Structure-specific binding of $[\text{Co}(\text{phen})_2(\text{HPIP})]^3+$ to a DNA duplex containing sheared G:A mismatch base pairs

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A B S T R A C T

The binding of a Co(II) complex to the decanucleotide $d\text{(CCGAATGAGG)}_2$ containing two pairs of G:A mismatches was studied by 2D-NMR, UV absorption, and molecular modeling. NMR investigations indicate that racemic $[\text{Co}(\text{phen})_2(\text{HPIP})]\text{Cl}_3$ binds the decanucleotide by intercalation: the HPIP ligand selectively inserts between the stacked bases from the minor groove at the terminal regions and from the major groove at the sheared region. Further, molecular modeling revealed that the recognition shows strong enantioselectivity: the $\Lambda$'-isomer preferentially intercalates into the $\text{T}_6\text{G}_7:\text{A}_5\text{A}_4$ region from the DNA major groove, while $\Delta$'-isomer favors the terminal $\text{C}_1\text{C}_2:G_9\text{G}_10$ region and intercalates from the minor groove. Detailed energy analysis suggests that the steric interaction, especially the electrostatic effect, is the primary determinants of the recognition event. Melting experiments indicate that binding stabilizes the DNA duplex and increases the melting temperature by 9.5 °C. The intrinsic binding constant of the complex to the mismatched duplex was determined to be $3.5 \times 10^5$ M⁻¹.

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1. Introduction

Nucleotide misincorporation during DNA synthesis yields non-complementary base pairs or mismatches within the DNA helix. Base pair anomalies resulting from DNA damage are generally subject to processing by mismatch repair [1]. However, the efficiency of mismatch recognition and repair depends both on the nature of the mismatch and on the flanking DNA sequences [2–12]. If uncorrected, mismatches are fixed as mutations during the subsequent round of DNA replication. Sheared side-by-side G:A pairing has a structure that is too wide to insert into matched B-form DNA; this instead results in an integrated helix; in addition the conformation of the sheared G:A base pairs is a quasi-helical duplex which weakens enantomer selectivity for DNA binding. In the present study we have used 2D-NMR, UV absorption, and molecular modeling to explore the binding of the complex to the decanucleotide $d\text{(CCGAATGAGG)}_2$ (Fig. 1) that contains two pairs of sheared G:A mismatches. We report that binding shows high enantioselectivity: $\Lambda'$-[Co(phen)$_2$(HPIP)]Cl$_3$ intercalates from major groove adjacent to the sheared mismatches while $\Delta'$-[Co(phen)$_2$(HPIP)]Cl$_3$ binds from the minor groove within the terminal regions of the duplex.

2. Experimental

2.1. Materials

The oligonucleotide $d\text{(CCGAATGAGG)}$ was purchased from GenScript Corporation, Nanjing. Oligomer single-strand concentration was calculated from high-temperature absorbance and single-strand extinction coefficient calculated as reference [14]. When duplex formed, the concentration was approximately a half of that of single-strand. The duplex was dissolved in 0.5 ml buffer containing 20 mM NaCl, 5 mM Na$_2$HPO$_4$, 5 mM NaH$_2$PO$_4$ and 0.05 mM TMSP. For imino proton studies, sample was dissolved into 90% H$_2$O/10% D$_2$O. For non-exchangeable proton studies, sample was dissolved into the phosphate buffer and repeatedly lyophilized from 99.8% D$_2$O in a speed vacuum, and then dissolved in 0.50 ml 99.996% D$_2$O with the concentration 1 mM. The complex was synthesized...
as previously reported [15]. Stock solution of the complex was prepared in D$_2$O (unbuffered) and added to the decanucleotide in 10 μl aliquots until a metal complex-to-duplex ratio (R) of 1 was achieved. The final duplex concentration was approximately 0.98 mM.

Absorbance vs. temperature melting curves were measured at 260 nm on a Hewlett Packard HP-8453 chemstation spectrometer by controlling the temperature of the sample cell with a Shimadzu circulating bath, and the intrinsic binding constant $K$ of the complex was determined according to Eq. (1) [16], through a plot of [DNA]/([DNA] - $e_a$) vs. [DNA].

$$\frac{[DNA]}{[DNA] - e_a} = \frac{1}{K(e_b - e_t)} \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, $e_a$, $e_t$, and $e_b$ are, respectively, the apparent extinction coefficient ($A_{DNA}/[M]$), the extinction coefficient ([DNA]/[M] = 0) for free metal complex and the extinction coefficient for the metal complex in the fully bound form ([DNA]/[M] = ∞). In plots of [DNA]/([DNA] - $e_a$) vs. [DNA], $K$ is given by the ratio of the slope to the intercept, $e_b$ is obtained from the slope and a measured value of $e_t$.

2.2. NMR measurements

NMR measurements were made on Bruker DRX-600 spectrometers and analyzed on a silicon graphics workstation running xwinmr. Proton chemical shifts were referenced to an internal TMSP standard.

All NMR spectra were recorded at approximately 20°C. Spectra recorded in 90% H$_2$O/10% D$_2$O were collected using a binomial 1:3:3:1 pulse sequence for solvent suppression. Two-dimensional phase sensitive NOESY spectra in 99.996% D$_2$O were recorded by 1:3:3:1 pulse sequence for solvent suppression. Two-dimensional TOCSY spectra were accumulated with 2048 data points in $t_2$ for power presaturation during the relaxation delay. Phase sensitive $^{13}$P resonance was achieved by low-power presaturation during the relaxation delay. The spectrum indicates that only the terminal residues failed to form stable base pairs, with four imino resonances being observed. It is therefore believed that the oligonucleotide is predominantly present as a stable duplex in our experiments. Both the imino protons at 10.40 ppm and the downfield $^{31}$P resonances (0.38 ppm and 0.12 ppm) (Supplementary material Fig. S1) indicate that two pairs of sheared G:A mismatch base pairs exist within the d(CCAGATGAGG)$_2$ duplex [21–25].

3. Results and discussion

3.1. Assignment of the proton resonances of d(CCAGATGAGG)$_2$

The $^1$H NMR resonances of the free oligonucleotide acid were assigned from a combination of NOESY and TOCSY experiments according to standard methods [18–20]. The imino resonances in the NMR spectrum of the oligonucleotide dissolved in 90% H$_2$O/10% D$_2$O were examined to determine the extent of nucleotide base pairing. The spectrum indicates that only the terminal residues failed to form stable base pairs, with four imino resonances being observed. It is therefore believed that the oligonucleotide is predominantly present as a stable duplex in our experiments. Both the imino protons at 10.40 ppm and the downfield $^{31}$P resonances (0.38 ppm and 0.12 ppm) (Supplementary material Fig. S1) indicate that two pairs of sheared G:A mismatch base pairs exist within the d(CCAGATGAGG)$_2$ duplex [21–25].

Table 1

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<th>$\Lambda$-Isomer</th>
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<td>H$_2$9/C/H$_5$ (M)</td>
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<td>A$_{12}$/G/H$_1$/H$^+$ (S)</td>
<td>A$_{12}$/G/H$_1$/H$^+$ (B)</td>
</tr>
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</table>
3.2. NMR analysis of [Co(phen)$_2$(HPIP)]$^{3+}$ binding to mismatched DNA

The resonances of the free complex were assigned based on the COSY spectrum (see Ref. [13] and Supplementary material Fig. 2S and Table S1). The resonances from the phen moiety are easily distinguished from HPIP protons by integration of the resonances. Within each ring system the resonances were then assigned to particular protons from the COSY spectrum. One set of resonances from two ancillary ligand phen moieties indicates the symmetrical structure of the complex, therefore it is not possible unambiguously to distinguish the resonances of protons 5,4,3,2 from 6,7,8,9. We assigned these protons to H5/6, H4/7, H3/8, and H2/9, respectively. H2/9 was coupled with H3/8 by vicinal $^3$J$_{H6-H3}$ 8.0 Hz while this was coupled with H4/7 by long-range $^4$J$_{H4-H7}$. In conse-

Fig. 2. 600 MHz $^1$H NMR spectra of [Co(phen)$_2$(HPIP)]$^{3+}$–d(CCGAATGAGG)$_2$ at the ratio (R) of metal complex-to-duplex 1 (0.98 mM duplex) in the aromatic region.

Fig. 3. Expansion of the NOESY spectrum (300 ms mixing time) of free- and [Co(phen)$_2$(HPIP)]$^{3+}$-bounded-d(CCGAATGAGG)$_2$ at a metal complex-to-duplex ratio of 1. (a and b) The aromatic to the sugar H1$'$ region of free- and complex-bounded-duplex and (c and d) the aromatic to the sugar H2'/H2$''$ region of free- and complex-bounded-duplex. The NOEs from the cobalt complex resonances to the decanucleotide are labeled. × indicates the disappearance of the intrastrand sequential NOEs.
quence, the coupling constant from H2/9–H3/8 was larger than that from H2/9–H4/7, and the cross-peak from H2/9–H3/8 was stronger than that from H2/9–H4/7. The resonance from H4/7 was assigned by its downfield chemical shift 9.07 ppm with respect to reference [12,26,27]; the resonance from H2/9 (7.65 ppm and 7.72 ppm) H3/8 (7.89–7.96 ppm) could also be confirmed from the COSY spectrum. H5/6 (8.48 ppm) failed to couple with other protons and showed a single peak. The resonances from HPIP were similarly assigned. The assignment for the duplex-bound complex was based on TOCSY and NOE experiments (see Supplementary material Fig. S4) and referenced to the free complex. The resonances at approximately 8.56–8.39 ppm were assigned to H5/6 which failed to couple with other protons. H4/7 was assigned through the strong NOE between approximately 9.12–8.97 ppm and H5/6. Accordingly, the resonances from H2/9 (7.70–7.55 ppm) and H3/8 (7.94–7.84 ppm) could also be assigned by the intensities of the cross-peaks with H4/7. Similarly, the protons of HPIP within the other ring system were assigned by the combination of NOE and TOCSY experiments. H12 (9.19 ppm and 8.80–8.63 ppm) was located at downfield and showed no NOE cross-peak with H5/6. H11 (7.91–7.81 ppm) and H10 (7.58–7.53 ppm) was assigned by their $^3$J (8.5 Hz) and $^4$J coupling with H12. The protons H13–H16 constitute another spin-couple system and were assigned by TOCSY. The proton sequence in chemical shift was also referenced to the previously reported hexanucleotide-bound-[Co(phen)$_2$(HPIP)]$^{3+}$ [13], [Ru(phen)$_2$(DPQ)]$^{2+}$ [26] and [Ru(phen)$_2$(DPPZ)]$^{2+}$ [27]. The possible chemical shift differences for intercalated ligand protons are restricted to within 0–1.6 ppm upfield from the location of the free ligand resonances [28,29]. Addition of [Co(phen)$_2$(HPIP)]$^{3+}$ to d(CCGAATGAGG)$_2$ induced large upfield chemical shift changes for the ligand HPIP (especially H11,12,13,14,15,16), while small shifts were observed for the ancillary ligand phen (Fig. 2). This behavior is consistent with preferential oligonucleotide binding of the HPIP ligand by HPIP (Table 1), suggesting that there may be two binding sites.

However, new NOE cross-peaks from HPIP to minor groove protons were also observed; these included H14–G$_7$H$_2$ (K), G$_9$H$^\prime$ (J), G$_9$H$^\prime$ (L), and H$_{16}$–G$_7$H$_2$ (H). Concomitantly, NOEs appeared from phen H2/9 to major groove protons including C$_2$H$_5$ (G) and C$_5$H$_5$ (M). These NOE cross-peaks indicated that the complex binds the decanucleotide by intercalation between the stacked bases proximal to the mismatch region, with the HPIP ligand selectively inserted from the minor groove and extending into the major groove. The disappearance of sequential intranucleotide NOEs provides further evidence; this includes A$_5$H$_8$–A$_4$H$_3$ (B) and A$_4$H$_2$–G$_9$H$_2$ (L). The imino resonance spectrum in 90% H$_2$O/10% D$_2$O indicates that the double-stranded structure of the decanucleotide is maintained after [Co(phen)$_2$(HPIP)]$^{3+}$ binding. Moreover, the two shoulder peaks neighboring the T$_6$ imino resonance reveal that the region neighboring T$_6$ is affected by addition of the complex (Supplementary material Fig. S3).

### 3.3. Thermal denaturation profiles of the duplex containing two sheared C:A mismatches

Thermal denaturation profiles for the free- and metal complex-bound-duplex (3.3 μM for each strand) were measured in the same buffer as for the NMR experiment. The absorption of the sample was monitored at 260 nm from 15 °C to 60 °C in the absence or presence of the Co(III) complex. Measurements were carried out at a complex concentration of 33 μM. The melting temperature ($T_m$) of the duplex was determined as the temperature crossing the melting curve at the median of two straight lines drawn for

![Fig. 4](image-url) Effect of the Co(III) complex on the melting temperature of d(CCGAATGAGG)$_2$ (3.3 μM) with a complex-to-duplex ratio of 10:1. d(CCGAATGAGG)$_2$ (circle) and [Co(phen)$_2$(HPIP)]$^{3+}$-d(CCGAATGAGG)$_2$ (square).

![Fig. 5](image-url) Changes in the UV spectra of [Co(phen)$_2$(HPIP)]$^{3+}$ in the PBS buffer with various amounts of the mismatched oligonucleotide (0–5 equiv). [Co] = 5 μmol/L and [DNA][Co] = 0, 1.0, 1.5, 3.0, 4.0, and 5.0.
the single-strand and duplex regions in the melting curve. As shown in Fig. 4, the binding of the complex increases the \( T_m \) of d[CCGAATGAGG]2 from 25 °C to 34.5 °C. The increase in \( T_m \) provides strong support for the intercalation of [Co(phen)2(HPIP)]3+ into the duplex, and further proves that the oligonucleotide adopts a double-stranded configuration under our experimental conditions.

### 3.4. Electronic absorption titration

The interaction between the complex and the mismatched oligonucleotide was investigated using absorption spectra. The electronic absorption spectra of the complex in the absence and the presence of the duplex are illustrated in Fig. 5. Addition of DNA results in a decrease in the peak intensities. Hypochromism suggested that there is a strong stacking interaction between the electronic state of the intercalated chromophore and that of the DNA bases, a general observation for the binding of intercalating molecules to DNA [33,34]. The intrinsic binding constant is \( 3.5 \times 10^5 \text{ M}^{-1} \) based on the hypochromism at 280 nm.

### 3.5. Molecular modeling

To acquire detailed binding information we carried out a computational examination of interactions between the Co(III) complex and the duplex using molecular modeling. The results of this calculation are detailed in the Supplementary material. A significant difference in calculated potential energies indicates that the \( \Lambda \)-isomer preferentially inserts from the major groove adjacent to the mismatches, while the \( \Delta \)-isomer preferentially inserts from the minor groove within the terminal regions of the duplex. Moreover, the complex of the \( \Lambda \)-isomer with the duplex may be more stable than of the complex formed with the \( \Delta \)-isomer. When optimal models were investigated we found that \( \Lambda \)-isomer has the potential to alter the conformation of the sheared DNA, converting the G7A8:A4G3 base pairs from sheared to parallel forms (Fig. 6). By combining the optimal binding models we were able to assign most of the intermolecular NOEs between the metal complex and duplex.

Molecular modeling reveals that the interaction of the Co(III) complex with the mismatched DNA shows obvious enantioselectivity for complex formation, groove-selectivity, and site-specificity for sheared DNA. This finding is consistent with our previous work [35–41] and detailed energy analysis is also consistent with

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**Table 2**

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**Table 3**

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the results obtained. The sub-items for total energy at optimal depths in the minor groove for the Δ-isomer and those in the major groove for the Λ-isomer are depicted in Tables 2 and 3. As shown in the tables, steric (non-bond) items are more influential for DNA recognition than are the internal items as the former is of greater magnitude than the latter. For non-bond interactions the electrostatic energy is 10^2–10^3 times as large as the VDW energy, and determines the magnitude of the total energy. As shown in Table 3, when the Δ-isomer intercalates into the major groove, the Λ-isomer intercalates from the minor groove and NOESY spectrum of d(CCGAATGAGG)2

\[ \text{The imino proton and sugar H1'–H1 NMR and } ^{31}\text{P NMR spectra of the free and complex-bound-duplex. Contour plots of the TOCSY and NOESY spectrum of d(CCGAATGGG)2–bounded-complex: H1' NMR chemical shift data for [Co(phen)_2(HPIP)]^3+ Bound to d(CCGAATGGG). The data on the molecular modeling. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2009.02.006.} \]

References


4. Conclusions

The following conclusions may be drawn from the results reported here. First, for racemic [Co(phen)_2(HPIP)]^3+–DNA interactions, selective intercalation of the metal complex into DNA was observed, with HPIP as the intercalating moiety. Second, binding shows obvious enantioselectivity, site-specificity and groove-selectivity: the Δ-isomer recognizes the mismatched region from the major groove, while the Λ-isomer recognizes the terminal region from the minor groove. Third, the steric interaction, especially the electrostatic effect, is the primary determinants of the recognition event. Fourth, the binding of the complex stabilizes the duplex conformation of the mismatched DNA.

The interactions between racemic [Co(phen)_2(HPIP)]^3+ and an oligonucleotide duplex were studied through NMR combined with molecular modeling. NMR investigations reveal the acting mode while molecular modeling indicates the likely sites of interaction for the different isomers. Overall, the results of this study demonstrate that the octahedral metallointercalator Λ-[Co(phen)_2(HPIP)]^3+ can recognize mismatched regions within a DNA duplex; this finding may have applications in the design of mismatch-specific chemical agents.

5. Abbreviations

phen 1,10-phenanthroline
TMSP 3-trimethylsilyl-[2,2,3,3-D4] propionate
SGI Silicon Graphics, Inc.
NOESY nuclear overhauser effect spectroscopy
COSY correlated spectroscopy
TOCSY total correlation spectroscopy
Total total energy
VDW van der Waals energy
Elect electrostatic energy
non-bond non-bond energy (the sum of the VDW and Elect) which describes the steric interactions;
internal the internal energy which describes bond properties

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