Study on the Binding of Base-Mismatched Oligonucleotide d(GCGAGC)₂ by Cobalt(III) Complexes

Huili Chen,[a] Pin Yang,*[a] Caixia Yuan,[a] and Xiaohua Pu[a]

Keywords: Mismatched DNA / Cobalt / Binding /Oligonucleotides

The binding of [Co(phen)₂(HPIP)]Cl₃ and [Co(phen)₂-(DPQ)]Cl₃ to the oligonucleotide d(GCGAGC)₂ containing two sheared G:A mispairs has been studied by NMR spectroscopy for the first time. For both the complexes, a considerable number of intermolecular NOEs were observed in NOESY spectra of the cobalt-complex-bound hexanucleotide. The results suggest that [Co(phen)₂(HPIP)]Cl₃, with HPIP, intercalates between the G₃:A₄ base pairs from the minor groove and extends to the major groove, and [Co(phen)₂(DPQ)]³⁺ binds to the terminal G₅:C₆ region from two directions. ³¹P NMR spectroscopy indicates that [Co(phen)₂-(HPIP)]Cl₃ binding induces a change in the phosphate backbone in the region of the mismatched base pairs, while [Co(phen)₂(DPQ)]³⁺ does not cause obvious changes in the backbone. Melting experiments indicate that [Co(phen)₂-(HPIP)]Cl₃ stabilizes the double-strand structure, while [Co(phen)₂(DPQ)]Cl₃ cause the duplex to untwist.

Introduction

Base mismatches arise naturally in the life cycle of a cell as a result of either polymerase error or DNA damage. Under most circumstances the cell corrects these mismatches by using a complex repair system to prevent mutations in the genetic code. Experimental systems for the recognition of mismatches are of particular interest in cases where this repair system is not functioning efficiently and allows errors in the DNA sequence to persist. However, the efficiency of mismatch error recognition and repair depends both on the nature of the mispair and on the flanking DNA sequence. For example, the type of GA pairs depends entirely on the context, i.e. the proceeding base pair, head-to-head GA pairing in a (NAGATN)₂ sequence switches to the more stable sheared side-by-side GA pairing when the context is changed to (NCGAGN)₂ or (NTGAAN)₂, while no duplex is formed (or only G:A bulges occur) in a (NGGACN)₂ context. Recognizing these “abnormal” structures at the atomic level presents new challenges. The sheared side-by-side G:A pairing has similar stabilities to the standard Watson–Crick base pairs. Up to now, specific recognition of this mispair has not been reported. According to the reported paper, the GpA phosphodiester link converts a B₁ conformation of normal B-form DNA into a B₁₁ conformation, which results in a downfield shift of the GpA ³¹P resonance. Furthermore, the adenosine swings away from intrastrand stacking on the preceding guanine and participates in cross-strand stacking with the adenine of the opposite strand (Scheme 1). Molecular modeling shows the two stacking guanines lie in the major groove, and the two stacking adenines lie in the minor groove.

Metallocomplexes of the general form [M(phen)₂L]ⁿ⁺ have contributed to our understanding of fundamental nucleic acid recognition. So far, most studies are limited to spectroscopic methods. However, it is difficult to clarify the details of the interaction by the phenomenological information, such as red shift, blue shift, hyperchromicity and hypochromicity etc. Hence, more accurate methods, such as two-dimensional NMR spectroscopy, are needed to describe the interaction between DNA and the metallocomplex.

In this paper, we report on a NMR spectroscopic study of two Co complexes that bind to d(GCGAGC)₂, which contains two sheared G:A mispairs. The results indicate that [Co(phen)₂(HPIP)]³⁺ indeed can selectively bind to the specific mispairs, and probably has the potential to repair the backbone in the region of the mispairs (Scheme I and Figure 1).

We tried to separate the racemic cobalt complex into the enantiomers using potassium antimonyl tartrate, but each isomer racemized in less than 24 hours; however, most of the important experiments could not be accomplished in such a short time. Further, it has been reported that the cobalt complex racemized rapidly in the presence of an oligonucleotide to generate a stable mixture of enantiomers.
Scheme 1. (a) d(GCGAGC)$_2$; (b) [Co(phen)$_2$(HPIP)]$^{3+}$-d(GCGAGC)$_2$.

**Results and Discussion**

### Assignment of the Proton Resonances of d(GCGAGC)$_2$

The $^1$H NMR resonances of the free oligonucleotide were assigned with the use of both NOESY and TOCSY experiments, according to well-established methods. The imino resonances in the NMR spectrum of the oligonucleotide dissolved in H$_2$O/D$_2$O (9:1) were examined to determine the extent of nucleotide base pairing. The spectrum indicates that only the terminal residue does not form a stable base pair, and two well-resolved imino resonances are observed. Hence, we believe that the oligonucleotide exists as double strands in our experiments. The imino proton at 10.36 ppm (Figure 2a) and the downfield $^{31}$P resonance (Figure 2b) indicates that the sheared G:A base pairs exist in d(GCGAGC)$_2$.

**DNA Binding by [Co(phen)$_2$(HPIP)]$^{3+}$**

The resonance signals of the free complex are assigned by using a combination of NMR experiments. The resonances from phen are easily distinguished from HPIP by the integral of each resonance. Within each ring system, the resonances are then assigned to particular protons with the use of the COSY spectrum. Because of the symmetrical structure of phen, on the NMR time scale, it is not possible to unambiguously distinguish the resonances of protons 5, 4, 3, 2 from 6, 7, 8, 9, respectively. We marked these protons...
as H5/6, H4/7, H3/8, and H2/9. H2/9 is coupled with H3/8 by vicinal $^3J_{H1,H}$, while it is coupled with H4/7 by long-range $^4J_{H1,H}$, so the coupling constant of H2/9–H3/8 is bigger than that of H2/9–H4/7, and the cross-peak for H2/9–H3/8 is stronger than that for H2/9–H4/7. The resonance for H4/7 has been assigned by its downfield chemical shift,\cite{24,25} hence, the resonance for H2/9 and H3/8 can also be confirmed from the COSY spectrum. The resonances for HPIP were assigned similarly.

The assignment of d(GCGAGC)$_2$-bound [Co(phen)$_2$(HPIP)]$^{3+}$ was also carried out with the help of COSY and NOESY spectra and previously reported hexanucleotide-bound [Ru(phen)$_2$(DPPZ)]$^{2+}$\cite{24,25} and [Ru(phen)$_2$(DPO)]$^{2+}$\cite{24,25}. Further, the possible chemical shifts for intercalated metal-ligand protons are restricted to within 0–1.6 ppm upfield from the location of the resonances for the free metal-ligand\cite{26,27}.

Addition of [Co(phen)$_2$(HPIP)]$^{3+}$ to d(GCGAGC)$_2$ induces large perturbations for the HPIP resonances (especially for H12, 13, 14, 15, 16), and much smaller perturbations for the ancillary phen ligand (Table 1 and Figure 3). This behavior is consistent with the fact that the ligand HPIP preferentially participates in binding with d(GCGAGC)$_2$. The magnitude and direction of the chemical shift change for the HPIP ligand protons are indicative of selective intercalation of this ligand.\cite{28,29} The interaction between the complex and d(GCGAGC)$_2$ leads to two sets of resonances from the ligand phen and one set of resonances from HPIP, which might be caused by the different spatial surrounding of the two ancillary ligands after HPIP intercalates with the oligonucleotide. Molecular modeling shows that after the complex intercalates into the sheared G:A region, the protons H13, 14, 15, and 16 from HPIP are all located in the ring plane formed by the two stacking adenines, and H12 lies in the ring plane formed by the two stacking guanines. The strong shielding from the purine rings causes these resonances to shift upfield to a different extent, the H8 and H2 protons of A4 and H8 of G3 are located in the side face of the aromatic ring of HPIP. In previous work,\cite{16} we observed that, after intercalation, the distance between the neighboring base pairs is doubled and the distances from the intercalating ligand plane to the two neighboring bases are similar to that between the neighboring base pairs before intercalation, so the ring current from HPIP has little effect on the resonances of these protons.

Addition of the metal complex to the hexanucleotide also induces significant broadening of the resonances of d(GCGAGC)$_2$ due to intermediate exchange on the NMR time scale (Figure 3B); this is, again, consistent with intercalation.\cite{24,29} In one- and two-dimensional spectra of this complex bound to the oligonucleotide, two G3H1’ and G1H1’ protons are evident, which were assigned to be the free form in the lower field and the bound form in the higher field according to previously published results,\cite{25} and the exchange between the two forms is slow on the NMR time scale.

The NOESY spectrum of d(GCGAGC)$_2$ with added [Co(phen)$_2$(HPIP)]$^{3+}$ (1:1) was recorded at 20 °C with a mixing time of 300 ms.\cite{24,25} For the nucleotide helix, in addition to the expected intraduplex sequential NOEs, a number of intermolecular NOE cross peaks between [Co(phen)$_2$(HPIP)]$^{3+}$ and d(GCGAGC)$_2$ are observed (Table 2). Of note are the strong NOE cross peaks between the HPIP H14 and H16 protons and G3H2’, G3H2’, and G3H8, and the NOE cross peaks between H16 and G3H3’ (see Figure 4 and Figure S1). The medium-intensity NOEs between H10–A1H2’, H10–A1H2’, H10–A3H3’ are also observed. Molecular modeling indicates that G3H2’/H2’, G3H8, G3H3’, A4H2’/H2’, and A3H3’ are all located in the DNA major groove, and the distance between the protons from the intercalated ligand to the neighboring nucleotide may be less than 5 Å, therefore some additional NOEs also appear, such as H16–C2H2’, between H15 and C6H5, C6H6, C2H2’/H2’, G3H2’/H2’, and between H13 and C2H2’/H2’ and G2H2’/H2’. In addition, the NOEs between phen and the protons located in the DNA minor groove are also clear, such as H3/8–A4H4’, H3/8–A4H5’/H5’’, and H2/9–G3H1’. All of these data strongly suggest that the complex inserts into the sheared G3A region from the minor groove A4 region, and the HPIP system spans the stacked base pairs and extends into the major groove G3 region. As the metal complex binds by intercalation, a selective loss in intensity of the sequential NOEs might be expected because the distance between the stacked bases at the intercalation site will significantly increase. Obviously, the disappearance of the intrastrand sequential NOEs in the sheared G:A region was detected (Figure 4), such as A4H8–G3H2’/H2’, A4H8–G3H3’, G1H2’/H2’–A1H2’/H2’, and simultaneously, the intensity of the sequential A4H8–G3H4’ NOE apparently diminished. However, the binding of the complex might result in a change in the DNA backbone, and some unexpected NOEs also appeared, such as A4H1’–G3H4’, A4H1’–G1H2’/H2’, G3H1’–A1H2’/H2’, and between A1H2 and G3H1’, G3H1’, C6H5, G2H2’, and A3H2’/H2’.

The two imino resonances in the NMR spectrum recorded in H$_2$O/D$_2$O (9:1) indicate that the double-strand structure of the hexanucleotide is maintained after [Co(phen)$_2$(HPIP)]$^{3+}$ binding. Only small shifts (<0.05ppm)

<table>
<thead>
<tr>
<th>Table 1. $^1$H NMR chemical shift data for [Co(phen)$_2$(HPIP)]$^{3+}$ bound to d(GCGAGC)$_2$.</th>
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<tbody>
<tr>
<td>Ligand proton</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>HPIP H16</td>
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<tr>
<td>H15</td>
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<td>H14</td>
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<td>H13</td>
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<td>H12</td>
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<td>H11</td>
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<tr>
<td>H10</td>
</tr>
<tr>
<td>Phen H5/6</td>
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<td>H4/7</td>
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<td>H3/8</td>
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<td>H2/9</td>
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</table>
**Figure 3.** 500 MHz $^1$H NMR spectra of [Co(phen)$_2$(HPIP)]$^{3+}$-d(GCGAGC)$_2$ with a metal complex to duplex ratio (R) of 1 (0.98 mm duplex) in 10 mM phosphate (pH 7) containing 20 mM NaCl. (A) aromatic region: (a) [Co(phen)$_2$(HPIP)]$^{3+}$, (b) [Co(phen)$_2$(HPIP)]$^{3+}$-d(GCGAGC)$_2$, (c) d(GCGAGC)$_2$; (B) sugar H$^{1}$ region: (a) [Co(phen)$_2$(HPIP)]$^{3+}$-d(GCGAGC)$_2$, (b) d(GCGAGC)$_2$.

**Table 2.** The NOE peaks between the complex and the oligonucleotide.

<table>
<thead>
<tr>
<th>Metal-complex Hexanucleotide protons</th>
<th>Protons</th>
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<tbody>
<tr>
<td>H16</td>
<td>G$_1$H8, G$_1$H3', G$_1$H2'/H2''', C$_6$H2''</td>
</tr>
<tr>
<td>H15</td>
<td>G$_1$H8', C$_6$H1', C$_2$H5, G$_2$H2'/H2''', C$_6$H6, C$_2$H2'/H2''</td>
</tr>
<tr>
<td>H14</td>
<td>G$_1$H8, G$_1$H2'/H2''</td>
</tr>
<tr>
<td>H13</td>
<td>G$_1$H2'/H2'', C$_6$H2'/H2''</td>
</tr>
<tr>
<td>H10</td>
<td>A$_4$H2'/H2'', C$_6$H3'</td>
</tr>
<tr>
<td>H3/8</td>
<td>A$_6$H4', A$_5$H5'/H5''</td>
</tr>
<tr>
<td>H2/9</td>
<td>G$_1$H1'</td>
</tr>
</tbody>
</table>

[a] The NOE is very weak.

and a little broadening were observed upon the addition of the complex (Figure S2).

**DNA Binding by [Co(phen)$_2$(DPQ)]$^{3+}$**

The resonance assignment of the free- and bound [Co(phen)$_2$(DPQ)]$^{3+}$ was similar to that of [Co(phen)$_2$(HPIP)]$^{3+}$ (Figure 5A). The two resonances from the DPQ H12 proton indicate at least two binding orientations for the binding of the metal complex to the helix, and the exchange rate between the two forms is slow. It was not possible to assign unambiguously the H13 resonance because of the overlap with the H4/7 resonances. For the same reason as for [Co(phen)$_2$(HPIP)]$^{3+}$, we marked phen protons as H5/6, H4/7, H3/8, and H2/9.

Addition of [Co(phen)$_2$(DPQ)]$^{3+}$ to d(GCGAGC)$_2$ also induces a large upfield chemical shift of the resonance for the DPQ H12 proton. By contrast, resonances from phen exhibit only a small shift, suggesting that the DPQ preferentially binds.

Addition of the metal complex also induces a significant broadening and selective shifts of the hexanucleotide resonances (Figure 5). Especially, significant upfield shifts are observed for the protons located in the major groove in the terminal G$_1$C$_2$/G$_5$C$_6$ region, such as H8 of G$_1$ and G$_5$, H2' of G$_1$, and H5 and H2' of C$_6$. Similarly, H1' protons of G$_1$ and G$_5$, which are located in the DNA minor groove, also show upfield shifts.

The NOESY spectrum of d(GCGAGC)$_2$ with added [Co(phen)$_2$(HPIP)]$^{3+}$ (1:1) was recorded at 20 °C with a mixing time of 300 ms. Figure 6 shows the aromatic to the sugar H$^{1}$, H$^{3}$ region (a) and the sugar H$^{2}$/H$^{2'}$ region (b). In addition to the expected intraduplex sequential NOE cross peaks, a number of intermolecular NOE cross peaks between the metal complex and the duplex are also observed (Table 3). Of note are the NOE cross peaks between the complex and the terminal protons located in the major groove, such as H13–C$_6$/C$_2$H$^{2'}$, H13–C$_6$H$^{3'}$, H12–C$_6$/C$_2$H$^{2'}$, H12–C$_6$H$^{5'}$, H11–G$_5$H$^{2'}$, H10–C$_6$/C$_2$H$^{2'}$, and H10–C$_6$H$^{5'}$. Further, the NOE cross peaks between the phen H4/7 protons and the minor groove H1' protons of C$_6$/C$_2$ are also observed. It was not possible to assign unambiguously the NOE cross peaks between the protons of the complex and the minor groove protons H4'/H5'/5' of C$_6$, C$_2$, G$_1$, and G$_5$ (a-g) because of the overlap of these resonances. These data strongly suggest that the cobalt complex binds to the DNA in the terminal G$_1$C$_2$/G$_5$C$_6$ region from two directions, one from the minor and the other from the major groove. The intensity of the sequential NOEs from C$_6$H$^{6}$–G$_5$H$^{3}$' and G$_1$H$^{8}$–C$_2$H$^{3}$' decrease with the binding of the metal complex. Additionally, the different degrees of broadening of the hexanucleotide resonances, induced by the addition of [Co(phen)$_2$(DPQ)]$^{3+}$, hinders the observation of a selective partial loss of the sequential NOE.

From the imino resonances, we confirm that the bound oligonucleotide still has a duplex conformation (Figure S2).
Binding of Base Mismatched Oligonucleotide d(GCGAGC)$_2$ by Cobalt(III) Complexes

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**Figure 4.** Expansion of the NOESY spectrum (300 ms mixing time) of [Co(phen)$_2$(HPIP)]$^{3+}$ and d(GCGAGC)$_2$ (1 mM) with a metal complex to duplex ratio of 1. It shows the peaks for the hexanucleotide and metal complex from the aromatic (6.5–9.0 ppm) to the sugar (a) H2’/H2‘’ region, and (b) H1’, H3’, H4’ and H5’/H5‘ region. The NOEs between the cobalt complex and the hexanucleotide are labeled. × indicates the disappearance of the intrastrand sequential NOEs in the sheared G:A region.

**Figure 5.** 500 MHz $^1$H NMR spectra of [Co(phen)$_2$(DPQ)]$^{3+}$-d(GCGAGC)$_2$ with a metal complex to duplex ratio (R) of 1 (0.98 mM duplex) in 10 mM phosphate (pH 7) containing 20 mM NaCl. (A) aromatic region: (a) [Co(phen)$_2$(DPQ)]$^{3+}$, (b) [Co(phen)$_2$(DPQ)]$^{3+}$-d(GCGAGC)$_2$, (c) d(GCGAGC)$_2$; (B) sugar H1’ region: (a) [Co(phen)$_2$(DPQ)]$^{3+}$-d(GCGAGC)$_2$, (b) d(GCGAGC)$_2$.

**$^{31}$P NMR Spectroscopy**

$^{31}$P NMR spectroscopic studies of oligonucleotide bound by intercalators provide direct information on any backbone distortion which accompanies any interaction.[10] Unlike $^1$H nuclei, for which upfield movement of the ligand resonances is caused by increases in shielding due to penetration into the ring currents of the base stack, the movement of $^{31}$P nuclei in oligonucleotides is governed by changes in the torsion angles about the phosphate linkage.[31–33] As shown in Figure 7, the free oligonucleotide exhibits a sharp peak at 1.1 ppm, far away from the other resonances, which is typical for sheared G:A base pairs with a B$_{II}$ conformation.[8–12] When the oligonucleotide is bound by [Co(phen)$_2$(HPIP)]$^{3+}$, the peak shifts upfield, and a new peak appears.
Figure 6. Expansion of the NOESY spectrum (300 ms mixing time) of [Co(phen)2(DPQ)]3+ and d(GCGAGC)2 (0.98 mm) with a metal complex to duplex ratio of 1, showing the peaks from the aromatic to hexanucleotide sugar (a) H1’, H3’ region, and (b) H2’/H2’’ region. The NOEs between the cobalt complex and the hexanucleotide are indicated.

Table 3. The NOE peaks between [Co(phen)2(DPQ)]3+ and the oligonucleotide.

<table>
<thead>
<tr>
<th>Metal-complex protons</th>
<th>Hexanucleotide protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>H13</td>
<td>C6H2/C6H3’</td>
</tr>
<tr>
<td>H12</td>
<td>C6/C6H2’, C6H5</td>
</tr>
<tr>
<td>H11</td>
<td>G7H2/H2’’</td>
</tr>
<tr>
<td>H10</td>
<td>C6H2/C6H2’, C6H5</td>
</tr>
<tr>
<td>H4/7</td>
<td>C6H1’</td>
</tr>
<tr>
<td>H2/9</td>
<td>C6H5, C6H2/C6H2’</td>
</tr>
</tbody>
</table>

[a] C6H2’ cannot be distinguished from C6H2’’.

little peak appears on the right of it. We speculate that it belongs to another resonance from 4P. The upfield shift indicates the change in the BII conformation of the phosphate backbone in the G3A4 region. In combination with the 1H NMR spectroscopy, we conclude that HPIP selectively inserts into the sheared G:A region. A few new peaks also appear in the upfield region; this occurs as a result of the intercalation of the complex into the G:A region, which causes the distortion of the neighborhood phosphate backbone. The addition of [Co(phen)2(dpq)]3+ shows no obvious change in the 31P NMR resonances of the duplex.

Melting Experiments

Other strong evidence for the binding of the complexes to the duplex was obtained from DNA melting studies.[34] Intercalation of small molecules into the double helix is known to increase the helix melting temperature (Tm), the temperature at which the double helix is denatured into single-stranded DNA. For d(GCGAGC)2, the transition tem-
perature can be determined by monitoring the absorbance at 254 nm as a function of temperature. As shown in Figure 8, both the Co complexes have a significant effect on $T_m$. The melting temperature of d(GCGAGC)$_2$ increases from 35 °C to 40 °C with [Co(phen)$_2$(HPIP)]$^{3+}$, whereas it decreases to about 30 °C with [Co(phen)$_2$(DPQ)]$^{3+}$. The increase in $T_m$ provides strong support for the intercalation of [Co(phen)$_2$(HPIP)]$^{3+}$ into the duplex, while [Co(phen)$_2$(DPQ)]$^{3+}$ binds to the duplex but does not intercalate.

Conclusion

The study on the site selective interaction between an octahedral metal complex and the mismatched oligonucleotide by NMR spectroscopy has not been reported up until now. Considering that the distortion of the backbone of DNA with sheared G:A mispairs weakens the selective binding of the enantiomer, we have attempted to study the interaction of an oligonucleotide d(GCGAGC)$_2$ with a racemic cobalt complex and have obtained some significant results.

In the NOESY experiments with d(GCGAGC)$_2$, many NOEs are observed between the metal complexes and the hexanucleotide. For [Co(phen)$_2$(HPIP)]$^{3+}$, as these NOEs are predominantly between HPIP and the major groove protons and between phen and the minor groove protons in the sheared G:A region, we conclude that the ligand HPIP inserts into the adenine base stacking from the minor groove, and extends to the guanine stacking in the major groove. The result of $^{31}$P NMR spectroscopic study indicates that the binding of the complex induces a change in the B$_1$ conformation of the phosphate backbone in the G$_3$A$_4$ region to a B$_2$ conformation. In this sense, the complex has the potential to repair the phosphate backbone in the sheared G:A region. For [Co(phen)$_2$(DPQ)]$^{3+}$, as the NOEs are predominantly from the terminal G$_1$C$_2$/G$_5$C$_6$ sequence, it is concluded that the complex binds to the oligonucleotide by two modes, one is from the major and the other is from minor groove in the G:C region. The $^{31}$P NMR spectroscopic study indicates that addition of the complex does not induce an obvious change in the conformation of the DNA. Melting experiments indicate that [Co(phen)$_2$(HPIP)]Cl$_3$ stabilizes the double-strand structure, while [Co(phen)$_2$(DPQ)]Cl$_3$ causes the duplex to untwist.

The difference in the binding properties between the two complexes could be because of the width of the ligand. The wider minor groove and the narrower major groove in the mispair region is exactly suitable for HPIP to insert between the sheared G:A base pairs.

Overall, the results of this study demonstrate that the octahedral metallointercalator [Co(phen)$_2$(HPIP)]$^{3+}$ can intercalate into the oligonucleotide d(GCGAGC)$_2$ in the sheared G:A region, which could have potential application in the design of mismatch-specific chemical agents.

Experimental Section

Materials: The oligonucleotide d(GCGAGC)$_2$ was purchased from AuGCT Biotechnology Co. Ltd, Beijing. The concentration was determined from the absorbance at 254 nm, and the single strand extinction coefficients were calculated from mononucleotide and dinucleotide data of a nearest-neighbor approximation.[20] The oligonucleotide was dissolved in phosphate buffer (pH 7.0, 0.50 ml of a 10 mm solution), containing NaCl (20 mM) and TMSP (0.05 mM). For the imino proton studies, the sample was dissolved into H$_2$O/ D$_2$O (9:1). For the non-exchangeable proton studies, the sample was dissolved into the phosphate buffer and repeatedly lyophilized from 99.8% D$_2$O in a speed vacuum, and then dissolved in D$_2$O (0.50 ml of a 99.96% solution), with a concentration of 1 mM. The complexes were synthesized as previously reported.[17] Stock solutions of the metal complexes were prepared in D$_2$O (unbuffered) and added to the hexanucleotide in 10ul aliquots until a metal complex to hexanucleotide duplex ratio of 1 was achieved.

The absorbance spectra were recorded with a Hewlett Packard HP-843 chemstation spectrometer. The DNA melting experiments were carried out by controlling the temperature of the sample cell with a Shimadzu circulating bath while monitoring the absorbance at 254 nm.

NMR Spectroscopic Measurements: NMR measurements were made with Bruker DRX-500 and DRX-300 spectrometers and analyzed with a silicon graphics workstation running Xwin-nmr. Proton chemical shifts were referenced to an internal TMSP standard. Phosphate chemical shifts were referenced to an external 85% H$_3$PO$_4$ standard.

All NMR spectra were recorded at approximately 20 °C. Spectra recorded in H$_2$O/D$_2$O (9:1) were collected by using a binomial 1:3:3:1 pulse sequence for solvent suppression. Two-dimensional phase-sensitive NOESY spectra in 99.96% D$_2$O were recorded by the TIPPI method, using 2048 data points in $t_2$ (over a spectral width of 5000 Hz) for 256 $t_1$ values with a pulse repetition delay 2 s. Suppression of the residual HDO resonance was achieved by low-power presaturation during the relaxation delay. Phase-sensitive TOCSY spectra were accumulated with 2048 data points in $t_2$ for 256 $t_1$ values with a pulse repetition delay 2 s.

Acknowledgments

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