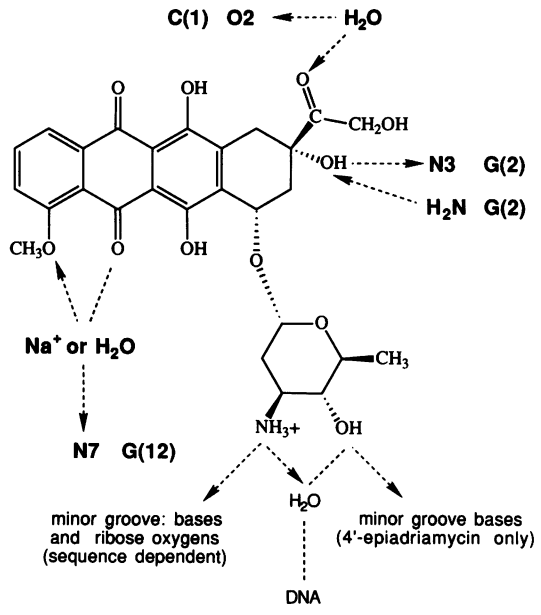

Fig. 3. Schematic diagram showing the hydrogen-bonding interactions of daunomycin-type anthracyclines with DNA. Hydrogen bonds are dashed lines. The arrows indicate the directions of proton donation in the hydrogen bonds. Interactions that are conserved throughout the series are highlighted with boldface type.

anthracycline. This continuous hydrophobic zone is highlighted in Figure 5A. Spermine molecules in these complexes form extensive van der Waals contacts with this hydrophobic zone. In addition, in some complexes, the amino groups of spermine form hydrogen bonds to the DNA.

Daun-AT:spermine interactions. In this complex, the spermine molecule is aligned roughly parallel to the sugar-phosphate backbone (Figure 5B), interacting with one strand of the duplex (29). One end of the spermine molecule interacts primarily with pyrimidines and the other end primarily with purines as shown schematically in Figures 6A and 7A. The spermine molecule in this complex forms two direct hydrogen bonds to the DNA. Spermine N10 forms a hydrogen bond to N7 of residue A(9) and spermine N14 forms a hydrogen bond to N7 of G(8). In addition, the spermine molecule forms several indirect hydrogen bonds *via* mediating water molecules to the backbone of the DNA.

The spermine molecule forms van der Waals contacts primarily with the hydrophobic zone described above. Beginning at one end of the spermine molecule, spermine N1 contacts C5 of residue C(11), spermine C2 and C3 contact N4 of residue C(11) and spermine C3 and N5 both contact the 5-methyl group of residue T(10). Spermine C7 and C8 contact O4 of residue T(10). The remainder of the spermine molecule interacts with two adjacent purines. Spermine C9 contacts N7 of residue A(9) while three atoms, N10, C11 and C12 contact N6 of the same residue. Spermine C12 and C13 both contact N7 of residue G(8) and spermine N14 contacts C8 of the same residue. In addition, the spermine interacts with the anthracycline. Spermine C2 is near and C3 of daunomycin although the distances (3.7 Å) exceed van der Waals criteria. However, the proximity is sufficient to exclude water from contact with C1, C2 and C3 of the daunomycin chromophore. In the ternary complex only a single water molecule is in contact with the hydrophobic zone.

Table II. Hydrogen-bonding contacts between DNA and first shell water molecules

Water molecule	DNA atom	distance (Å)	angle (degrees)	to third atom
22W01	C(1)N4	3.16	169	C(1)C4
22W06	C(1)N4	2.57	92	C(1)C4
17W04[1]	C(1)02	2.65	141	C(1)C2
17W08	C(1)05'	3.23	108	C(1)C5'
24W11	G(2)N7	2.61	135	G(2)C8
25W01	G(2)O2P	2.69	133	G(2)P
24W02	G(2)O2P	3.23	125	G(2)P
24W06	A(3)N7	2.61	131	A(3)C8
27W07	A(3)O1P	3.17	123	A(3)P
21W09[2]	T(4)O2	2.91	119	T(4)C2
23W05	T(4)O4	2.95	158	T(4)C4
29W02	C(11)O1P	2.69	156	C(11)P
26W05	C(11)O2P	3.28	118	C(11)P
22W02[3]	G(12)N7	2.83	120	G(12)C8
22W01	G(12)O6	2.48	162	G(12)C6
30W06	G(12)N2	3.26	146	G(12)C2
30W05[4]	G(12)N2	3.28	89	G(12)C2
	G(12)N3	2.60	122	G(12)C2
	G(12)O1'	3.40	146	G(12)C4'
	G(12)O3'	2.74	89	G(12)C3'
31W02	G(12)O2P	2.64	168	G(12)P

1) Water molecule 17W04 is also bound to O13 of 4'-epiadriamycin (Table III, below).
2) Water molecule 21W09 is also bound to N3' and O4' of 4'-epiadriamycin (Table III, below).
3) Water molecule 22W02 is also bound to O4 and O5 of 4'-epiadriamycin (Table III, below)
4) The N2-30W05-N3 angle equals 40°.

Table III. Hydrogen-bonding contacts between 4'-epiadriamycin and first shell water molecules

drug atom	water molecule	distance (Å)	angle (degrees)	to third atom
O13	17W04[1]	3.34	161	C13
O14	18W09	3.25	99	C14
O4	22W02[2]	3.02	129	C4
O5	22W02[2]	3.11	131	C5
N3'	21W09[3]	3.18	109	C3'
O4'	21W09[3]	3.09	115	C4'
	26W07	3.18	107	C4'

1) Water molecule 17W04 is also bound to C(1)O2 (Table II).
2) Water molecule 22W02 is also bound to G(12)N7 (Table II).
3) Water molecule 21W09 is also bound to T(4)O2 (Table II).

Adri-AT:spermine interactions. The detailed conformation and interactions of the spermine molecule bound to the adri-AT complex are different from those in the daun-AT complex. Unlike in the daun-AT complex, the spermine molecule in the adri-MT complex does not form direct hydrogen bonds to the DNA. However, many other general characteristics of the interactions of spermine are similar in the two complexes. In both complexes the spermine molecule forms several water mediated hydrogen bonds to the DNA.

In the adri-MT complex the spermine molecule is aligned roughly perpendicular to the helical axis (Figure 5C), spanning the major groove and interacting directly with both strands of the duplex. This spermine molecule maintains extensive van der

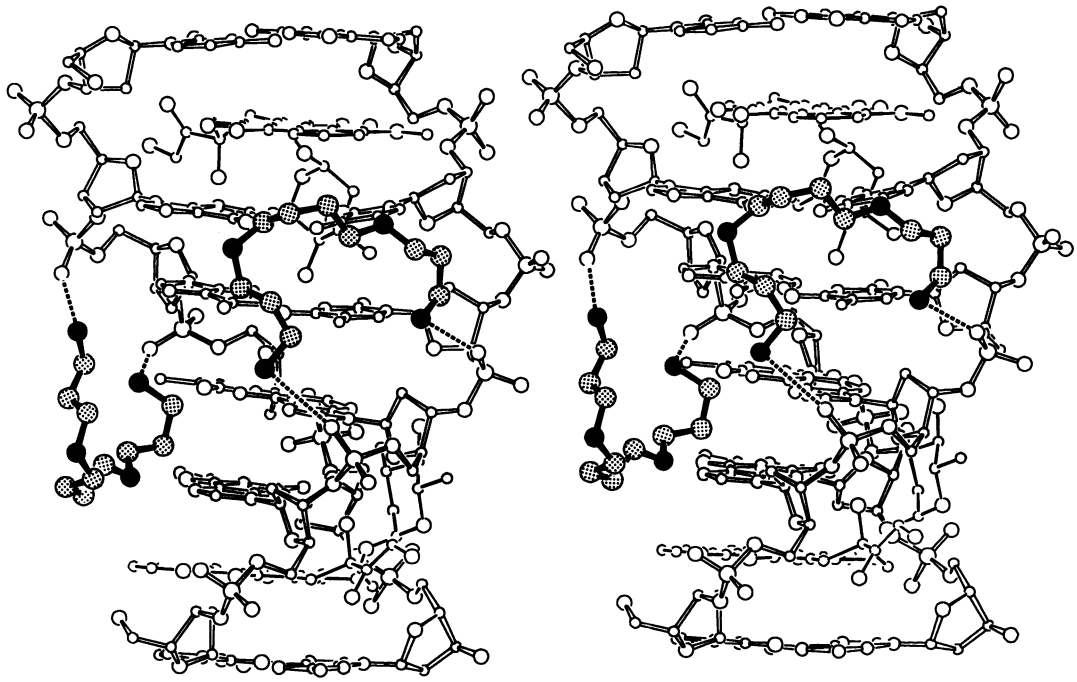

Fig. 4. Stereoview ORTEP representation of spermine molecules bound in the major groove of the epia-AT complex. The DNA is drawn with hollow bonds, 4′-epiadriamycin is drawn with thin solid bonds and spermine is drawn with thick solid bonds. The nitrogen atoms of spermine are black and the carbon atoms are stippled. The hydrogen bonds linking the spermine with the DNA are drawn with dashed lines.

Waals contacts, primarily with the hydrophobic zone (Figures 6B and 7B). Spermine N1 and C2 contact C5 of residue C(11) while spermine C3 and N5 contact 5-methyl of residue T(10). Spermine C8 and C9 contact O4 of the residue T(10) of the same strand of the duplex, while spermine C12 contacts N7 of residue G(2) of the other strand. As in the daun-AT complex, in the adri-AT complex the spermine molecule interacts simultaneously with the DNA and the anthracycline. Spermine C2 contacts C3 of the anthracycline. In the adri-AT:spermine complex, as in daun-AT, only a single water molecule is in contact with the hydrophobic zone.

Epia-AT:spermine interactions. The detailed conformation and interactions of the spermine molecule in the epia-AT complex are different from those in either the daun-AT or adri-AT complexes. In the epia-AT complex, one end of the spermine molecule interacts directly with the deoxyribose-phosphodiester backbone. The molecule arcs towards the center of the major groove and returns to the same backbone (Figures 4 and 5D). The spermine molecule in this complex forms two different hydrogen bonds to the DNA, both from terminal amino groups of spermine to phosphate oxygens of DNA (Figure 4). One hydrogen bond is from N1 to a phosphate oxygen of residue T(10) (2.5 Å) and the other from N14 to a phosphate oxygen of residue A(9) (3.5 Å). The latter hydrogen bond is weak and is slightly longer than our hydrogen bond criteria.

Other interactions of spermine with epia-AT are qualitatively similar to those with adri-AT and daun-AT. This spermine interacts extensively with the hydrophobic zone (Figures 6C and 7C) and essentially wraps around the 5-methyl group of T(10). Spermine N1 contacts 5-methyl of residue T(10) and C2′ of residue A(9). Spermine C2 contacts the phosphate oxygen, 5′O and C6 of residue T(10). Spermine N5 contacts C5 and N4 of C(11) while spermine C6 contacts the same N4. Spermine C13 contacts the 5-methyl of residue T(10). As in the previously described complexes, the spermine interacts simultaneously with the DNA and the anthracycline. Spermine C7 contacts C2 of 4′-epiadriamycin. In the epia-AT complex, no water molecules are in contact with the hydrophobic zone.

DISCUSSION

In three DNA:anthracycline complexes, all with d(CGATCG), spermine binds in the major groove of the DNA. Many general characteristics of the interactions of spermine are consistent throughout this series of complexes even though the detailed conformation and contacts of spermine vary from complex to complex. As described below, the structures of these complexes support the importance of charge−charge interactions in the binding of spermine with the association driven by release of other bound ions. However, hydrophobic contributions to stability are also significant.

Spermine binds near the floor of the major groove in these ternary complexes. X-ray crystallographic studies suggest that mono- and polycations always bind to DNA such that their positive charge resides near the floors of the major or minor grooves. Positive charge is located near the floor of a groove in the three dimensional crystal structures of DNA complexed with (a) intercalators which place positive charge in the major (43) or in the minor groove (29,38,39,42) (b) minor groove binding compounds (44), and (c) spermine in the major groove of A-DNA (28). Theoretical calculations indicate the floors of the grooves are regions of high electronegative potential (45,46). Thus it would appear that favorable charge−charge interactions in the grooves are a predominant factor in stability and conformation of DNA complexes in general. The favorable charge−charge interactions do not require, and seldom accompany, direct hydrogen bonds between positively-charged groups and negatively-charged phosphate groups.

These ternary complexes demonstrate that DNA can

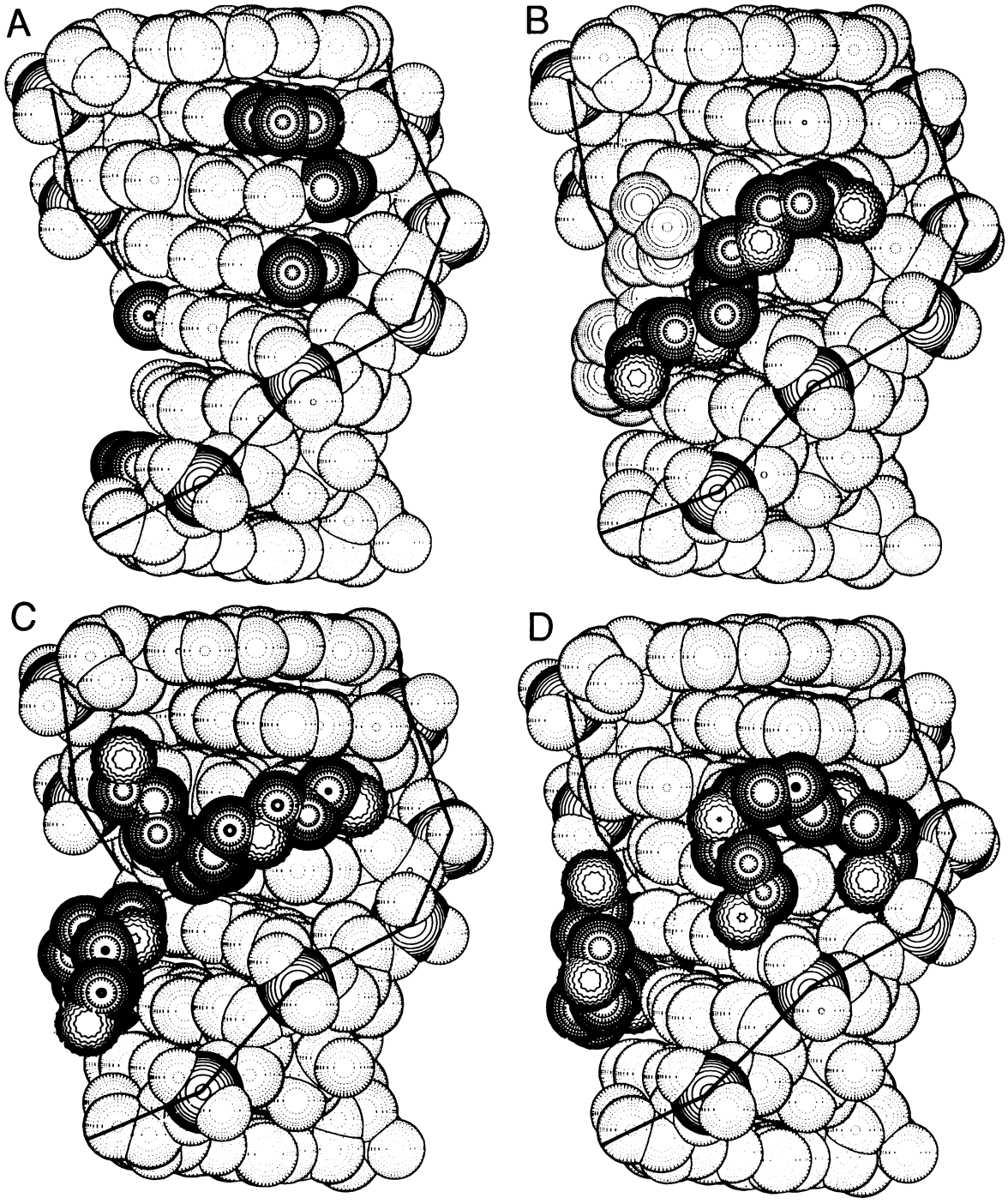

Fig. 5. The hydrophobic zone and three spermine:anthracycline:DNA complexes. Space filling representation of the major groove of (A) the epia-AT complex, minus the spermine molecules, but with the hydrophobic zone highlighted, (B) the daun-AT:spermine complex, (C) the adri-AT:spermine complex, and (D) the epia-AT:spermine complex. Phosphorous atoms are marked with solid circles. In (A) the hydrophobic zone and in (B-D) the spermine carbon atoms are marked with circles with radial lines. The spermine nitrogen atoms in (B-D) are marked with wavy circles. For clarity the spermine molecule on the left side of (B) is marked by lighter, dotted circles. The DNA backbones are traced with solid lines.

accommodate positively-charged ligands simultaneously in both grooves. The minor groove contains the positively charged sugar of the anthracycline and spermine binding there is precluded by charge–charge and steric repulsion. However, charge–charge repulsion between the spermine in the major groove and the anthracycline sugar in the minor groove, through the relatively low dielectric core of the DNA duplex, would be a destabilizing influence on the complex. A constant sum of the attractive and repulsive charge–charge interactions is the probable reason why the total region of space occupied by the two symmetry-related spermine molecules per complex is nearly constant throughout the series of complexes described here.

In addition to charge–charge interactions, hydrophobic interactions (47) contribute to stability of the ternary

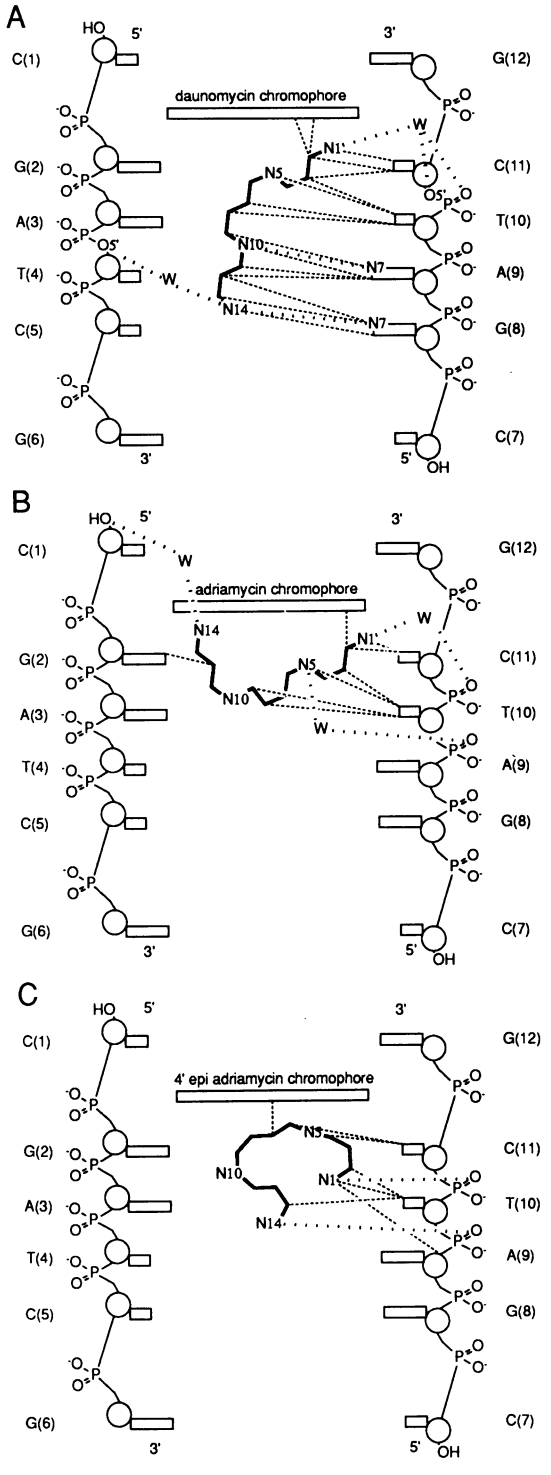

Fig. 6. Schematic diagrams of the spermine molecules bound to DNA:anthracycline complexes. (A) the daun-AT complex, (B) the adri-AT complex, (C) the epia-AT complex. Hydrogen bonds are drawn with dashed lines and van der Waals contacts with dotted lines. Atoms of the DNA that form hydrogen bonds to spermine molecules are identified by atom type. For clarity, van der Waals contacts between the spermine and the backbone atoms are omitted in (C) but are listed in 7C below.

spermine:anthracycline:d(CGATCG) complexes (also see reference 28). In each complex, spermine forms van der Waals contacts with a continuous hydrophobic zone composed of the 5-methyl and C6 of residue T(10), C5 and C6 of residue C(11) and C1, C2 and C3 of the anthracycline (Figure 5A). At least one methylene group of each spermine molecule forms van der Waals contacts with the 5-methyl of a thymine. These contacts exclude water molecules from contact with the hydrophobic zone and with the methylene groups of the spermine. In these ternary complexes, few water molecules (from zero to one per spermine) are in contact with the hydrophobic zone. From a comparison with DNA:anthracycline complexes without bound spermine molecules (39), it appears that 5–7 water molecules are displaced from the hydrophobic zone by a single spermine molecule suggesting considerable stabilization of the complexes by hydrophobic effects. Hydrophobic stabilization of DNA:spermine complexes appears to be general phenomena. The methylene groups of a spermine molecule bound to the deep (*i.e.*, major) groove of an A-DNA octamer also form van der Waals contacts with pyrimidines (28), and presumably exclude water.

It is generally believed that an important component of specificity in biomolecular interactions arises from complementary arrangements of hydrogen bond donors and acceptors. This is especially true in nucleic acid double helices. However, in our short series the interactions of spermine may also be sequence-specific with little or no contribution from hydrogen-bonding. Spermine molecules bind with specificity for the major groove of anthracycline complexes with d(CGATCG) but not for d(CGTACG). Spermine appears to bind to TpC·Drug (5′–3′) steps, but not to ApC·Drug steps. The continuous hydrophobic zone found in the d(CGATCG):anthracycline complexes (Figure 5A) may form a specific spermine binding site to which spermine can bind in a variety of conformations. The types of interactions observed in DNA:spermine complexes are also observed in DNA:protein complexes. When phage 434 repressor binds to its DNA operator, non-polar contacts with methyl groups of thymines make important contributions to recognition (48). Thus specificity in biomolecular interactions arises not only from hydrogen bonds but also from the shapes of hydrophobic surfaces. Spermine does not generally form direct hydrogen bonds to the DNA in these ternary complexes. The number of direct hydrogen bonds in this series of complexes varies from zero to two per spermine molecule. Direct hydrogen bonds between spermine and the DNA do not appear to contribute significantly to stability or specificity of the complexes.

Recently it was proposed that water-mediated hydrogen bonds are important in determining specificity of the *trp* repressor with its DNA operator sequence (49). The highest specificity in water-mediated hydrogen bonds should be achieved when the ligand and/or the DNA form more than one hydrogen bond to the intermediate water molecule. When this multidentate hydrogen-bonding condition is met, both the location of the water molecule and its hydrogen bond donor/acceptor interactions can be restrained in the complex. Under such conditions a water molecule may be considered a surrogate functional group of either the DNA or the ligand. There are two instances where a water molecule acts as a surrogate functional group of 4′-epiadriamycin by forming two hydrogen bonds to the anthracycline and a third hydrogen bond to the DNA (Figure 2). A water molecule binds to both O4 and O5 of the chromophore and also to N7 of residue G(12). A second water molecule binds to both N3′ and O4′ of the anthracycline sugar and also to O2 of residue T(4). Although spermine forms water-mediated hydrogen bonds to deoxyriboses, bases and backbone phosphate groups, these generally do not involve multidentate interactions. Thus the water mediated hydrogen bonds of spermine with DNA do not appear to be sequence-specific.

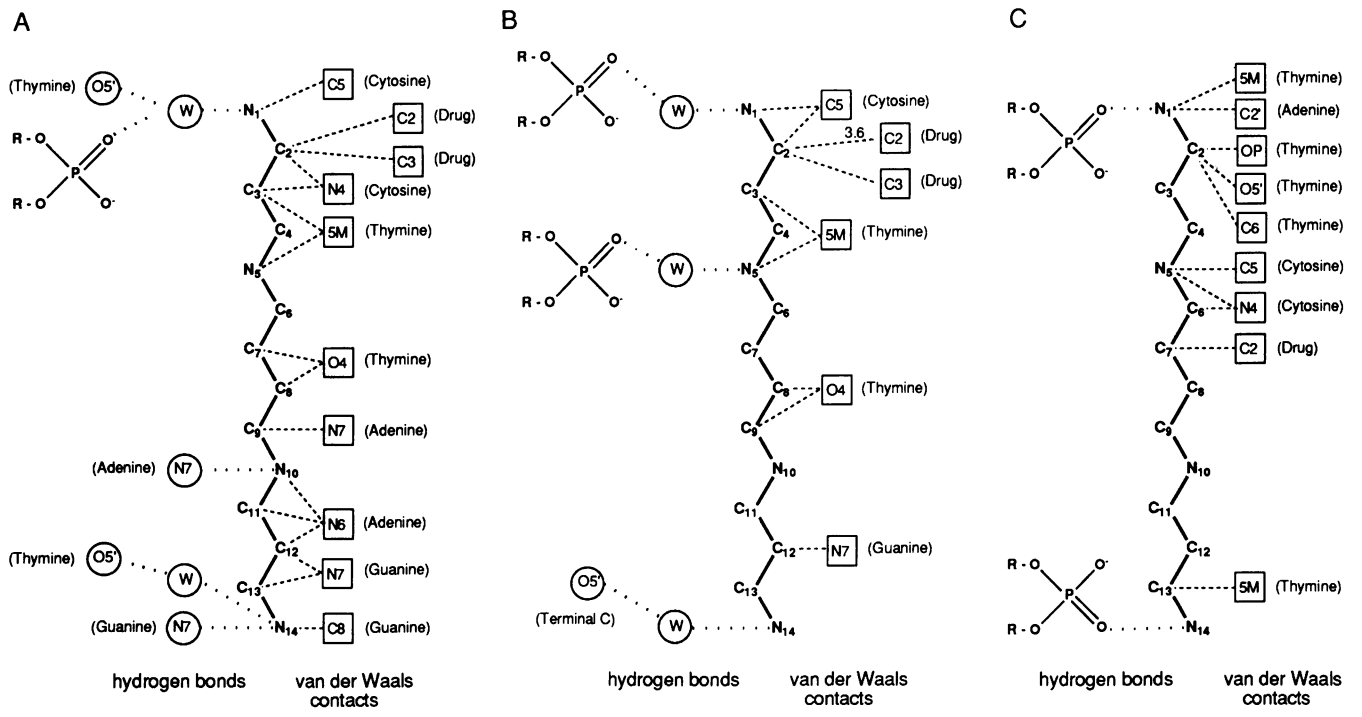

Fig. 7. Schematic diagrams illustrating the interactions of spermine with (A) the daun-AT complex, (B) the adri-AT complex, and (C) the epia-AT complex. Hydrogen bonds are listed on the left and van der Waals contacts are listed on the right of each schematic.

However, water-mediated interactions do influence the conformation and interactions of spermine bound to these DNA:drug complexes. The only covalent differences between the three complexes involve the hydroxyl groups of the anthracyclines. Adriamycin differs from daunomycin by addition of a hydroxyl group to the 14-position and 4′-epiadriamycin differs from adriamycin by inversion of the stereochemistry at the 4′-position. This inversion of stereochemistry swings the 4′-hydroxyl towards the DNA to form a hydrogen bond with the floor of the minor groove. The distinguishing hydroxyls are found in the minor groove, remote from the location of the spermine molecules. However, through a reorganization of the solvent, they are the most likely cause of the differences observed in the spermine molecules. Whether such solvent-mediated effects are related to biological functions and clinical properties of anthracyclines remains an interesting but unanswered question.

It may be possible to resolve the question of whether DNA:spermine complexes are delocalized and dynamic or static and site-specific. The results here suggest a third possibility, that spermine and DNA form complexes that are both site-specific and dynamic. These complexes are clearly site-specific as indicated by the consistency in the interactions with the hydrophobic zone and by the sequence selectivity. However, it would appear that a spermine molecule could easily flip between the conformation in the daun-AT complex and that in the adri-AT and the epia-AT complex, supporting the notion that in solution the complexes are dynamic. The series of crystal structures with and without bound spermine further suggest that as spermine fluctuates in solution, and even dissociates from the DNA, the DNA remains relatively static.

ACKNOWLEDGEMENTS

We thank Drs Nassim Usman and Martin Elgi for helpful suggestions and Stephen Scaringe for synthesis of the DNA. This research was supported by grants from the National Institutes of Health, the American Cancer Society, the National Science Foundation, the Office of Naval Research and the National Aeronautics and Space Administration. LDW is supported by a National Institutes of Health Postdoctoral Fellowship.

REFERENCES

1. Morris,D.R. (1981) In Morris,D.R. and Marton,L.J. (eds), *Polyamines in Biology and Medicine*. Marcel Dekker, New York, pp. 223–242.
2. Pegg,A.E. and McCann,P.P. (1982) *Am. J. Physiol.* **243**, C212–C221.
3. Tabor,C.W. and Tabor,H. (1984) *Annu. Rev. Biochem.* **53**, 749–790.
4. Igarashi,K., Sakamoto,I., Goto,N., Kashiwagi,K., Honma,R. and Hirose,S. (1982) *Arch. Biochem. Biophys.* **219**, 438–443.
5. Meers,P., Hong,K., Bentz,J. and Papahadjopoulos,D. (1986) *Biochem.* **25**, 3109–3118.
6. Tabor,H. (1962) *Biochem.* **1**, 496–501.
7. Mandel,M. (1962) *J. Mol. Biol.* **5**, 435–441.
8. Bloomfield,V.A. and Wilson,R.W. (1981) In Morris,D.R. and Marton,L.J (eds), *Polyamines in Biology and Medicine*. Marcel Dekker, New York, pp. 183–206.
9. Thomas,T.J. and Bloomfield,V.A. (1984) *Biopolymers* **23**, 1295–1306.
10. Gosule,L.C and Schellman,J.A. (1978) *J. Mol. Biol.* **121**, 311–326.
11. Chattoraj,D.K., Gosule,L.C. and Schellman,J.A. (1978) *J. Mol. Biol.* **121**, 327–337.
12. Wilson,R.W. and Bloomfield,V.A. (1979) *Biochem.* **18**, 2192–2196.
13. Widom,J. and Baldwin,R.L. (1980) *J. Mol. Biol.* **144**, 431–453.
14. Crothers,D. and Sen,D. (1986) *Biochem.* **25**, 1495–1503.
15. Sen,D. and Crothers,D.M. (1986) *Biochem.* **25**, 1495–1503.
16. Behe,M.J. and Felsenfeld,G. (1981) *Proc Natl. Acad. Sci. USA* **78**, 1619–1623.
17. Chen,H.H., Behe,M.J. and Rau,D.C. (1984) *Nucl. Acids Res.* **12**, 2381–2389.
18. Braunlin,W.H., Strick,T.J. and Record,M.T., Jr. (1982) *Biopolymers* **21**, 1301–1314.
19. Wemmer,D.E., Srivenugopal,K.S., Reid,B.R. and Morris,D.R. (1985) *J. Mol. Biol* **185**, 457–459.
20. Manning,G.S. (1978) *Q. Rev. Biophys.* **11**, 179–246.
21. Tusboi,M. (1964) *Bull. Chem. Soc. Jpn.* **37**, 1514–1522.
22. Liquori,A.M., Costantino,L., Crescenzi,V., Elia,V., Giglio,E., Puliti,R.,

de Santis Savino,M. and Vitagliano,V. (1967) *J. Mol. Biol.* **24**, 113–122.
23. Feuerstein,B.G., Pattabiraman,N. and Marton,L.J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5948–5952.
24. Zakrzewska,K. and Pullman,B. (1986) *Biopolymers* **25**, 375–392.
25. Feuerstein,B.G., Basu,H.S. and Marton,L.J. (1988) In Zappia,V. and Pegg,A.E. (eds), *Progress in Polyamine Research*. Plenum Press, New York, pp. 517–523.
26. Quigley,G.J., Teeter,M.M. and Rich,A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 64–68.
27. Gessner,R.V., Frederick,C.A., Quigley,G.J., Rich,A. and Wang,A.H.-J. (1989) *J. Biol. Chem.* **264**, 7921–7935.
28. Jain,S., Zon,G. and Sundaralingam,M. (1989) *Biochem.* **28**, 2360–2364.
29. Frederick,C.A., Williams,L.D., Ughetto,G., van der Marel,G.A., van Boom,J.H., Rich,A. and Wang,A.H.-J. (1990) *Biochem.* **29**, 2538–2549.
30. Geiger,L.E. and Morris,D.R. (1980) *J. Bacteriol.* **141**, 1192–1198.
31. Pingoud,A. (1985) *Eur. J. Biochem.* **147**, 105–109.
32. D'Orazi,D., Fracassini,D.S. and Bagni,N. (1979) *Biochem. Biophys. Res. Commun.* **90**, 362–367.
33. Morgan,J.E., Calkins,C.C. and Matthews,H.R. (1989) *Biochem.* **28**, 5095–5106.
34. Pommier,Y., Zwelling,L.A., Kao-Shan,C.-S., Whang-Peng,J. and Bradley,M.O. (1985) *Cancer Res.* **45**, 3143–3149.
35. Hsiang,Y.-H. and Liu,L.F. (1988) *Cancer Res.* **48**, 1722–1726.
36. Acramone,F. and Penco,S., (1988) In Lown,J.W. (ed.), *Anthracycline and Anathracenedione Based Anticancer Agents*. Elsevier, New York, pp. 1–53.
37. Brown,J.R. (1983) In Neidle,S. and Waring,M.J. (eds), *Molecular Aspects of Anti-Cancer Drug Action*. Macmillan Press, London, pp. 57–92.
38. Williams,L.D., Egli,M., Ughetto,G. van der Marel,G.A. van Boom,J.H., Quigley,G.J., Wang,A.H.-J., Rich,A. and Frederick,C.A. *J. Mol. Biol.* in press.
39. Quigley,G.J., Wang,A.H.-J., Ughetto,G., van der Marel,G.A., van Boom,J.H. and Rich,A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7204–7208.
40. Hendrickson,W.A. and Konnert,J. (1981) In Srinivasan,R. (ed.), *Biomolecular Structure, Conformation, Function and Evolution*. Pergamon Press, Oxford, pp. 43–57.
41. Jones,T.A. (1978) *J. Appl. Crystallogr.* **11**, 268–272.
42. Moore,M.H., Hunter,W.N., Langlois, d'Estaintot,B. and Kennard,O. (1989) *J. Mol. Biol.* **206**, 693–705.
43. Williams,L.D., Egli,M., Gao,Q., Bash,P., van der Marel,G.A. van Boom,J.H., Rich,A. and Frederick,C.A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2225–2229.
44. Coll,M., Frederick,C.A., Wang,A.H.-J. and Rich,A. (1987) *Proc. Natl. Sci. USA* **84**, 8385–8389.
45. Lavery,R. and Pullman,B. (1981) *Nucl. Acids Res.* **9**, 4677–4688.
46. Lavery,R. and Pullman,B. (1985) *J. Biomolec. Struct. Dynam.* **2**, 1021–1032.
47. Tanford,C. (1980) *The Hydrophobic Effect*. John Wiley and Sons, New York.
48. Aggarwal,A.K., Rodgers,D.W., Drottar,M., Ptashne,M. and Harrison,S.C. (1988) **242**, 899–907.
49. Otwinowski,Z., Schevitz,R.W., Zhang,R.-G., Lawson,C.L., Joachimiak,A., Marmorstein,R.Q., Luisi,B.F. and Sigler,P.B. (1988) *Nature (London)* **335**, 321–329.
50. Johnson,C.K. (1976) ORTEP II, Oak Ridge National Laboratory, Report RNL-5138, Oak Ridge, Tennessee.