Bifunctional Metal Complexes

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2D NMR Spectroscopic Evidence for Unprecedented Interactions of *cis*-[Rh_2(dap)-(μ-O_2CCH_3)$_2$(η^1-O_2CCH_3)(CH_3OH)](O_2CCH_3) with a DNA Oligonucleotide: Combination of Intercalative and Coordinative Binding **

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Coordination binding to nucleic acid bases[1,2] and intercalation between DNA bases[3–5] are two major interactions that metal complexes establish with DNA. These binding modes

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cause changes in the DNA structure that can affect cellular processes and may result in cytotoxicity and cell death. Most metal complexes, including the well-known anticancer drug cisplatin,[1] are mononuclear complexes that coordinatively bind to DNA purines. There are, however, a few cases of inorganic or organometallic antitumor agents that incorporate more than one metal unit, for example, by introducing a hydrocarbon chain or polypeptide unit to form a multifunctional complex with improved binding affinity to DNA. Examples of systems under study are polynuclear platinum complexes,[6–12] compounds with one or more intercalative binding units,[3,13,14] and metal complexes with bifunctional coordinative and intercalative binding units.[8–12,15–20] There are also reports of intercalative and coordinatively bound organometallic ruthenium(II) anticancer complexes that possess both an intercalating arene group and a labile chloride ligand.[21–23]

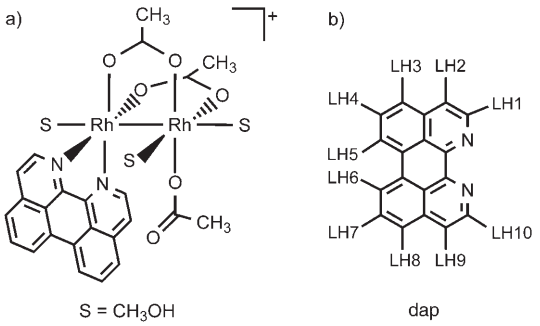
Among the many metal complexes that are known to exhibit antitumor activity are dirhodium compounds that establish coordinative interactions with DNA[24,25] with a preference for the N7 sites of adenine and guanine.[25,26] Recently, an entirely new class of dirhodium(II) carboxylate complexes with intercalating groups has been developed jointly by Dunbar, Turro, and co-workers as potential agents for photochemotherapy.[27] Of specific interest to the present study are complexes that contain a chelating intercalating ligand on one of the two Rh atoms and two *cis* labile ligands on the second Rh center, a situation that poises the compounds to act as dual intercalative/coordinative binding agents for DNA.

To date, most studies on metal complexes that show combined coordinative and intercalative interactions with DNA have made use of UV/Vis and CD spectroscopies as well as thermostability measurements to probe these interactions. To our knowledge, however, there are no NMR spectroscopic investigations of such adducts to probe the precise mode of binding to DNA oligonucleotides. Herein, we report a 2D NMR spectroscopic investigation of a new dirhodium complex binding to a DNA 12 mer by combined intercalative and coordinative interactions.

The compound *cis*-[Rh_2(dap)(μ-O_2CCH_3)$_2$(η^1-O_2CCH_3)-(CH_3OH)](O_2CCH_3) (**1**; dap = 1,12-diazaperylene),[28] as shown in Scheme 1, was selected for the present study owing to the fact that it contains the intercalating dap ligand on one Rh atom and a labile, monodentate acetate group on the other Rh center. Compound **1** was incubated with d(CTCTCAACTTCC) (AA12 mer) in a 1:1 ratio in water at 37 °C (see the Supporting Information). The major dirhodium–DNA adduct was isolated by combined reverse-phase and anion-exchange HPLC chromatography.

The complementary strand d(GGAAGTTGAGAG) (TT12 mer) was added to the isolated single-stranded dirhodium adduct (see the Supporting Information) and the major dirhodium double-stranded (ds) DNA adduct Rh_2(dap)-(O_2CCH_3)$_2$–AA12 mer_ds was investigated by 2D NMR spectroscopy, which revealed several interesting features. First, the DQF-COSY spectrum of the major adduct shows noticeable changes in the chemical shifts of the protons of residues C5 and C8 of the dirhodium adduct as compared to the DQF-COSY spectrum of the unmodified AA12 mer_ds (see the Supporting Information). Second, overall upfield shifts were observed for the resonances of the ligand protons upon metal binding. The most significant shifts were observed for the LH9 and LH10 protons ($\approx$1 ppm), which are located on the opposite side of the dangling acetate group in **1**. Finally, it was possible to complete the NOESY "walk" with the cross-peaks of the aromatic protons of the bases to the H1′ protons of the sugar rings for the metalated double-stranded DNA. The connections between the A6 and A7, and the T18 and T19 residues, however, were not clear, which is an indication of disruption of this base step (see the Supporting Information). The absence of the respective H2 and H8 proton correlations of the A6 and A7 residues in other regions of the NOESY spectrum, which normally give very strong NOESY cross-peaks in double-stranded B-DNA (Figure 1), also corroborates disruption of this base step.

The previous observations imply that the distance between the A6–T19 and the A7–T18 base step is longer than that in normal B-DNA due to the insertion of the dap group between these bases. The latter is fully supported by the prevalent NOE cross-peaks of the H2 and H8 protons of A6 and A7 residues to the dap protons (Figure 2). If the dirhodium compound was merely coordinatively bound to either the A6 or A7 residues, the dap ligand would be located on either the major- or minor-groove of DNA. In this case, the NOE correlation peaks of the dap protons would be



Scheme 1. Structures of a) *cis*-[Rh_2(dap)(μ-O_2CCH_3)$_2$(η^1-O_2CCH_3)-(CH_3OH)](O_2CCH_3) and b) numbering scheme for the dap ligand.

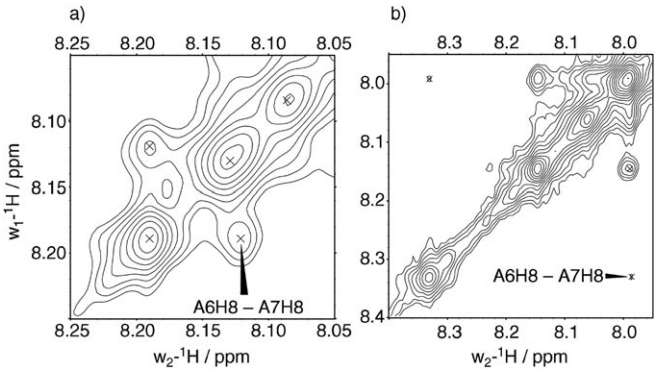


Figure 1. Comparison of the sizes of the NOE correlation peaks between A6H8 and A7H8 in double-stranded DNA oligonucleotides. a) Unmodified AA12 mer_ds, b) Rh_2(dap)(O_2CCH_3)$_2$–AA12 mer_ds.

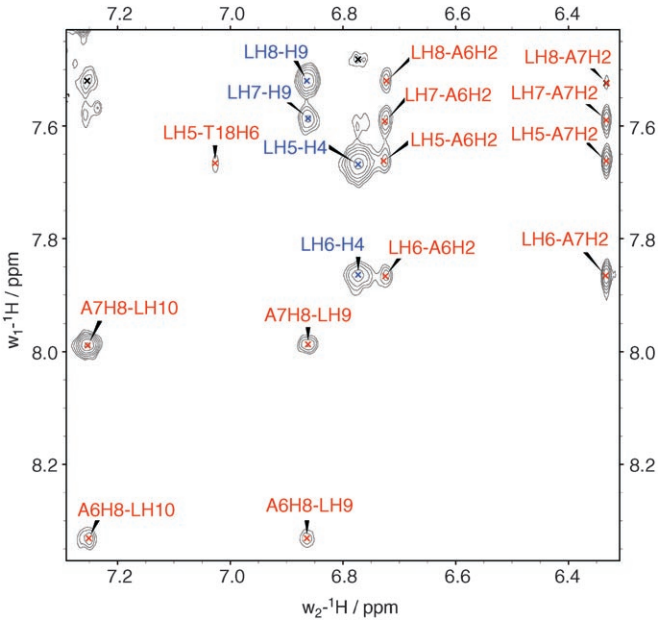


Figure 2. Expansion of the NOESY spectrum of $Rh_2(dap)(O_2CCH_3)_2$–AA12 mer_ds in D_2O at 15 °C. The blue labels indicate the NOE cross-peaks between dap protons and the red labels between the aromatic protons of the AA12 mer and the dap protons.

observable with either the H8 or H2 protons, but not with both, because the H2 and H8 protons are located on opposite sides of the double-stranded DNA. As NOE cross-peaks are observed for both H2 and H8 with the dap protons (Figure 2), the NOESY data provide solid evidence for the intercalation of the dap ligand between the A6 and A7 residues.

Apart from the intercalation between DNA bases in $Rh_2(dap)(O_2CCH_3)_2$–AA12 mer_ds, the NMR spectroscopic data support coordinative binding of the adenine residue A6 to the dirhodium unit. The assignment of the N7 atom of residue A6 as the binding site of the dirhodium complex, instead of the N7 atom of residue A7, is mainly due to the downfield chemical shift of the H8 proton of A6 ($\Delta\delta =$ 0.14 ppm) in contrast to the upfield shift ($\Delta\delta = -0.12$ ppm) of the H8 proton of the A7 residue. The downfield shift of A6H8 is rather small when compared with the dirhodium adduct from the reaction of $Rh_2(O_2CCH_3)_4$ with the same AA12 mer ($\Delta\delta = 0.55$ ppm)[29] in which the dirhodium unit binds to the N7 atom of the A6 residue. In the present case, however, the change in chemical shift for A6H8 reflects both the downfield shift induced by the coordinative binding of the metal on the protons near the metal-binding site (inductive effect)[30] and the upfield shift induced by the intercalation effect on the protons located above or below the intercalator, due to the ring current of the aromatic group.[31] On the other hand, pure upfield shifts due to intercalation are evident for the H2 protons of the A6 and A7 residues of the dirhodium adduct when compared to the AA12mer_ds ($\Delta\delta = -0.51$ and -1.24 ppm, respectively), as well as for the LH9 and LH10 protons with upfield shifts of ≈ 1 ppm as compared to **1** (see the Supporting Information). As compared to the LH9 and LH10 protons, however, the upfield shifts of protons LH1 and LH2 are relatively small ($\Delta\delta = -0.13$ to -0.19 ppm; see the Supporting Information). This may be due to the fact that the dap ligand intercalates more towards the AA12 mer rather than the complementary strand owing to coordination bonding to the N7 site of residue A6. Based on the watergate-NOESY data of the dirhodium–DNA adduct at different temperatures, the coordinative binding and intercalation appear to disrupt the structure more severely at the downstream end of the DNA oligonucleotide duplex, for example, residues A7, C8, and T18. The intensity of the NOE cross-peak of the A7H2–T18N3H pair is much weaker than that of A6H2–T19N3H, and the former nearly disappears when the temperature is raised from 7 °C to 15 °C (see the Supporting Information).

The aforementioned 2D NMR data corroborate coordinative binding and intercalation of **1** in the AA12 mer_ds as proposed in Figure 3. The combined effects of coordinative

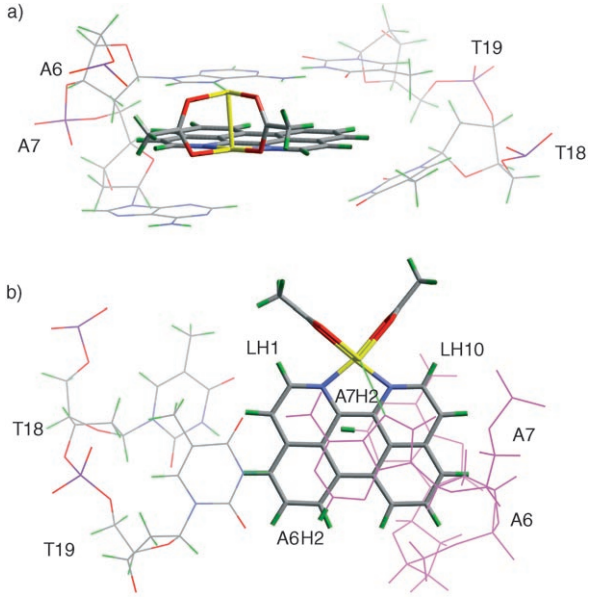


Figure 3. Proposed binding model for $Rh_2(dap)(O_2CCH_3)_2$–AA12 mer_ds with a combination of intercalative and coordinative binding. a) Side view and b) top view along the DNA axis of the oligonucleotide duplex.

and intercalative binding on the physical properties of the AA12 mer are also evident from the changes in the T_m (melting temperature) value of $Rh_2(dap)(O_2CCH_3)_2$–AA12 mer_ds ($\Delta T_m = -6.0$ °C; see the Supporting Information) as compared to the dirhodium adduct ($Rh_2(O_2CCH_3)_3$–AA12 mer_ds), which involves only coordination binding at the same site ($\Delta T_m = -22.9$ °C),[29] to cisplatin coordination binding ($\Delta T_m = -13.5$ °C),[32] or to metallointercalators ($\Delta T_m = 16$ °C).[33] Thus, in the present dirhodium–DNA adduct, the effects of coordination binding appear to dominate over the effects of intercalation for both the chemical shift of A6H8 and T_m value changes.

Owing to the octahedral coordination environment of both Rh atoms, the equatorial coordination binding to the N7 atom of the A6 residue renders the dap group capable of stacking with the adenine bases without causing any bending

of the aromatic groups, as observed in the case of a rhenium(II) biphenyl compound.[21,22] Furthermore, this dual binding of the dirhodium complex lends stability to the metal–DNA adduct.

In conclusion, we have shown that the dirhodium dap complex has the dual capability of intercalative and coordinative binding, which is an exciting new direction for designing dirhodium compounds that bind tightly and specifically to DNA. Future studies on this type of dirhodium compounds[34] with double-stranded DNA are in progress and the results will be reported in due course.

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