Interactions between an Anthracycline Antibiotic and DNA: Molecular Structure of Daunomycin Complexed to d(CpGpTpApCpG) at 1.2-Å Resolution†

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ABSTRACT: The crystal structure of a daunomycin–d(CGTACG) complex has been solved by X-ray diffraction analysis and refined to a final R factor of 0.175 at 1.2-Å resolution. The crystals are in a tetragonal crystal system with space group $P4_12_12$ and cell dimensions of $a = b = 27.86$ Å and $c = 52.72$ Å. The self-complementary DNA forms a six base pair right-handed double helix with two daunomycin molecules intercalated in the d(CpG) sequences at either end of the helix. Daunomycin in the complex has a conformation different from that of daunomycin alone. The daunomycin aglycon chromophore is oriented at right angles to the long dimension of the DNA base pairs, and the cyclohexene ring A rests in the minor groove of the double helix. Substituents on this ring have hydrogen-bonding interactions to the base pairs above and below the intercalation site. O9 hydroxyl group of the daunomycin forms two hydrogen bonds with N3 and N2 of an adjacent guanine base. Two bridging water molecules between the drug and DNA stabilize the complex in the minor groove. In the major groove, a hydrated sodium ion is coordinated to N7 of the terminal guanine and the O4 and O5 of daunomycin with a distorted octahedral geometry. The amino sugar lies in the minor groove without bonding to the DNA. The DNA double helix is distorted with an asymmetrical rearrangement of the backbone conformation surrounding the intercalator drug. The sugar puckers are C1,C2′-endo, G2,C1′-endo, C11,C1′-endo, and G12,C3′-exo. Only the C1 residue has a normal *anti*-glycosyl torsion angle ($\chi = -154°$), while the other three residues are all in the high anti range (average $\chi = -86°$). This structure allows us to identify three principal functional components of anthracycline antibiotics: the intercalator (rings B–D), the anchoring functions associated with ring A, and the amino sugar. The structure–function relationships of daunomycin binding to DNA as well as other related anticancer drugs are discussed.

The anthracycline antibiotics daunomycin (daunorubicin, Figure 1) and closely related adriamycin (doxorubicin) are currently widely used in cancer chemotherapy (Henry, 1979; Crooke & Reich, 1980). Daunomycin is used for the treatment of acute leukemia, while adriamycin is used in treating various solid tumors. Both compounds have an aglycon chromophore containing four fused rings and an amino sugar (Figure 1). They have been the subject of intensive chemical and biological research since their discovery over 20 years ago. More than 500 compounds of this type have been synthesized or isolated from nature and tested for biological activity (Henry, 1979; Crooke & Reich, 1980). These agents are believed to act by binding to DNA and are known to inhibit both DNA replication and transcription (diMarco et al., 1974; Neidle, 1979). There are indications that the inhibition is associated with a direct interaction of the drugs with DNA (Crooke & Reich, 1980).

Using a highly sensitive microspectrofluorometer, it was possible to record the fluorescence emission spectra of adriamycin at the single-cell level on the basis of the fluorescence properties of the drug (Manfait et al., 1982). In sensitive adriamycin-treated cells, but not with adriamycin-resistant leukemic cells, it can be demonstrated that the drug does not interact with anything during its cytoplasmic transit but it ultimately intercalates in the presence of nuclear DNA in a way similar to that observed when adriamycin is bound to calf thymus DNA (Manfait et al., 1982). This experiment supports the notion that the cellular target of the anthracycline antibiotics is the DNA of the nucleus.

It has been shown in solution studies that the aglycon chromophore intercalates between DNA base pairs (diMarco et al., 1974; Neidle, 1979). However, the complexity of the molecule makes it difficult to discern the exact mode of interaction between daunomycin and DNA. For example, whether the amino sugar resides in the minor groove or the major groove of the DNA double helix when daunomycin is bonded to DNA remained uncertain for a long time.

In solution it has been shown that the binding of daunomycin to DNA probably involves three distinct steps on the basis of the results of equilibrium binding and kinetic studies (Chaires et al., 1985). First step is a rapid outside binding, followed by intercalation, and it is then followed by conformational changes of either drug, DNA, or both. In addition, high-resolution nuclear magnetic resonance (NMR) studies have been carried out on the binding of daunomycin to DNA, including poly(dA-dT), $d(pTpA)_3$, and $d(CGm^5CGCG)$ (Patel & Canuel, 1978; Phillips & Roberts, 1980; Neumann et al., 1985). Daunomycin has also been shown to be a potent effector of the equilibrium between right-handed B-DNA and left-handed Z-DNA (Chaires, 1985).

Several anthracycline antibiotics have been crystallized and their structures determined by X-ray diffraction analysis. These include three crystal forms of daunomycin (Anguili et al., 1971; Neidle & Taylor, 1977; Courseille et al., 1979), carminomycin (von Dreele & Einck, 1977), nogalomycin (Arora, 1983), and steffimycin B (Arora, 1985). All of them showed very similar structures with ring A in a half-chair conformation and with the C8 atom most out of plane in ring A.

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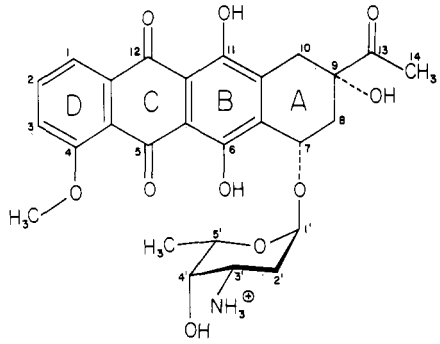

FIGURE 1: Molecular formula of daunomycin. There are four fused rings in the aglycon chromophore; they are unsaturated (B–D) while the semisaturated ring A has an amino sugar attahced. Adriamycin is 14-hydroxydaunomycin.

Some years ago, a model was proposed for the interaction of daunomycin and DNA on the basis of X-ray fiber diffraction data and molecular model building (Pigram et al., 1972). Although this model could explain some features of the drug–DNA interactions, it still left many questions unanswered.

In order to visualize the precise manner in which this important class of anticancer drugs interacts with DNA molecules, we have carried out an X-ray crystal structure analysis on a complex of daunomycin with a self-complementary DNA hexamer fragment, d(CpGpTpApCpG) or d(CGTACG). The structure has been solved and refined to 1.2-Å resolution. A six base pair fragment of double-helical DNA was found with two molecules of daunomycin bound to it, plus 166 water molecules and two hydrated sodium ions. The structure reveals the manner in which the stereochemistry of the antibiotic is required for DNA binding and the way in which DNA accomodates a fairly complex drug molecule such as daunomycin. A preliminary report of this structure at 1.54-Å resolution has been published (Quigley et al., 1980).

Materials and Methods

Oligonucleotide Synthesis and Crystallization. The DNA hexamer d(CGTACG) was synthesized by an improved solution-phase phosphotriester technique in which hydroxybenzotriazole was used as an activating reagent (van der Marel et al., 1981). After the deblocking of the protecting groups, the crude product was loaded onto a Sephadex G-50 column for purification. The final product was judged to be over 95% pure by high-performance liquid chromatography (HPLC) analysis. Daunomycin was purchased from Sigma Chemical Co.

Crystals were grown from a solution containing 2 mM DNA hexamer (single strand), 2 mM daunomycin, 15 mM $MgCl_2$, 10 mM spermine, 30 mM cacodylate buffer (pH 6.5), and 5% 2-methyl-2,4-pentanediol (MPD) by equilibrating with a 30% MPD (v/v) reservoir. After a few weeks, bright red-orange, tetragonal, rod-like crystals appeared. Spectroscopic analysis of the dissolved crystal revealed a 1:1 ratio of hexanucleotide to daunomycin. X-ray diffraction studies indicated a tetragonal crystal system with space group $P4_12_12$ or $P4_32_12$, $a = b = 27.86$ Å, $c = 52.72$ Å. The calculated density of the crystal ($d_{calcd} = 1.37$ g/cm^3) is consistent with the asymmetric unit containing one hexanucleotide and one daunomycin molecule. Intense meridional reflections near 3.3-Å resolution along the c axis strongly suggested that the nucleotide bases were stacked perpendicular to that direction. By dividing the length of the c axis (52.72 Å) by 3.3 Å, we obtained a number close to 16. From this we inferred that there were two complexes of DNA hexamer plus two daunomycins stacked end-over-end along the c axis. Furthermore, it has been shown previously that simple intercalators have a preference for pyrimidine–phosphate–purine site, in particular the CpG site (Tsai et al., 1977; Neidle et al., 1977; Wang et al., 1978). A model was constructed with two daunomycins intercalated between the two CpG sites of the hexamer duplex, as shown schematically in Figure 2A. In this model, the nucleotides are numbered from C1 to G6 in one strand and C7 to G12 in the other strand with C1 based paired to G12 etc. The two daunomycins are numbered D13 and D14. This model was then placed into the lattice ($P4_12_12$) with the molecular 2-fold axis coinciding with the crystallographic 2-fold axis along the diagonals of the tetragonal unit cell. This is shown schematically in Figure 2B where the dotted circles represent the end view of the elongated daunomycin–hexamer complexes.

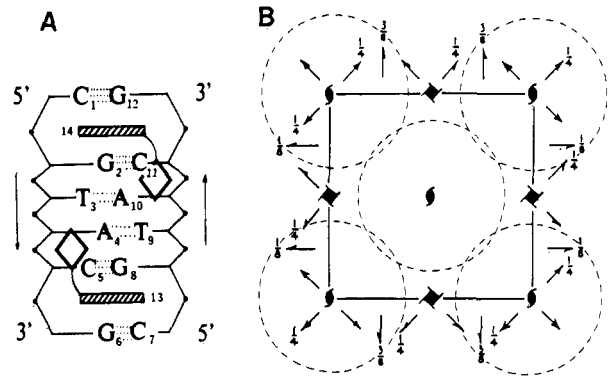

FIGURE 2: (A) Schematic drawing of a side view of the daunomycin–d(CGTACG) complex. The aglycon chromophores of the daunomycin are shown as hatched bars, which are intercalated between the CpG sequences, while the amino sugars are represented by the diamond figures. The nucleotides are numbered C1 to G6 along one strand and C7 to G12 in the complementary strand with C1 base paired to G12 etc. The daunomycins are numbered D13 and D14. There is a 2-fold axis located in the center between the TpA step. The c axis is vertical. (B) Diagrammatic view down the c axis depicts the packing arrangement of the drug–DNA complexes in the unit cell with space group $P4_12_12$. The molecular 2-fold axis coincides with the crystallographic 2-fold axis at $z = 0$, which runs diagonally across the square. The symmetry-related 2-fold axis at $z = {}^1/_4$ relates the two complexes, which are stacked end-to-end along the 2-fold screw axis in the c direction. The solvent channels are in the areas near the 4_1 axes.

Data Collection and Structure Refinement. Initially, three-dimensional data were collected on a Nicolet P3 X-ray diffractometer to a resolution of 1.5 Å at 15 °C in an ω scan mode. The crystal was mounted in a glass capillary tube sealed with a droplet of mother liquor. A total of 3428 reflections were collected, of which 2108 were observed at the $2\sigma(F)$ level. This data set was used for the structure determination. Later, it was discovered that, by a lowering of the temperature of the crystal to −15 °C, the mosaicity of the crystal improved from $\Delta\omega_{1/2} = 0.7°$ to $\Delta\omega_{1/2} = 0.3°$, where $\Delta\omega_{1/2}$ is the peak width of the ω scan at half-height of the peak. This marked improvement in the crystal mosaicity provided a significantly better data set to a resolution of 1.2 Å ($2\theta = 85°$ with Cu Kα radiation). All the reflections in a complete octant ($h \geq 0$, $k \geq 0$, $l \geq 0$) of the reflection sphere out to 1.2 Å were recorded, which amounted to 13 258 reflections, of which 9238 were judged to be observed at the $2.0\sigma(F)$ level. Intensity data were reduced to structure factor amplitudes by use of the Lorentz and polarization correction (Stout & Jensen, 1968). Empirical ψ curve absorption corrections were also applied.

The initial refinement was carried out by a constrained least-squares Konnert–Hendrickson refinement program (Hendrickson & Konnert, 1979) to an R value of 20% with the $2\sigma(F)$ data collected at room temperature. At that stage,

the refined structure revealed all of the atoms of the DNA and the daunomycin plus 40 water molecules in the asymmetric unit. Because daunomycin has a positive charge on the amino group of the amino sugar moiety at neutral pH, this makes the hexamer–daunomycin complex have eight net negative charges. However, we were not able to locate the remaining positively charged counterions in the lattice. This raised the possibility that the actual space group of the crystal may be of lower symmetry, namely, $P4_1$ instead of $P4_12_12$.

With the improved low-temperature data set, we decided to carry out two independent refinements by assuming the space group to be $P4_1$ or $P4_12_12$. These two refinements produced comparable final R factors and indistinguishable final electron-density Fourier maps regardless of the space group. This confirms the correct space group to be $P4_12_12$.

In the final stage of refinement, the root mean square (rms) deviation of the bond distances from the ideal values is 0.034 Å for the nonaromatic bonds and 0.018 Å for the aromatic bonds. The final R factor for the independent 5133 reflections (in space group $P4_12_12$) with $F > 3\sigma(F)$ (F is the structure factor amplitude) in the range 10.0–1.18 Å is 17.5%. In addition to the 83 water molecules, one hydrated sodium ion was found to coordinate with the guanine as well as daunomycin molecule.

The identity of the sodium ion has been assigned on the basis of its surrounding atoms. The electron density at the site of the assigned sodium ion is an isolated, well-resolved peak with a temperature factor of $B = 27.8$ Å^2. It has six atoms within 3.10-Å distance (G6 N7, 2.77 Å; D14 O4, 3.03 Å; D14 O5, 3.10 Å; W3, 2.90 Å; W4, 3.04 Å; W5, 2.77 Å) forming a somewhat distorted octahedral sphere. In the structure of two crystal forms of the sodium salt of the ionophore X537A (lasalocid A), the averaged Na–O distance is 2.5 Å (Wang, 1974). The observed distances are somewhat longer here, indicating weaker coordination interactions. Moreover, the geometry around this peak makes it unlikely that it is a water molecule. It is also unlikely that a magnesium ion would fit in here as the magnesium ion is known to have an even shorter coordination bond (2.0 Å). Consequently, we concluded that the most probable candidate for this peak is a sodium ion. It is possible that a potassium or calcium ion could form a better coordination complex at this site.

Although the spermine molecule is necessary for the crystallization, no spermine molecule could be identified unambiguously in the final electron-density map despite a rather well-refined structure. This suggests that the spermine molecule is disordered in the large solvent channels (10 × 15 Å) along the c axis as shown schematically in Figure 2B. The crystal contains about 40% solvent.

Hydrogen atoms were not included in the refinement; instead, they were generated at their predicted positions on the basis of the type of atoms to which the hydrogen atoms are attached. The final atomic positional and thermal parameters are deposited in Cambridge Data Bank.

RESULTS

Conformation of Daunomycin Molecule. The bond distances and angles of the daunomycin molecule are generally within the limit of accepted values except a few which will be described later. In the aglycon part of the molecule (see Figure 1), the B and D rings are the most aromatic part with an averaged C–C distance of 1.39 Å. The C ring is in a quinonoid structure with O5 and O12 possessing a keto property, while O6 and O11 are of phenolic type. The distances between O5 and O6 and between O11 and O12 are 2.44 and 2.62 Å, respectively. Thus, they presumably from intramolecular hydrogen bonds. The aromatic part of the aglycon is quite planar with a rms distance of 0.03 Å for the least-squares plane calculated from all the atoms of rings B, C, and D without the exocyclic atoms. If all the exocyclic oxygens (O4, O5, O6, O11, O12) as well as C7 and C10 are included in the calculation, the rms distance is 0.035 Å. The methyl group (C21) in the methoxy side chain is also in plane with a deviation of 0.04 Å. The orientation of the methoxy group is such that the methyl group is pointed away from O5 atom and protrudes into the solvent region.

Table I: Selected Torsion Angles (deg) in Daunomycin Molecule[a]

notation	this work	A	B	C
ring A				
C20–C7–C8–C9	−31.6	−47.8	−48.2	−42.8
C7–C8–C9–C10	54.3	62.0	57.9	62.3
C8–C9–C10–C19	−50.7	−45.2	−38.4	−60.5
C9–C10–C19–C20	26.3	17.7	13.8	39.4
C10–C19–C20–C7	−4.6	−4.6	−4.6	−25.4
C19–C20–C7–C8	7.6	20.1	20.4	29.4
glycosyl linkage				
C8–C7–O7–C1′	92.6	117.0	124.9	102.4
C20–C7–O7–C1′	−144.2	−119.5	−113.8	−120.0
C7–O7–C1′–O5′	−86.2	−69.9	−67.5	−88.8
C7–O7–C1′–C2′	143.7	166.9	167.4	161.8
amino sugar				
O5′–C1′–C2′–C3′	−47.6	−49.6	−53.8	−54.9
C1′–C2′–C3′–C4′	41.7	50.1	55.7	61.5
C2′–C3′–C4′–C5′	−46.2	−54.5	−61.2	−64.7
C3′–C4′–C5′–O5′	51.4	59.1	61.0	63.3
C4′–C5′–O5′–C1′	−54.3	−61.2	−58.8	−64.5
C5′–O5′–C1′–C2′	55.2	55.3	56.5	60.0

[a] The atomic coordinates were taken from the following references: von Dreele and Einck (1977) for A; Neidle and Taylor (1977) for B, and Anguili et al. (1971) for C.

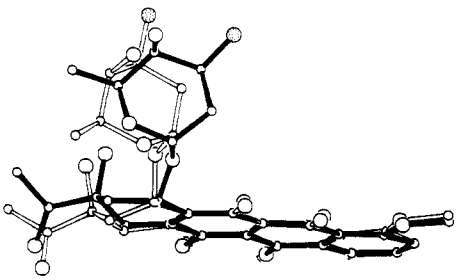

FIGURE 3: Comparison of the conformation of daunomycin in the complex (filled bonds) with the daunomycin cyrstallized by itself [open bonds, coordinates are taken from Neidle and Taylor (1977)]. The two molecules are superimposed by a least-squared fitting with corresponding atoms in rings B–D. It can be seen that ring A has a different conformation in the complex which produces different dispositions of the various substituents of ring A including O7, O9, and the acetyl group. In addition, the torsion angles around the O7–C1′ bond in the amino sugar of the two molecules vary by 20° (see Table I).

The torsional angles of the daunomycin molecule in the complex are listed along with those from three other daunomycin derivatives in Table I. It can be seen that the conformation of the A-ring system of the drug in the complex is rather unusual. The torsional angles around C19–C20 and C7–C20 are −4.6° and 7.6°, respectively. This is associated with the fact that in this ring all the atoms except C9 are almost in plane with a rms distance of 0.03 Å. The C9 atom is displaced by 0.59 Å in the same direction as the amino sugar relative to the plane of the aglycon (see Figure 3). In this conformation O9 is nearly perpendicular or axial to the plane of the aglycon molecule. However, O7 is in a slightly displaced axial position in which the O7 atom is projected further away from the A ring. This has the effect that the O7 and O9 atoms can no longer form an intramolecular hydrogen bond in con-

trast to those observed in other crystal structures of anthracycline antibiotics.

The torsion angle in the glycosyl linkage of the daunomycin molecule is also different from those found in other related structures. The value in C7–O7–C1′–C2′ is 143.7°, which is significantly lower than that of the bromo derivative of daunomycin (161.8°) (Anguili et al., 1971), daunomycin pyridine salt (167.4°) (Neidle & Taylor, 1977), and carminomycin (166.9°) (von Dreele & Einck, 1977). It should be noted that this low torsional angle provides a favorable conformation between the aglycon and the amino sugar such that they form a right-handed chiral molecule. The difference in conformation between daunomycin in this complex and other daunomycins crystallized by themselves can be seen in Figure 3, which compares two daunomycins with a least-squares superposition of the aglycon parts.

The amino sugar is in a chair conformation with all the side chains pointed away from the aglycon. However, it is interesting to note that the torsional angles about the C1′–C2′ and C2′–C3′ bonds in the six-membered ring are lower than the expected ideal gauche values (near 60° or −60°). The C1′–C2′–C3′–C4′ and O5′–C1′–C2′–C3′ angles are 41.7° and −47.6°, respectively. These low values may be associated with the fact that the C2′ atom has a short van der Waals contact with the DNA molecule. This will be discussed later.

Overall Conformation of Drug–DNA Complex. As shown diagrammatically in Figure 2A, two daunomycin molecules intercalate their aglycon chromophores between the CpG sites at both ends of the hexamer duplex. Figure 4 shows a series of van der Waals diagrams of the daunomycin–DNA hexamer complex. In Figure 4A, the drug molecules have been removed to show the vacated intercalation sites. Here, the molecular 2-fold axis is horizontal in the plane of the paper. The minor groove of the double helix is on the upper left side of the figure, whereas the major groove is on the lower right side of the figure. In Figure 4B, the daunomycin molecules (shaded) fill the minor groove of the double helix with their amino sugars while the aglycon chromophores intercalate between two GC base pairs with ring D protruding well into the major groove. This can be seen clearly in Figure 4C,D. Figure 4C is a view looking along the molecular 2-fold axis into the major groove where ring D of the daunomycin molecules hangs out into the solvent region. The central four nucleotides remain in a B-DNA-like conformation with their base pairs exposed to solvents. On the contrary, the minor groove of the hexamer duplex is almost completely occupied by the amino sugars of the daunomycin molecules as shown in Figure 4D. Only the two central AT base pairs are partially accessible to the solvent region, whereas the inner two GC base pairs are completely blocked. The hydrophilic (hydroxyl and amino) functional groups of the amino sugar can be seen pointing away from the DNA molecule and projecting into the solvent regions. Figure 5 shows the stereoscopic skeletal drawings of the complex viewed from different orientations.

Conformation of DNA. The DNA molecule in the complex adopts a distorted right-handed double-helical structure with Watson–Crick base pairing. The molecule possesses a 2-fold axis relating the two strands of the DNA molecules. This 2-fold axis coincides with the crystallographic dyad axis. There are two daunomycin molecules that interact with the DNA at either end of the duplex at the CpG sites.

The torsional angles of the sugar–phosphate backbone in the DNA molecules are listed in Table II along with those of other conformations for comparison. It can be seen that they in general fall in the region of a B-type DNA conformation

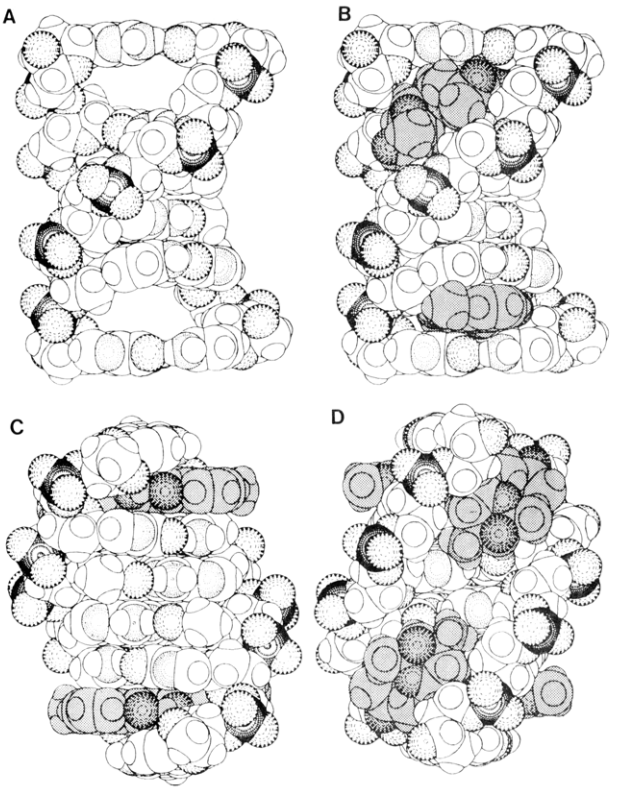

FIGURE 4: van der Waals diagrams of the daunomycin–d(CGTACG) complex. (A) DNA hexamer alone. This is viewed perpendicular to the molecular 2-fold axis, which lies horizontally in the plane of the paper. The minor groove of the distorted right-handed B-DNA is at the upper left of the figure, while the major groove is at the lower right. (B) The drug–DNA complex viewed from the same direction as in (A). The amino sugar of the daunomycin (shaded) fills the minor groove of the double helix. Ring D of the intercalated aglycon chromophore skewers through the base pairs and protrudes into the major groove. (C) A view looking down the 2-fold axis from the major groove. The degree of penetration of the aglycon ring through the base pairs is evident. (D) A view looking down the 2-fold axis from the minor groove. The amino sugars of the daunomycins largely fill the minor groove of the double helix. Note that the disposition of the sugar relative to the aglycon keys the daunomycin for a right-handed helix.

with modifications in the local area where it accommodates the drug molecules. The phosphodiester linkage along the DNA backbone have the following conformations going from the 5′ to 3′ direction: $g^-g^--tg^--g^-g^--g^-g^--tg^-$, where t and g^- stand for trans and gauche$^-$, respectively (Saenger, 1984). For comparison, idealized B-DNA and A-DNA have a g^-g^- conformation.

It is interesting to note that the trans–gauche$^-$ conformation in the G2-p-T3 step is associated with a change in the β angle of T3 (β = 138.5°), while that of the C5-p-G6 step is associated with a change in the ϵ angle of C5 (ϵ = −104.3°). Apart from these deviations, it is quite striking that the DNA sugar–phosphate backbone maintains a fairly uniform range of conformation throughout the molecule which has been perturbed by the rather complex daunomycin molecule. The averaged torsion angles along the DNA backbone are α = −68° (5°), β = 176° (7°), γ = 45° (5°), δ = 137° (9°), ϵ = −156° (11°), and ζ = −81° (6°), excluding the two angles mentioned above.

The phosphate groups on either side of the daunomycin aglycon have different conformations. This is associated with the asymmetric shape of the daunomycin molecule. When the daunomycin molecule intercalates between the base pairs in DNA, it creates a nonsymmetric environment around the daunomycin molecule due to the skewered orientation of the

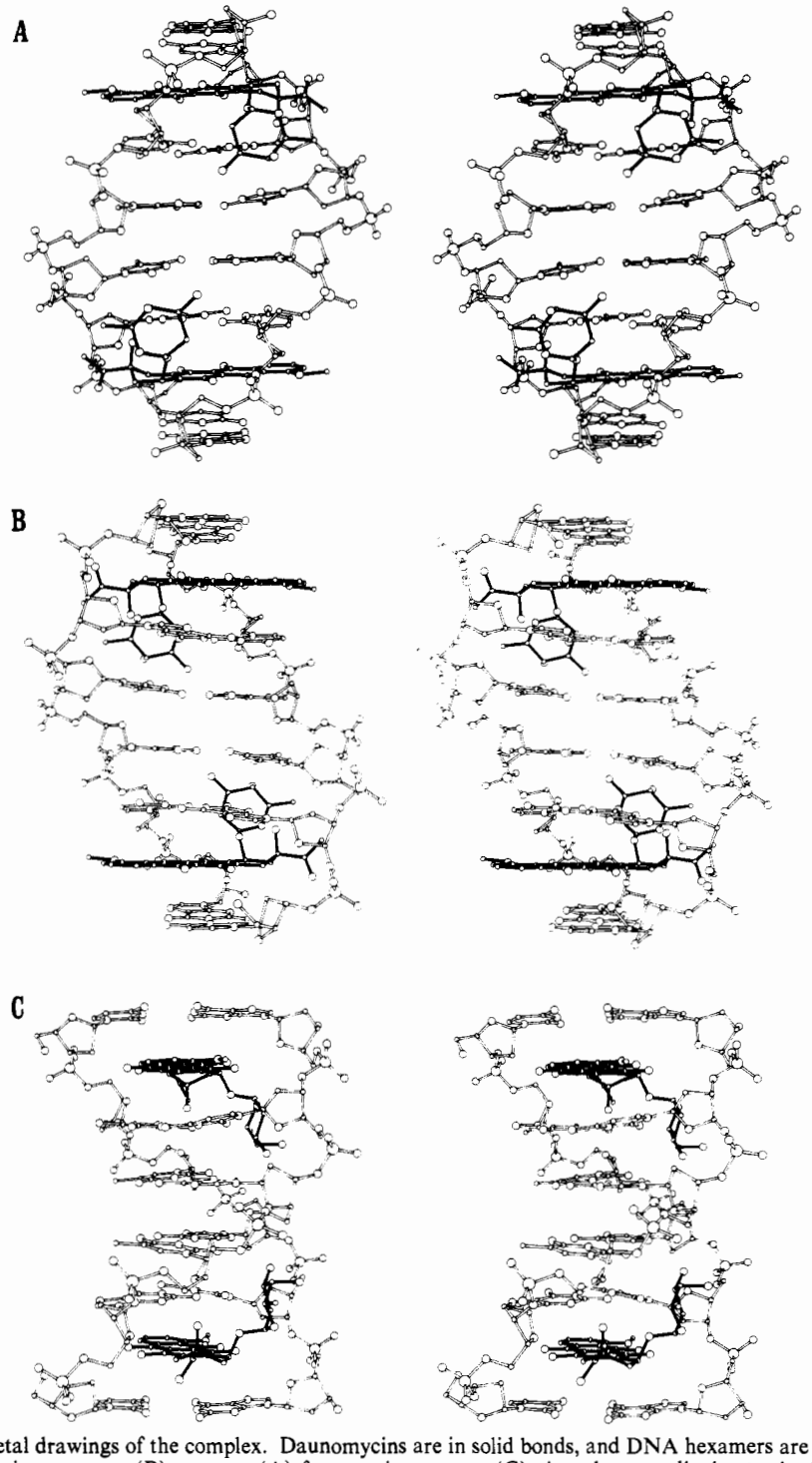

FIGURE 5: Stereoscopic skeletal drawings of the complex. Daunomycins are in solid bonds, and DNA hexamers are in open bonds. (A) Viewed down the 2-fold axis from minor groove; (B) same as (A) from major groove; (C) viewed perpendicular to the 2-fold axis.

Table II: Torsion Angles (deg) along Oligonucleotide Backbone and Glycosyl Angle (deg)[a]

	torsion angles						
	α	β	γ	δ	ϵ	ζ	χ
residue							
C1			45.5	141.6	−131.7	−67.5	−154.2
G2	−68.8	175.9	36.4	146.1	−149.8	177.5	−85.6
T3	−49.1	138.5	50.7	116.1	−173.6	−84.7	−136.1
A4	−81.3	−177.8	59.3	131.5	−169.2	−90.7	−102.4
C5	−61.3	173.0	31.5	143.3	−104.3	171.5	−87.7
G6	−67.7	174.0	47.3	144.3			−86.1
B-DNA[b]	−63	171	54	123	−169	−108	−117
B-DNA[c]	−41	136	38	139	−133	−157	−102
A-DNA[c]	−90	−149	47	83	−175	−145	−154

[a] Torsion angles along the backbone of the polynucleotide are defined as $P\overset{\alpha}{-}O5'\overset{\beta}{-}C5'\overset{\gamma}{-}C4'\overset{\delta}{-}C3'\overset{\epsilon}{-}O3'\overset{\zeta}{-}P$ and χ is the glycosyl angle. [b] From Dickerson and Drew (1981). [c] From Arnott et al. (1980).

Table III: Sugar Conformations[a]

residue	pucker	type	P	τ_m	τ_1	τ_2	τ_3	τ_4	τ_0
C1	C2′-endo	C2′-endo	156.9	37.3	37.0	−34.1	21.2	3.0	−25.9
G2	C1′-endo	C3′-exo	151.5	46.3	44.5	−40.7	22.4	9.8	−36.2
T3	O1′-endo	C2′-endo	104.8	29.7	22.9	−7.6	−10.7	25.4	−29.7
A4	C2′-endo	C2′-endo	146.9	35.4	36.8	−29.7	15.1	8.5	−29.4
C5	C1′-endo	C2′-endo	135.6	41.3	43.3	−29.5	11.0	17.7	−39.1
G6	C3′-exo	C2′-endo	181.8	33.8	27.2	−33.8	28.7	−11.2	−9.5

[a] P and τ_m are pseudorotation angle and amplitude, respectively, whereas τ_0 and τ_4 are the internal torsion angles of the deoxyribose ring as defined in Saenger (1984).

aglycon chromophore in the double helix. The daunomycin molecule has two sides with respect to the long direction of the molecule; one side has the bulky amino sugar moiety while the other side consists of exocyclic oxygen atoms only. The phosphate group facing the amino sugar side has a phosphodiester conformation of trans–gauche$^-$ while the other phosphate remains at a more regular gauch$^-$–gauche$^-$ conformation.

The pucker of the six deoxyribose rings is generally in the C2′-endo class. The detailed conformational parameters of the deoxyriboses in the DNA molecule is listed in Table III. The actual puckers are as follows: C1, C2′-endo; G2, C1′-endo–C3′-exo; T3, O1′-endo–C2′-endo; A4, C2′-endo; C5, C1′-endo–C2′-endo; G6, C3′-exo–C2′-endo. Thus, there is no simple mixed pucker pattern of C3′-endo(3′–5′)C2′-endo type found in the nucleotides on either side of the simple intercalators (Wang et al., 1978).

The two nucleotides (T3 and A4) in the center of the molecule adopt slightly different sugar conformations. The deoxyribose ring of T3 has a O1′-endo pucker with pseudorotation parameters $P = 104.8°$ and $\tau_m = 29.7°$ (Saenger, 1984). This is to be compared with the values of $P = 150°$ and $\tau_m = 32°$ in B-DNA. The deoxyribose ring of A4 has a more regular pucker of C2′-endo with $P = 146.9°$ and $\tau_m = 35.4°$. Thus, it appears that the conformation of the T3-A4 nucleotide pair is also affected by the insertion of the daunomyocin chromophore into the dCpG sequence one base pair away. However, one cannot rule out the intrinsic sequence-dependent conformation associated with dTpA sequence. It has been shown that the dT-dA nucleotide has different conformations in poly(dA-dT) from several solution and crystallographic studies (Viswamitra et al., 1978; Shindo et al., 1979; Vorlickova & Kypr, 1985).

In this complex the glycosyl χ values of the DNA molecule, which measure the rotation of the base around the sugar, vary somewhat. In B-DNA, χ is about −117°; in A-DNA it is −154°. Here the χ angles are as follows: C1, −154.2°; G2, −85.6°; T3, −136.1°; A4, −102.4°; C5, −87.7°; G6, −86.1°. It can thus be seen that the DNA backbone uses a nonsymmetrical mechanism to adopt to the complex daunomycin molecule. In the backbone on the C1pG2 side, the 5′ deoxycytidine residue changes the glycosyl angle from a high anti (−117° in B-DNA) to a low anti value (−154.2°). At the same time, by adjusting the ϵ angle from a near trans (−169° in B-DNA) to a somewhat lower value (−131.7°), it allows the adjacent bases to separate from 3.4 to 6.8 Å.

On the C5pG6 side, both nucleotide units maintain the glycosyl angles at high anti values. But, by changing the ϵ value from −169° to a near gauche (−104.3°) conformation in the C5 residue, it is possible to separate the neighboring C5-G6 bases to 6.8-Å distance. This can be achieved by coupling it with the rotation of the phosphodiester linkage from a normal gauche$^-$–gauche$^-$ conformation to a trans–gauche$^-$ one.

The base pairs in this complex also have some distortions. For example, the dihedral angle between G2 and C11 is 18°,

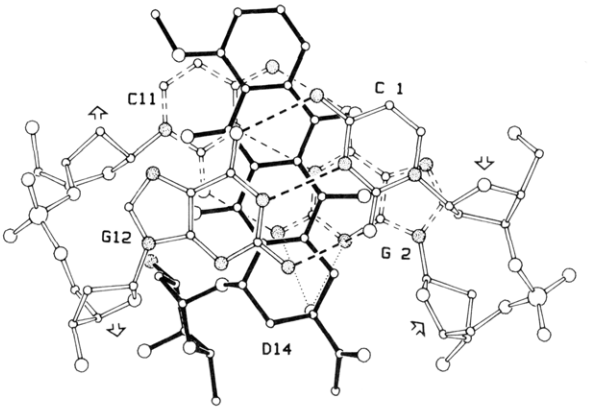

FIGURE 6: View of the daunomycin molecule (dark bonds) and its surrounding nucleotides from a direction perpendicular to the base plane. The adjacent C1-G12 base pair is closer to the viewer, while the G2-C11 base pair is further away. The conformation of the DNA backbone is not symmetrical in the two strands of the duplex. The center of the lower G2-C11 base pair is moved up toward the major groove relative to the C1-G12 pair. This is partly due to the overcrowding of the amino sugar with the C11 residue. Arrows are used to show the compression and expansion of the DNA backbone.

and it is buckled, not propeller twisted, in the center of the base pair.

This structure clearly illustrates the flexibility of the DNA backbone, which can be used to accommodate a complex intercalator. In previous crystal structures of simple intercalator–nucleic acid complexes, the nucleic fragments were dinucleoside monophosphate without exception (Tsai et al., 1977; Neidle et al., 1977; Wang et al., 1978). This raises a question concerning the possible end effects of such short nucleic acid fragments.

Interactions between DNA and Daunomycin. A view showing the stacking interactions of the anthracycline chromophore perpendicular to the bases is presented in Figure 6. This shows the manner in which the aglycon chromophore skewers two adjoining base pairs, lying almost at right angles to the long axis between the base pairs. The stacking of the bases upon each other is shown in Figure 7. In Figure 7a the stacking of the C·G pair over the A·T pair is shown. This is somewhat similar to that anticipated for a B-DNA purine–pyrimidine sequence, but there is significant deviation from the pseudo 2-fold symmetry relating the backbones of the B-DNA molecules. Furthermore, the "helix axis" of the molecule is not in the same position in the two base pairs as would be expected for a perfect B-DNA conformation, and the G2-C11 base pair appears to be translated toward the major groove relative to T3-A10 by about 1.3 Å. This represents an alteration in the base stacking one removed from the intercalative site. An opposite translation of the "axis" of the G2-C11 base pair relative to the C1-G12 pair is also seen around the intercalator in Figure 6. It appears as if daunomycin binding has "pushed" the G2-C11 base pair away from its unperturbed position. The stacking of the A.T base

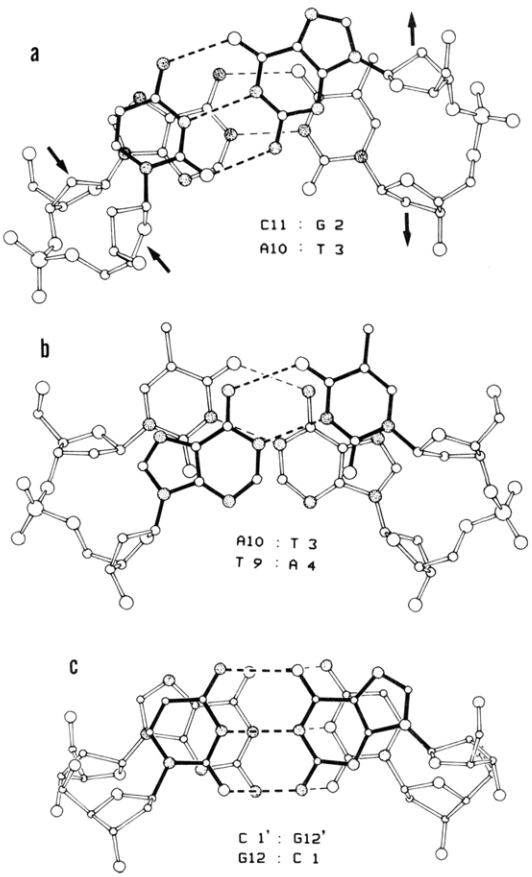

FIGURE 7: Base-pair stacking. (a) The G·C base pair over the A·T base pair. Note the compression of the backbone at the A10-p-C11 step on the left side (arrows). This compensates the opposite movement of the C11-p-G12 step observed in Figure 6. (b) The A·T base pair over the 2-fold-related T·A base pair at the center of the duplex. There is very little inter- and intrastrand base overlap here. (c) The terminal C·G base-pair stacking over the crystallographic 2-fold related G·C base pair from an adjacent duplex.

pair over the two-fold related T.A base pair at the center of the molecule is shown in Figure 7b. This pair lies around the dyad axis and shows stacking typical of pyrimidine–purine sequence in B-DNA. It can be seen that there are very little interstrand as well as intrastrand base–base overlaps typical of a T_pA step in B-DNA. Figure 7c shows the stacking between two molecules in which the C-G end base pair of one molecule is stacking over the end G-C base pair of its neighbor along the *c* axis. The base pairs are stacked over each other in a relatively parallel fashion with large intrastrand base–base overlap.

The unwinding angle is the extent to which adjacent base pairs are unwound at other than the 36° associated with unperturbed B-DNA. In this structure, the unwinding angle for the two base pairs on either side of the intercalator is close to 0° (Figure 6); the next two base pairs (Figure 7a) have an unwinding angle of approximately 8°. This is associated with a distorsion of the backbone that appears to be more extended in the strand on the right side and slightly compressed in the left strand. An opposite effect of extension and compression is seen around the intercalator (Figure 6). Therefore, the total unwinding of the DNA double helix is estimated to be about 8° per daunomycin molecule. It is interesting that the modifications in twist are not found around the intercalation site but rather are one base removed.

One of the striking features of the anthracycline antibiotics is that changing a number of constituents produces only moderate or no influence on its biological activity. However,

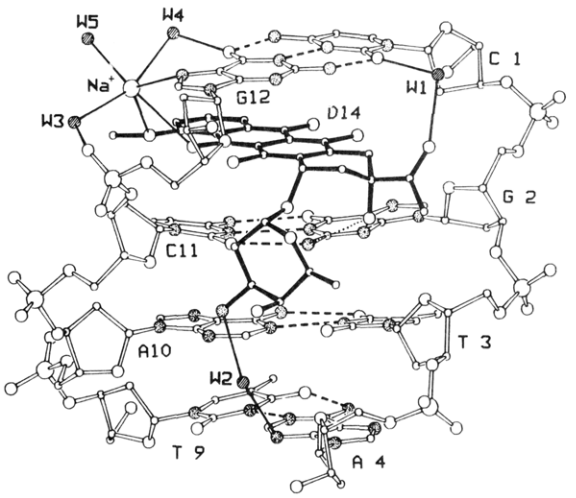

FIGURE 8: Diagram of daunomycin (D14) intercalated into d-(CGTACG) showing intermolecular interactions. Four base pairs of the hexamer are shown. Hydrogen bonds in the base pairs are represented by dashed lines. O9 of daunomycin forms two hydrogen bonds to base G2 by donating one hydrogen to N3 atom while receiving one from N2. There are two bridging waters (W1 and W2) that form hydrogen bonds between O13 of daunomycin and O2 of C1 and between N3′ of daunomycin and N3 of A4, respectively. A sodium ion is found to coordinate to N7 of G12, O4 and O5 of daunomycin, and three water molecules forming a distorted octahedral arrangment in the major groove and further stabilizing the complex.

some substituents seem to be important. This is particularly true of the hydoxyl group on C9, which appears to be essential for activity. It is thus of great interest to find that this hydroxyl oxygen O9 is within hydrogen-bonding distance of two nitrogen atoms (N3 and N2) of guanine base G2. The distance between O9 and N3 is 2.61 Å, indicating a strong hydrogen bond. In addition, the amino group N2 of the same guanine is also withing good hydrogen-bonding distance of O9 (2.91 Å). This proton on guanine N2 is normally found with a water molecule bonding to it. As there is no room for a water molecule here, the presence of O9 within hydrogen-bonding distance suggests that a hydrogen bond is made in this case. Therefore, this essential O9 hydroxyl group is involved with two hydrogen bonds to a guanine residue adjacent to the chromophore: donating one to N3 and receiving one from N2. This type of paired hydrogen bonding is quite specific for hydroxyl groups, and it could be a model for the interaction of the serine side chain of proteins with DNA, for example.

Another hydrogen-bonding system is seen on the other side of the aglycon ring involving the C13 carbonyl oxygen (Figure 8). This carbonyl oxygen is within hydrogen-bonding distance to a bridging water molecule (W1), which itself is hydrogen bonded to carbonyl O2 of the cytosine ring C1 in the base pair above the intercalator. The angle between the carbonyl oxygen O13, the water molecule, and the carbonyl O2 of C1 is 107°, very close to that which is normally found for water molecules. This water molecule, which appears strongly in the electron-density map, may also stabilize insertion of the unsaturated chromophore into DNA. The detailed geometry of daunomycin ring A thus appears to play an important role in its interaction with the minor groove of the double helix.

The amino group on the amino sugar ring has been implicated in electrostatic interactions between the daunomycin and the phosphate group of the nucleic acid (Gabbay et al., 1976). In this complex, however, we do not observe such direct interactions. Instead, the sugar N3′ atom of the daunomycin molecule is strongly hydrogen bonded to two water molecules, the W2 and W13, and possibly to the O2 of residue C5 (C11) (Tables IV and V). The water molecule W2 shows up

Table IV: Hydrogen Atoms in Close Contact between Daunomycin and DNA

DNA	daunomycin	distance (Å)	DNA	daunomycin	distance (Å)
C1 O2	H1C10	2.72	A4 HC2	N3′	2.96
C1 H1C2′	HO11	2.97	A4 HC2	H1N3′	2.11
G2 N3	H2C10	2.76	C5 O2	H1C2′	2.36
G2 N2	HC3′	2.71	C5 O2	H1C3′	2.56
G2 O1′	H2C10	2.44	C5 O2	H3N3′	2.62
G2 H1N2	O9	2.31	C5 C1′	H2C2′	2.92
G2 H1N2	O7	2.71	C5 O1′	H3N3′	2.38
G2 H2N2	HC3′	2.35	C5 HC1′	O5	2.95
G2 H2N2	H3N3′	2.43	C5 HC1′	O6	2.79
G2 HC4′	C14	2.98	C5 H1C2′	O5	2.57
G2 HC4′	H1C14	2.26	C5 HC1′	HO6	2.26
G2 HC4′	C13	2.84	C5 HC1′	H2C2′	1.99
G2 HC4′	O13	2.74	G6 N2	H1C10	2.97
T3 O2	HC4′	2.61	G6 N3	HC7	2.58
T3 O2	H2N3′	2.97	G6 O1′	HC7	2.95
T3 O1′	H2C14	2.81	G6 O1′	HC1′	2.52
T3 C5′	H2C14	2.91	G6 C4′	HC1′	2.52
T3 H1C5′	C14	2.94	G6 H1C5′	C2′	2.89
T3 H1C5′	H2C14	2.00	G6 H1C5′	H2C2′	2.23
A4 N3	H1N3′	2.97	G6 H1C5′	HC1′	2.41
A4 N3	H2N3′	2.93	G6 H1N2	H1C8	2.43
A4 C2	H1N3′	2.82			

Table V: First-Shell Water Molecules

water	complex	distance (Å)	water	complex	distance (Å)
W1	C1 O2	2.73	W20	C1 N4	2.90
W1	D O13	2.88	W21	G6 O2P	2.89
W2	A4 O1′	3.32	W23	T3 O2P	2.34
W2	A4 N3	3.07	W24	C1 O5′	3.03
W2	D N3′	2.69	W25	G6 N2	3.12
W7	G6 N3	2.92	W27	D O4′	3.20
W8	A4 O2P	2.65	W29	D O12	2.78
W9	T3 O1P	2.78	W30	G6 O1P	2.87
W10	G2 O2P	3.10	W31	D O5′	2.85
W11	A4 N7	2.93	W32	G2 O6	3.30
W12	C5 N4	2.76	W33	T3 O1P	3.26
W13	D N3′	2.80	W36	G2 N7	2.70
W14	A4 O1P	2.66	W39	G2 O1P	3.17
W14	A4 O5′	3.32	W40	C5 N4	3.27
W15	T3 O4	2.72	W44	C5 O1P	3.22
W16	C5 O1P	2.89	W44	G6 O3′	3.20
W16	C5 O5′	3.32	W48	C5 O20	2.84
W17	D O4′	2.75	W50	C5 O2P	2.59
W17	D O5′	3.03	W57	D O4′	3.35
W18	A5 O2P	2.55	W60	G6 O3′	2.98
W19	C1 O5′	2.73	W67	G2 O2P	2.40

strongly in the electron-density map with a temperature factor of $B = 23.4$ Å^2, indicating a tightly bonded water molecule, and it is within hydrogen-bonding distance to O1′ (3.32 Å) and N3 (3.07 Å) atoms of nucleotide A4. Although the distance between W2 and N3 of A4 is somewhat long and the angle between the W2–N3 vector and the best plane of the adenine base of A4 is 142°, there are only the three hydrogen-bonding donors and acceptors around W2. It is likely N3′ is at least weakly hydrogen bonded to N3 of A4. O1′ of deoxyribose is usually a somewhat "inert" atom in the DNA molecule in that the O1′ atoms do not participate in hydrogen-bonding interactions. However, since there is no other water in the vicinity of either O1′ or N3 of the A4 residue, it is reasonable to believe that this water molecule is held at that position with weak hydrogen-bonding interactions.

Another important interaction involves a sodium ion that is coordinated to the N7 position in deoxyguanosine G12. This sodium ion appears to have a somewhat distorted octahedral coordination around it is shown in Figure 8. The six coordination sites are the G12 N7 (2.77 Å), O4 (3.03 Å), and O5 (3.10 Å) of daunomycin and three water molecules, W3, W4, and W5 (Figure 8). It should be pointed out that the water molecules W3 and W4 stabilize the position of the sodium ion

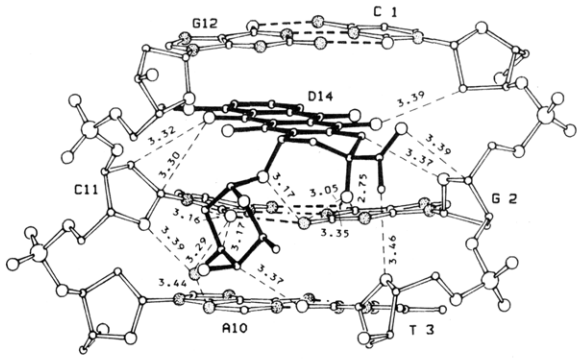

FIGURE 9: Diagram showing the close van der Waals contacts (<3.5 Å) between daunomycin and DNA.

by bridging to atoms in the DNA molecule. W3 is hydrogen bonden to O6 of the guanine base of the G12 residue, while W4 is only 2.23 Å from O1P of the G12 residue, indicating a strong hydrogen bond.

Thus the complex is stabilized by several hydrogen-bonding interactions between daunomycin and the DNA molecule. In the major groove, a coordination-involved sodium ion and bridging waters are used. In the minor groove, two types of hydrogen bonds are found. One is the direct hydrogen bond between the O9 hydroxyl daunomycin and the N3 and N2 atoms of the guanine ring, while the other type involves bridging waters.

In addition, there are several nonbonded van der Waals interactions (Figure 9, also listed in Table IV) that may be important in stabilizing the complex. The hydrogen atom H′ at the equatorial position of the C10 atom of daunomycin approaches the O1′ of G2 at a distance of 2.44 Å. The H3′ atom of the amino sugar of daunomycin has a close contact (2.62 Å) to O2 of C11, while the distance between H1′ and O1′ of G12 is 2.52 Å. Several other close contacts are between the hydrogen atoms of daunomycin and DNA. For example, the distance between H2 of daunomycin C2′ and H1′ of C11 is 1.99 Å. Figure 9 summarizes all the van der Waals contacts (<3.5 Å) involving non-hydrogen atoms between DNA and daunomycin. Table IV lists many of the close proton–proton distances between daunomycin and the DNA molecule. These distances can be used to evaluate the nuclear Overhauser effect (NOE) in the nuclear magneic resonance (NMR) study of

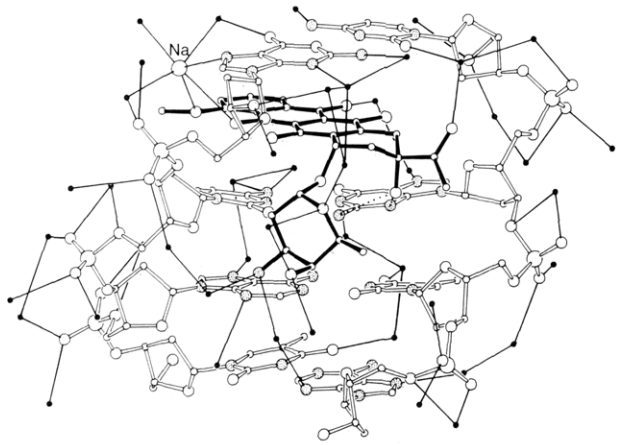

FIGURE 10: Water molecules in the first hydration shell of the complex are shown. They are represented as small solid circles connected with hydrogen bonds (thin lines) to other atoms.

the daunomycin–DNA complex in solution.

Solvent Molecules. There are 83 water molecules that have been located during the structure refinement. Some of these water molecules have high temperature factors ($B > 50$ Å^2), and they are in general located in the large solvent channels. They are likely to be disordered. However, most of the first-shell hydration water molecules are very well ordered. Table V summarizes all the first-shell water molecules. They can be seen clearly from the final electron-density map in which well-resolved solvent peaks are hydrogen bonded to various hydrophilic sites on DNA molecules. In particular, the water molecules that are hydrogen bonded to base pairs tend to be more ordered than those hydrogen bonded to sugar–phosphate backbone. This observation has also been found in the high-resolution structure of different Z-DNA crystals (Wang et al., 1979; Wang & Rich, 1985; Ho et al., 1985).

The G2-C11 base pair is devoid of hydrogen-bonded solvent molecules in the minor groove side as may be expected due to the complete shielding from the amino sugar of daunomycin. Similarly, O2 of residue T3 does not have solvent molecules near it as it is also partially blocked away by the amino sugar as can be seen in Figure 4.

Most of the exocyclic oxygens of the intercalated anthracycline chromophore are not associated with any water molecule except O4 and O$_5$ oxygens, which are coordinated to the sodium ion. O12 has one water bonded to it. This is not surprising as the aglycon is completely sandwiched between two GC base pairs. Figure 10 shows a number of first-shell water molecules as solid black dots with thin lines connecting them to atoms within hydrogen-bonding distance.

Lattice Interactions. One of the major lattice interactions is the end-on-end stacking of the complexes along the crystallographic *c* axis. This stacking interaction is shown in Figure 7c, where it can be seen that the 2-fold symmetry-related terminal C1-G12 base pairs have large base–base overlaps. In this manner, a long column of the daunomycin–DNA hexamer complexes is built along the *c* axis, and this is schematically illustrated in Figure 2B, where the columns are depicted as dotted circles. These columns of complexes are associated laterally across the diagonal of the tetragonal unit cell forming criss-cross sheets of complexes. This lateral side to side interaction is stabilized by two symmetry-related hydrogen bonds involving the terminal O3′ hydroxyl groups of G6 (and G12) to the phosphate oxygen atom of the T3pA4 (and T9pA10) sequence (Figure 11). Between these sheets of complexes are the solvent channels that run through the entire unit cell of the crystal.

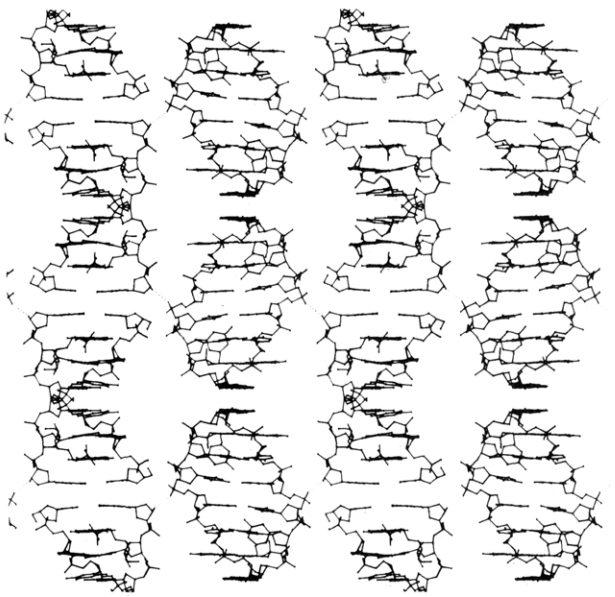

FIGURE 11: Interactions of the complexes of daunomycin–DNA in the crystal lattice. The molecules are stacked end to end along the *c* axis (vertical direction) forming a long column. These columns are associated laterally by hydrogen bonds (dotted lines) between O3′ of G6 (and G12) to O1P of A4 (and A10).

Discussion

The crystal structure of the daunomycin–d(CGTACG) complex has been determined by X-ray analysis and refined at quite high resolution (1.2 Å). This allows us to have confidence in discussing the conformational details derived from the present crystal structure. It is interesting to note that by lowering of the temperature of the crystal to −15 °C the diffraction resolution of the crystal is markedly improved. This can be probably attributed to the fact that the organization of first-shell hydration structure becomes substantially more ordered, thereby influencing and improving the second-shell hydration structure. This structure is one of the best refined structures of an oligonucleotide or a drug–DNA complex. Only the atomic-resolution Z-DNA structures of d(CG)$_3$ (Wang et al., 1979) and d(CGCGTG) (Ho et al., 1985) have better resolution. However, even such high-resolution structure have limitations in that certain features of the structure remain unresolved. For example, no spermine molecule could be found in the unit cell. They may be disordered in the solvent channels.

This structure represents one of a growing number of crystal structures of oligonucleotides complexed to drugs. Up to the present time, over thirty crystal structures have been determined by X-ray diffraction analysis of oligonucleotides with the molecules forming B-DNA, A-DNA, or Z-DNA conformations. In addition, the structures of a number of antitumor drug–DNA complexes have been determined. These include the bis-intercalator antibiotics triostin A and echinomycin (Wang et al., 1984, 1986; Ughetto et al., 1985; Quigley et al., 1986), the minor groove binding drug netropsin (Kopka et al., 1985), actinomycin D (Sobell et al., 1971; Takusagawa et al., 1982), and the *cis*-diamminedichloroplatinum(II) adduct of d(pGpG) (Sherman et al., 1985).

The significance of this study is that it augments the observations that a right-handed segment of DNA can have significant variations both in backbone geometry and sugar puckering associated with the accommodation of a large intercalator molecule with an amino sugar. The base stacking around the central two A·T base pairs (Figure 7b) looks similar to that seen in B-DNA, but there are modifications in the

conformation of the backbone. This suggests that there may be changes in conformation of the right-handed double helix that are dependent on sequence or on the local environment. This has been amply demonstrated in the crystal structures of triostin A–d(CGTACG) complex and triostin A–d(GCGTACGC) complex in which the base pairs adjacent to the intercalated quinoxaline rings are in the Hoogsteen geometry (Wang et al., 1984, 1986; Ughetto et al., 1985; Quigley et al., 1986). It should be noted that in this complex daunomycin molecule also adjusted its conformation (Figure 3) in order to fit tightly into the double helix.

The distortions to the DNA double helix associated with the daunomycin intercalator are complex as shown in Figures 6–8. It can be seen that the sugar puckering around the intercalator site does not have the common C3′-endo(3′–5′)-C2′-endo mixed pucker pattern that has been seen for simple intercalators. Furthermore, the bases adopt different glycosyl torsion angles to accommodate the daunomycin molecules. It is interesting to note that three nucleotide residues (G2, C11, and G12) around the intercalator have the high anti conformation, while the C1 residue is in the low anti conformation. This asymmetric distortion of two strands of DNA backbone conformation across the intercalation is also reflected in the base-stacking geometry. In general, there is an unwinding of the double helix when an intercalator is bonded to DNA molecules. Ethidium ion has been found to unwind DNA double helix by 26°. In the present structure we see two kinds of effects. Around the intercalator we see a modification such that, instead of unwinding, the base pair G2-C11 moves laterally toward the major groove. This is particularly evident on the C11-p-G12 step. This translation is probably associated with the presence of the amino sugar in the minor groove and the tight hydrogen bonding between daunomycin O9 and the G2 base. There are several close van der Waals contacts between the amino sugar and the cytosine O2 as well as the deoxyribose O1′ of C11 (Figure 9). Instead of having unwinding associated with the bases surrounding the intercalator, there is unwinding associated with the base pair one removed from the intercalator. The total unwinding of the DNA due to daunomycin molecule is 8° as measured from the present structure. This result correlates reasonably well with solution studies on superhelical DNA in which the unwinding associated with daunomycin has been estimated to be near 11° (Neidle, 1979). The observed modifications of the geometry of the double helix and the helix unwinding associated with daunomycin and other drugs such as triostin A and echinomycin suggest that there are not likely to be general rules that can be applied for all intercalators. Instead, different effects are seen, and many will be quite specific to the individual drug.

The aglycon chromophore of the daunomycin intercalates at the CpG sequences of the d(CGTACG) duplex by skewering the double helix in an interesting way such that ring D of daunomycin actually protrudes out on the major groove side and ring A on the minor groove side. An interaction of this type had been suggested earlier by Gabbay et al. (1976). The methoxy group of ring D in the major groove participates in the coordination of the sodium ion; this may provide some stability for the complex. Carminomycin, also an effective antitumor drug (Henry, 1979), has a hydroxyl group at the C4 positoin of ring D, indicating that the methyl group is not essential. On the other hand, the substituents on ring A seem to play a key role in the interactions of bases on either side of the intercalator in the minor groove. The O9 hydroxyl group of ring A forms two hydrogen bonds to the G2 residue in the minor groove (Figure 6). This suggests that the daunomycin binding to DNA may have a slight preference for G residues. However, a strong interaction involving hydrogen bonding to N3 could still be there if adenine were present, even though the other interaction would be missing. Likewise, O2 of a pyrimidine might play a role similar to that of N3. In a similar way, the interaction associated with the bridging water molecules and the carbonyl O13 could occur with various bases other than cytosine in that site.

In structure–activity studies, modifications of the groups attached to C9 produced profound effects (Henry, 1979). The interactions of the daunomycin O9 hydroxyl group with DNA provide an important stabilizing force for the complex and sequence preference for the double helix as described above. In addition, the amino sugar projecting to the lower left (Figure 8) with the acetyl group oriented toward the upper right provides a right-handed twist that may fit well into a right-handed double helix. This may explain the observation that daunomycin can effectively shift the equilibrium from the left-handed Z-DNA to the right-handed intercalated B-DNA conformation in solution (Chaires, 1985).

The size of the interaction between daunomycin and DNA accounts well for the limit, in solution, of one daunomycin per three base pairs (Neidle, 1979). In Figure 4D, it can be seen that two daunomycin amino sugars fill the minor groove almost fully. From this we can expect that anthracycline antibiotics with more than one sugar, such as cinerubin A (Crooke & Reich, 1980) or aclacinomycin A (Crooke & Reich, 1980), can also bind to the right-handed B-DNA double helix with their oligosaccharide moiety running along the minor groove covering several base pairs.

This structure explains satisfactorily the results of solution NMR studies of the interactions between daunomycin and DNA. The chemical shifts of the drug protons due to the antibiotic complex formation suggest that ring B and/or ring C of the intercalated aglycon chromophore overlaps with adjacent base pairs, but ring D does not overlap significantly with the base pairs and in fact extends out from the double helix into the solvent regions. One can now utilize the geometrical informations provided by this structure (for example, in Table V) to be references for a structural analysis of the daunomycin–DNA complex with the nuclear Overhauser enhancement (NOE) data by high-resolution two-dimensional NMR studies in solution.

Adriamycin, which is 14-hydroxydaunomycin, is closely related to daunomycin structurally. However, adriamycin is used in treating a number of tumors that are quite distinct from those against which daunomycin is active. We are interested to know whether an extra hydroxyl group on adriamycin would affect the binding of the drug to the DNA double helix. Recently, we have determined the crystal structure of a complex between adriamycin and a related DNA hexamer, d(CGATCG), and it is almost isostructural with the present structure (unpublished results). The O14 hydroxyl group is hydrogen bonded to a water molecule that is in turn hydrogen bonded to a nearby phosphate group. Therefore, it seems that the difference in the in vivo biological activity between daunomycin and adriamycin may not be related to their mode of binding to the DNA double helix.

On the basis of this structure, we can start to address the question of how other related anthracycline compounds may bind to DNA? For example, carminomycin, which lacks a methyl group at the C4 position as compared to daunomycin, is expected to bind to DNA in an analogous manner. Another example, *N*-(trifluoroacetyl)adriamycin 14-valerate (AD32) is a semisynthetic adriamycin analogue currently used in

clinical trials. The introduction of the trifluoroacetyl group at the N3′ position in the amino sugar of adriamycin (causing a loss of positive charge) and the hydrophobic valerate group increases its lipophilicity. Although it has been shown that AD32 binds poorly to DNA, the structure here suggests that it is not due to a steric hindrance of the new substituents as they can be accommodated into the structure easily (see Figure 8 for the positions of N3′ and C14).

It is interesting to note that even more complex anthracyclines such as aclacinomycin A (Oki, 1980) can also be accommodated into the structure without significant rearrangements in conformation. The methylcarboxylate group at C10 position on the aglycon ring A is in a configuration such that the chirality at C10 allows the methylcarboxylate group to project away from the DNA molecule. If C10 had an opposite chirality, the methylcarboxylate group would have a severe van der Waals clash with the G2 residue in this structure (Figure 8). As described earlier, the trisaccharide of aclacinomycin A can be fitted easily in the minor groove of the double helix.

The finding in this structure of a metal ion coordinating to both daunomycin and DNA molecules simultaneously, thereby creating a ternary complex, is interesting in that studies have been carried out showing that various metal ions such as Cu(II), Fe(III), Pd(II) ions can interact with daunomycin strongly (Spinelli & Dabrowiak, 1982; Beraldo et al., 1985; Fiallo & Granier-Suillerot, 1986), and these may be important in the biological activity of the drug. It should be pointed out that many of oxygen atoms (O5, O6, O11, O12) of the daunomycin that are putatively involved in the metal ion binding are not available for the metal interactions since those oxygen atoms are shielded between the base pairs and the backbones of the DNA molecule. Therefore, the octahedral coordinate site observed in this structure may in fact be an optimal binding site for various metal ions and can contribute significantly to the binding of daunomycin to DNA. This can be tested experimentally.

It has been shown that daunomycin exerts its antitumor activity by inhibiting both DNA replication and transcription (diMarco et al., 1974; Neidle, 1979). The present structure provides some insights into the possible mechanism of this activity. Daunomycin binds to DNA double helix tightly by intercalating the aglycon chromophore between base pairs. It is held in place by interactions between substituents on ring A and the minor groove of the helix. This provides an anchor to hold the amino sugar in the minor groove, where it sits with its functional hydroxyl and amino groups facing out such that they may interact with polymerases. This may prevent or retard the action of polymerases. More recently, it has been suggested that the cytotoxic effect of daunomyocin may be related to the generation of a dead-end complex of topoisomerase II induced by the drug (Tewey et al., 1984; Pommier et al., 1985). The present structure provides a detailed picture of the distorted DNA double-helical conformation at the daunomycin binding sites where they may be the high-afffinity sites for topoisomerase II which may be complexed tightly due to the distortions in the DNA molecules.

In summary, this high-resolution structure allows us to visualize many fine details of the molecular interactions between an important anticancer drug—daunomycin—with its cellular target, DNA. The nonplanar substituents in ring A of the daunomycin chromophore interact through hydrogen bonding with the double helix and provide an anchoring function. In the molecules of the anthracycline antibiotic family, such as daunomycin and adriamycin, there are three important functional components in the molecule: the intercalator (rings B–D), the anchoring function associated with ring A, and the amino sugar. The dissection of the functional properties associated with the anticancer drug molecule may help us develop a more systematic approach in thinking about how to target distinct modifications in the drug molecule. For example, we could add further substituents in the anchoring part, which will increase the hydrogen-bonding capability of the molecule. By analogy with adriamycin, this may give rise to an altered specificity toward cancer cells of the new agents.

High-resolution structural studies of other antitumor drugs such as the quinoxaline antibiotics (triostin A and echinomycin) also provide us with similar valuable information (Wang et al., 1984, 1986; Ughetto et al., 1985; Quigley et al., 1986). These antitumor drugs possess analogs functional components: the intercalators (quinoxaline rings), the anchoring function (cyclic peptide backbone), and the amino acid side chains.

By solving more structures of this type—antitumor drug bound to the DNA receptor—there may emerge a picture in which general rules governing the biological activities of various drugs can be obtained. These rules can then be used to design molecules with improved activities of existing compounds or with the desired functions to create a totally new class of compounds.

Registry No. Daunomycin–d(CGTACG) complex, 77064-60-9; duanomycin, 20830-81-3.

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