Use of Selective Deuteration and ^{1}H NMR in Demonstrating Major Groove Binding of Δ-[Ru(phen)$_2$dppz]$^{2+}$ to d(GTCGAC)$_2$

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Received July 26, 1994

The nucleic acid binding properties of Ru(II) polypyridyls have been studied extensively using a variety of spectroscopic and photophysical techniques.[1-6] Among those which are octahedral metallointercalators, dipyridophenazine (dppz) complexes of ruthenium bind DNA with the highest affinity[2,4,6] ($K_a \geq 10^6$ M^{-1}) and exhibit unique "light switch" behavior.[2] Quenched in aqueous solution, [Ru(phen)$_2$dppz]$^{2+}$ and [Ru(bpy)$_2$dppz]$^{2+}$ photoluminesce upon intercalation into double-stranded DNA, where substantial protection of the phenazine nitrogens from solvent is afforded.[2a,7] Biexponential decays in luminescence for enantiomerically pure [Ru(phen)$_2$dppz]$^{2+}$ bound to DNA indicate that both Δ and Λ enantiomers intercalate into DNA through multiple binding orientations.[6,8] An understanding of the geometry of [Ru(phen)$_2$dppz]$^{2+}$ bound to duplex DNA is central to the development of new complexes with improved binding affinity and specificity and to the application of the Ru(II) complex as a luminescent probe of nucleic acids. However, no direct structural data are available for dppz complexes bound to double helical DNA. ^{1}H NMR spectroscopic experiments have been difficult due to the intermediate exchange kinetics at ambient temperature and to the abundance of overlapping resonances in the aromatic region. Moreover, information regarding site specificity and groove location is not accessible through metal-mediated cleavage of DNA.[9] Here we utilize selective deuteration of the coordinating ligands of [Ru(phen)$_2$dppz]$^{2+}$ as a means of simplifying ^{1}H NMR spectra of this dppz complex bound to oligonucleotide DNA. This approach yields results which establish the intercalation of Δ-[Ru(phen)$_2$dppz]$^{2+}$ into DNA from the major groove.

One-dimensional ^{1}H NMR spectra of Δ-[Ru(phen)$_2$dppz]$^{2+}$ with d(GTCGAC)$_2$ collected at various temperatures demonstrate the exchange behavior of this system (supplementary material). Slow exchange is achieved for most resonances at 280 K.[10] Analysis of these data yields an exchange rate no faster than 65 s^{-1} at 300 K.[11] This rate is slower than that observed for [Rh(phen)$_2$phi]$^{3+}$ [12] (phi = 9,10-phenanthrenequinone diimine) and must be attributed to the different stacking with the base pairs imparted by the extended dppz ligand.

Upon the addition of d(GTCGAC)$_2$, easily observed resonances assigned to the 4′,7′ protons of the dppz ligand of [Ru(phen)$_2$dppz]$^{2+}$ undergo upfield changes in chemical shift (–0.17 to –0.80 ppm) consistent with intercalation (Figure 1). Conversely, chemical shift changes for resonances assigned to

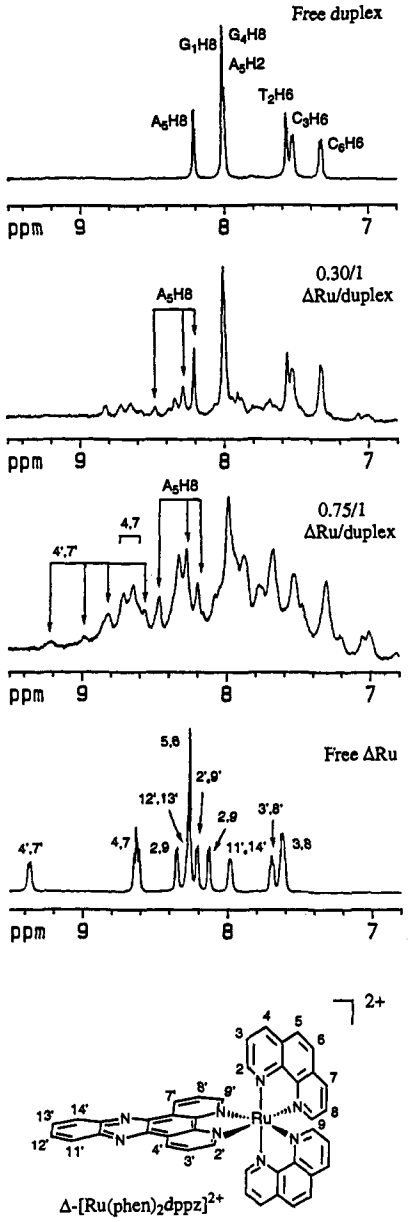

Figure 1. 500 MHz ^{1}H NMR spectra of Δ-[Ru(phen)$_2$dppz]$^{2+}$–d(GTCGAC)$_2$ at metal:duplex ratios of 0:1, 0.30:1, 0.75:1, and 1:0 collected at 280 K. The growth of bound A$_5$H8 resonances with increasing Δ-[Ru(phen)$_2$dppz]$^{2+}$ is indicated. The locations of bound 4′,7′ and 4,7 proton resonances are also highlighted. The oligonucleotide[12] and [Ru(phen)$_2$dppz]$^{2+}$ [8] were prepared as previously described. Resolution of enantiomers was achieved on Sephadex SP-25 using potassium antimonyl tartrate as a chiral eluent. DNA samples contained 0.5 mM duplex, 25 mM NaCl, 10 mM sodium phosphate, pH 7.0, in 100% D$_2$O. The free metal complex sample contained 0.5 mM [Ru(phen)$_2$dppz]$^{2+}$ in the same buffer. Chemical shifts are reported relative to TMSP at 280 K. The proton labeling scheme for the metal complex is indicated in the schematic of the structure.

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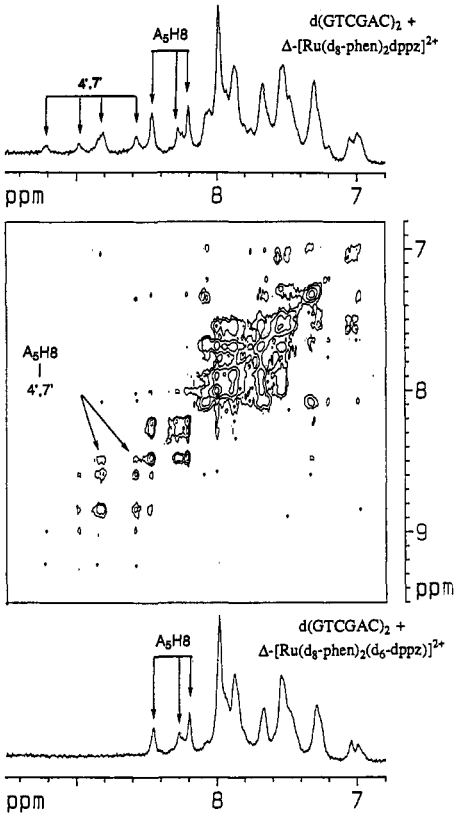

Figure 2. One- and two-dimensional ^{1}H NMR spectra of selectively deuterated Δ-[Ru(phen)$_2$dppz]$^{2+}$ bound to d(GTCGAC)$_2$. Shown is a contour plot of the aromatic–aromatic region of a 300 ms NOESY spectrum at 280 K of 1 mM d(GTCGAC)$_2$, 0.75 mM Δ-[Ru(phen-d_8)$_2$dppz]$^{2+}$ in 10 mM sodium phosphate, 25 mM NaCl, pH 7.0, in 100% D_2O with TMSP as a standard. Intermolecular NOEs are indicated. These NOEs are also evident with a mixing time of 150 ms. A 1D projection of the aromatic region is shown above. A 1D spectrum of Δ-[Ru(phen-d_8)$_2$(dppz-d_6)]$^{2+}$–d(GTCGAC)$_2$ collected under identical conditions is shown at the bottom. Selective deuteration of the phenanthroline portion of the dppz ligand eliminates the 4′,7′ protons and thus the accompanying intermolecular NOEs (data not shown).

4,7 protons of the ancillary phenanthroline ligands are minimal (0.00 to +0.11 ppm). These data indicate that Δ-[Ru(phen)$_2$dppz]$^{2+}$ intercalates into duplex DNA selectively through the dppz ligand, which is consistent with other spectroscopic studies.[2] A similar selectivity in chemical shift change is apparent with Δ-[Rh(phen)$_2$phi]$^{3+}$ bound to DNA.[12]

The application of perdeuterated phenanthroline in the assembly of Δ-[Rh(phen-d_8)$_2$dppz]$^{2+}$ simplifies the spectra and permits us to focus on the activities of the dppz ligand. In one-dimensional spectra of this complex bound to the oligonucleotide, three unique A$_5$H8 resonances are evident, as well as at least four detectable peaks for the bound 4′,7′ protons (Figure 2). For assignments, see supplementary material. These data indicate that at least two binding sites or modes exist for the complex,[13] a result which is consistent with luminescence studies.

Two-dimensional NOESY spectra (Figure 2) indicate that at least two intercalative modes occur from the major groove.[14] In spectra of Δ-[Ru(phen-d_8)$_2$dppz]$^{2+}$ with duplex, NOE crosspeaks are detected between a bound A$_5$H8 resonance (8.48 ppm) and upfield-shifted dppz protons (4′,7′, 8.82 and 8.58 ppm). Using deuterated dppz[15] to form Δ-[Ru(phen)-d_8)$_2$(dppz-d_6)]$^{2+}$, a complex in which only the protons distal of the phenazine nitrogens (11′–14′) remain, these metal ligand resonances and thus the intermolecular NOEs are eliminated. Therefore, the proximal protons of the intercalated dppz ligand (i.e., those located closest to the Ru(II) center) are responsible for interactions with A$_5$H8, and the approach of Δ-[Ru(phen)$_2$dppz]$^{2+}$ to the oligonucleotide is from the major groove.[16] These results establish the feasibility of applying ^{1}H NMR spectroscopy to structural studies of [Ru(phen)$_2$dppz]$^{2+}$–DNA interactions.

While minor groove binding to DNA predominates as a mode of interaction among small molecules,[17] association from the major groove is observed for square-planar and octahedral metallointercalators.[1,12,18–20] These complexes possess a range of shapes, sizes, DNA site affinities, and specificities. The extension of this list to [Ru(phen)$_2$dppz]$^{2+}$ prompts conjecture that a major groove orientation is a general binding characteristic of metallointercalators.

Acknowledgment. We are grateful to the NIH (GM33309) for their financial support and to the NSF for a postdoctoral fellowship (C.M.D.).

Supplementary Material Available: Figure depicting the variable temperature behavior of Δ-[Ru(phen)-d_8)$_2$dppz]$^{2+}$ with d(GTCGAC)$_2$; contour plot of the base-H2′2″ region of the NOESY spectrum of Δ-[Ru(phen-d_8)$_2$dppz]$^{2+}$ bound to d(GTCGAC)$_2$ (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(13) Assuming that the binding of one end of the palindromic oligonucleotide does not perturb the other end, one asymmetric binding event is represented by one shifted A$_5$H8 resonance and a resonance at the unperturbed A$_5$H8 chemical shift. The detection of another bound A$_5$H8 indicates that at least one additional binding mode/site exists. Analogously, the inequivalence of the 4′ and 7′ protons of the bound complex produces two peaks per binding mode/site. The presence of both symmetric and canted geometries would account for the several upfield shifts seen.

(14) The geometry of the dppz ligand and the location of A$_5$H8 in the helix preclude NOEs to both 4′ and 7′ protons simultaneously.

(15) dppz-d_6 was synthesized from the oxidation of phen-d_8 to 5,6-phendione-d_6, which is subsequently condensed with *o*-phenylenediamine to form the ligand.[8]

(16) An NOE is also observed between the 2′,9′ protons of bound Δ-[Ru(phen-d_8)$_2$dppz]$^{2+}$ and 2′2″ DNA sugar protons (supplementary material).

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