

## Enantiospecific Palindromic Recognition of 5'-d(CTCTAGAG)-3' by a Novel Rhodium Intercalator: Analogies to a DNA-Binding Protein

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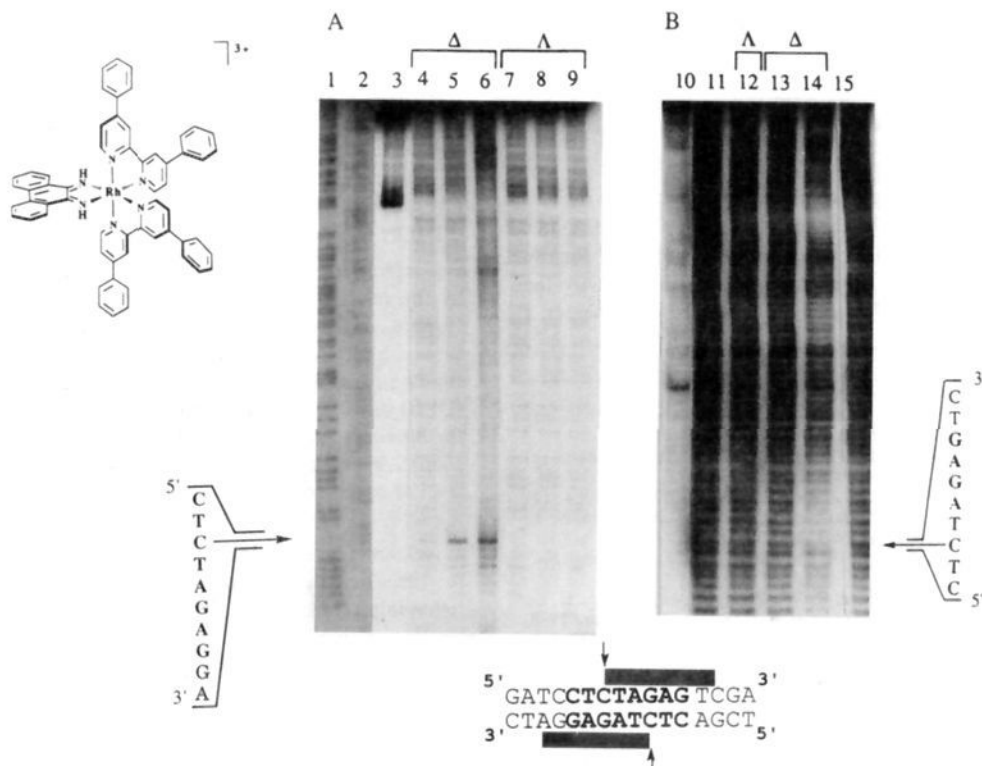
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We are exploring principles of protein–DNA recognition<sup>1</sup> through the design of model 9,10-phenanthrenequinonediimine (phi) complexes of rhodium(III) which bind site-selectively to DNA.<sup>2</sup> [Rh(phi)]<sup>3+</sup> complexes intercalate in the DNA major groove through the phi ligand<sup>3</sup> and upon photoactivation promote strand scission via abstraction of the deoxyribose C3'-H atom.<sup>4</sup> Here we report on the metal complex [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> (DPB = 4,4'-diphenyl-2,2'-bipyridyl) (Figure 1), which mimics DNA-binding proteins<sup>5</sup> in DNA site-specificity and affinity.

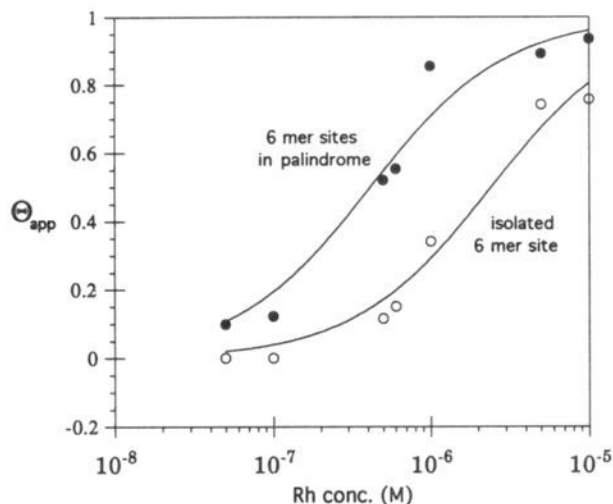
[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> was synthesized,<sup>6</sup> and complete resolution of the metal complex into its Δ and Λ enantiomers was achieved<sup>7,8</sup> using a DNA–cellulose column.<sup>9</sup> The Δ isomer is retained tightly to the DNA–cellulose column, while the Λ enantiomer elutes at

low ionic strength. A high level of enantioselectivity is also seen in metal-induced DNA photocleavage (Figure 1). Specific photocleavage is induced by Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> (≤1 μM) at the highlighted cytosine in the self-complementary sequence 5'-CTCTAGAG-3' and at the corresponding cytosine on the complementary strand (data not shown). In photocleavage experiments conducted on whole plasmids at low resolution, Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> cleaves only at the 5'-CTCTAGAG-3' site; in high-resolution screening of several 200-bp fragments, related sequences such as 5'-CCCCAGGG-3' and 5'-TTCCGGAG-3' are not cleaved. In comparison, Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> does not promote detectable cleavage, even at metal complex concentrations as high as 10 μM.<sup>10</sup> With MPE-Fe<sup>2+</sup>, a distinct 3'-shifted footprint<sup>12,13</sup> at the 5'-CTCTAGAG-3' site is also observed for Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> but not for Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>. Therefore, specific DNA cleavage by the complex at 5'-CTCTAGAG-3' is associated with specific DNA binding. These results are consistent with modeling studies that predict that Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>, but not the Λ isomer, matches the helical symmetry of B-DNA.<sup>11</sup>

The sequence-specificity of Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> was examined quantitatively by photocleavage on a double-stranded oligonucleotide containing the 5'-CTCTAGAG-3' palindrome (which may be considered as two overlapping 5'-CTCTAG-3' 6-mer sites on opposing strands),<sup>14</sup> an isolated 5'-CTCTAG-3' 6-mer site, and the central 5'-TCTA-3' 4-mer site. Binding isotherms (Figure

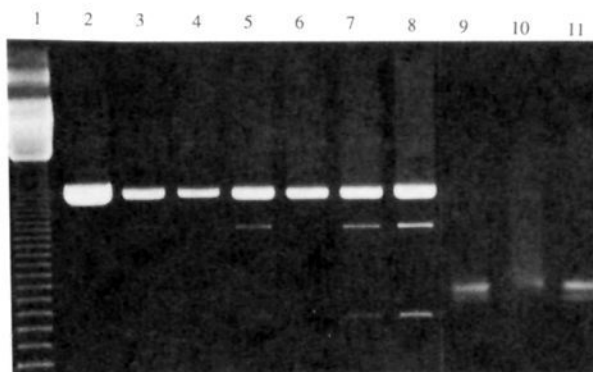


**Figure 1.** DNA binding and photocleavage by Δ- and Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>. Autoradiogram of an 8% polyacrylamide gel showing cleavage (A) and MPE-Fe<sup>2+</sup> footprinting (B) reactions on a 140-base-pair [3'-<sup>32</sup>P]- and [5'-<sup>32</sup>P]-end-labeled *Eco*RI/*Pvu*II fragment of pUC18,<sup>20</sup> respectively. All reaction mixtures contained [32P]-end-labeled fragment and 50 μM (in nucleotides) linearized pUC18 plasmid in 50 mM sodium cacodylate, pH 7.0. (A) Lanes 1–9: Photocleavage of the [3'-<sup>32</sup>P]-end-labeled fragment by Δ- and Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>. Cleavage reactions were carried out as previously described.<sup>2b</sup> Lanes 1 and 2, Maxam–Gilbert A+G reaction and C+T reactions; lane 3, fragment irradiated for 8 min in the absence of rhodium complex; lanes 4–6 and 7–9, fragment irradiated for 8 min in the presence of 0.1, 1.0, and 10 μM Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> and Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>, respectively. (B) Lanes 10–15: Footprinting Δ- and Λ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> bound to DNA using MPE-Fe<sup>2+</sup>, (MPE = methidiumpropyl-EDTA) on the [5'-<sup>32</sup>P]-end-labeled fragment. Lane 10, untreated fragment; lanes 11–15, fragment incubated for 1 h at ambient temperature with 0 μM, 2.0 μM Δ-, 1.0 μM Δ-, 2.0 μM Δ-, and 0 μM rhodium complex, respectively, and subsequently reacted with 0.5 μM MPE-Fe<sup>2+</sup>, 0.5 mM Na ascorbate at 4 °C for 1 h. Note that cleavage by the rhodium complex at the 5'-CTCTAGAG-3' site corresponds to a 3'-shifted footprint. A schematic illustration of Δ-[Rh-(4,4'-diphenyl-2,2'-bipyridyl)<sub>2</sub>phi]<sup>3+</sup> is shown as is the recognition site. The sites of cleavage on each strand are depicted by the arrows and regions which are footprinted by the shaded bars. At low concentrations, only one cleavage and footprint site is observed for Δ-[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>; only at a concentration 1000-fold higher are weaker cleavage sites and corresponding footprints evident.



**Figure 2.** Binding isotherms for  $\Delta$ -[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> to sites on a 28-mer double-stranded oligonucleotide, showing fractional cleavage ( $\theta$ ) as a function of total rhodium concentration (Rh<sub>T</sub>). The fractional cleavage  $\theta$  is defined as the ratio of  $I_{\text{site}}$  (amount of cleavage at recognition site) to  $I_{\text{sat}}$  (apparent maximum cleavage at recognition site). Binding curves were obtained by fitting<sup>15</sup> the photocleavage data to the expression  $\theta = (K_a)(\text{Rh}_T)/[1 + (K_a)(\text{Rh}_T)]$ . Saturation in cleavage was not obtained at the 4-mer site at these rhodium concentrations. The data for rhodium binding to the palindromic site fit better to the Hill equation, where  $n$ , the Hill coefficient, equals 1.4 ( $K_{\text{Hill}} = 1.6 \times 10^9$ ). The oligonucleotides 5'-GTGACTCTAGAGTCTATGACTCTAGTCT-3' (strand I) and 5'-AGACTAGAGTCATAGACTCTAGAGTCAC-3' (strand II) were synthesized on an ABIDNA synthesizer using phosphoramidite chemistry, labeled, and isolated using established protocols.<sup>20</sup> Polyacrylamide gels were quantitated using a Molecular Dynamics phosphorimager.

2), determined<sup>15</sup> through photocleavage and quantitative phosphorimetry, yield  $K_a = (2.4 \pm 0.6) \times 10^6 \text{ M}^{-1}$  for each 6-mer site in the palindrome and  $K_a = (0.4 \pm 0.1) \times 10^6 \text{ M}^{-1}$  for the isolated 6-mer site. This enhancement in binding affinity for both 6-mer sites in the palindrome as compared to the isolated



**Figure 3.** [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> as an inhibitor of *Xba*I cleavage of linearized pUC18 at the unique 5'-CTCTAGAG-3' site. Linearized plasmid was incubated with racemic rhodium complex in 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, and 50 mM NaCl, pH 7.9, at a nucleotide/Rh ratio of 13:1. Lane 1, 123-bp markers; lane 2, linearized pUC18; lanes 3 and 4, *Xba*I cleavage of pUC18 in the absence and presence of 500 nM [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>, respectively; lanes 5 and 6, *Xba*I cleavage of linearized pUC18 in the absence and presence of 100 nM [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>, respectively; lanes 7 and 8, *Xba*I cleavage of linearized pUC18 in the absence and presence of 100 nM [Rh(phi)<sub>2</sub>bpy]<sup>3+</sup>, respectively; lanes 9-11, restriction enzyme *Ssp*I cleavage of linearized pUC18 in the presence of 0, 0.5, and 0.1  $\mu\text{M}$  [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup>.

6-mer site indicates that two bound rhodium complexes may cooperatively interact on DNA,<sup>16</sup> which would account for the high specificity we observe for the 8-mer site.<sup>18</sup>

Docking studies reveal that two  $\Delta$ -[Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> may intercalate between the central CT step of each 5'-CTCTAG-3' site by canting<sup>19</sup> to one strand of the helix so as to promote aromatic interactions between the phenyl groups of one complex and the bipyridyl rings of the adjacent complex. Significantly, a few DNA-binding proteins also appear to dimerize across a central CTAG site.<sup>5</sup>

Furthermore, [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> specifically inhibits cleavage by the restriction endonuclease *Xba*I at this palindromic site (Figure 3). In contrast, no specific inhibition of *Ssp*I cleavage is seen, and [Rh(phi)<sub>2</sub>bpy]<sup>3+</sup>, a sequence-neutral intercalator,<sup>4</sup> shows no similar inhibition. These results illustrate that [Rh(DPB)<sub>2</sub>phi]<sup>3+</sup> sequence-specifically competes with the enzyme for binding to DNA. Importantly, this work demonstrates that a small, synthetic metal complex is able to bind to DNA with a level of specificity that mimics DNA-binding proteins.

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**Supplementary Material Available:** A computer graphics model depicting two rhodium complexes bound to the 8-base-pair site (2 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.

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