A trigonal form of the idarubicin:d(CGATCG) complex; crystal and molecular structure at 2.0 Å resolution

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ABSTRACT

The X-ray crystal structure of the complex between the anthracycline idarubicin and d(CGATCG) has been solved by molecular replacement and refined to a resolution of 2.0 Å. The final *R*-factor is 0.19 for 3768 reflections with $F_o \geq 2\sigma(F_o)$. The complex crystallizes in the trigonal space group *P*3₁ with unit cell parameters $a = b = 52.996(4)$, $c = 33.065(2)$ Å, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$. The asymmetric unit consists of two duplexes, each one being complexed with two idarubicin drugs intercalated at the CpG steps, one spermine and 160 water molecules. The molecular packing underlines major groove–major groove interactions between neighbouring helices, and an unusually low value of the occupied fraction of the unit cell due to a large solvent channel of ~30 Å diameter. This is the first trigonal crystal form of a DNA–anthracycline complex. The structure is compared with the previously reported structure of the same complex crystallizing in a tetragonal form. The geometry of both the double helices and the intercalation site are conserved as are the intramolecular interactions despite the different crystal forms.

INTRODUCTION

Anthracyclines have been the subject of intensive research and many different techniques have been applied to characterize the chemistry, the biological activity and pharmacological properties of this family of antitumor antibiotics (1,2). Anthracyclines are biologically active by several cytotoxic mechanisms (3) although the precise detail of this activity remains undetermined. They participate in DNA intercalation, DNA topoisomerase II and helicase inhibition, aerobic and anaerobic redox activation, and are also able to associate with membranes. Their clinical properties are linked to the variety of substituents on the

anthracycline chromophore and small alterations of their functional groups have been shown to influence clinical efficacy (1).

Crystallographic research supplied accurate structural details concerning the anthracyclines intercalation into the DNA duplex (4). The first studies involved daunomycin and adriamycin which are considered as representative members of the group (for example see 5). Anthracycline intercalation at different DNA sequences were characterized (6,7) and the more recently developed antibiotics, such as 4'-epiadriamycin and idarubicin, were also studied (8–11). The latter two represent the new generation of compounds developed in attempts to improve medicines. Idarubicin in particular has attracted attention in recent studies. It was first synthesized by Arcamone *et al.* (12) and its clinical and pharmacological studies were reported by Daghestani *et al.* (13). Its intercalation properties were analysed with the sequences d(CGATCG) (8) and d(TGATCA) (11) at high resolution, in an isomorphous tetragonal crystal form. As part of our research program investigating anthracycline–DNA interactions, we obtained a new crystal form (trigonal) of the DNA hexanucleotide-d(CGATCG) co-crystallized with idarubicin, (schematic drawing given in Fig. 1). We present details of the crystallographic analysis and the molecular structure of the complex in this new crystal form, and compare our results with those relative to the tetragonal form.

MATERIALS AND METHODS

Chemical synthesis and crystallization

Idarubicin was kindly provided by Farmitalia Carlo Erba Laboratories, Milan, Italy. The hexamer d(CGATCG) was synthesized by phosphoramidite methodology (14) on an Applied Biosystems 381A DNA synthesizer. After cleavage from the resin and deprotection, the crude material was purified by ion-exchange chromatography and reverse-phase HPLC. Crystals were grown at 277 K by vapour diffusion from droplets sitting in Corning glass depression plates (15). Red hexagonal prisms grew over 6 months, from 22 µl droplets which initially contained 2.4 mM oligonucleotide (single strand concentration), 2 mM idarubicin, 136 mM magnesium chloride, 27 mM sodium

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Figure 1. Ball and stick representation of idarubicin. The aglycone chromophore consists of three unsaturated (B, C, D) rings and one saturated ring (A) covalently bonded to an amino-sugar at the 7-position. The functional groups are indicated.

cacodylate (pH 6.5), 4 mM spermine tetrachloride plus 9% v/v 2-methyl-2,4-pentanediol (MPD). The solution was equilibrated against a reservoir of 50% aqueous MPD.

Data collection

A crystal of dimensions $0.45 \times 0.35 \times 0.25$ mm was sealed in a glass capillary with a droplet of mother liquor. Data ($-28 < h < 28$, $0 < k < 28$, $0 < l < 18$; $2\theta_{\max} = 46^\circ$ corresponding to $1.97 \text{ \AA}$ resolution) were collected at room temperature (298 K) on a Rigaku AFC-5 diffractometer with X-rays from an RU200 rotating anode (graphite monochromator Cu $K\alpha$: $\lambda = 1.54178 \text{ \AA}$, 50 kV and 100 mA settings; 0.5 mm focal spot). The crystal-to-detector distance was set at 400 mm. A continuously evacuated beam tunnel was in place to reduce adsorption by air. The lattice parameters ($a = b = 52.996(4)$, $c = 33.065(2) \text{ \AA}$, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$, $V = 80424 \text{ \AA}^3$) were determined from a least-squares fit of 20 reflections with $13.5^\circ < 2\theta < 28^\circ$.

Laue group considerations and the identification of systematic absences ($00l$, $l \neq 3n$) suggested possible trigonal space groups. Reflections for which $I < 8\sigma(I)$ were scanned in triplicate to improve counting statistics for the weaker reflections. Excluding standards, 7882 reflections were measured with ω scans of 1° at a speed of $4.0^\circ/\text{min}$ (16). Three standard reflections were monitored every 150 measurements throughout data collection. Intensities were corrected for linear decay of 3.4% in addition to corrections for Lorentz and polarization factors. An empirical absorption correction was also applied (17) with a minimum transmission factor of 0.65 and an average value of 0.83. Software for data collection and processing was provided by the Molecular Structure Corporation, Texas, USA.

Merging equivalents in $P3_1$ and $P3_121$ space groups results in R -symmetries of 6.1 and 9.8%, respectively. Structure solution and refinement procedures identified the correct space group as $P3_1$. In this space group, the molecular arrangement only slightly differs from that which would correspond to a $P3_121$ packing by the fact that one spermine molecule only binds one of the two

DNA complex molecules. 7276 unique reflections of which 3768 have $F_o \geq 2\sigma(F_o)$ were used in the refinement.

Structure determination and refinement

Most anthracycline-hexanucleotide complexes crystallize in $P4_12_12$ (approximate unit cell dimensions of $a = b \approx 28 \text{ \AA}$, $c \approx 54 \text{ \AA}$), with a single strand of hexanucleotide and one drug in the asymmetric unit. At present, there are 21 entries in the Nucleic Data Base, (18). In these structures, the duplex is formed by a crystallographic 2-fold symmetry operation, the global helix conformation and the anthracycline intercalation site are similar. We used one of these [PDB entry code 1D38, (8)], which presents the same DNA sequence and the same drug, as the starting model to perform molecular replacement calculations.

Similarities in the c unit cell dimension in the tetragonal form and the a and b parameters of the trigonal form suggest a comparable arrangement (i.e. two end-to-end stacked duplexes, each one containing two intercalated idarubicin molecules) along these directions.

A DNA duplex with two intercalated idarubicin molecules was generated by applying the $(1 - y, 1 - x, 1/2 - z)$ crystallographic symmetry operation to Gao and Wang's asymmetric unit (8). A second duplex was built from the first one by applying the $(1 - x, 1 - y, 1/2 + z)$ crystallographic symmetry operation. The pair of idarubicin-d(CGATCG) complexes constituted the starting model. A real space rotation search, using the *X-PLOR* package (19), was employed to determine the orientation of the model. Data from 15 to $4 \text{ \AA}$ resolution and $F \geq 2\sigma(F)$ were used in the calculation. The Patterson maps were computed by placing the model in an artificial orthogonal $P1$ unit cell measuring $100 \times 100 \times 50 \text{ \AA}$. Patterson vectors were limited to the 15 000 largest, the length of which ranges from 5 to $15 \text{ \AA}$ (20).

A Patterson correlation refinement of the 80 best solutions was carried out. The refinement consists of 10 cycles of rigid-body minimization, each duplex being considered as an independent entity. The actual solution (number 15 of the rotation function) reaches a correlation factor of 0.32 with eulerian angles: $\theta_1 = 177.5^\circ$, $\theta_2 = 77.5^\circ$, $\theta_3 = 121.5^\circ$.

The translation search was carried out by computing the standard linear correlation coefficient between $(E_{\text{obs}})^2$ and $(E_{\text{calc}})^2$. The starting, step-size and ending values for the grid search along x and y directions were respectively 0, 0.02 and 1 in fractional coordinates. No search was made along the c direction since the origin is undefined in $P3_1$. However, the origin was fixed so that the average value of the z fractional coordinates for the two duplexes is equal to $1/3$ in order to be in agreement with the $P3_121$ space group origin. The maximum for the translation function was relatively high (7σ). The non-existence of residual density above 1σ in $(F_o - F_c)$ maps indicates the absence of other molecules, and suggests that our starting hypothesis (two idarubicin-duplex complexes in the asymmetric unit) was the correct one. At this stage, the R -factor was 36% for all data in the $10\text{--}4 \text{ \AA}$ resolution range.

The structure was refined with *X-PLOR*, using energy minimization with the Powell-method conjugate gradient, and further completed with the restrained least squares method of Konnert and Hendrickson (21), using *NUCLSQ* (22). Electron density ($2F_o - F_c$) and difference density ($F_o - F_c$) maps were calculated and displayed on a Silicon Graphics 4D25TG using *TURBO-FRODO* (23). Examination of the $(F_o - F_c)$ density maps,

Figure 2. The spermine molecule, superimposed on its associated omit map at 3 r.m.s. contour level, is in the close vicinity of the sugar-phosphate backbone.

computed in the first stages of the refinement, clearly showed a spermine molecule (Fig. 2). Solvent molecules were gradually added on the criteria that they displayed well-shaped peaks in ($F_o - F_c$) density maps and reasonable hydrogen-bonding geometry with neighbouring functional groups. There were numerous peaks in the difference maps that represent disordered solvent sites, but we decided not to include these in the final refinement as their refined thermal parameters were larger than $55 \text{ \AA}^2$.

The final refinement stage, including 160 water molecules per asymmetric unit, converged at $R = 0.19$, for 3768 reflections in the resolution range of 10–2.0 $\text{\AA}$.

The r.m.s. deviations from standard geometry are 0.025 and 0.068 $\text{\AA}$ in bond lengths for sugar/base and phosphate groups respectively, 0.040 and 0.037 $\text{\AA}$ in the corresponding bond angles. The average thermal parameters are 23, 18 and 9 $\text{\AA}^2$ for phosphate, sugar and base groups, and 8, 21 and 31 $\text{\AA}^2$ for idarubicin, spermine and water molecules. Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (reference: T5647), and are available in machine-readable form from the Protein Data Bank at Brookhaven.

RESULTS AND DISCUSSION

The asymmetric unit consists of two duplexes of d(CGATCG), four idarubicin, one spermine and 160 water molecules. Nucleotides are labelled C(1) to G(6) on strand I, G(7) to C(12) on strand II for the first duplex, and C(13) to G(18) on strand I, G(19) to C(24) on strand II for the second duplex. The idarubicin molecules are labelled IDR(25) and IDR(26) in the first duplex, IDR(27) and IDR(28) in the second one. The spermine molecule, defined as SPM(29), is located in the major groove of the first duplex. Both duplexes are distorted by the chromophore intercalated at both CpG steps. A representation of the asymmetric unit is given in Figure 3. IDR(25) and IDR(26) intercalate between

Figure 3. The two (CGATCG) independent duplexes constituting the asymmetric unit. The intercalated idarubicin molecules are displayed in green, the spermine molecule is coloured in blue.

C(5)–G(8)/G(6)–C(7) and C(1)–G(12)/G(2)–C(11); IDR(27) and IDR(28) between C(17)–G(20)/G(18)–C(19) and C(13)–G(24)/G(14)–C(23). An example of one idarubicin molecule in its intercalation site, with associated electron density, is displayed in Figure 4. The long axis of each aglycone is nearly perpendicular to that of the base pairs, and the drug spans the two grooves of the helix. The amino-sugars are located in the minor groove of each duplex, while ring D of the chromophore protrudes in the major groove.

Figure 5 shows the overlay of the asymmetric unit duplexes. The low corresponding r.m.s. value (0.3 $\text{\AA}$), all atoms including idarubicin molecules taken into consideration, emphasizes the similarity of each duplex. It also suggests an upper limit to the error in atomic coordinates. A comparable result is deduced from the r.m.s. value (0.6 $\text{\AA}$) obtained when superimposing one of the duplexes with Gao and Wang's tetragonal molecule (Fig. 6). This

Figure 4. One of the intercalation site shown with associated electron density in a $(2F_o - F_c, \alpha_{calc})$ map. The contour level is 1.2 r.m.s. the electron density in the unit cell.

Figure 5. Stereoview showing the overlap of complexed duplex 1 (bases 1–12) with duplex 2 (bases 13–24). Duplex 1 in thick lines; duplex 2 in thin lines.

observation suggests that the conformation of the drug–DNA complex is essentially independent of the crystal packing forces.

DNA conformation

The overall structure can be considered as B-type DNA. The sugar conformations are mainly *C2'-endo* or similar and, excluding the intercalation steps, the average rise per base pair in each duplex is 3.6 Å. Geometrical parameters of the helices are given in Table 1. The sugar–phosphate backbone and glycosyl torsion angles were obtained with *NEWHEL93* (24).

The binding of the drug molecule modifies the DNA conformation. As noted by Gao and Wang in the description of the tetragonal form, a classical character of the intercalation concerns the geometrical parameters of the double helix base pairs.

(i) In each duplex, the A·T base pairs not involved in the intercalation display a low buckle angle, which differs from those of the base pairs affected by the drug intercalation. The terminal C·G base pairs are slightly less buckled (mean angle = 8°) than the internal one (mean angle = 17°).

(ii) At the intercalation steps, the idarubicin molecules unwind the DNA. It is particularly noticeable for helical twist angles between A(3)–T(10)/T(4)–A(9) which decrease by almost 7.5° from the B-DNA standard value of 36°.

(iii) The idarubicin molecules induce a higher rise at the intercalation sites, with observed values going over 5 Å.

(iv) Finally, we would like to emphasize the propellor twist angles associated with the central A·T base pairs (around 9°) not involved in the intercalation process. These values are higher than those usually observed in anthracycline–hexanucleotide complexes (2 or 3°) and could be explained by the fact that, in each duplex of this trigonal form, the base pairs are independent and not related by a 2-fold screw axis symmetry.

Sugar–phosphate backbone

The sugar–phosphate backbone conformation is rather regular, with limited deviations (particularly at the intercalation site) from the standard B-DNA conformation.

Figure 6. Stereoview showing the overlap of the trigonal complexed duplex 1 (thick lines) with the tetragonal complexed duplex (thin lines) described by Gao and Wang (8).

Regarding the bases involved in the intercalation, the average ϵ and ζ angle values (248° and 169°) are calculated excluding the values taken by the first cytosine and last guanosine bases of each strand, which are affected by the stacking forces generating, as discussed below, quasi-continuous helical rods. These mean values are close to those (250° , 168°) obtained for the tetragonal complex and differ from the values (191° , 252°) observed in the canonical B-DNA dodecamer structure d(CGCGAATTCGCG) (25). Such modifications, already observed in other anthracycline-hexanucleotide complexes, appear to be a general feature.

The usual ζ/α phosphodiester linkage conformation of B-DNA ($-sc/-sc$) is observed for most residues in both duplexes. However, there are a few exceptions where ζ values for residues G2, G8, G14, G20 and residues C5, C11, C17, C23 are decreased to averaged values of 180° ($\pm ap$) and 159° ($+ap$) respectively.

In each case, these alterations occur with other angular shifts. The ϵ angles for residues C1, C7, C13, C19 are increased to 248° ($-ac$), and the mean glycosyl linkage is rotated in order to adopt a low anti conformation ($\chi = 197^\circ$). The mean ϵ for G2, G8, G14, G20 is increased to 236° ($-ac$) from the $-ap$ usual conformation. In a similar way, the average β value for A9 and A21 (142°) ($+ac$) is smaller than found for the other residues (164°) ($+ap$).

Conformation and intercalation site of idarubicin

The conformation of idarubicin molecules in their intercalation sites of d(CGATCG) closely resembles those observed in related complexes. The chromophore is inserted in a 'head-on' fashion with the long axis of the aglycone moiety oriented at right angles to the long axis of adjacent base pairs, and with ring D protruding in the major groove. The amino-sugar (daunosamine) nestles in the minor groove, its three substituents pointing away from the bases, towards the outside of the duplex. Table 2 lists, for the four drugs, the glycosyl angles depicting the orientation of the

amino-sugar. The torsion angles around the glycosyl linkage are similar in the two duplexes. The average values for C8-C7-O7-C1' and C7-O7-C1'-C2' dihedral angles, $77^\circ/149^\circ$, are in agreement with those found in the tetragonal form, $85^\circ/146^\circ$, or in other anthracycline-hexanucleotide complexes.

The hydrogen bond interactions of the drugs with, either the oligonucleotide or solvent molecules, are mentioned in Table 3. The standard and most important contacts between an anthracycline drug and its intercalation site are conserved in the trigonal structure. This is particularly true: (i) for the N3' atoms of the amino-sugars which are directly hydrogen-bonded to the same 3 acceptor groups (O2 of a thymine, O2 and O4' of the adjacent cytosine) in the minor groove. (ii) for the O6, O7 and O9 atoms of the chromophore which are always in close contact with the internal C-G base pair, at each intercalation step.

Spermine conformation and contacts

The spermine molecule nestles in the major groove of the first duplex, and is roughly parallel to the sugar-phosphate backbone of strand I. It establishes numerous interactions (Table 4) either via direct hydrogen bonds between its terminal ammonium groups and phosphate groups [N1....O1P(A3) and N14....O1P(G6)], or via close van der Waals contacts between some of its methylene groups and T4 or C5. This tight interaction between the spermine and duplex I is reinforced by several water bridges.

Moreover, via extra van der Waals contacts, this spermine molecule connects one DNA molecule with a symmetry related one, and therefore contributes to the cohesion of the structure discussed below.

Crystal packing

In the tetragonal crystals, the double helices are stacked on top of each other to form quasi-continuous helical rods, parallel to the

Table 1. Geometrical properties of base pair and base pair steps

Duplex 1	Step	Roll	Tilt	PrTw	Buckle	Slide	Twist	Rise
C1-G12	1	0.8	1.8	-1.3	-6.6	1.1	35.4	5.8
G2-C11	2	-0.1	-3.6	-2.1	19.6	1.3	34.5	3.9
A3-T10	3	5.0	-0.3	-9.1	-2.5	-0.5	28.5	3.1
T4-A9	4	-0.5	3.2	-9.1	-5.5	0.8	33.8	3.7
C5-G8	5	0.8	-4.0	-0.6	-15.7	0.8	34.1	5.6
G6-C7				2.3	10.2			
Av.		-0.8	-0.6	-3.3	-0.1	0.7	33.3	4.4
Sd.		2.4	3.2	4.7	12.8	0.7	2.7	1.2

Duplex 2	Step	Roll	Tilt	PrTw	Buckle	Slide	Twist	Rise
C13-G24	1	-0.3	4.4	1.2	-3.2	1.1	33.2	5.7
G14-C23	2	3.0	-4.2	-1.4	19.4	1.2	34.2	4.0
A15-T22	3	-2.6	-0.8	-7.9	2.9	-0.3	32.6	3.3
T16-A21	4	-2.4	0.9	-9.5	-6.9	0.7	30.5	3.6
C17-G20	5	-0.9	-2.2	-1.8	-16.2	0.8	35.4	5.4
G18-C19				1.8	11.7			
Av.		-0.7	-0.4	-2.9	1.3	0.7	33.2	4.4
Sd.		2.3	3.3	4.7	12.9	0.6	1.8	1.1

Tetragonal form	Step	Roll	Tilt	PrTw	Buckle	Slide	Twist	Rise
C-G	1	-0.6	-0.4	4.4	-13.5	0.8	35.5	5.1
G-C	2	-0.2	-2.6	0.2	16.6	0.3	32.3	3.5
A-T	3	0.7	0.0	-2.3	7.2	-0.5	32.2	3.4
T-A	4	-0.2	2.6	-2.3	-7.2	0.3	32.3	3.5
C-G	5	-0.6	0.4	0.2	-16.6	0.8	35.5	5.1
G-C				4.4	13.5			
Av.		-0.2	0.0	0.8	0.0	0.4	33.6	4.1
Sd.		0.5	1.8	3.1	14.3	0.5	1.8	0.9

Helical parameters are calculated with the *NEWHEL93* routine using the 1989 Cambridge Nomenclature convention (24). The helix axes are calculated using all C1' and N9 of purines and N1 of pyrimidines.

*Calculated from PDB entry code 1D38 (8).

Table 2. Glycosyl torsion angles for idarubicin molecules

torsion angles	IDR25	IDR26	IDR27	IDR28	mean	IDA
C8 C7 O7 C1'	71	83	70	86	77	84
C20 C7 O7 C1'	193	203	189	207	198	209
C7 O7 C1' C2'	145	149	156	147	149	151
C7 O7 C1' O5'	267	272	284	271	273	274

IDA is the drug molecule intercalated in Gao and Wang's tetragonal structure (8).

c direction. The shortest distance between helix axes is equal to 19.9 Å. Interhelix contacts primarily involve the close approach of phosphate backbones from different adjacent helices.

In the trigonal arrangement, quasi-continuous helical rods lay in planes perpendicular to the *c* axis. The interplanar-distance (11 Å) is 1/3 of the *c* parameter. Such crystal packing, viewed along the *c* direction, shows quasi-continuous helices passing over the others, with an angle of 120° between the helical axes. Within a given plane, the distance between nearest parallel helix axes is 45.9 Å (*a* sinγ). The so-formed arrangement (Figure 7): (i) induces the occurrence of an ~30 Å diameter channel (29% of the cell volume) parallel to the *c* axis, centered at the unit cell origin, which explains the low value (30%) of the occupied fraction of

Table 3. Hydrogen bonding contacts around the idarubicin molecules

IDR25			IDR26		IDR27		IDR28		IDA	
Drug atom	Bonded atom	dist (Å)	Bonded atom	dist (Å)	Bonded atom	dist (Å)	Bonded atom	dist (Å)	Bonded atom	Dist (Å)
N3'	O2(T4)	3.3	O2(T10)	3.5	O2(T16)	2.8	<i>O2(T22)</i>	<i>3.7</i>	O2(T4)	3.4
	O2(C5)	3.2	O2(C11)	3.0	O2(C17)	3.4	<i>O2(C23)</i>	3.3	O2(C5)	3.0
	O4'(C5)	2.7	O4'(C11)	3.2	O4'(C17)	3.5	<i>O4'(C23)</i>	<i>3.6</i>	O4'(C5)	3.2
	W26	2.8	W25	3.4	W17	3.5			W24	3.4
O9	N2(G8)	3.3	N2(G2)	2.8	N2(G20)	2.9	N2(G14)	3.4	N2(G2)	3.3
	N3(G8)	3.2	N3(G2)	2.7	N3(G20)	2.7	N3(G14)	3.0	N3(G2)	2.9
									O4'(A3)	3.5
O7	N2(G8)	3.3	N2(G2)	3.3	N2(G20)	3.0	N2(G14)	3.4	N2(G2)	3.2
O6	O2(C5)	3.1	O2(C11)	3.5	O2(C17)	3.1	O2(C23)	3.4	O2(C5)	3.4
O13	W80 ¹	2.8			<i>W132</i>	<i>3.9</i>			O4(G2)	3.4
									W13 ⁷	3.2
									W14 ⁸	3.4
O4'			W25 ³	3.3	W17 ⁴	3.4				
O5					W63 ⁵	3.0				
O12			W77 ^{5*}	2.7	W15	3.0	W136 ⁶	3.0		

IDA is the drug molecule intercalated in Gao and Wang's tetragonal form (8). An asterisk stands for symmetry related molecule.

Distances and contacts (>3.5 Å), written in *italic characters*, are only given for comparison.

Water molecules labelled with an exponent number are bonded to a second atom. The corresponding distances and contacts are listed below.

¹Distance W80–O2(C7) = 2.62 Å

²Distance W13–O2(C19) = 2.62 Å

³Distance W25–N3'(IDR26) = 3.42 Å

⁴Distance W17–N3'(IDR27) = 3.46 Å

⁵Distance W77*–N1(SPM29) = 3.32 Å

⁶Distance W136–O5'(C13) = 2.77 Å

⁷Distance W13–O2(C1) = 2.79 Å

⁸Distance W14–N2(G6) = 2.76 Å

Table 4. Hydrogen bonding and van der Waals contacts involving the spermine molecule

Spermine atom	dist (Å)	bonded atom	dist (Å)	bonded atom
Direct Hydrogen bond				
N14	2.9	O1P(G6)		
Water bridges				
N1	3.3	W37*	3.4	O6(G2)*
	3.3	W77	2.7	O12(IDR26)*
	3.4	W130	3.2	W37*
	3.4	W130	3.1	O1P(A3)
N5	3.5	W78*	3.2	O1P(T4)
van der Waals contacts				
C4	3.6	C5(C1)*		
C6	2.9	O1P(T4)		
C7	3.4	C5(C5)		
C9	3.6	O1P(C5)		
C11	3.0	O1P(C5)		
C11	3.5	C2'(C17)*		
C12	3.3	C2'(C5)		
C12	3.5	C2'(C17)*		
C13	3.0	O1P(C17)*		

An asterisk stands for symmetry related molecules.

the unit cell and (ii) leads to major groove–major groove interactions between closest duplexes, (i.e. along the *c* direction).

The most intimate intermolecular contacts (Table 5) are between: (i) the terminal C7–G6 base pair of a unit and the C13–G24 base pair of the next stacked one generating, in the *ab* planes, the quasi-continuous helices, (ii) the outer chromophore rings D and the floor of the major groove in the other helix, (iii)

Figure 7. Schematic representation of the unit cell packing, displayed using the *RIBBONS* software package (26). The main channel is in the picture center. Quasi-continuous helical rods of a given plane are identical in color. Along the projection direction, the molecular arrangement between adjacent planes obeys the 3-fold screw axis symmetry.

Table 5. Intermolecular contacts shorter than van der Waals distances

Atom 1	Atom 2	dist (Å)
End to end stacking interactions :		
Symmetry operation : x, y, z - 1, 0, 0		
C5(G6)	C4(C13)	3.4
N3(G6)	C2(C13)	3.4
C2(C7)	N3(G24)	3.4
C2(C7)	C4(G24)	3.6
C4(C7)	C4(G24)	3.6
C4(C7)	C6(G24)	3.6
C4(C7)	C5(G24)	3.4
C5(C7)	C8(G24)	3.6
N4(C7)	C6(G24)	3.3
Side by side interactions :		
Symmetry operation : y, x, y, z+1/3 - 1, 0, 0		
O6(G6)	C3(IDR27)	3.3
C2(IDR25)	O6(G12)	3.3
C3(IDR25)	O6(G18)	3.4
C4(SPM29)	C5(C1)	3.6
C8(SPM29)	O1P(G18)	3.0
C9(SPM29)	O1P(G18)	3.2
C11(SPM29)	C2(C17)	3.5
C12(SPM29)	C2(C17)	3.5
C13(SPM29)	O1P(C17)	3.0
Symmetry operation : y, x, x, z+2/3 - 1, 1, 1		
C5(C1)	C4(SPM29)	3.6
O6(G12)	C2(IDR25)	3.3
C2(C17)	C11(SPM29)	3.5
C2(C17)	C12(SPM29)	3.5
O1P(C17)	C13(SPM29)	3.0
O1P(G18)	C8(SPM29)	3.0
O1P(G18)	C9(SPM29)	3.2
O6(G18)	C3(IDR25)	3.4
C3(IDR27)	O6(G6)	3.3
Symmetry operation : y, x, y, z+1/3 - 1, 1, 0		
O6(G24)	C2(IDR27)	3.3
Symmetry operation : y, x, x, z+2/3 - 0, 1, 1		
C2(IDR27)	O6(G24)	3.3

the phosphate-sugar backbones via the spermine molecules or water molecule bridges.

The crossover between superimposed quasi-continuous helical rods takes place at the end-to-end stacking junctions. In each asymmetric unit, the interhelix junction twist angle is small ($\sim 13^\circ$) due to the unwinding of the helix by the two intercalated drugs. It leads, in each duplex, to a widening of the major groove (15.5 Å instead of 12.7 Å at the inner ApT steps), favouring the

close observed interaction with the second helix, which also presents such geometrical properties by symmetry operations.

This particular packing, which obeys a 3-fold screw symmetry, is stabilized by the drug intercalation which, nevertheless, is identical to that found in the tetragonal complexes. We would like to emphasize that, in all other drug-hexamer complexes already studied, no particular geometrical constraint prevents the packing described here, packing that we think is due to the crystallization conditions, in particular the higher salt concentrations used.

That the molecular structures of this anthracycline-DNA complex remain unchanged despite differences in crystallisation conditions and crystal forms is encouraging. It suggests that the structures observed are a true representation of anthracycline-DNA interactions.

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