Structure of d(ITITACAC) complexed with distamycin at 1.6 Å resolution

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The crystal structure of the DNA octamer d(ITITACAC)$_2$ complexed with distamycin has been determined at 1.6 Å resolution and refined to a final R_{work} and R_{free} of 17.0 and 20.7%, respectively. Two molecules of distamycin bind to the DNA duplex in an antiparallel side-by-side fashion. Each drug molecule covers five base pairs of the DNA duplex, with its amide groups hydrogen-bonding to bases in the proximal DNA strand. These two antiparallel drug molecules are stacked together with the pyrrole rings of one molecule stacking against the amide groups of the other. The present structure emphasizes the features of alternating DNA octamers in interaction with distamycin.

1. Introduction

The interaction between DNA and drugs is of great importance in molecular biology and medicinal chemistry. Drugs that target nucleic acids have wide applications in nucleic acid recognition, regulation of biological processes and the development of therapeutic agents against cancers and virus-related diseases. After the first DNA–netropsin complex was solved (Kopka *et al.*, 1985), crystal structures of a series of 1:1 DNA–drug complexes have been reported with other natural antibiotics such as distamycin and synthetic drugs (Wang & Teng, 1990; Kopka & Larsen, 1992). Structural studies on the 1:1 DNA–drug complexes demonstrated that netropsin and distamycin molecules interact with both DNA strands through hydrogen bonds and van der Waals interactions with sequence specificity for A·T base pairs (Kopka *et al.*, 1985; Coll *et al.*, 1987). The 2:1 side-by-side binding mode of distamycin was elucidated in NMR (Pelton & Wemmer, 1989, 1990) and crystallographic studies (Chen *et al.*, 1994, 1997; Mitra *et al.*, 1999). This new binding mode opened up avenues for designing new drugs with higher affinity (Mrksich & Dervan, 1993) and altered specificity (Geierstanger *et al.*, 1996).

The first crystal structure of a 2:1 DNA–drug complex was d(ICICICIC)–distamycin (Chen *et al.*, 1994), where I stands for inosine. In order to investigate whether the observed 2:1 binding mode of distamycin is pertinent only to the unnatural I·C base pair, we have systematically substituted I·C base pairs with A·T base pairs. The results have implied that the binding mode of distamycin does not change with these substitutions (Chen *et al.*, 1997; Mitra *et al.*, 1999). Here, we further study the binding behavior of distamycin with another alter-

nating DNA octamer d(ITITACAC). The present study indicates that distamycin does not change its binding mode on the substitution of A·T for I·C base pairs, reinforcing the common feature of the binding interaction of distamycin in alternating DNA octamers.

2. Experimental

The DNA octamer d(ITITACAC) was synthesized by the solid-phase phosphoramidite method on an Applied Biosystems DNA synthesizer. The DNA was cleaved off from the column using 2 ml 37% ammonium hydroxide and incubated in the same solution at 328 K overnight. The lyophilized sample was suspended in 25 µl 2.5 *M* ammonium acetate and precipitated with 2 ml absolute ethanol at 248 K. The lyophilized precipitate was used for crystallization without further purification. Crystals were grown by the hanging-drop vapor-diffusion method in the presence of 1 m*M* DNA (single-stranded concentration), 30 m*M* sodium cacodylate buffer pH 7.0, 0.1 m*M* cobalt hexamine chloride and 1 m*M* distamycin hydrochloride, equilibrated against 60% MPD in the reservoir. Crystals grew to dimensions of $0.30 \times 0.30 \times 0.50$ mm in a few weeks. One of the large crystals was mounted on a thin glass capillary for intensity-data collection on an in-house R-AXIS IIc imaging plate equipped with a Rigaku copper rotating-anode generator and a graphite mono-chromator ($\lambda = 1.5418$ Å). The rotating-anode generator was operated at 50 kV and 100 mA. The crystal-to-detector distance was 60 mm. The intensity data were collected at room temperature and the crystal did not decay during data collection. A data set consisting of 45 frames with an oscillation angle of 3° and an exposure time of 30 min per frame was

collected and processed using the software supplied by the manufacturer (Molecular Structure Corporation). The crystal data are given in Table 1.

The crystal structure of the present DNA sequence is isomorphous to the monoclinic crystal form of d(ICATATIC) (Chen *et al.*, 1997) and therefore the coordinates of both the DNA molecule and distamycin were used directly in refinement with *X-PLOR*

(Brünger, 1992). 10% of the data were chosen randomly for R_{free} calculation from the beginning of the refinement. Rigid-body refinement with 475 reflections $>2\sigma(F)$ in the resolution range 8.0–3.0 Å lowered R_{work} to 0.290. Positional and thermal *B*-factor refinement gave an R_{work} of 0.264 for 1248 reflections in the resolution range 8.0–2.0 Å. After confirming with omit maps, the bases were changed to the correct sequence for

Table 1
Crystal data and refinement statistics of d(ITITACAC).

Values in parentheses are for data in the highest resolution bin (1.7–1.6 Å).

| Crystal data	
Crystal system	Monoclinic
Space group	*C*2
Unit-cell parameters	
a (Å)	33.26
b (Å)	25.05
c (Å)	28.30
β (°)	124.2
Asymmetric unit	1 DNA strand and 1 distamycin
Volume per base pair (Å³)	1218
Resolution (Å)	10.0–1.6
No. of unique reflections	2737 (381)
Data completeness (%)	92.0 (88.8)
R_{sym} (%)	4.6 (16.2)
Refinement results	
Resolution range used (Å)	10.0–1.6
No. of reflections used [$\geq 2\sigma(F)$]	2520
No. of water molecules	36
R_{work} (%)	17.0
R_{free} (%)	20.7
R.m.s. deviations from ideal geometry	
Parameter file	param11.dna
Bond length (Å)	0.006
Bond angle (°)	1.3
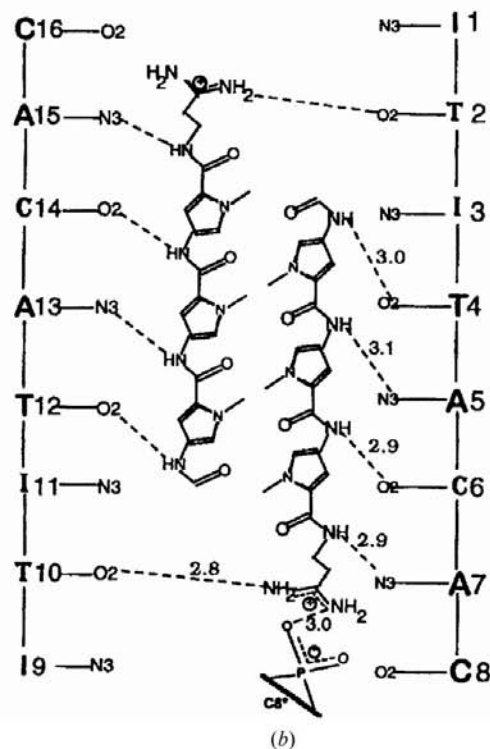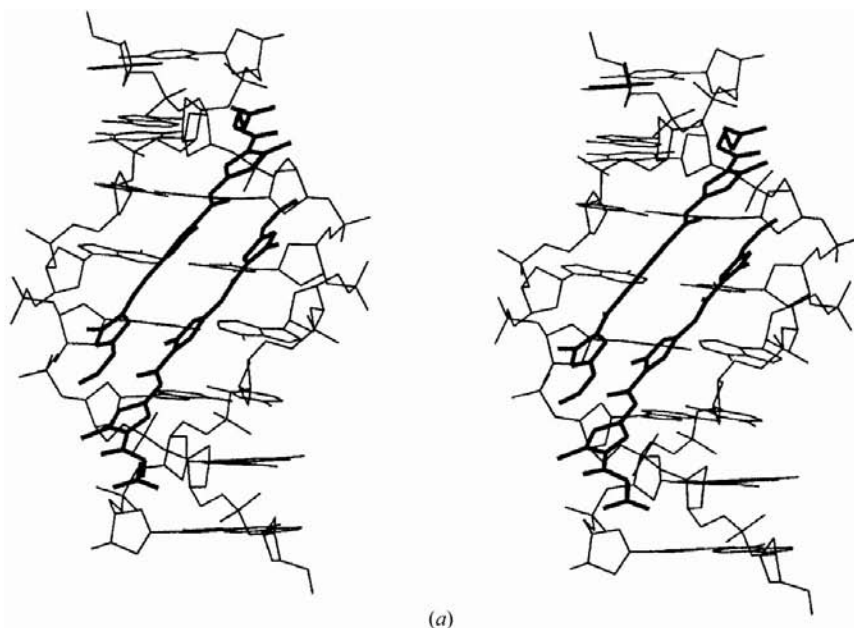
Figure 1
(*a*) A stereoview of the d(ITITACAC)–distamycin complex. (*b*) Hydrogen-bonding scheme of the present DNA–distamycin complex.

simulated annealing. The refinement was then continued, increasing the resolution to 1.6 Å. Water molecules were located in the $|F_o - F_c| > 3\sigma$ and $|2F_o - F_c| > \sigma$ electron-density maps and included in subsequent refinement. The refinement converged to a final R_{work} and R_{free} of 0.170 and 0.207, respectively, for 2520 reflections [$\geq 2\sigma(F)$] in the resolution range 10.0–1.6 Å. Table 1 summarizes the refinement statistics for the present structure.

3. Results and discussion

3.1. Overall conformation

The DNA octamer d(ITITACAC)–distamycin complex crystallized in the monoclinic space group *C*2 with one DNA strand and one distamycin molecule in the asymmetric unit. This strand and its symmetry-related strand form a right-handed B-form duplex with two distamycin molecules binding in the minor groove in a side-by-side pattern (Fig. 1). The minor-groove widths, determined as the shortest P—P distance across the groove, are quite uniform, varying from 7.3 to 7.5 Å. The major-groove widths change from 10.9 Å at the end of the duplex to 9.3 Å in the center. The average distance between intra-stranded adjacent P atoms is 6.7 Å, the same as the typical value for B-DNA. The average helical twist is 35°, corresponding to 10.3 bp per turn. The helical twists exhibit a regular alternation, with the purine–pyrimidine

Table 2
Crystal structures of alternating DNA octamers complexed with distamycin.

NDB ID	Sequence	Space group	Binding mode	Reference
GDHB25	d(ICICICIC)	$P4_122$	2:1	Chen *et al.* (1994)
GDLB49	d(ICATATIC)	$C2$	2:1	Chen *et al.* (1997)
GDLB50	d(ICATATIC)	$P4_122$	2:1	Chen *et al.* (1997)
GDLB51	d(ICITACIC)	$P4_122$	2:1	Chen *et al.* (1997)
GDH060	d(GTATATAC)	$P2_1$	2:1	Mitra *et al.* (1999)
DD0043	d(ITITACAC)	$C2$	2:1	Present study

steps under-twisted (average 28°) and the pyrimidine–purine steps over-twisted (average 45°). The base stacking is intra-stranded. The low twist angles at the I1–T2 and I3–T4 steps correspond to good intra-stranded base stacking, while the high twist angles at the T2–I3 and T4–A5 steps correspond to poor intra-stranded base stacking. The helical rise of the purine–pyrimidine step is large and that of the pyrimidine–purine step is small, consistent with the observation of 'low twist, high rise' in many crystal structures. The central T–A step exhibits a roll angle of −16°, opening the base pair towards the major groove. The sugar puckers adopt C2'-endo and C1'-exo geometries.

3.2. Distamycin interaction

The four amide groups of the drug molecule make hydrogen bonds alternately to the O2 of pyrimidine and N3 of purine in the fragment of 5'-TACA-3' on the proximal DNA strand (Fig. 1*b*). The N9 group of the propylamidinium tail makes a hydrogen-bonding interaction with the O2 group of thymine of the distal strand, while the N8 group interacts with an anionic phosphate O atom of C8 of a symmetry-related molecule. N9 is the only part of the drug molecule that makes a hydrogen bond to the distal strand. In addition to the hydrogen-bonding interaction between the drug molecule and the DNA, another major contribution to the stability of the DNA–drug complex comes from the van der Waals interaction between the drug and the sugar-phosphate backbone of the DNA molecule. Some of the atoms in the sugar rings of DNA are in van der Waals contacts with the drug molecule. The two molecules of distamycin expand the minor groove of the DNA to 7.4 Å, similar to previous results (Chen *et al.*, 1994, 1997; Mitra *et al.*, 1999).

Comparison with other DNA octamer–distamycin complexes in Table 2 shows that distamycin adopts the same 2:1 side-by-side binding mode, with the four amide groups binding to the fragment 5'-Py4-Pu5-Py6-Pu7-3' (where Py is thymine or cytosine, Pu is adenine or inosine and the number represents the position of the nucleotide). This result indicates that the 2:1 binding mode is characteristic of the sequence and distamycin, irrespective of the crystal packing fashion or the A·T and I·C components.

3.3. Hydration and crystal packing

There are 36 water molecules in the present structure. 67% of the water molecules directly interact with the anionic O atoms in the phosphate groups. An anionic O atom usually has more than one water molecule bound to it. Some water bridges have been observed between adjacent anionic O atoms in the phosphate groups. The major groove is much more hydrated than the minor groove owing to the presence of the drug molecules. In the major-groove edge, all of the functional groups of the central four bases are hydrated. In the minor-groove edge, only the two terminal I·C base pairs are hydrated, which is the same as in other previous structures (Chen *et al.*, 1997). The hydration in the major groove is more similar to the monoclinic form of d(ICATATIC) than its tetragonal form (Chen *et al.*, 1997).

The DNA duplexes are stacked on top of each other, forming helical columns along the *c* axis in a 5',5'/3',3' pattern, with the O4 atom of one cytosine stacking on the ring of another cytosine and with a little overlap of the six-membered rings of two inosines. This pattern has also been observed in the crystal structure of d(ICATATIC) in space group $C2$ (Chen *et al.*, 1997). A water molecule in the junction plays an important role in stabilizing the crystal packing, connecting O4' (I1) (3.1 Å) and O5' (I1) (3.3 Å) in one duplex with the N3 (I1) (3.0 Å) and O4 (T2) (3.2 Å) in the adjacent duplex.

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