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Structure of 11-Deoxydaunomycin Bound to DNA Containing a Phosphorothioate

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The anthracyclines form an important family of cancer chemotherapeutic agents with a strong dependence of clinical properties on minor differences in chemical structure. We describe the X-ray crystallographic solution of the three-dimensional structure of the anthracycline 11-deoxydaunomycin plus d(CGTsACG). In this complex, two drug molecules bind to each hexamer duplex. Both the drug and the DNA are covalently modified in this complex in contrast with the three previously reported DNA–anthracycline complexes. In the 11-deoxydaunomycin complex the 11 hydroxyl group is absent and a phosphate oxygen at the TpA step has been replaced by a sulfur atom leading to a phosphorothioate with absolute stereochemistry *R*. Surprisingly, removal of a hydroxyl group from the 11 position does not alter the relative orientation of the intercalated chromophore. However, it appears that the phosphorothioate modification influenced the crystallization and caused the 11-deoxydaunomycin–d(CGTsACG) complex to crystallize into a different lattice (space group *P*2) with different lattice contacts and packing forces than the non-phosphorothioated DNA–anthracycline complexes (space group $P4_12_12$). In the minor groove of the DNA, the unexpected position of the amino-sugar of 11-deoxydaunomycin supports the hypothesis that in solution the position of the amino sugar is dynamic.

1. Introduction

The anthracyclines, including daunomycin and adriamycin, form an important family of chemotherapeutic agents. The strong dependence of the clinical properties of anthracyclines on minor differences in chemical structure has led to biological testing of a large number of natural and synthetic analogues (Brown, 1983; Acramone & Penco, 1988). Although interactions with other cellular targets may play a role in selective cytotoxicity (Aubel-Sadron & Londos-Gagliardi, 1984), the combined biochemical evidence indicates that anthracyclines act at the DNA level by intercalating into duplex DNA (Calendi *et al.*, 1965; Reinert, 1983) and blocking the processes of replication and transcription (Zunino *et al.*, 1974; Mizuno *et al.*, 1975). A variety of biochemical evidence has shown that daunomycin binds preferentially to alternating pyrimidine-purine tracts (Du Vernay *et al.*, 1979; Phillips *et al.*, 1978; Chaires, 1983) and appears to have some specificity for certain triplet sequences containing C·G base-pairs (Plumbridge & Brown, 1977; Chen *et al.*, 1985; Chaires *et al.*, 1987). X-ray diffraction patterns of oriented fibers of DNA plus daunomycin provided the first evidence that the aglycone intercalated into *B*-like DNA (Pigram *et al.*, 1972).

The interactions of daunomycin and adriamycin (14-hydroxyl daunomycin) with duplex DNA have been revealed on a more detailed level by X-ray crystallographic structural solutions of single crystals (Quigley *et al.*, 1980; Wang *et al.*, 1987; Frederick *et al.*, 1989; Moore *et al.*, 1989); daunomycin bound to two closely related hexamers, d(CGTACG) and d(CGATCG), (complexes referred

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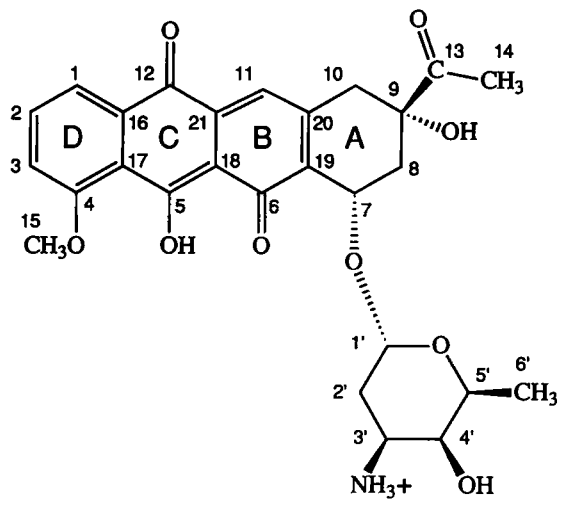

Figure 1. 11-Deoxydaunomycin in the 5-hydroxy, 6-keto tautomer.

to here as daun–TA and daun–AT, respectively) and adriamycin bound to d(CGATCG) (a complex referred to as adri-AT). In each of these complexes, two drugs bind to a hexamer DNA duplex with the chromophore intercalated in the same manner at the CpG steps on each end of the helix and with the amino sugar resting in the minor groove. Throughout the series of complexes, the interactions and orientations of the amino sugar are relatively variable while those of the chromophore are constant.

This report describes the three-dimensional structure of 11-deoxydaunomycin (Fig. 1) bound to d(CGTsACG), a complex referred to here as 11dd–TsA. This drug, 11-deoxydaunomycin, like daunomycin and adriamycin, is an anthracycline composed of a relatively planar aglycone plus a positively charged amino sugar. Both the drug and the DNA are covalently modified in the 11dd–TsA complex in ways not previously studied for this class of intercalator. On the DNA, a phosphate oxygen of the adenylate residue has been replaced by a sulfur to produce a phosphorothioate derivative containing a chiral phosphorus with the absolute stereochemical configuration *R*. On the drug, the hydroxyl group on the 11 position of daunomycin is absent. The 11dd–TsA complex was crystallized in a different lattice (space group *P*2) than the previous complexes (space group $P4_12_12$) and, therefore, demonstrates structural features that are not dependent on lattice contacts and packing forces.

2. Experimental Procedures

The self-complementary DNA hexamer was synthesized by the phosphotriester method (van der Marel *et al.*, 1981) and purified by Sephadex G50 chromatography. The final product was judged to be greater than 95% pure by high performance liquid chromatography. 11-Deoxydaunomycin was kindly supplied by Farmitalia Carlo Erba, Milan, Italy. Crystals were grown at room temperature in sitting drops using the vapor diffusion technique. The crystallization mother liquor contained 1·5 mM-DNA (single strand concentration), 20 mM-sodium cacodylate buffer (pH 6·0), 6·0 mM-$MgCl_2$, 1·5 mM-spermine and 20% 2,4-methyl-pentane-diol. The drops were equilibrated against a reservoir of 30% 2,4-methyl-pentane-diol. The crystals grew from a solution containing drug to duplex DNA in a ratio of 2 : 1. Plate-like crystals began to appear within 3 weeks and grew to a size of about 0·15 mm × 0·5 mm × 0·7 mm. Their space group was determined to be monclinic *P*2 (lattice constants $a = 17{\cdot}73$ Å, $b = 19{\cdot}13$ Å, $c = 26{\cdot}36$ Å (1 Å = 0·1 nm) and $\beta = 98{\cdot}01°$). The *P*2 space group is relatively rare (Mighell *et al.*, 1983; Dohohue, 1985) and, therefore, initial data were collected in a cell with a doubled *b* axis of 38·26 Å. However, careful examination of the reflections confirmed the original *P*2 cell. Data to a resolution of 1·5 Å were collected on a Nicolet P3 diffractometer at 10°C using an ω scan mode. The structure was solved with the rotational/translational search program Ultima (Rabinovich & Shakked, 1984) using the co-ordinates of the daun–TA complex, minus the solvent molecules as a starting model. Refinement was performed with the Hendrickson–Konnert constrained least squares refinement procedure (Hendrickson & Konnert, 1981) as modified for nucleic acids (by G.J.Q.). A total of 2129 unique reflections with $F_{obs} > 2{\cdot}0$ sigma (F_{obs}) were included in the refinement, 349 of them lying between 1·50 and 1·70 Å.

One drug molecule and one strand of the DNA duplex make up the asymmetric unit with the DNA duplex hexamer plus 2 drug molecules adopting crystallographic 2-fold symmetry. The structure was refined using 2 paired strands and 2 drugs, the atomic positions of which were averaged after each group of refinement cycles to maintain the 2-fold symmetry. The sulfur atom was refined as an oxygen until the final stages of refinement. At that time a shell of difference density surrounding one oxygen atom clearly indicated the presence of the sulfur. Solvent molecules were located from a series of difference Fourier maps and gradually added, always in symmetry-related pairs (except for the single water on a 2-fold axis) as the refinement continued. The final refinement of the 11dd–TsA complex included 36·5 unique water molecules per asymmetric unit (i.e. per DNA strand) and had a root mean square deviation in bond lengths from ideal values of 0·038 Å and an *R*-factor of 22·4 %. The atomic co-ordinates have been deposited in the Brookhaven Protein Databank (entry number ID14).

3. Results

(a) *Conformation and interactions of 11-deoxydaunomycin*

The 11-deoxydaunomycin intercalates at the CpG steps on each end of the helical duplex formed by d(CGTsACG), as shown by the skeletal drawing in Figure 2. The orientation of the intercalated 11-deoxydaunomycin aglycone within the DNA (between the C(1)·G(12) and G(2)·C(11) base-pairs) is slightly skewed from the perpendicular of the long axis of the base-pairs. The stacking of the 11-deoxydaunomycin aglycone between the two terminal base-pairs can be observed from a different perspective in Figure 3 (the aglycone is shaded). In this view the complex has been rotated so that the

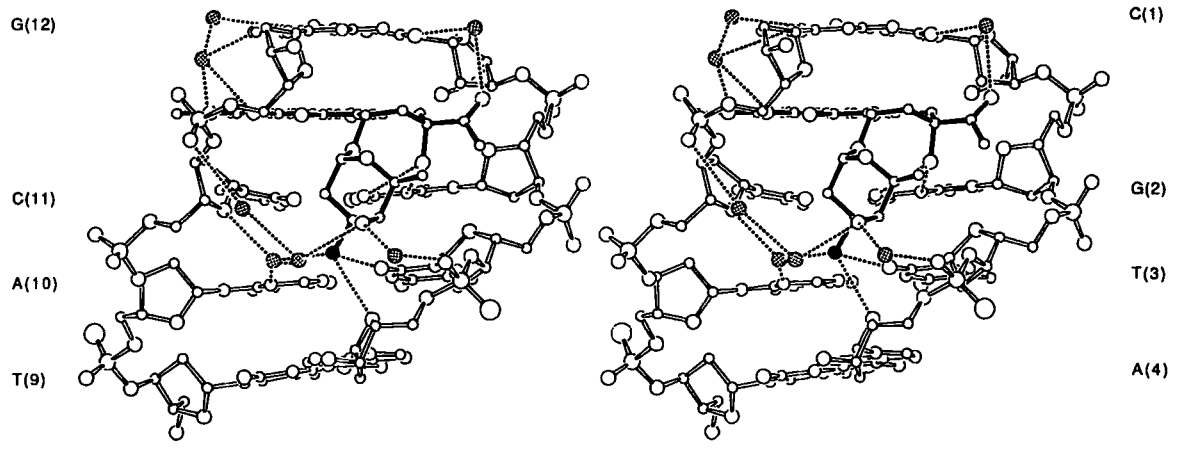

Figure 2. ORTEP stereo view of the 11-deoxydaunomycin–d(CGTsACG) complex. The DNA, with labelled residues, is drawn with open bonds. The 11-deoxydaunomycin is drawn with filled bonds. Hydrogen bonds involving the drug are drawn as broken lines. The water molecules are highlighted with stippling and the positively charged N-3′ of 11-deoxydaunomycin is shaded. The sulfur atom at the TpA step is slightly larger than the phosphate oxygens.

three planar rings (B to D) of the drug define the plane of the paper. The base-pairs above (thick open bonds), and below (thin bonds), are projected onto this plane. The 9-hydroxyl group on the A ring forms the only direct hydrogen bonds from the aglycone to the DNA. These two hydrogen bonds, to N-2 and N-3 of G(2) (3·05 Å and 2·87 Å, respectively), are nearly identical in the 11dd–TsA complex to their counterparts in other anthracycline complexes (average for three previous complexes is 3·04 Å and 2·65 Å, respectively).

Certain indirect hydrogen bonds are also observed in this complex. In the minor groove a single water molecule links O-13 of the drug to O-2 of residue C(1). Similarly, in the major groove the other end of the aglycone forms indirect hydrogen bonds between oxygens O-4 and O-5 of the aglycone and N-7 of G(12). This solvent molecule is co-ordinated by only four neighboring atoms and has therefore been assigned as a water molecule, although in previous complexes co-ordination by six neighboring atoms suggested that this bridging solvent molecule could be a sodium ion (Wang *et al.*, 1987; Frederick *et al.*, 1989). The hydrogen bonding distances of 2·78 Å to N-7 of G(12), 2·88 Å to O-4 of 11-deoxydaunomycin, 2·96 Å to O-5 of 11-deoxydaunomycin, and 2·82 Å to a second water molecule are consistent with the assignment of this peak as a water molecule and are rather long for co-ordination to an ion.

It appears that 11-deoxydaunomycin binds to DNA as the 5-hydroxy, 6-keto tautomer. The tautomeric states of the N-7 of G(12) and the O-4 of 11-deoxydaunomycin are fixed as hydrogen bond acceptors. The bridging water molecule (with a capacity to donate two and accept two hydrogen bonds) donates hydrogen bonds to the N-7 of residue G(12) and the O-4 of the aglycone. Therefore, in the hydrogen bond to O-5 of the aglycone (2·96 Å) the bridging water molecule would be accepting a proton, requiring that the aglycone bind as the O-5-hydroxy tautomer as shown in Figure 1.

In the 11dd–TsA complex, as in other crystalline

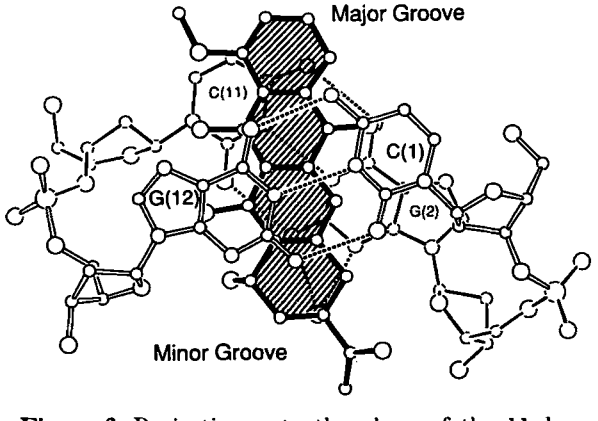

Figure 3. Projection onto the plane of the 11-deoxydaunomycin chromophore. The terminal C(1)·G(12) base-pair is drawn with thick open bonds. The aglycone of 11-deoxydaunomycin, drawn with thick continuous lines, is shaded, and the lower base-pair (G(2)·C(11)) is drawn with thin lines. Hydrogen bonds are drawn with broken lines.

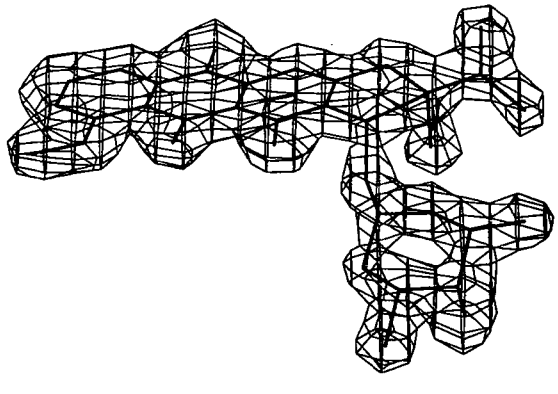

Figure 4. Electron density from a $2F_o - F_c$ Fourier map in the vicinity of 11-deoxydaunomycin.

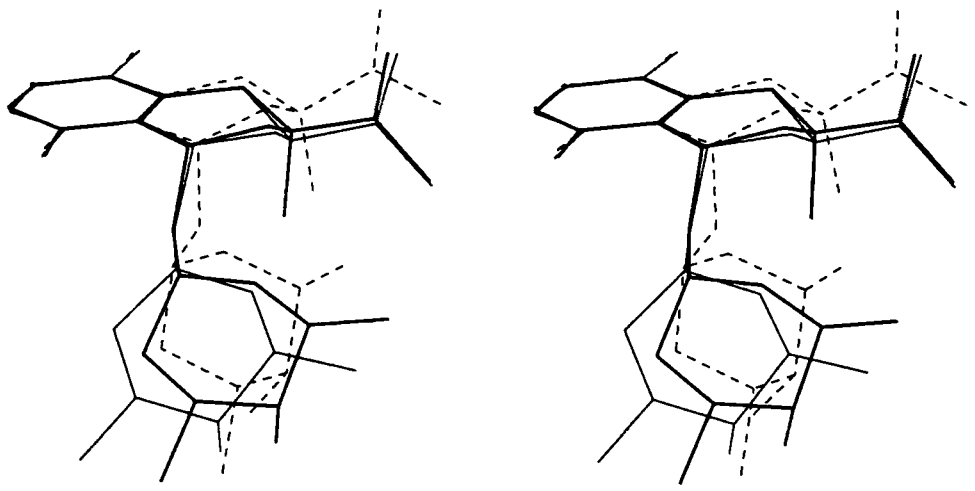

Figure 5. Stereo view of the A ring and amino sugar moieties of daunomycin alone (broken lines), 11dd–TsA (thick lines) and daun–TA (thin lines) with the 3 chromophores superimposed.

environments of daunomycin, the A ring is in a half-chair conformation with the essential 9-hydroxyl group in the axial position. The final electron density map ($2F_o - F_c$) surrounding 11-deoxydaunomycin is shown in Figure 4. The conformation of 11-deoxydaunomycin in the 11dd–TsA complex is different from that of daunomycin alone (Neidle & Taylor, 1977; von Dreele & Einck, 1977) as well as from that in the daun–TA complex (Wang *et al.*, 1987). The three different conformations in the three different environments can be seen in Figure 5 and selected torsion angles are shown in Table 1. With the exception of moderate differences in two torsion angles, the conformation of the A ring is the same in the two DNA complexes. In both DNA–drug complexes, the conformation of the A ring is severely constrained by the two hydrogen bonds from the 9-hydroxyl group to the minor groove of the DNA. The two torsion angles, which are moderately different in 11dd–TsA from daun–TA, are C-11–C-20–C-10–C-9 and C-20–C-10–C-9–C-8. The changes in these torsion angles by 12° and 8°, respectively, have the combined effect of shifting C-9 in the 11dd–TsA complex slightly up towards the plane of the chromophore (by 0·2 Å).

The largest differences in the conformation of the drug in the three environments are in the glycosidic linkage. The torsion around the C-7–O-7 bond is similar in the two DNA complexes while in contrast, that around the O-7–C-1′ bond is similar in 11dd–TsA and daunomycin alone. Thus, the relative position of the amino sugar is different in each environment (Fig. 5). The conformation of the drug in the 11dd–TsA complex is a hybrid of the conformations of daunomycin alone and in the daun–TA complex.

The amino sugar of 11-deoxydaunomycin forms

Table 1

Selected torsion angles (degrees) in anthracycline crystal structures

	Daunomycin†	11-Deoxydaunomycin plus d(CGTsACG)‡	Daunomycin plus d(CGTACG)§
A ring			
C-11–C-20–C-19–C-7	180	178	178
C-11–C-20–C-10–C-9‖	−170	−164	−156
C-20–C-10–C-9–C-8‖	−40	−39	−51
C-10–C-9–C-8–C-7	58	53	54
C-9–C-8–C-7–C-19‖	−48	−34	−31
C-8–C-7–C-19–C-6‖	−157	−172	−177
C-6–C-19–C-20–C-10	171	−179	180
Glycosidic linkage			
C-19–C-7–O-7–C-1′‖	−114	−150	−144
C-8–C-7–O-7–C-1′‖	125	91	93
C-7–O-7–C-1′–C-2′‖	167	167	144
C-7–O-7–C-1′–O-5′‖	−67	−68	−86

† From the crystal structure of daunomycin alone (Neidle & Taylor, 1977).
‡ This paper.
§ From the crystal structure of the daunomycin/d(CGTACG) complex (Wang *et al.*, 1987).
‖ Indicates torsion angles that vary with environment.

both direct and indirect hydrogen bonds to the minor groove of the DNA (Fig. 2). The positively charged amino nitrogen (N-3′) of the drug forms two direct hydrogen bonds to the DNA, the first to O-4′ of A(4), (3·09 Å) and the second to O-2 of T(3), (3·19 Å). The N-3′, *via* a single bridging water molecule, also forms indirect hydrogen bonds to both the endocyclic N-3 of A(10) and to O-4′ of C(11). The O-4′ of the amino sugar forms indirect hydrogen bonds *via* intermediate water molecules to phosphate oxygens of both strands. One water molecule that forms a hydrogen bond to a phosphate oxygen is connected simultaneously to both the N-3′ and the O-4′ of the amino sugar. Between the amino sugar of 11-deoxydaunomycin and the DNA, there is also a single van der Waals' contact in the 11dd–TsA complex. The C-3′ of the drug is located 3·10 Å from the O-2 of T(3). No direct or indirect interactions are observed between the drug and the sulfur atom of the DNA.

(b) *Conformation of the DNA*

The 11dd–TsA complex follows the pattern of conformational adjustments observed in previously determined intercalated structures. The DNA helix of this anthracycline complex is surprisingly regular, with only moderate deviations in backbone torsion angles and sugar puckers from standard *B*-conformation, even at the intercalation step. The individual base-pair geometries as well as helical twist angles from one base-pair to the next are reasonably similar to what is seen in *B*-DNA. Neither the change in the position of the amino sugar nor the switch of an oxygen to a sulfur to form a phosphorothioate have significantly altered the conformation of the DNA. In the 11dd–TsA complex there are 10·5 residues per turn (10·9; values in parentheses represent the corresponding values from the daun–TA complex) and the average distance from C-6 to C-8 across the base-pairs is 9·7 Å (9·8 Å). The distances from phosphorus to phosphorus across the minor groove (less the van der Waals' radius of the phosphate group, 5·8 Å) are 10·8 Å, 12·1 Å and 11·9 Å (10·7 Å, 11·7 Å and 11·8 Å) for residues G(2) to G(12), T(3) to C(11) and A(4) to A(10), respectively.

However, there are some significant deviations from standard *B*-DNA conformations. In the 11dd–TsA complex, as in previous DNA–anthracycline complexes, the G(2)·C(11) base-pair has been displaced towards the major groove. The sugar–phosphate backbone and glycosyl torsion angles of 11dd–TsA as shown in Table 2 are generally similar to those of the other DNA–anthracycline complexes. Two ε, ζ torsion pairs are in the *B*I conformation (*trans*, *gauche*–) (Dickerson *et al.*, 1982), two are in the *B*II conformation (*gauche*–, *trans*) and one pair (in *gauche*–, *gauche*–) is different from *B*-DNA geometry. In contrast, in the daun–TA complex, two of the ε, ζ pairs (in *trans*, *trans* and *gauche*–, *gauche*–) are different from standard *B*-DNA geometry. In addition, in the 11dd–TsA complex, α, β and γ of residue T(3); γ of residue A(4); and α, β and γ of residue G(6) are significantly different from those in the daun–TA complex. In spite of these differences, the overall conformation of the DNA is similar.

To wrap around the intercalated chromophore, the terminal C(1)·G(12) base-pair buckles by −6·6° (−8·2°) while on the other side of the intercalation site the G(2)·C(11) base-pair buckles by 16·1° (16·4°). The sugar puckers of the 11dd–TsA complex are C-2′-*endo* or close to that conformation (Table 3).

(c) *Lattice Interactions*

The 11dd–TsA complex has crystallized in a different lattice (space group *P*2) from the previous DNA–anthracycline complexes (space group $P4_12_12$). In the $P4_12_12$ lattice, the DNA forms a continuous helix (Fig. 6). However, in the *P*2 lattice one 5′-terminal cytosine is stacked on the 5′-terminal cytosine of the next duplex forming a 5′–5′/3′–3′ junction. This packing differs from the previously observed packing arrangement in the $P4_12_12$ lattice, where the 5′-terminal cytosine of one duplex is stacked on the 3′-terminal guanine of the next duplex in a head-to-tail orientation. Certain direct and indirect hydrogen bonds help to stabilize the crystal lattice by connecting adjacent extended columns of helices. In the 11dd–TsA complex, the terminal 3′ hydroxyl group of one duplex forms a

Table 2

Sugar–phosphate backbone and glycosyl torsion angles (degrees) of the 11dd–TsA complex

Residue	α	β	γ	δ	ε	ζ	χ
C(1)	—	—	61	134	−108	−74	−169
G(2)	−57	168	42	149	−119	164	−92
T(3)	78	−144	−135	149	−175	−74	−135
A(4)	180	155	151	133	−151	−81	−132
C(5)	−75	168	40	128	−108	166	−93
G(6)	9	160	−19	146	—	—	−76
B-DNA	−63	171	54	123	−169	−108	−117

Backbone torsion angles are defined as P-α-O-5′-β-C-5′-γ-C-4′-δ-C-3′-ε-O-3′-ζ-P, the glycosidic torsion angle is χ.

Table 3

Sugar conformations

Residue	Pseudorotation phase angle (degrees)	Puckering mode
C(1)	149	C-2′-*endo*
G(2)	157	C-2′-*endo*
T(3)	174	C-2′-*endo*
A(4)	158	C-2′-*endo*
C(5)	141	C-1′-*exo*
G(6)	188	C-3′-*exo*

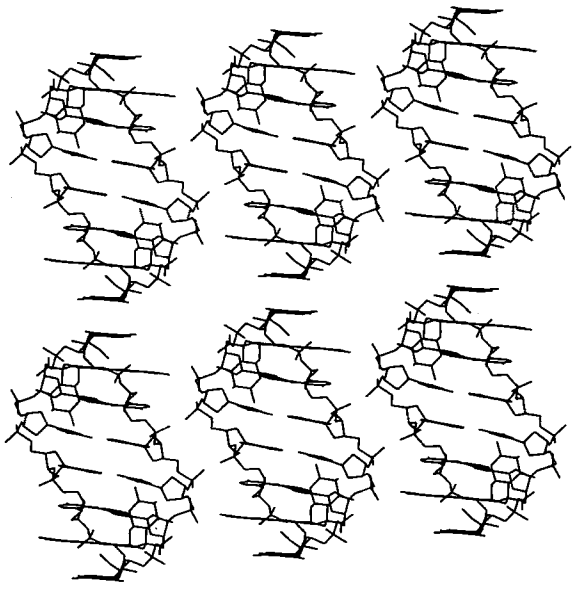

Figure 6. Crystal packing of the 11dd–TsA complex viewed down the *b* axis.

hydrogen bond to the phosphate oxygen of residue G(8) of another duplex. The sulfur atom is also involved in contacts that may be important to crystal stability. A sulfur from one duplex forms hydrogen bonds to a water that, in turn, forms hydrogen bonds to the sulfur in another duplex.

4. Discussion

In the search for improved chemotherapeutic agents, a large number of daunomycin derivatives have been isolated and synthesized (Brown, 1983; Acramone & Penco, 1988). This paper describes the three-dimensional structure of 11-deoxydaunomycin bound to the DNA hexamer, d(CGTsACG). Structures of adriamycin or daunomycin bound to d(CGATCG), and daunomycin bound to d(CGTACG), were previously reported from this laboratory (Quigley *et al.*, 1980; Wang *et al.*, 1987; Frederick *et al.*, 1989) and by Kennard and coworkers (Moore *et al.*, 1989). The complex between 11-deoxydaunomycin and d(CGTsACG) reported here (11dd–TsA) is different from the previously described DNA–anthracycline complexes (daun–TA, daun–AT and adri–AT) in four ways. In the 11dd–TsA complex: (1) the intercalated chromophore is chemically modified; (2) the DNA is chemically modified; (3) the position and interactions of the amino sugar are different; and (4) the crystal lattice and packing interactions are different. Nonetheless, the DNA conformation, including backbone torsion angles and sugar puckers, of the 11dd–TsA complex in the *P*2 lattice is reasonably similar to that of other DNA–anthracycline complexes in the $P4_12_12$ lattice. The position and orientation of the anthracycline chromophore, intercalated at the CpG steps, is also the same in all four complexes. The similarity in conformation of DNA–anthracycline complexes in different crystallographic environments suggests that the observed conformations are near an energy minimum and would predominate in solution. These results indicate that the conformations of crystallized DNA–anthracycline complexes are not severely affected either by the chemical modifications or by lattice forces.

The aglycone of 11-deoxydaunomycin stacks in a position nearly identical to the aglycones of other DNA–anthracycline complexes even though the dipolar interactions between the chromophore and the adjacent base-pairs have been altered by removing a hydroxyl group from the chromophore. Thus, dipolar interactions of the 11-hydroxyl group do not appear to determine the orientation of the aglycone within the DNA. One end of the aglycone is fixed by two direct hydrogen bonds from the O-9 directly to the DNA. These two hydrogen bonds are highly preserved in all four complexes and form in such a way that dG can be distinguished, within the minor groove, from any other nucleotide. The position of this end of the aglycone may also be stabilized by an indirect hydrogen bond (*via* a bridging water molecule) from the O-13 carbonyl oxygen of the drug to O-2 of residue C(1). This bridging interaction is also preserved in each complex. The other end of the aglycone appears to be held in place by a solvent molecule acting as a surrogate functional group (i.e. an arm of the drug). A water molecule (or a sodium ion in some other DNA–anthracycline complexes) binds to two oxygens of the chromophore in nearly the same fashion in all four complexes. This surrogate functional group binds to the major groove of the DNA by forming a direct hydrogen bond to the N-7 of G(12) and an indirect hydrogen bond *via* a second intermediate water molecule to the O-6 of the same residue. The persistence of such solvent molecules in a variety of crystallographic environments underscores their importance in maintaining the stability of DNA–drug complexes and indicates that bound solvent molecules are an intrinsic part of such complexes.

The identification of this surrogate functional group in the 11dd–TsA complex as a water molecule rather than an ion leads to an unexpected conclusion regarding the tautomeric state of 11-deoxydaunomycin. As described above, in the DNA complex, 11-deoxydaunomycin appears to adopt the 5-hydroxy, 6-keto tautomer (Fig. 1). The tautomeric states of anthracyclines crystallized alone, daunomycin (Courseille *et al.*, 1979) and the closely related carminomycin I (von Dreele & Einck, 1977), were found to be 5-keto, 6-hydroxy tautomers. Thus, binding to DNA may induce a switch in the tautomeric state of the drug.

The position and interactions of the anthracycline amino sugar are different in the 11dd–TsA complex in contrast to the three previously described DNA–anthracycline complexes. Throughout this series of complexes, the position of the amino sugar is variable. We have reported that although the

amino sugar extends into the minor groove in each DNA–anthracycline complex, the interactions with the DNA are dependent on sequence (Frederick *et al.*, 1989). This positively charged moiety appears to form a tighter complex with d(CGATCG) than with d(CGTACG). In daun–TA, the amino group does not form direct hydrogen bonds to the DNA while in daun–AT and adri–AT the amino sugar is shifted towards the floor of the minor groove so that the N-3′ forms hydrogen bonds to the bases of one strand of the DNA duplex. The position of the amino sugar in 11dd–TsA is shifted towards that in the daunomycin crystal structure. Further, the positively charged N-3′ forms direct hydrogen bonds to the bases of the other strand of the duplex. In a fashion similar to previous DNA–anthracycline complexes, the amino sugar of 11dd–TsA also forms indirect hydrogen bonds with phosphate oxygens.

There are three possible causes for the different position of the amino sugar in the 11dd–TsA complex. (1) The modification of the DNA (i.e. the switch of an oxygen to a sulfur forming a phosphorothioate) could influence the position of the amino sugar. The character of a phosphorothioate is somewhat different from that of the usual DNA phosphate (Saenger, 1984). The P–S bond is 0·3 Å longer than the P–O bond and sulfur has a significantly larger van der Waals' radius than oxygen. In addition, sulfur is more polarizable and less electronegative than oxygen. Therefore, the position of the positively charged amino sugar could be altered either directly by differences in electrostatic interactions with the phosphorothioated compared to normal DNA or indirectly, by differences in hydration. Our observation that the sulfur does not significantly affect the conformation of the DNA is consistent with a previous report describing the structure of a mutiply phosphorothioated analog of $d(GC)_3$ (Cruse *et al.*, 1986). This duplex crystallizes in the *B*-conformation with no obvious structural perturbations resulting from the switch of several oxygens to sulfurs. (2) Crystal packing forces could also influence the position of the amino sugar. This could only be an indirect effect of different hydration in the different lattices as the amino sugar is not involved in direct lattice contacts. (3) Finally, the chemical modification of the aglycone, i.e. the removal of the 11-hydroxyl group of daunomycin, could influence the position of the amino sugar. However, this possibility is unlikely as in the region of this modification the conformations and interactions of all four complexes are similar.

If we assume that the conformation of daunomycin crystallized alone predominates in solution, then the change in conformation of daunomycin associated with the formation of the daun–TA complex would be greater than that associated with the formation of the 11dd–TsA complex. However, it is reasonable to suggest that in DNA complexes in solution the amino sugar may flip between the conformations observed in the different lattices and possibly between other conformational states as well. Such mobility of anthracycline sugars in DNA complexes is consistent with the inability of minor groove footprinting agents to detect anthracyclines bound to DNA (van Dyke *et al.*, 1982; Fox, 1988).

It appears that the phosphorothioate caused the 11dd–TsA complex to crystallize in a different lattice from previously crystallized DNA–anthracycline complexes. A total of eight different complexes of bi-component anthracyclines (i.e. daunomycin type) bound to hexamer DNA duplexes of varying sequence, cytosine methylation states and drug modification have been crystallized (unpublished results). With the exception of the complex containing a phosphorothioate, all have crystallized into the $P4_12_12$ lattice. Sulfur substitution of DNA shows promise as a method for influencing packing (this paper) and promoting growth of DNA crystals that are otherwise difficult to obtain (Cruse *et al.*, 1986).

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