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Structure of a B-form DNA/RNA chimera (dC)(rG)d(ATCG) complexed with daunomycin at 1.5 Å resolution

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The crystal structure of a DNA/RNA chimera (dC)(rG)d(ATCG) complexed with the anticancer drug daunomycin has been determined at 1.5 Å resolution with R_{work} and R_{free} of 19.7 and 23.3%, respectively, for 2767 reflections. The complex crystallizes in space group $P4_12_12$, with unit-cell parameters $a = b = 28.05$, $c = 53.16$ Å, and contains one nucleic acid strand and one daunomycin molecule in the asymmetric unit. To our knowledge, this is the first crystal structure of a DNA/RNA chimera complexed with an intercalating drug. The DNA/RNA chimera adopts the B-form helical conformation, with the 2′-hydroxyl group in the major groove of the duplex, forming hydrogen bonds to N7 and the anionic phosphate oxygen of its 3′-side adenine. The present results indicate that the replacement by the ribose sugar in the DNA sequence does not change the geometry and intercalation pattern of daunomycin. A model of B-form RNA has been built based on the present structure. The model indicates that the interactions of the 2′-hydroxyl groups in the B-form duplex depend on their 3′-side nucleotides.

1. Introduction

Interactions between nucleic acids and drug molecules have been studied for several decades in order to search for the principles that govern the specificity of drug targeting. These drug molecules are usually classified into two different categories: minor-groove binders and intercalators. Targeting of specific sequences has produced much progress with minor-groove binders. Different lexitropsins have been designed to target different DNA-specific sequences, with pyrrole rings being specific for A·T base pairs, while imidazole rings are specific for G·C base pairs (Kopka *et al.*, 1985). The 2:1 binding mode in the minor groove provides a way for drug molecules to distinguish the A·T base pair from the T·A base pair and the G·C base pair from the C·G base pair (Kopka *et al.*, 1997; Kielkopf, Baird *et al.*, 1998; Kielkopf, White *et al.*, 1998; White *et al.*, 1998). Intercalating drugs display different interaction modes with nucleic acids. Instead of making hydrogen-bonding interactions in the minor groove, intercalating drugs essentially slide between two adjacent base pairs, with their planar aromatic or heteroaromatic rings stacking with the base pairs (Quigley *et al.*, 1980). Accordingly, the vertical separation between the two base pairs increases and the duplex is partially unwound. Intercalators generally have a preference for 5′-pyrimidine–purine-3′ for binding, with the exception of actinomycin D, which has a high affinity for GpC steps (Krugh, 1994).

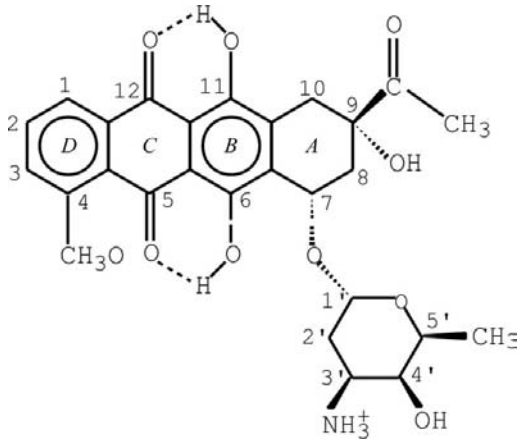

Figure 1
Molecular formula of daunomycin.

Table 1
Crystal data and refinement parameters of the CgATCG–daunomycin complex.

Space group	$P4_12_12$
Unit-cell parameters (Å)	$a = b = 28.05$, $c = 53.16$
Asymmetric unit contents	1 strand and 1 daunomycin
Resolution range (Å)	10.0–1.5
No. of reflections [$F > 2\sigma(F)$]	2767
Final R_{work}/R_{free} (%)	19.7/23.3
Volume per base pair (Å^3)	1337
Parameter file	dna-rna_rep.param
Deviations from ideal geometry	
Bond length (Å)	0.014
Angle (°)	1.5
Dihedral angles (°)	17.5
'Improper' angles (°)	1.9
Final model	
Nucleic acid atoms	120
Water molecules	40

Daunomycin is a cytotoxic anthracycline antibiotic that has been used in the treatment of acute leukemia (Crooke & Reigh, 1980). It is composed of a tetracyclic aglycon chromophore and an amino sugar (Fig. 1). The antitumor activity of daunomycin is related to the intercalation of the aglycone chromophore between the DNA bases, which causes inhibition of DNA replication and RNA transcription (Arcamine, 1981; Di Marco *et al.*, 1974; Neidle, 1979). X-ray crystallographic studies indicate that daunomycin binds to DNA through the insertion of the tetracyclic aglycon chromophore between CG and TG base steps, with the amino sugar in the minor groove (Quigley *et al.*, 1980; Wang *et al.*, 1987; Moore *et al.*, 1989; Frederick *et al.*, 1990; Nunn *et al.*, 1991). These structures provided the exact intercalation sites and details of the interaction between DNA and intercalators. Recently, bis-daunomycin was designed to improve the binding affinity for DNA sequences (Hu *et al.*, 1997).

DNA/RNA chimeras play an important role in biological processes, such as in Okazaki fragments in DNA replication. Recently, chimerical structures have been observed in reverse-splicing processes (Yang *et al.*, 1996) and used as synthetic therapeutic oligomers (Cole-Strauss *et al.*, 1996). Our laboratory has continuously been studying the structural effects of the ribose sugar upon DNA molecules (Ban *et al.*, 1994*a*,*b*; Wahl & Sundaralingam, 2000; Pan & Sundaralingam, 2003). We have found that replacement of the ribose sugar in only one nucleotide in DNA sequences can convert the helical form from B to A (Ban *et al.*, 1994*a*; Wahl & Sundaralingam, 2000). Interestingly, the chimeras IcICICIC and IcIcICIC (where upper-case letters represent DNA and lower-case letters represent RNA) complexed with the minor groove-binding drug distamycin still adopt the B-form helical conformation (Chen *et al.*, 1995). As an attempt to systematically study of the effect of the ribose sugar in DNA structures, we decided to study the conformation of DNA/RNA chimeras bound with intercalating drugs. As daunomycin binds in the CG step in CGATCG (Moore *et al.*, 1989), we substituted the deoxyribose sugar with a ribose sugar in the intercalating site G2. In this paper, we describe the crystal structure of the complex at 1.5 Å resolution, discuss the interactions of the 2′-hydroxyl groups in the B-form helix and also propose a model for a B-form RNA duplex.

2. Materials and methods

2.1. Synthesis, crystallization and data collection

The DNA/RNA chimera CgATCG was synthesized using our in-house Applied Biosystem 391 automatic DNA synthesizer on a 1 µmol scale using solid-state phosphoramidite chemistry. The product was cleaved off from the column with a 3:1(*v*/*v*) mixture of 37% ammonium hydroxide: absolute ethanol, followed by deprotection for 1 h in a hot-water bath at 328 K. The crude solution was then lyophilized to dryness and the resulting pellet was dissolved in 100 µl tetrabutylammonium fluoride (0.1 *M* in THF). The solution was kept at room temperature for 24 h in the dark to deprotect the 2′-OH and 100 µl saturated ammonium acetate solution was added. The oligonucleotide was recovered by absolute ethanol precipitation at 253 K, purified by FPLC and crystallized by the hanging-drop vapor diffusion-method at room temperature. Typical conditions used were 2 m*M* oligonucleotide (single-strand concentration), 40 m*M* sodium cacodylate buffer pH 7.0, 24 m*M* $MgCl_2$, 2 m*M* daunomycin and 2%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD) equilibrated against 60% MPD in the reservoir. Tetragonal rod crystals grew to dimensions of 0.4 × 0.4 × 0.8 mm after one week. 15 frames of intensity data were collected on a Rigaku imaging-plate system operating at 50 kV and 100 mA with Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å) by the oscillation method with 30 min exposure time for each frame. The data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). A total of 15 870 reflections to 1.5 Å were collected from the crystal, of which 2778 reflections (75% completeness) were unique. The crystallographic data are given in Table 1.

2.2. Structural solution and refinement

The present crystal is isomorphous to the all-DNA counterpart d(CGATCG)–daunomycin (Moore *et al.*, 1989; unit-cell parameters $a = b = 27.98$, $c = 52.87$ Å compared with

$a = b = 28.05$, $c = 53.16$ Å in the present crystal). Therefore, the coordinates of the DNA hexamer were used as the initial model for refinement using *CNS* (Brünger *et al.*, 1998). Manual fitting and modification of the hexamer and daunomycin were performed using *CHAIN* (Sack & Quiocho, 1992). In order to remove the conformational bias of the starting model, simulated annealing (heating to 3000 K and then slow cooling to 300 K) was employed, giving an R_{work} and an R_{free} of 29.8 and 33.4%, respectively. $|F_o - F_c|$ maps calculated at this stage clearly showed the position of the daunomycin. Further positional and *B*-factor refinement with daunomycin lowered the R_{work} and R_{free} to 25.3 and 29.3%, respectively. At this stage, the $|F_o - F_c|$ maps clearly revealed the electron density for the O2′ atom at g2. 40 water molecules were added stepwise to the complex from the electron-density maps ($|F_o - F_c| > 3\sigma$, ($|2F_o - F_c| > \sigma$), giving a final crystallographic R_{work} and R_{free} of 19.3 and 23.3%, respectively, for the data [10.0–1.5 Å, $F > 2\sigma(F)$] using 2767 unique reflections. The refinement parameters are listed in Table 1.

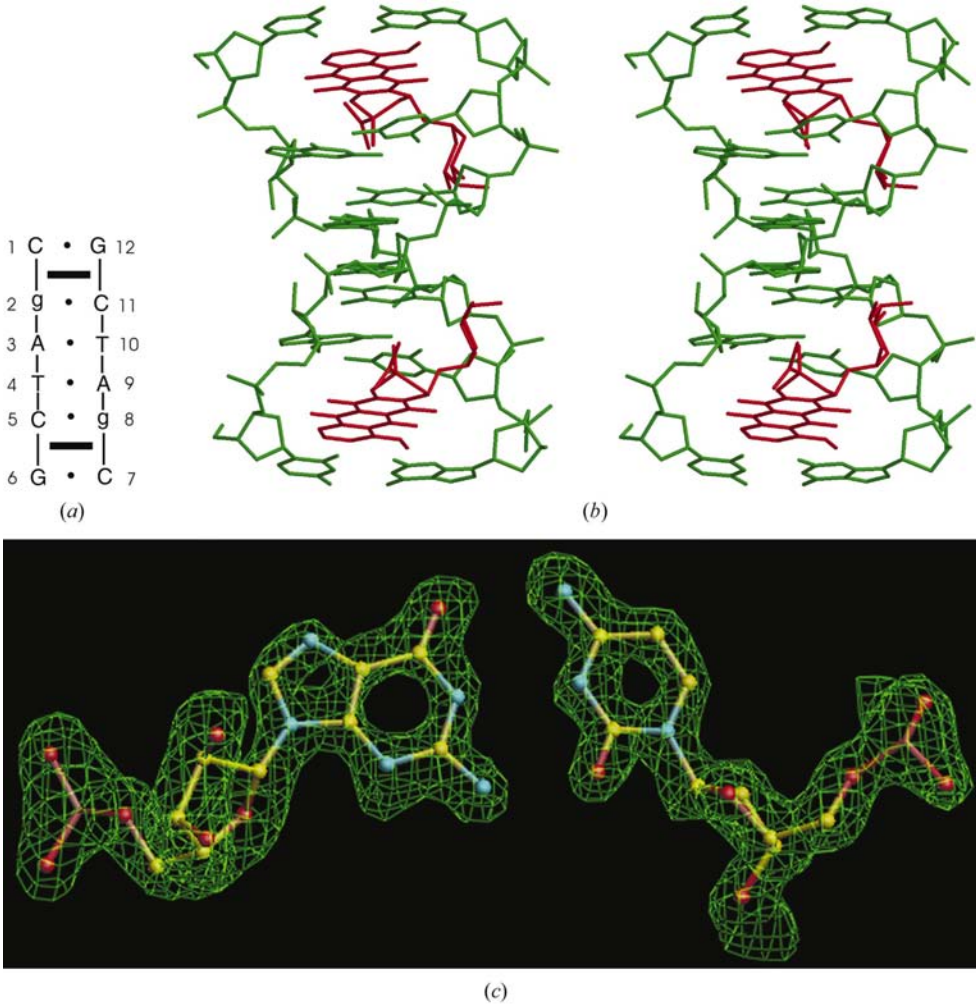

Figure 2
(*a*) Numbering scheme and diagram of the present DNA/RNA chimera with two daunomycin molecules intercalating at CG steps. (*b*) Stereoview of the present structure complexed with two daunomycin molecules (red). (*c*) g2–C11 base pair superposed with the $|2F_o - F_c|$ map at the 1σ level.

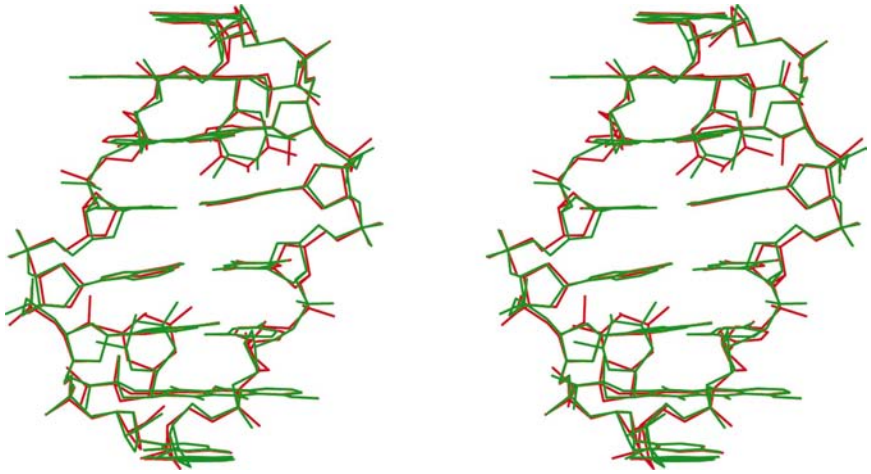

Figure 3
Stereoview of the superposition of (CgATCG)–daunomycin (in red) and d(CGATCG)–daunomycin (in green).

3. Results and discussion

3.1. Overall structure

The present structure has a single chimera strand and one molecule of daunomycin in the asymmetric unit. This independent strand and its symmetry-related strand form a distorted right-handed duplex with daunomycin intercalating at the Cg step (Figs. 2*a* and 2*b*). Fig. 2(*c*) displays a representative electron-density map of the structure. This is the first observation of a DNA/RNA chimera bound by intercalating drugs. C1 adopts the C2′-*endo* sugar pucker. The central four nucleotides g2, A3, T4 and C4 adopt the C1′-*exo* sugar pucker and G6 adopts the C3′-*exo* sugar pucker. All of these sugar puckers belong to the B-form family. The base-pair planes

are perpendicular to the helical axis. The distance between adjacent intrastrand P atoms at each base step ranges from 6.4 to 6.9 Å, close to the typical B-DNA value of 6.7 Å; the intercalation of daunomucin does not disturb the adjacent intra-strand phosphorus distance in the intercalating sites. However, the helical rise at the intercalation site is 7.3 Å, more than twice the average value of 3.1 Å for the other steps in the structure. The duplexes pack on the adjacent duplexes along the *c* axis, with a twist angle of 16° between terminal base pairs, forming pseudocontinuous infinite columns typical of B-DNA duplexes. In addition to the good stacking between the terminal bases, the crystal packing is further stabilized by the hydrogen bonds between O3′ of G6 (G12) and O2P of T4 (T10) (2.9 Å) in the adjacent columns. Therefore, the chimera adopts the B-form conformation with distortions at the intercalation sites.

Superposition of the nucleic acid atoms in the present structure with those of the d(CGATCG)–daunomycin complex (Moore *et al.*, 1989) shows that these two complexes are similar in structure and intercalating geometry, with an r.m.s. deviation of 0.8 Å (Fig. 3). The major difference appears in the orientation of the amino sugar in daunomycin, implying that the amino sugar is the flexible part in daunomycin and its

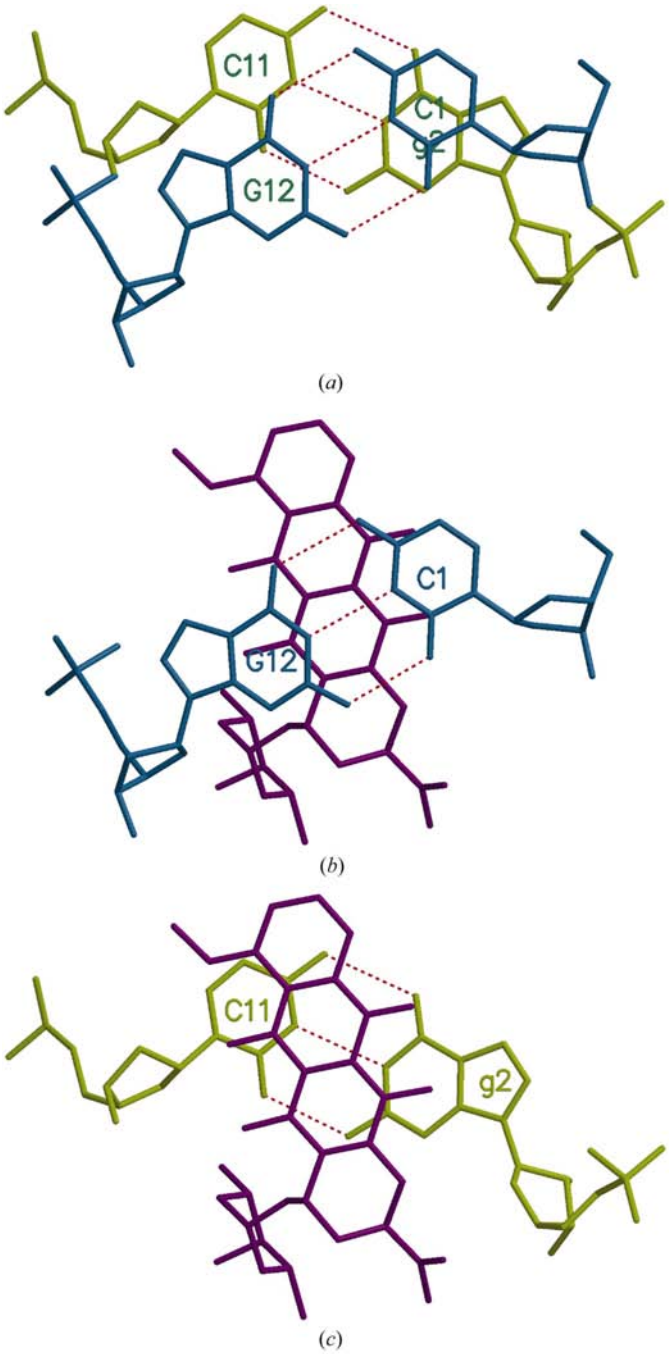

Figure 4
(*a*) Base stacking between C1–G12 and g2–C11 base pairs; (*b*) base stacking between the C1–G12 base pair and daunomycin rings; (*c*) base stacking between the daunomycin rings and the g2–C11 base pair.

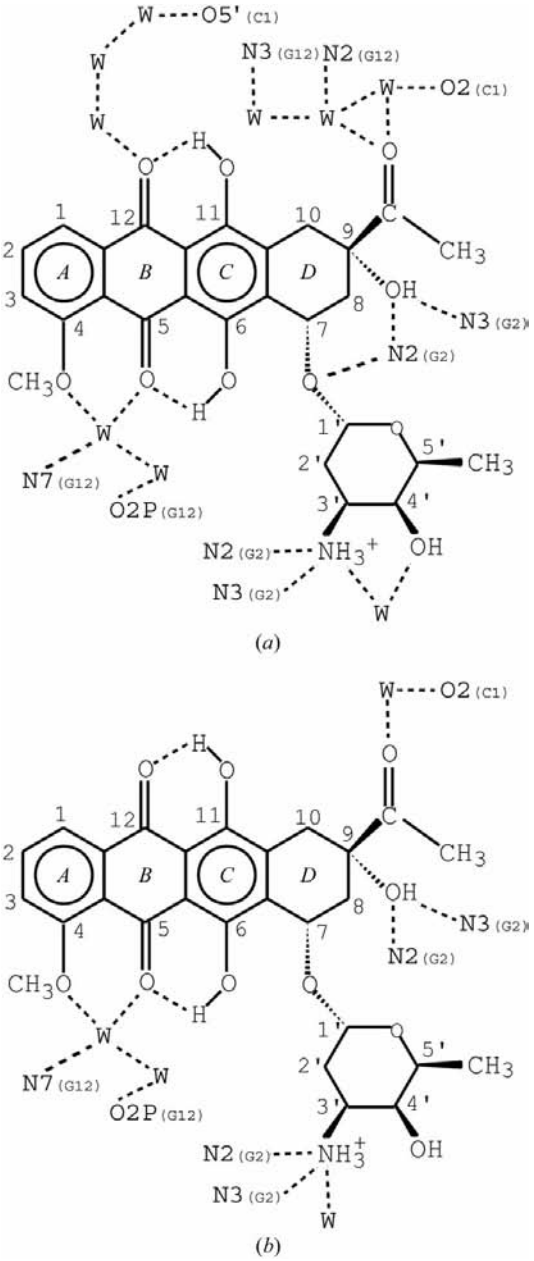

Figure 5
Schematic diagrams showing details of the interactions of daunomycin. (*a*) (CgATCG)–daunomycin complex; (*b*) d(CGATCG)–daunomycin complex.

orientation can be changed to achieve optimal interaction with nucleic acids. Differences between the two structures are also observed in the phosphate groups, which have r.m.s.d.s greater than 1.7 Å, except for the phosphates between C1 and g2 (r.m.s.d. of 0.2 Å). Superposition with a further three structures of d(CGATCG) complexed with various daunomycin derivatives (Frederick *et al.*, 1990; Gao & Wang, 1991; Saminadin *et al.*, 2000) also gives r.m.s.d.s of less than 0.8 Å, indicating that daunomycin and its derivatives have similar effects on the geometry of the DNA duplex.

3.2. Daunomycin binding to DNA/RNA chimera

Daunomycin intercalates between the base pairs C1–G12 and g2–C11 and C7–G6 and g8–C5 with a rise of 7.4 Å and a twist angle of 35° for the base pairs. The aglycon chromophore of daunomycin is orientated at nearly 90° to the long axis of the base pairs, while the amino sugar and ring *A* are located in the minor groove of the helix and ring *D* protrudes into the major groove (Fig. 4). The rings of daunomycin do not show much overlap with the base pairs. However, it displays overlap of the carbonyl group and hydroxyl group with the rings in the base pairs and also overlap of the rings with the amino groups of the base pairs. In addition to the stacking, daunomycin also interacts with the chimera through direct hydrogen bonds and water-mediated hydrogen bonds (Fig. 5). Bases g2 and G12 are involved in seven of the ten hydrogen bonds with daunomycin, indicating the important role of guanine in the interaction with daunomycin. Of the seven interactions with daunomycin, three interactions involve N2 of guanine: two direct hydrogen-bonding interactions with N2(g2) and one water-mediated interaction with N2(G12). Thus, it may be inferred that substitution of inosine for guanine will reduce the binding affinity of daunomycin.

Comparison with the CGATCG–daunomycin complex shows that most of the interactions between DNA and drug are conserved except for some water-mediated hydrogen-bonding interactions (Fig. 5). In other words, the replacement of the ribose sugar in the intercalating sites of the DNA does not change the binding preference or the interaction patterns of daunomycin. This may be related to the fact that the 2′-hydroxyl group stays in the major groove and does not make any interactions with the daunomycin. Similar results have been observed with the minor-groove-binding drug distamycin (Chen *et al.*, 1995).

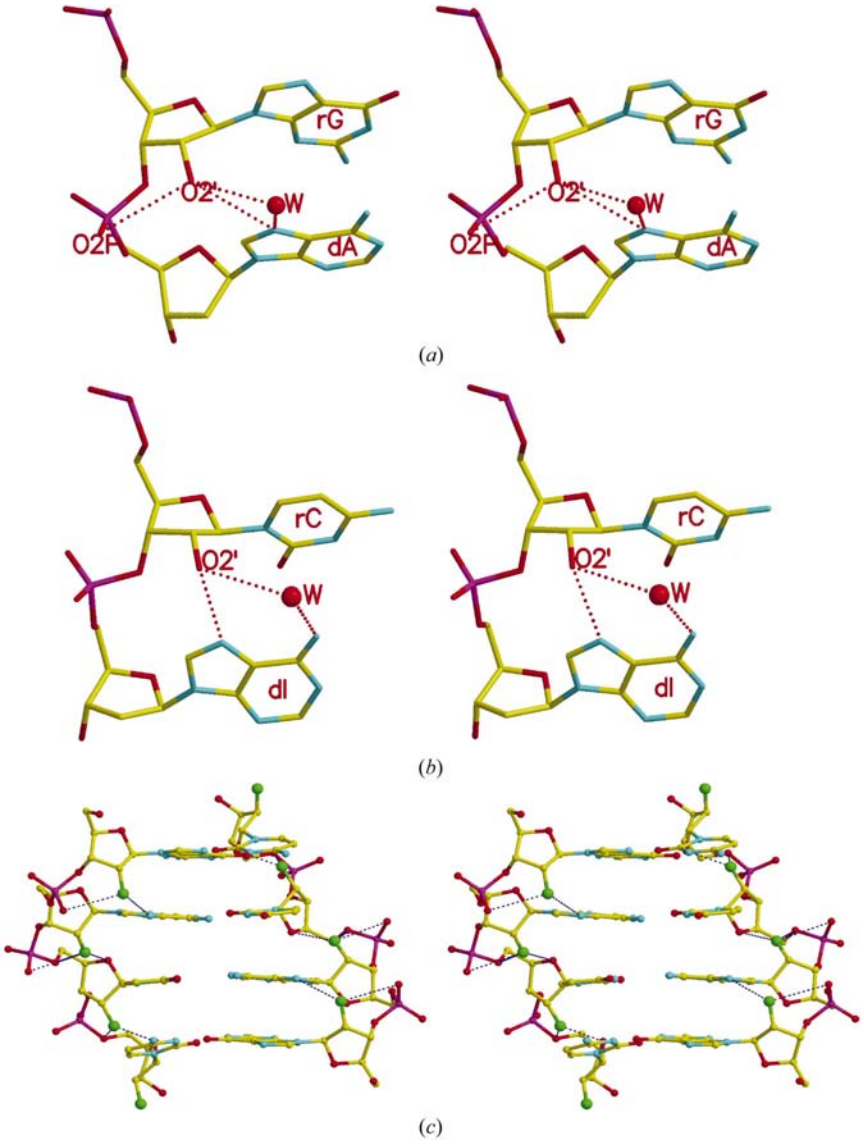

Figure 6
(*a*) The interactions of the 2′-hydroxyl group in the gA step in the present structure. (*b*) The interactions of the 2′-OH in the cI step in the IcICICIC–distamycin complex. (*c*) A model of the B-form RNA duplex.

3.3. Hydration

Of the 40 water molecules located in the present structure, 32 are in the first hydration shell and eight are in the second hydration shell. The numbers of water molecules that directly interact with the minor groove, the major groove and the phosphate groups are 9, 11 and 12, respectively. There is little difference in the hydration in these three different regions. The usual spine of hydration in the minor groove of B-DNA is broken at the amino sugar of daunomycin. The water bridges that connect the O2P of phosphate groups are also broken at the g2 nucleotide where daunomycin intercalates. Hydration also stabilizes the intercalation of daunomycin into the DNA duplex. Eight water molecules directly interact with daunomycin and four water bridges connect the drug molecule and the nucleic acid base atoms.

Table 2
Hydrogen-bonding interactions of 2′-hydroxyl groups in B-form RNA.

(*a*) Interactions observed in the B-form RNA model.

2′-Hydroxyl group	Atoms	Distance (Å)
O2′(g1)	O2P(a2)	3.2
O2′(g1)	N7(a2)	3.2
O2′(a2)	O2P(u3)	3.0
O2′(a2)	O4′(u3)	3.1
O2′(a2)	O5′(u3)	2.4
O2′(u3)	O4′(c4)	2.4
O2′(u3)	O5′(c4)	2.5

(*b*) Generalized rules for the interactions in the B-form RNA duplex.

Base step	3′-side atoms that 2′-OH may interact with
5′-Pu–Pu-3′	N7, O2P
5′-Py–Pu-3′	N7
5′-Pu–Py-3′	O4′, O5′, O2P
5′-Py–Py-3′	O4′, O5′

3.4. 2′-Hydroxyl groups in the B-form duplexes

The 2′-hydroxyl group of g2 in the present structure stays in the major groove of the duplex, forming hydrogen bonds with N7(A3) (3.2 Å) and O2P (3.2 Å). A water bridge also connects the 2′-hydroxyl group and N7(A3): 2′-OH—H_2O (3.1 Å)—N7(A3) (2.8 Å) (Fig. 6*a*). In the A-form duplex, the 2′-hydroxyl groups always stay in the minor groove and may be involved in hydrogen bonds to the O4′ and O5′ atoms in the sugar of the 3′-side residue in the minor groove (Ban *et al.*, 1994*a*,*b*; Dock-Bregeon *et al.*, 1993). To date, only three crystal structures have been determined that adopt the B-form duplex in the presence of 2′-hydroxyl groups in the sequence: IcICICIC and IcIcICIC complexed with the minor-groove binder distamycin A (Chen *et al.*, 1995) and CgATCG with the intercalator daunomycin (this study). The 2′-hydroxyl groups in these three structures are located in the major groove and are involved in interactions with the N7 atom and the anionic oxygen of phosphate groups (Figs. 6*a* and 6*b*). These results demonstrate that 2′-hydroxyl groups adopt completely different interaction patterns in the B-form duplexes.

Even though the 2′-hydroxyl groups in the B-form duplexes are located in the major groove, they still show different interactions in different sequences. In the alternating sequences IcICICIC and IcIcICIC, strong hydrogen bonds (2.8–2.9 Å) have been observed between the 2′-OH group and the N7 atom of the 3′-purine residue (Chen *et al.*, 1995) (Fig. 6*b*). A water molecule also bridges the 2′-OH and the O6/N6 of inosine. The 2′-hydroxyl groups do not have any interactions with phosphate groups. This is different from our observation in the present structure. The difference may result from the different base stacking in the adjacent base step. In the sequences IcICICIC and IcIcICIC, the base step cI has a very large twist angle (44°) and thus the 2′-OH is located very close to the zenith N7 of the next purine and forms a strong hydrogen bond. The 2′-OH is also close to O6/N6 of the next purine and a water-mediated hydrogen bond can be formed to stabilize the 2′-OH group. In the present structure, the base step gA has a comparatively low twist angle (29°) and the 2′-OH is placed far away from the N7 of next purine and near the phosphate group (Fig. 6*a*). Furthermore, the 2′-OH can only approach N7 from the C8 side. Thus, 2′-OH cannot approach N7 of A3 without clashing with C8 H. Therefore, the 2′-OH group in the present structure interacts comparatively weakly with both anionic phosphate oxygen (3.2 Å) and N7(A3) (3.2 Å).

3.5. Structural implications for the B-form RNA model

The present structure, together with the previous chimeras complexed with the minor-groove binder distamycin A (Chen *et al.*, 1995), shows that chimeras can adopt the B-form conformation when they interact with either minor-groove binders or intercalators. These results indicate that the interaction of drug molecules overwhelms the effect of the ribose sugar upon duplexes. Similarly, it is expected that the interactions of proteins may convert DNA/RNA chimeras from an A-form duplex to B-form or B-form-like structures.

Based on the B-form conformation and the interaction of the 2′-hydroxyl groups in alternating and non-alternating sequences, a model of B-form RNA duplexes can be constructed. Fig. 6(*c*) shows a B-form RNA model built by placing the 2′-hydroxyl group into the deoxyribose sugars in the central four base pairs of the present structure. The 2′-hydroxyl group is placed by superposition of the sugar in g2 on the sugars in the other deoxyribose residues. No short contacts have been observed in the model. The methyl group on the thymine base was removed so that the sequence becomes r(GAUC) or gauc. The figure shows the hydrogen-bonding interactions of the 2′-hydroxyl group and Table 2 gives details of the interactions. It is clear from the model that the 2′-hydroxyl groups have different interactions in the different base steps. Combined with the interactions in the base step 5′-Py–Pu-3′ observed in IcICICIC and IcIcICIC (Chen *et al.*, 1995), we obtained the interactions of the 2′-hydroxyl group in all four possible base-step combinations. Table 2(*b*) gives the atoms in the 3′-side nucleotides with which the 2′-hydroxyl groups may interact. The interactions of the 2′-hydroxyl group mainly depend on the 3′-side nucleotides. If the 3′-side nucleotide is a purine (Pu), the 2′-hydroxyl group will interact with the N7 atom of the purine base; if the 3′-side nucleotide is a pyrimidine (Py), it will interact with sugar atoms O4′ and O5′. If the 5′-side nucleotide is a purine, it may also interact with the anionic oxygen of the phosphate group. These phenomena may be explained by the following. A 3′-side purine offers a relatively hydrophilic pocket formed by the N7 atom of the base and the O2P anionic oxygen of the phosphate group and may form hydrogen bonds with N7. A 3′-side pyrimidine creates a more hydrophobic environment owing to the C5–C6 edge of the pyrimidine base and may only make interactions with O4′ and O5′ of the sugars. Even though it is reasonable, the present B-RNA model certainly requires further structural investigation to be verified.

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