NMR Evidence for Specific Intercalation of Δ-Rh(phen)$_2$phi^{3+} in [d(GTCGAC)$_2$]

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The anchoring of metal complexes in the major groove of DNA through intercalation has been increasingly useful in the shape-selective design of novel metal complexes which bind DNA with high sequence-selectivity.[1-3] Toward that goal, direct structural information regarding this intercalative interaction is essential. Phenanthrenequinone diimine (phi) complexes of Rh(III) bind avidly ($K_b \geq 10^7$) to DNA by intercalation in the major groove.[2] Here we report the first ^{1}H-NMR studies of Δ-Rh(phen)$_2$phi^{3+} [4,5] bound to an oligonucleotide. These studies provide direct structural evidence for specific intercalation by this octahedral complex in the major groove of DNA.

^{1}H-NMR experiments were performed at 500 MHz with the hexamer [d(GTCGAC)$_2$].[6] Photocleavage experiments (Figure 1A) on this oligonucleotide indicate enantioselective targeting of the 5′-CG-3′ step by Δ-Rh(phen)$_2$phi^{3+}.[7] Temperature-dependent studies indicated that the metal complex is in intermediate exchange on the NMR time scale at 300 K. Fast-exchange conditions are observed at 320 K,[8] where two-dimensional (2-D) experiments were performed.[9,10] Complete assignment of free Rh(phen)$_2$phi^{3+} and the free duplex protons were made using COSY and NOESY experiments.[11] Dramatic effects on the chemical shifts of the rhodium complex were observed on binding to DNA (Table I). The protons of the phi ligand are markedly shifted upfield, almost by 1 ppm, while the phenanthroline ligand protons are shifted only slightly. Spectra taken in H_2O indicate significant upfield shifting and broadening of the base-paired imino protons. These shifts are consistent with the preferential partial intercalation of the phi ligand with the phenanthroline ligands serving in ancillary positions.[12-14]

More compelling evidence for the intercalation mode comes from the observation of a disruption in the sequential NOE connectivities. In right-handed B-DNA duplexes, the base proton (H8 or H6) exhibits NOEs to its own and 5′-flanking sugar H1′ and H2′2″ protons, allowing an NOE walk from the 5′- to the 3′-end of the oligonucleotide.[11,16] In Figure 1B, the base-H2′2″ region from the NOESY spectrum of Δ-Rh(phen)$_2$phi^{3+}–[d(GTCGAC)$_2$] is shown. In this region it is possible to perform the NOE walk along the entire oligonucleotide. However, the intensities of the internucleotide NOEs between G_4-H8 and C_3-H2′2″ protons at the 5′-CG-3′ step are particularly weak. This result is expected if the rhodium complex is intercalated in the 5′-CG-3′ step, since helical unwinding would increase the distance between these protons to ≥5 Å.[17] Thus this result provides direct evidence, consistent with the photocleavage study, for intercalation at this specific base step. It is noteworthy that the intensities of the internucleotide NOEs at the 5′-TC-3′ and 5′-GA-3′ steps are also weaker than those in the corresponding free duplex, which suggests some intercalation at these steps as well.[7]

Also observed in the NOESY spectrum are many intermolecular NOEs between the metal complex and the DNA duplex.[10] Figure 1B includes an intermolecular NOE between the phi (h) and the thymine methyl group (TMe). The observation of this NOE puts a constraint on one binding mode such that the rigid complex intercalates from the major groove.[18] Other examples of intermolecular NOEs which we observe and which require

Table I: ^{1}H-NMR Chemical Shift[a] Changes in Δ-Rh(phen)$_2$phi^{3+} upon Binding to DNA

ligand protons[b]	in D_2O buffer[c,d] (ppm)	in 1:1 complex with [d(GTCGAC)$_2$][c,d] (ppm)	change in shift upon binding (ppm)
phen			
c(4)	9.02	8.98	–0.04
a(2)	8.87	9.05	0.18
e(7)	8.86	8.88	0.02
d(5,6)	8.38	8.37	–0.01
g(9)	8.03	7.88	–0.15
b(3)	8.14	8.19	+0.05
f(8)	7.82	8.00	0.18
phi			
h(1,8)	8.21	7.63	–0.58
k(4,5)	8.14	7.41	–0.73
j(3,6)	7.69	6.81	–0.88
i(2,7)	7.47	6.64	–0.83

[a] All spectra were recorded at 500 MHz on a Bruker AMX500. [b] The labeling scheme for the complex is indicated in Figure 1B. Also given in parentheses is the standard numbering schemes for each ligand. [c] Chemical shifts are reported relative to TSP (±0.02 ppm) at 320 K. [d] This sample contained 3 mM Δ-[Rh(phen)$_2$phi]Cl$_3$ in 50 mM sodium phosphate buffer (uncorrected pD = 7), 0.2 M NaCl in 100% D_2O.

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(5) Enantiomers were separated by the method of chiral ion-exchange chromatography using tris(L-cysteinsulphinato)cobaltate(III) as the chiral eluent on a CM-sephadex column. The method used was similar to that of Yoshikawa et al.: *Coord. Chem. Rev.* **1979**, *28*, 205–229.

(6) The Δ-isomer enantioselectively targets 5′-pyr-pyr-pur-pur-3′ sites. See Pyle, A. M.; Morii, T.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 9432.

(7) Photocleavage experiments at millimolar concentrations indicate preferential binding at the 5′-CG-3′ step; however, at these concentrations cleavage at other sites is also detectable.

(8) The free duplex ([duplex] = 3 mM) melts at 323 K in 50 mM sodium phosphate buffer (D_2O) containing 0.2 M NaCl and increases by 15° in the presence of the metal complex.

(9) NOESY experiments were also performed at different mixing times (100–400 ms).

(10) Full details will be reported subsequently.

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(14) In studies[15] with Δ-Ru(phen)$_3^{2+}$ bound to an oligonucleotide, the peripheral phenanthroline ligand protons (4,7 and 5,6) are shifted upfield approximately 0.3 ppm, which is significantly greater than the corresponding shift for the ancillary phenanthroline protons in this study. The shift of 0.3 ppm for Ru(phen)$_3^{2+}$ is wholly consistent with intercalation of one of three phenanthroline ligands in the complex, which is averaged through fast exchange.

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(18) The family of intermolecular NOEs we observe, however, point to more than a single intercalative binding mode. For example, besides seeing NOEs between the φ ligand protons and G_4-H2′2″ and C_3-H2′2″ protons, an NOE between the φ protons and T_2-H2′2″ protons is observed. In addition, in a different salt-dependent fashion, an NOE between a phenanthroline proton and the A_5-H2 proton is observed.

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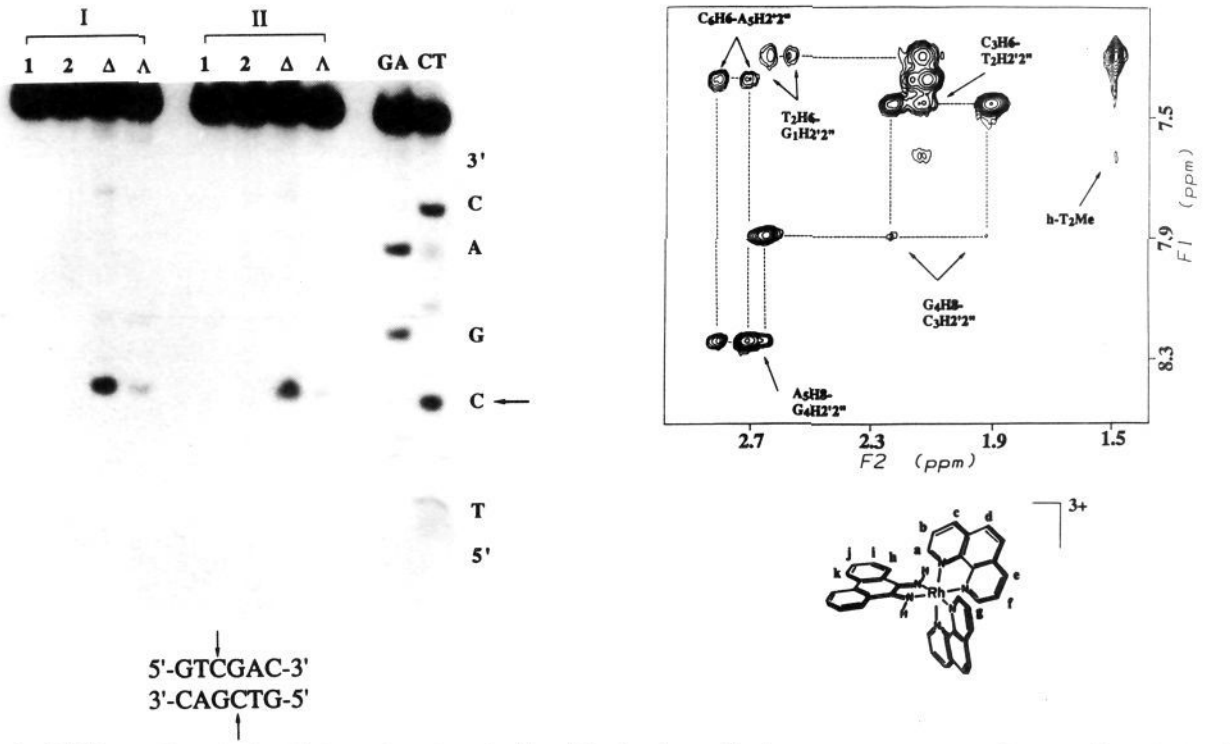

Figure 1. (Left) Autoradiograph of a 20% denaturing polyacrylamide gel showing site-specific cleavage by Δ-Rh(phen)$_2$phi^{3+} on the 5′-^{32}P-end-labeled hexamer. Experiments I were performed at concentrations of 50 μM Rh complex and 500 μM hexamer–nucleotide, and Experiments II were performed at concentrations of 25 μM Rh complex and 500 μM hexamer–nucleotide. Lane 1: 5′-end-labeled oligomer without rhodium or light. Lane 2: 5′-end-labeled strand with irradiation but no rhodium. Lane Δ: 5′-end-labeled oligomer irradiated in the presence of Δ-Rh(phen)$_2$phi^{3+}. Lane Λ: 5′-end-labeled oligomer irradiated in the presence of Λ-Rh(phen)$_2$phi^{3+}. Lane GA and CT: Maxam–Gilbert GA and CT specific reactions, respectively, on the 5′-end-labeled oligomer. All irradiations were performed for 5 min at 313 nm using a 1000-W Hg/Xe lamp and monochromator in 50 mM sodium phosphate buffer, pH 7.0. The oligonucleotides were synthesized on an Applied Biosystems 394B DNA synthesizer. Below is schematically illustrated (with arrows) the sites of Rh(phen)$_2$phi^{3+} photocleavage on the hexamer duplex. The 5′-asymmetry in cleavage is consistent with reaction in the major groove. (Right) Expanded NOESY contour plot (mixing time, 300 ms) of Δ-Rh(phen)$_2$phi^{3+}–[d(GTCGAC)]$_2$ (0.75:1,3 mM duplex) at 320 K. The sample was buffered with 50 mM phosphate containing 0.6 M NaCl (D_2O), uncorrected pD = 7.0. The contour plot correlates the base protons (7.0–8.5 ppm) to the H2′2″ and the thymine methyl protons (1.0–3.0 ppm). The labeled cross peaks correspond to the internucleotide NOEs between the base protons and the H2′2″ protons of the sugar residue of the 5′ nucleotide. Also indicated is an intermolecular NOE between a phi ligand proton (h) and the thymine methyl (T_2Me). The numbering scheme for the bases is given from the 5′-end of the nucleotide. Dotted lines illustrate the NOE walk along the oligonucleotide. Note the loss of intensity at the 5′-CG-3′step. Below is schematically illustrated the structure of Δ-Rh(phen)$_2$phi^{3+} and the proton labeling scheme.

binding from the major groove include C_3H5–phen (b), TMe–phen (a,b), and C_3H2′2″–phi (h).[19,21]

Substitution of the phi ligand for phenanthroline is useful in characterizing the intercalative binding mode. Other studies[22] have questioned intercalation by Ru(phen)$_3^{2+}$ primarily on the basis of the absence of disruptions in the NOE connectivities in a 2-D NMR experiment under conditions of low ionic strength. Substantial work has indicated binding to DNA by tris(phenanthroline) metal complexes through *two* binding modes, intercalation and groove binding.[15,23,24] The phi ligand, with its increased surface area for stacking, yields an increase in binding affinity by 3 orders of magnitude and, in the case of Rh(phen)$_2$phi^{3+}, an increase in sequence specificity over Ru(phen)$_3^{2+}$. This specificity permits the detection of site-specific perturbations in the DNA backbone by NMR. Furthermore, in a mixed ligand complex such as Rh(phen)$_2$phi^{3+}, the phenanthrolines primarily occupy ancillary positions rather than averaging between an intercalative and nonintercalative environment.[14] Using the intercalative model initiated with the tris(phenanthroline) metal complex and refined on the basis of structural experiments with the derivative phi complexes, new site-specific metal complexes anchored through intercalation in the major groove may now be envisioned.

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Supplementary Material Available: 1-D spectra of the metal complex bound to the oligonucleotide as a function of temperature; 2-D spectra showing the disruption in H1′–base connectivities (3 pages). Ordering information is available on any current masthead page.

(19) Intercalation in the major groove by octahedral metal complexes is consistent with (i) mechanistic studies on rhodium complexes which demonstrate the abstraction of the C3′-H atom;[2b] (ii) the order of binding affinity of complexes to helices with increasing access to the major groove;[1c,3b,20] and (iii) the selective loss of interaction by a rhodium complex through removal of a thymine methyl group in the major groove.[2c]

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