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DNA Metalloinsertors

Thread Insertion of a Bis(dipyridophenazine) Diruthenium Complex into the DNA Double Helix by the Extrusion of AT Base Pairs and Cross-Linking of DNA Duplexes**

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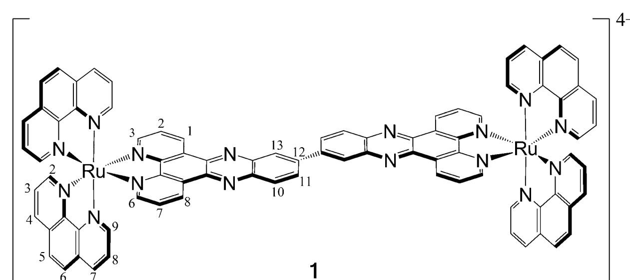
Abstract: *The crystal structure of the Δ,Δ enantiomer of the binuclear "light-switch" ruthenium complex [μ-(11,11′-bidppz)(1,10-phenanthroline)$_4$Ru$_2$]$^{4+}$ bound to the oligonucleotide d(CGTACG) shows that one dppz moiety of the dumbbell-like compound inserts into the DNA stack through the extrusion of an AT base pair. The second dppz moiety recruits a neighboring DNA molecule, and the complex thus cross-links two adjacent duplexes by bridging their major grooves.*

The field of DNA targeting has recently been enriched with several examples of molecules that recognize noncanonical DNA topologies[1]. The ability to recognize unique DNA conformations that occur in vivo in a similar way to protein–DNA recognition provides a potentially powerful means of manipulating DNA in living cells. A few examples of this type of DNA recognition by (mostly planar) synthetic molecules have been described, including binding to four-way[2] junctions and the recognition of quadruplex stacks by aromatic intercalator-type molecules.[3] Another example is the recognition of a three-way-junction DNA molecule by transition-metal complexes.[4] We report herein the crystal structure of the binuclear Ru compound **1** complexed with the DNA oligonucleotide d(CGTACG) at a resolution of 2.95 Å. The structure exhibits the cross-linking of adjacent duplexes by threading through the DNA base stack.

Complexes of transition metals with (hetero)aromatic ligands have been widely studied as DNA-binding probes and drug candidates during recent decades owing to their readily constructed 3D geometries and versatile photophysical properties.[5] Chiral ruthenium compounds comprising a dipyridophenazine (dppz) ligand, such as the monomer unit of **1** (Scheme 1), have been given particular attention, because



Scheme 1. Chemical structure of **1**, the Δ,Δ enantiomer of [μ-(bidppz)-(phen)$_4$Ru$_2$]$^{4+}$; phen = 1,10-phenanthroline, bidppz = 11,11′-bis-(dipyrido[3,2-*a*:2′,3′-*c*]phenazinyl).

they show a remarkable increase in luminescence intensity upon intercalation into DNA, a phenomenon known as the "light-switch" effect.[6] For mononuclear Ru–dppz compounds, it has been shown recently by X-ray crystallography that the dppz ligand can invade the base-pair stack either by intercalation or by insertion at a mismatched site, and that in the latter case, the extruded mismatched bases stack on the ancillary ligands.[7,8] Complex **1** has an extremely long DNA-dissociation half-life (estimated to be 38 h at 37 °C), which has been attributed to threading intercalation.[9] However, structural information on the threaded intercalated state of binuclear Ru compounds with the bidppz ligand is limited.

Our structure (see Figure S2 and Table 1 in the Supporting Information) shows that the large metalloinsertor **1** traverses the DNA double helix and displaces an AT base pair from the DNA stack to position the two bulky Ru centers on opposite groove sides of the double helix (Figure 1). The overall binding mode can be described as "thread insertion". The fact that the bulky coordinated metal center has to thread through a transient bubble in the base-pair stack explains why **1** has a very slow on and off rate for intercalation into a DNA double helix. There are no other examples of structures of compounds with similar on/off rates bound to DNA, and the structure is also the first visualized example of true metalloinsertion into regular base-paired DNA. The extrusion of an AT base pair is of particular interest, since experimental data show that **1** and related compounds have preference for long AT stretches.[10,11]

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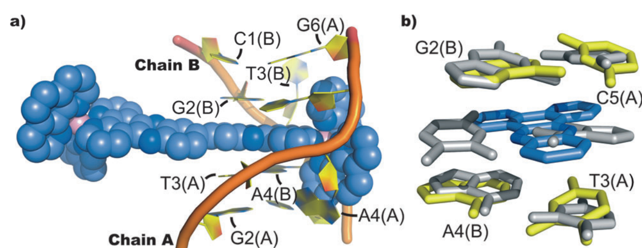

Figure 1. Structure of the complex between **1** and the oligonucleotide d(CGTACG). a) A single molecule binds a single DNA duplex and reveals the large area of the bridging biddpz ligand protruding from the DNA major groove and available for further interactions. b) Superposition on an idealized B-DNA molecule (gray) of the bases (yellow) stacked on the inserted dppz moiety (blue) of the bidppz ligand, showing how the dppz moiety substitutes for the bases extruded from the base stack.

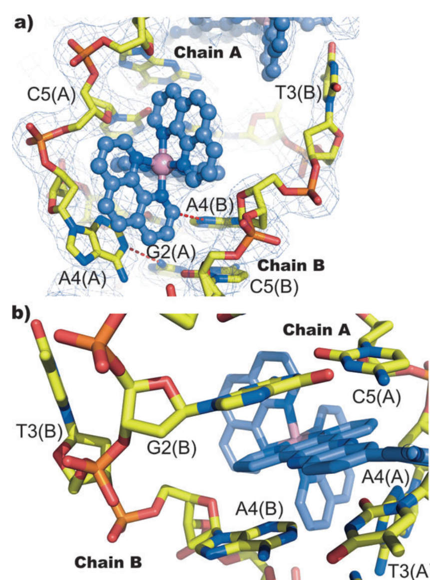

Figure 2. Details of the observed binding mode. a) The binding of the Ru center in the minor groove leads to substantial widening of the minor groove through the insertion of the phenanthroline moieties. b) As in (a), but viewed from the major groove.

Light-scattering studies in dilute solution showed the development of a pronounced light scattering of decreasing magnitude with increasing concentration of the oligonucleotide (see Figure S1 and methods description in the Supporting Information). These results are consistent with an equilibrium in solution between soluble 1:1 and 2:1 complexes of duplex and the ruthenium compound, whereby the former can dimerize to form a 2:2 complex which may be similar to the structure in the crystal and can subsequently slowly form higher-order structures. The strong effect of the concentration of the duplex on the formation of higher-order complexes thus provides indirect evidence that the interactions found in the crystal are of importance also in dilute solution.

The T3(B) and A4(A) bases (parenthesized A and B refer to the two DNA strands of the same duplex) that are extruded by the inserting dppz moiety fold back into the minor groove, where they form stacking interactions with the aromatic ring system of the phen ligands (Figure 2). Interestingly, this behavior supports the earlier suggestion that stacking interactions of the expelled base pair with ancillary ligands in the transition state for thread intercalation can explain the counterintuitive observation of a decrease in activation enthalpy with an increase in ligand size.[11] Atom N1 of the extruded A4(A) base and atom N2 of base G2(A) further downstream on the same strand form a hydrogen bond at a distance of 3.2 Å. The bulky Ru center and its phen ligands cause a significant widening of the minor groove from 10.7 for standard B-DNA to 15.6 Å.[12] Both phen ligands fully span the minor groove and make contacts with the ribose rings of both DNA strands (see Figure 2 A), but also interact with the bases. Thus, the C9 atom forms a C−H⋯N hydrogen bond with atom N3 of base A4(B) (C9⋯N3 distance: 3.0 Å). The phen ligands lie approximately parallel with the direction of the duplex. Although the backbone conformation is perturbed locally by the introduction of the Ru center, no significant bending of the duplex DNA occurs. The DNA duplex can be described as a distorted form of B-DNA and appears to be underwound (see Table S2 in the Supporting Information).

The structure shows considerable differences to the reported structure of the Λ enantiomer of the complex of the monomer [Ru(tap)$_2$dppz] (tap = 1,4,5,8-tetraazaphenanthrene) with the 10-mer d(TCGGCGCCGA),[13] in which one of the tap ligands partially insert into the base-pair stack of neighboring duplex molecules. More recent structures of the Λ enantiomer of [Ru(phen)$_2$dppz]$^{2+}$ by Niyazi et al.[7] and the simultaneous binding of both enantiomers of this compound to d(ATGCAT)$_2$[14] show that the Ru center nestles in the minor groove in a similar way to that observed in the structure described herein. Thus, a similar widening of the minor groove and a comparable degree of buckling in the stacking bases were found. However, these structures differ in fundamental aspects from the present bidppz structure. First of all, in the structures of the mononuclear variant, the ligand binds through intercalation and not insertion. Second, the phen ligands of both enantiomers stack onto one of the strands, in contrast to the binuclear compound described herein, which makes extensive contacts with both strands. As a result, the orientation of the dppz ligand protruding out from the major groove changes. This arrangement is expected to have consequences for the cross-linking properties of the molecules. It would therefore be interesting to see whether the Λ,Λ binuclear complexes are also able to cross-link adjacent duplexes. However, this aspect is outside the scope of the present study.

The recent structure described by Song et al. for the Δ enantiomer of monomeric [Ru(bpy)$_2$dppz] (bpy = 2,2′-bipyridine) bound to the mismatch-containing duplex d(CGGAAATTACCG)$_2$ showed both an insertion, with the mismatched A·A pair extruded, and an intercalation mode.[8] The extruded A bases lie in the minor groove and are involved in stacking interactions with the bpy ligands that resemble the interactions of the extruded non-mismatched AT bases with the phen ligands in our structure. The present structure thus confirms the stacking insertion of the dppz ligand, but demonstrates that a mismatched pair is not needed for insertion.

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In the structure described herein, the dppz moiety that protrudes out of the major groove at the insertion site binds to a neighboring duplex strand in a similar way to the inserted Ru center (Figure 3). The protruded dppz moiety associated with the second Ru center stacks onto the blunt end of the neighboring DNA duplex to place the second Ru center in the minor groove (Figure 3 A). Thus, **1** cross-links two adjacent DNA duplexes, which interact with each other through their major grooves, and have an angle of about 30° between their DNA axes (Figure 3 B). This interaction is possible because of the size of the binuclear molecule, in which the longer axis has

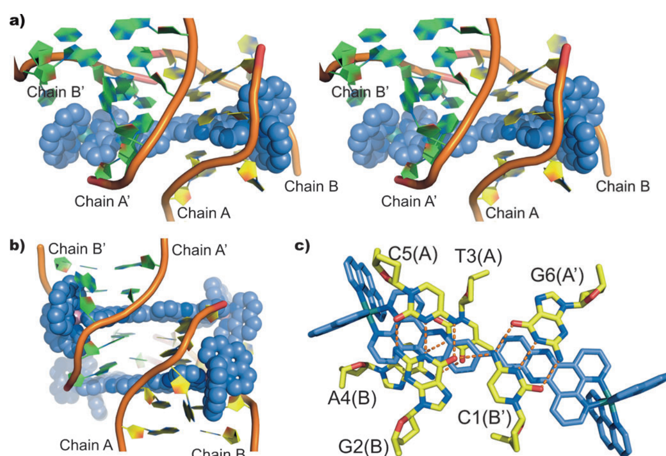

Figure 3. a) Stereoview of the binding mode displayed by the Ru compound. One half of the bidppz ligand inserts into the duplex DNA (strands with bases colored in yellow) and thus displaces an AT base pair. The other half binds to a nearby duplex (strands with bases colored in green). b) As in (a), but including the second molecule of **1** that cross-links the two duplexes. c) A view of the stacking interaction between the bidppz ligand and the interacting DNA bases.

a length of 29 Å and the Ru centers are 22 Å apart. Few examples exist of structures of cross-linked DNA; the most relevant are those of bisacridine derivatives, which consist of two acridine moieties connected by a flexible linker. Both acridine groups were shown to intercalate into neighboring DNA duplexes.[15] The main differences between the acridine-based molecules and the bis(dppz) Ru compound is that the DNA duplexes in the structure described herein are brought together in such a way that their major-groove sides confront one another, which is a feature not observed so far in cross-interactions. The two duplex DNA molecules that are bound by dppz moieties are held in place with a lower degree of freedom as compared to the bisacridine derivatives, thus restricting the interduplex angle to about 30°. This angle is in close agreement with the value of 40° estimated by experiment and quantum-chemical calculations.[11,16]

The two Watson–Crick (WC) base pairs C5(A)–G2(B) and G6(A′)–C1(B′) and their symmetry-related counterparts are located between the two bound molecules of **1** in both of the two neighboring strands (Figure 3 B,C). The stacking interactions of two adjacent strands with one molecule of **1** are shown in Figure 3 A (symmetry-related strands of A and B are denoted A′ and B′, respectively). The four base pairs from neighboring strands form a configuration reminiscent of a quadruplex (Figure 3 C). The bases C1(B′) and G2(B) of neighboring strands are in close contact and connected by hydrogen bonds between the O6 atom of G2(B) and the N4 atom of C1(B′). There is also close contact between the C1(B′) C5 atom and the G2(B) N7 atom, which are 3.4 Å apart. Therefore, a thymine base would not fit into the observed configuration, since the thymine methyl group would clash severely with the neighboring strand. The WC counterparts of C1(B′) and G2(B), that is, bases G6(A′) and C5(A), are also part of the quadruplex-like configuration, but do not make direct contacts. Interestingly, the minor-groove-to-minor-groove distance in the structure of the human *MYC* promoter region, which forms a quadruplex complexed to tetra(4-*N*-methylpyridyl)porphin[17] (20 Å), matches that between the two Ru centers of **1** (21.5 Å), thus suggesting that **1** may fit inside a quadruplex (see Figure S3). It has recently been shown that $[(Ru(phen)_2)_2tpphz]^{4+}$, a dinuclear Ru compound with a smaller bridging ligand than bidppz in compound **1**, shows enantioselective binding to G-quadruplexes from human telomeres.[18]

Several novel observations were made with respect to the structure reported herein. First, the structure showed the threading of compound **1** with on/off rates unequaled by any other known structure. Second, insertion threading at a non-mismatched WC base-pair site was observed directly for the first time. Third, the structure is the first example of the adjoining of two DNA duplexes at their major grooves. DNA structures displaying adjacent duplexes are ubiquitous in nature, for example, in nucleosomes, Holliday junctions, and quadruplexes. Our study opens the possibility of targeting some of these DNA configurations.

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