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Structure of the First Parallel DNA Quadruplex-Drug Complex

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The telomeric ends of chromosomes consist of tandem repeats of guanine-rich DNA sequences, in association with a number of functional and structural proteins.[1] Telomeric DNA is typically several kb in length. Almost all of this is double-helical, but a single-stranded overhang protrudes at the extreme 3′ terminus, of length ca. 100−200 bases in humans. Telomeric DNA sequences can associate strongly together, either inter- or intramolecularly, to form a variety of four-stranded quadruplex structures,[2] all containing stacked guanine-tetrads, as revealed in several crystal[3] and NMR[4] structures. There is a strong indication that the polarity of the strands in a quadruplex may be dependent on the nature of the sequences intervening between consecutive runs of guanines in a telomeric sequence, and on the counterion stabilizing the G-tetrads. Crystallographic studies have revealed that all strands are parallel[3a] in the intermolecular quadruplex formed from four molecules of d(TGGGGT), and in two quadruplexes formed from the human telomeric repeat d(TTAGGG).[3b]

It has been shown that ligands that interact with quadruplexes can also act as inhibitors of the enzyme telomerase,[5] whose function is to protect tumor cells against telomere loss during replication. Telomerase, which is expressed in 80−85% of tumor cells while being absent in normal somatic cells, is a potential tumor-selective target for chemotherapy. It catalyzes the synthesis of further telomeric DNA repeats onto the 3′ end of telomeres, contributing to the immortalization of human tumor cells. The facilitation of quadruplex formation and stabilization at the 3′ ends halts further DNA synthesis, and a number of quadruplex-binding molecules have been developed that are effective by this mechanism.[6] These molecules have planar, electron-deficient aromatic ring systems, together with acyclic substituent groups. It has been suggested on the basis of molecular modeling that these latter features impart selectivity toward quadruplex DNA structures.[7] The nature of ligand-quadruplex complexes is not well understood, and to date design studies have in large part relied on structural information from molecular modeling studies on model systems.[7] In particular, there has been controversy as to whether planar aromatic ligands such as acridines or anthraquinones are intercalated in the center of the stack of G4-tetrads in a quadruplex or are stacked on the exterior, as suggested by NMR studies.[8]

There is increasing evidence that the cellular activity of established duplex DNA-interacting anticancer drugs such as the anthracyclines doxorubicin and daunomycin also involves interaction with telomeric DNA,[9] possibly via quadruplex stabilization of the anthraquinone chromophore. These drugs lack the quadruplex selectivity of, for example, trisubstituted acridines, although they do also bind to quadruplex DNA in solution.[10] We have previously developed disubstituted amidoanthraquinones as a paradigm for ligand-quadruplex binding and obtained crystals of a complex with d(TGGGGT), although these only provided a disordered fiber diffraction pattern.[11] We have now obtained well-ordered crystals of a complex that diffract to high resolution and report here the first crystal structure determination of a parallel G4 quadruplex-drug complex employing the anticancer drug daunomycin (Figure 1). The complex was crystallized by the hanging-drop method in the monoclinic space group *C*2, with cell dimensions $a = 53.078$ Å, $b = 47.239$ Å, $c = 31.914$ Å, $\beta = 119.80°$. Intensity data were collected at SSRL to 1.17 Å resolution. A total of 202 689 reflections were measured, which reduced to 20 944 unique intensities with an R_{sym} of 5.5%. The structure was solved by molecular replacement using coordinates from the d(TGGGGT) crystal structure.[3a] The final R factor is 15.7%, with an R_{free} of 19.7%. Atomic coordinates and structure factors have been deposited in the Nucleic Acid and Protein Databases (ID codes DD0055 and 1O0K, respectively).

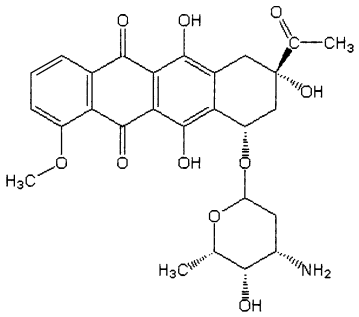

Figure 1. Structure of daunomycin.

The asymmetric unit contains four parallel d(TGGGGT) strands that form a discrete intermolecular quadruplex, together with three daunomycin molecules, three Na^+ cations, and 129 water molecules. The quadruplex itself comprises four G-tetrad units, each stacked 3.35 Å apart. A sodium ion is coordinated in bipyramidal antiprismatic geometry between each G-tetrad. This arrangement and associated nucleotide backbone conformations are almost identical to those found in the crystal structure of the native d(TGGGGT) quadruplex. As in that structure, the thymine bases are oriented away from the quadruplex into solvent regions of the lattice, and the electron density for four of the eight thymines was not observed. The complex is related by a crystallographic two-fold axis to another asymmetric unit, such that the two are stacked end-to-end, 5′ to 5′ (Figure 2). The interface between the two quadruplexes is filled by two layers of daunomycin molecules. No drug molecules are intercalated into the guanine core of the quadruplex. The six daunomycin molecules at the interface are arranged into two dyad-related sets of three coplanar molecules (Figure 3a). Each set of three daunomycins is stacked onto the 5′ end of the quadruplex where they make weak $\pi-\pi$ interactions with the guanines in the terminal tetrad (Figure 3b). The greater degree of chromophore overlap within the two daunomycin layers indicates stronger $\pi-\pi$ interactions than those between daunomycins and the G4 layer.

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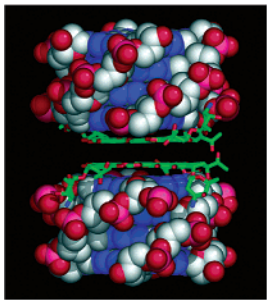

Figure 2. Structure of the daunomycin-d(TGGGGT) complex, showing the arrangement in the crystal lattice of two quadruplexes, in van der Waals space-filling mode, and stacked end-to-end. The daunomycin molecules are shown in green ball-and-stick representation. Several thymine residues have been removed to enhance clarity.

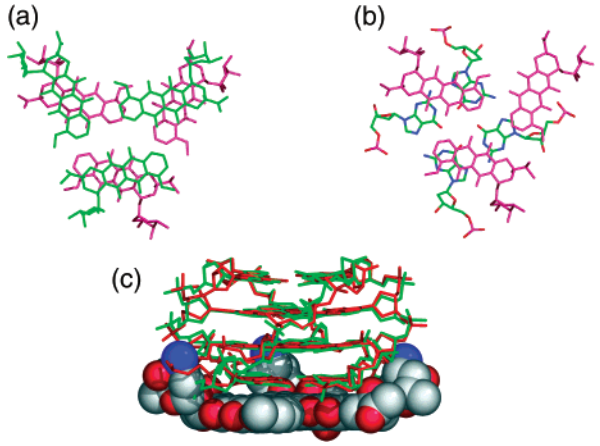

Figure 3. (a) The stacking interactions of the daunomycins in one layer (magenta) with the symmetry-related ones from the neighboring asymmetric unit (green). The dyad axis runs vertically through the center of the diagram. (b) The three daunomycin molecules (magenta) stacked onto the guanines at the 5′ end of the quadruplex. (c) View of the complex, showing the quadruplex (in red) and the daunomycin molecules as space-filling spheres. The native quadruplex structure[3a] (in green) has been least-squares fitted onto the present quadruplex.

Each trio of daunomycin molecules is held together in one layer by a cluster of van der Waals contacts. The daunomycin layer packs tightly onto the end of the quadruplex stack, with the daunosamine sugar moieties forming H-bonding interactions and/or van der Waals contacts with three of the four quadruplex grooves. The daunosamine sugar of the first daunomycin (DM25) is wedged tightly into its groove, and its cationic amine substituent (N3*) H-bonds to phosphate oxygens on both sides of the groove (N3*- -O2P_8, 2.79 Å; N3*- -O1P_16, 2.81 Å); the sugar of the second daunomycin (DM26) is not as deep in its groove, and H-bonds are found from its cationic amine and exocyclic OH groups to phosphate oxygens on just one side of the groove (N3*- -O2P_20, 2.68 Å, O4*- -O1P_18, 2.66 Å); there are no direct H-bonds between the daunosamine of the third daunomycin (DM27) and the quadruplex, although there are some water-mediated interactions. The binding of the daunosamine sugars into the quadruplex grooves has not resulted in any significant alteration to the widths of these grooves as compared to those in the native quadruplex. The only changes are some small (ca. 0.5 Å) movements in phosphate groups that are involved in H-bonding to the amine substituents. The backbone conformations of the two quadruplexes remain very similar (Figure 3c).

This crystal structure shows that daunomycin prefers to stack onto a terminal G-quartet rather than intercalate between the layers of the quadruplex. This is in accord with models from NMR studies.[8] We have recently solved the crystal structure of a complex between a disubstituted acridine and the dimeric intermolecular G-quadruplex formed by two strands of d(GGGGTTTTGGGG),[12] which has both parallel and antiparallel nucleotide strands. The acridine ligand in that structure is also bound at the end of the G-quartet stack. Further structural data are required before a definitive rule can be established regarding end-stacking versus intercalation. However, we note that the energetics of unstacking a G-quadruplex, once formed, must mitigate against the destacking and unwinding required if ligands were to intercalate between G-quartets, rather than at the ends, as we see here.

A dominant feature of parallel DNA quadruplexes[3a,b] is that they present the large surface area of their terminal G-quartet to the environment. We have shown that this is sufficiently large to simultaneously accommodate three daunomycin molecules. Substituted porphyrins[13] or the potent telomerase inhibitor telomestatin,[14] which cannot fit readily into the loops at the end of antiparallel quadruplexes, would also be easily accommodated into this arrangement.

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