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Structure of a stacked anthraquinone–DNA complex

The crystal structure of the telomeric sequence d(U^{Br}AGG) interacting with an anthraquinone derivative has been solved by MAD. In all previously studied complexes of intercalating drugs, the drug is usually sandwiched between two DNA base pairs. Instead, the present structure looks like a crystal of stacked anthraquinone molecules in which isolated base pairs are intercalated. Unusual base pairs are present in the structure, such as G–G and A–U^{Br} reverse Watson–Crick base pairs.

1. Introduction

There is presently great interest in drugs which may interact with G4 quadruplexes and thus interfere with telomere function. We have therefore attempted the cocrystallization of several peptide derivatives of anthraquinone and acridine drugs with the oligonucleotide d(TAGG) and its related sequence d(U^{Br}AGG), which is part of the human telomere sequence. In solution, it appears that this oligonucleotide may form guanine tetrads as determined by NMR (Kettani *et al.*, 1997). We have obtained adequate cocrystals with the drug *N,N'*-(9,10-dioxo-9,10-dihydroanthracene-2,7-diyl)-bis-*N,N*'-dimethylglycinamide, the structure of which is shown in Fig. 1.

The crystal structure that we have obtained is most unusual and unexpected. It does not contain any guanine tetrads, only guanine–guanine base pairs. It shows stacks of drug molecules in which some unusual base pairs are intercalated. We may think of this structure as DNA intercalated in a drug crystal, rather than the usual intercalation of a drug in a DNA duplex.

We should also note that there are very few previous studies of DNA–anthraquinone complexes. Gasper *et al.* (1998) determined the structure for an anthraquinone with a single side chain, which showed

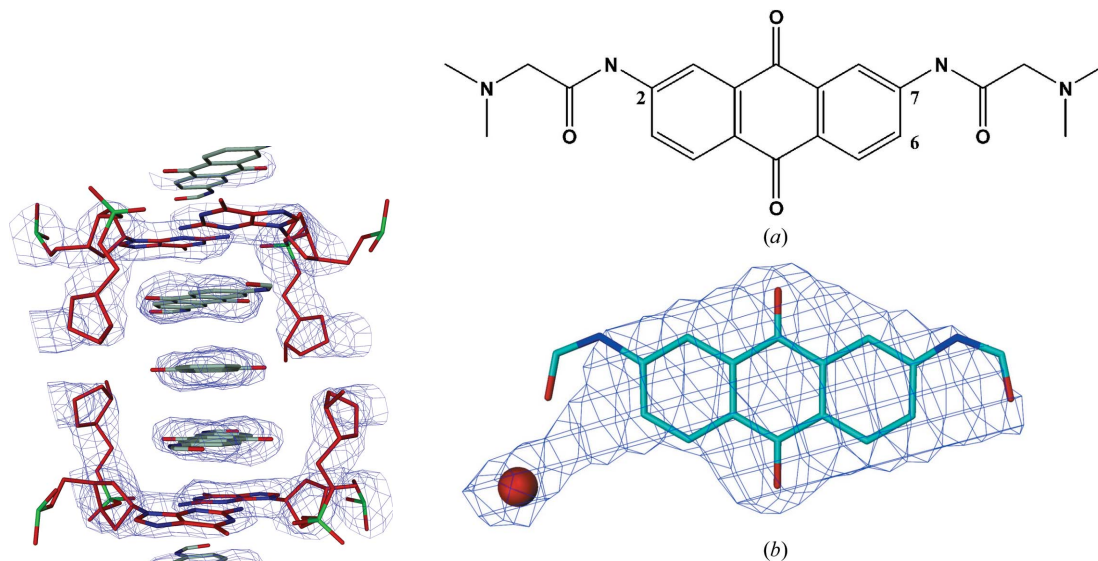


Figure 1
(a) Chemical structure of *N,N'*-(9,10-dioxo-9,10-dihydroanthracene-2,7-diyl)-bis-*N,N'*-dimethylglycinamide. For refinement, the terminal dimethylamino groups were removed. An OMIT electron-density map ($F_o - F_c$ at the 2σ level) of one of the drugs (*E*) and one associated water molecule is also shown (*b*).

a standard intercalation. In contrast, anthraquinones with two side chains show unusual stacking features and significant disorder (Yang *et al.*, 2000; Valls *et al.*, 2005).

In addition to the interest of this study in order to understand DNA–drug interactions, its unusual features may be helpful in the design of DNA nanostructures, a field that is currently under strong development.

2. Experimental

The anthraquinone drug (Fig. 1) was a gift from Professor S. Neidle. It was obtained using methods that are described elsewhere (Zagotto *et al.*, 2008). The drug was crystallized in association with the tetranucleotide dU^{Br}-dA-dG-dG. Crystals were obtained at 310 K in hanging drops (5 µl) using the following conditions: 0.8 mM DNA, 1.6 mM drug, 22 mM sodium cacodylate pH 7.0, 15 mM NaCl, 0.8 mM MgCl₂, 20% PEG 400. The reservoir contained 1 ml crystallization solution with a higher concentration of PEG 400. X-ray diffraction data were measured with cryocooling at 110 K on the former Spanish beamline BM14 at the European Synchrotron Radiation Facility. Data were measured from different crystals. Data from crystal 1 were collected at a wavelength of 0.9795 Å; it diffracted to a maximum resolution of 2.4 Å. However, only data to 2.8 Å resolution could be used owing to increased mosaicity in the higher resolution region. Data from crystal 2 were collected to 3.0 Å resolution at three different wavelengths in order to use multiple-wavelength anomalous dispersion (MAD) with bromine as the heavy atom. The wavelengths used were obtained from a fluorescence spectrum measured from the crystal. The data were integrated and scaled with the *HKL* suite (Otwinowski & Minor, 1997). A mosaicity of 0.7–1.2° was found. The space group turned out to be tetragonal

Table 1

Crystal data and refinement statistics.

Values in parentheses are for the last shell.

Wavelength (Å)	0.907
Temperature (K)	110
Space group	<i>P</i> 4 ₃ 2 ₁ 2
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 29.70, <i>c</i> = 60.88, α = β = γ = 90
Resolution range (Å)	28–2.8 (2.875–2.803)
Unique reflections	757
Free <i>R</i> factor reflections	41
Completeness (%)	99.01 (100)
Redundancy factor	8.9
<i>R</i> _{merge} [†]	0.060
Contents of asymmetric unit	2 DNA single chains, 3.5 anthraquinones, 1 magnesium, 6 water molecules
<i>R</i> _{work} [‡]	0.232 (0.430)
<i>R</i> _{free} [‡]	0.259 (0.164)
Mean <i>B</i> factors (Å ²)	
Wilson plot	48.9
DNA	44.1
Anthraquinone <i>C</i>	112.1
Anthraquinone <i>D</i>	81.6
Anthraquinone <i>E</i>	46.6
Anthraquinone <i>F</i>	35.3
Magnesium ion	94.0
Water molecules	101.8
R.m.s.d. bonds (Å)	0.006
R.m.s.d. angles (°)	1.769
R.m.s.d. chiral (Å ³)	0.059

[†] $R_{\text{merge}}(I) = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$ calculated for the whole data set. [‡] $R_{\text{work}} = \frac{\sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}||}{\sum_{hkl} |F_{\text{obs}}|}$. *R*_{free} is the *R* factor calculated for reflections used for cross-validation during refinement.

*P*4₃2₁2, with unit-cell parameters *a* = *b* = 29.7, *c* = 60.88 Å. Initial phases were obtained by MAD using the program *CNS* (Brünger *et al.*, 1998). Two Br atoms were detected. The model was manually built and the anthraquinones were inserted into the electron density. The model was then refined using the MAD phases with the *CNS* package

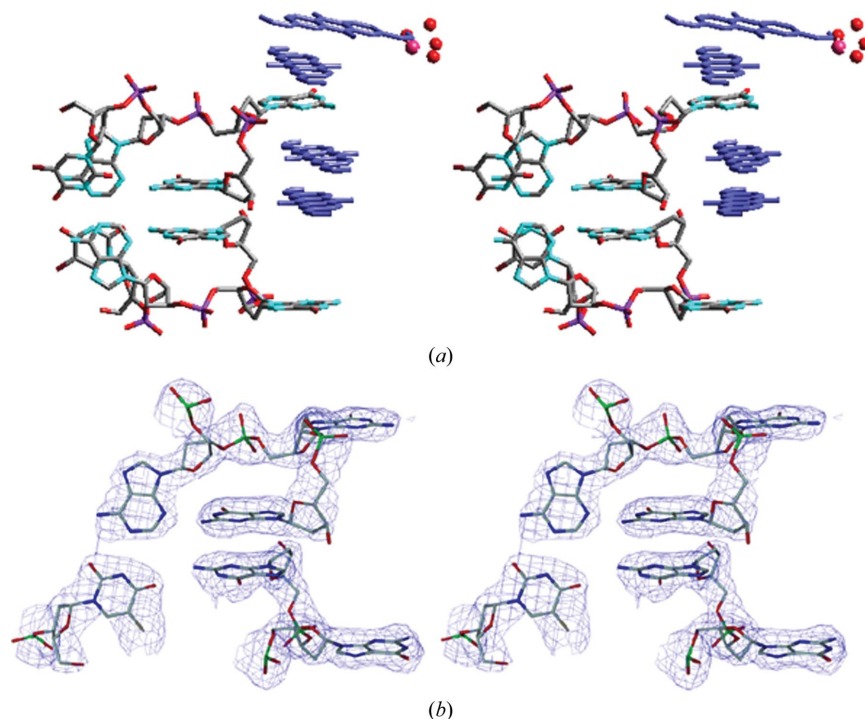


Figure 2

Stereoviews of (a) the asymmetric unit and (b) the electron-density map ($2F_o - F_c$ at the 1.2σ level) of one portion of the structure. The anthraquinone molecules correspond to *C*, *D*, *E* and *F* from top to bottom, as shown schematically in Fig. 4. Chain *A* is in the upper part, with chain *B* below. In the electron-density map one symmetry-related U^{Br} base is also shown. It forms a reverse Watson–Crick base pair with the adenine of chain *A*. Note that the two DNA molecules in the asymmetric unit have a practically identical conformation, with the exception of the U^{Br} residue. One magnesium ion (pink) with four coordinated water molecules (red) is also shown.

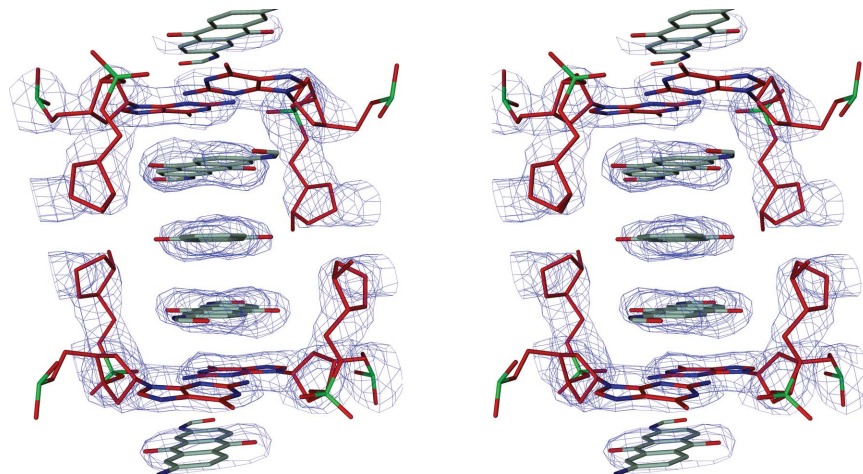


Figure 3

Stereoview of the electron-density map ($2F_o - F_c$ at the 1.2σ level) of the central anthraquinone *F* with its two neighbouring anthraquinones *E*. The figure illustrates that the planes of the drugs are clearly defined, although the side chains could not be located.

(Brünger *et al.*, 1998). Refinement was concluded with *REFMAC5* (Murshudov *et al.*, 1997) using data to 2.8 Å resolution. We used the standard dictionary found in *LIBCHECK* from the *CCP4* interface. Rigid-body refinement of *B* factors was performed using a single TLS group. The side chains of the drug were disordered and were partially eliminated for refinement, as shown in Fig. 1. The asymmetric unit and the electron-density map for one portion of it are presented in Fig. 2. Data-set and refinement statistics are shown in Table 1. Note that the R_{free} value in the last shell is very low. This anomaly was a consequence of the fact that only three reflections were used in order to calculate this value.

The position of the plane of the drugs was clearly defined as shown in Fig. 3. However, the side chains could not be located. A 180° rotation of the drug molecules around their main axis did not change their *R* factors. It appears that the drug molecules may have multiple positions in their average plane which prevent localization of the side chains. This fact is apparent from the large *B* factors of the drug molecules, as given in Table 1. Anthraquinone *F* sits on a dyad axis and was modelled with 50% occupancy. The two superimposed symmetric drugs in the same plane had their side chains in opposite orientations. As a result, their *B* factors (Table 1) are significantly smaller. It should also be noted that the exact position of a related anthraquinone drug studied at much higher resolution could not be accurately modelled (Valls *et al.*, 2005).

Attempts to model residual electron density in the neighbourhood of the anthraquinones did not help to locate the side chains. A significant region of missing electron density was found next to anthraquinone *C*, but the side chains could not be traced. A tentative magnesium ion was instead placed in this region, with four associated water molecules in an approximately octahedral coordination. Coordination was completed by an O atom from anthraquinone *C* and a phosphate O atom from a neighbouring asymmetric unit. However, their *B* factors were high, as shown in Table 1.

3. Results and discussion

This oligonucleotide has a partial sequence of human telomere repeat motifs. It has previously been studied by NMR (Kettani *et al.*, 1997) and formed a guanine tetrad. Therefore, we thought that it would be a good model in order to study the interaction of anthraquinone drugs

with telomere sequences. Instead, we found a complex structure in which several anthraquinone molecules are stacked together with several base pairs, as shown in Fig. 4. The asymmetric unit of this structure contains three and a half anthraquinone molecules and two oligonucleotide chains (Fig. 2). The DNA bases form guanine–

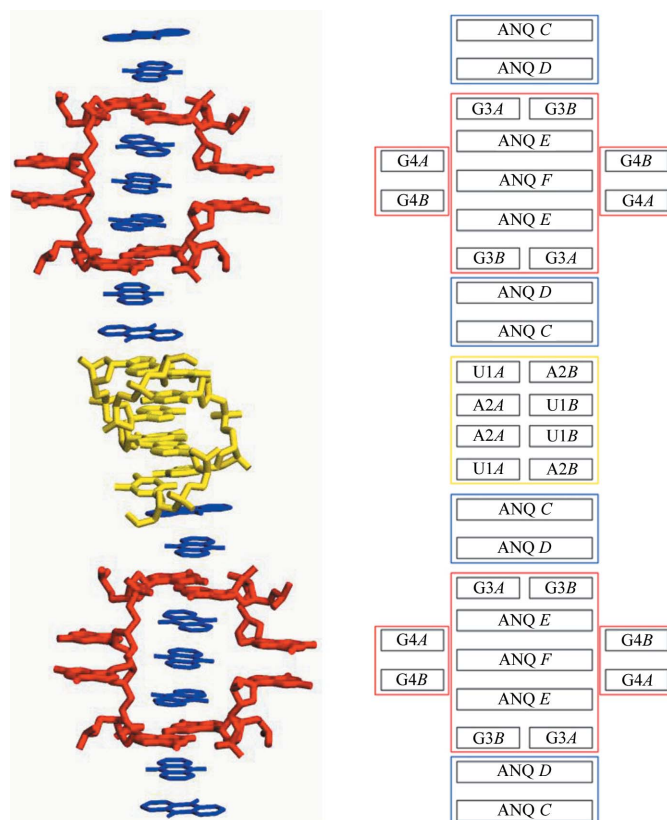


Figure 4

In the crystal the drugs (blue) are stacked together with G3–G3 (red) and A·U^{Br} base pairs (yellow). They form infinite columns, with neighbouring columns perpendicular to the plane of the figure. The codes for each base and drug (ANQ) are shown on the right. Each individual d(U^{Br}AGG) molecule is shared by two perpendicular columns in the crystal, with the guanines in one column and the U^{Br}A bases in a perpendicular column.

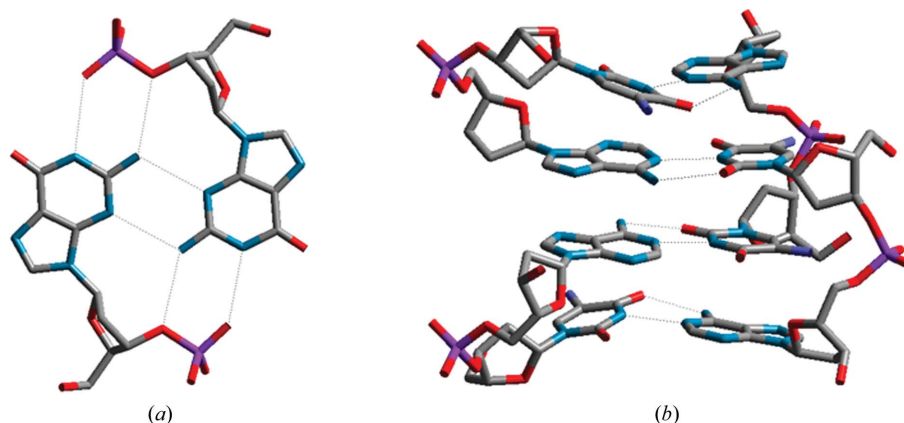


Figure 5

The DNA base pairs in the structure. G3-G3 pairs are shown in (a). A-U^{Br} pairs are shown in (b) and are reverse Watson-Crick in the centre and normal Watson-Crick at both ends. Chain A is on the left and chain B on the right. A dyad axis is present at the centre approximately in the plane of the drawing.

guanine, standard adenine-thymine and reverse adenine-thymine Watson-Crick base pairs. They are stacked among the anthraquinone residues. The plane of the anthraquinone drugs is clearly defined (Fig. 3), but the positions of the side chains could not be ascertained. The column of stacked anthraquinone/base pairs presents several dyad axes. Guanine 4 forms an independent group of stacked bases which lie outside the stacked columns, as indicated in Fig. 4. They interact through hydrogen bonds with the A-T base pairs in neighbouring stacked columns, as shown in Fig. 2. In the crystal, parallel sets of columns are surrounded by symmetry-related identical perpendicular columns. This work demonstrates that short oligonucleotides may adopt quite unexpected shapes. This structure contains several unusual features that are not found in longer oligonucleotides.

The DNA bases in the structure present hydrogen bonds that are not frequently found. Guanine 3 forms a base pair through hydrogen bonding of the N2 and N3 atoms, as shown in Fig. 5. Such an interaction is not found in guanine crystals (Thewalt *et al.*, 1971) or in guanine tetrads. However, it is present in the standard crystal structure of DNA dodecamers (Dickerson *et al.*, 1987). The guanine 3 base pair is further stabilized through hydrogen bonds with phosphate O atoms. Adenines and thymines in the present structure form an unusual stack of base pairs, as is also shown in Fig. 5. The two central base pairs form a reverse Watson-Crick system of hydrogen bonds. The other two A-T base pairs have standard Watson-Crick hydrogen bonds.

We have compared our structure with those found in some anthraquinone-derivative crystals available in the Cambridge Structural Database (Allen, 2002). The structure is strongly influenced by the size and polarity of the side chains attached to the anthraquinone rings. In general, anthraquinones with small lateral side chains pack as infinite stacked columns that are usually slanted (Kingsford-Adaboh & Kashino, 1995; Il'in *et al.*, 1975; Janczak, 1995; Fu & Brock, 1998), similar to the situation found here in anthraquinones *E* and *F* (Figs. 3 and 4). In some cases the inclination is very large (Popova *et al.*, 1975). In one case (de Ruyck *et al.*, 2006), although the side chains are small (two OH groups), the anthraquinone molecules are stacked in an approximate perpendicular fashion, as found here in anthraquinones *C* and *D* (Fig. 4). Cases of staggered stacking are also found (de Abreu *et al.*, 2002). Finally, in the presence of bulky side chains no stacking is found (Agbandje *et al.*, 1992). In summary, the variability of stacking features present in our structure is reminiscent of that

found in crystals of anthraquinone derivatives. Drawings of all stacking pairs in our structure are available in De Luchi (2008).

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