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The ligand-structure-selective binding of oligonucleotide by cobalt complexes

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ABSTRACT

The binding of two Co(III) complexes $[Co(phen)_2(DPQ)]^{3+}$ and $[Co(phen)_2(HPIP)]Cl_3$ [HPIP = 2-(2-hydroxyphenyl) imidazo [4,5-f][1,10] phenanthroline, DPQ = dipyrido[3,2-f:2′,3′-h]quinoxaline] to the normal base-paired decanucleotide $d(CCTAATTAGG)_2$ was studied by 2D NMR. The results indicate that the width of intercalating ligand has a large effect on the selectivity of binding site. For $[Co(phen)_2(HPIP)]Cl_3$, the complex binds the decanucleotide at C_2T_3:G_9A_8 and A_4A_5:T_7T_6 by intercalation from the minor groove, while $[Co(phen)_2(DPQ)]^{3+}$ intercalates into T_3A_4:T_7A_8 region from the minor groove. The conclusion was further proved by molecular modeling.

In the past decade, many octahedral Co(III) polypyridyl-type complexes have been synthesized as probes for DNA secondary structure, and their DNA-binding properties have been investigated mostly by UV–vis absorption spectra, emission spectra, viscosity measurements, circular dichroism spectra and gel electrophoresis experiments, etc. [1–12]. However, these methods are based on the properties of whole DNA molecules and could not provide the information about binding site. Two-dimensional NMR is powerful tool to detect DNA interactions and locate accurate binding sites, such as major/minor groove binding mode and base pairs involved in the complex intercalation [13–15].

In our previous work, we initially reported the binding of $[Co(phen)_2(DPQ)]^{3+}$ to hexanucleotide $d(GTCGAC)_2$ by 2D NMR and molecular simulations [16]. However, as DNA model, hexamer is too short to form an integrated duplex. Herein, as an extension of our work, we studied the binding of the complex $[Co(phen)_2(DPQ)]^{3+}$ and $[Co(phen)_2(HPIP)]^{3+}$ to the decanucleotide $d(CCTAATTAGG)_2$ by 2D NMR (Fig. 1). The results suggest that the two complexes, with different width of the intercalating ligand, bind to the decanucleotide at different sites. $[Co(phen)_2(DPQ)]Cl_3$, with narrower ligand DPQ, intercalates into T_3A_4:T_7A_8 region from the minor groove, while $[Co(phen)_2(HPIP)]^{3+}$, with wider ligand HPIP, intercalates into C_2T_3:G_9A_8 and A_4A_5:T_7T_6 region from the minor groove.

The ^1H NMR resonances of the free oligonucleotide acid have been assigned in the previous work [13]. The imino resonances spectrum indicates that only the terminal residues failed to form stable base pairs (Supplementary material Fig. S1). It is therefore believed that the oligonucleotide is predominantly present as a stable duplex in the experimental condition.

Addition of $[Co(phen)_2(HPIP)]^{3+}$ or $[Co(phen)_2(DPQ)]^{3+}$ to $d(CCTAATTAGG)_2$ induced large chemical shift changes for the ligand HPIP (especially H12, H13, H14, H15, H16) and DPQ (H11, H12, H13), while small shifts were observed for the ancillary ligand phen (Fig. 2). This behavior is consistent with preferential oligonucleotide binding of the ligand HPIP or DPQ by intercalation [13–15]. However, the chemical shift changes from the oligonucleotide are different for the two complexes binding. For $[Co(phen)_2(HPIP)]^{3+}$ binding, a large upfield (∼−0.771 ppm) of T_3Me in the major groove indicates the strong shied from the intercalating ligand, and reveals the binding site at T_3:A_8 region. While for $[Co(phen)_2(DPQ)]^{3+}$ binding, significant upfield shifts were observed for the particular protons located in the minor groove in T_3A_4/T_7A_8 region, such as A_4H1' (−0.07 ppm), T_7H1' (−0.09 ppm), T_7H2'' (−0.08 ppm) and A_8H2'' (−0.10 ppm), which imply that the complex intercalates into T_3A_4:T_7A_8 from the minor groove.

NOESY spectra of $d(CCTAATTAGG)_2$ bound to the complexes (1:1) was recorded respectively at 20 °C with mixing time of 300 ms (Fig. 3 and Supplementary material Tables S3–S4). In addition to the expected intraduplex sequential NOEs from the oligonucleotide, a number of intermolecular NOE cross-peaks between complexes to $d(CCTAATTAGG)_2$ were observed (Fig. 3). For $[Co(phen)_2(HPIP)]^{3+}$ binding, the intense NOE cross-peaks between phen and sugar are observed, including $H3/8–G_9H1'$ (A), $H3/8–T_3H1'$ (B), $H5/6–T_3H1'$ (E), $H5/6–A_8H1'$ (I), $H4/7–A_8H1'$ (J), $H4/7–T_3H1'$ (K). Because the sugar H1′ protons are located in the decanucleotide minor groove, these NOEs indicate that the phen

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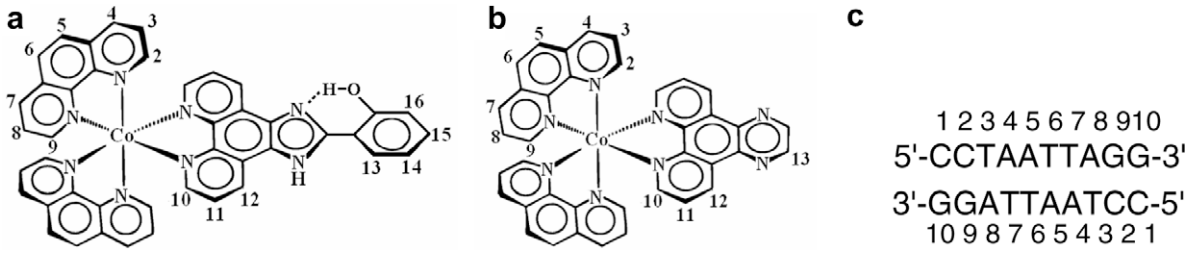

Fig. 1. The structure of $[Co(phen)_2(HPIP)]^{3+}$ (a) and $[Co(phen)_2(DPQ)]^{3+}$ (b) with atomic numbering; (c) the DNA sequence used in this work.

ligands are bound in the minor groove at the region C_2T_3:G_9A_8. This conclusion is further supported by the observation of NOE cross-peaks from phen and the decanucleotide H4′/H5′/5″ (protons located in the minor groove): H3/8–T_3H5′/5″ (C), H3/8–C_2H4′ (D), H5/6–T_3H4′ (G), H4/7–T_3H4′ (H). Therefore, the NOE data, combined with the upfield shift of T_3Me, indicate that the complex binds the decanucleotide by intercalation with the HPIP ligand selectively inserted from the minor groove and extended to the major groove between the stacked bases in C_2T_3:G_9A_8 region.

NOE cross-peaks from intercalating ligand HPIP to sugar protons were also observed; these included H11–A_5H1′ (M), H11–A_4H1′ (N), H11–T_6Me (O), and H11–A_5H2″ (P). Concomitantly, NOEs appeared from phen H5/6 to minor groove proton T_6H1′ (L) and A_5H4′ (F). These NOE cross-peaks indicated that the complex binds the decanucleotide by intercalation between the stacked bases in A_4A_5:T_7T_6 region, with the HPIP ligand selectively inserted from the minor groove. The disappearance of sequential intra-nucleotide NOEs A_5H2–A_4H1′ (Q) provides further evidence.

$[Co(phen)_2(DPQ)]^{3+}$ binding also induced a considerable number of intermolecular NOEs between the complex and d(CCTAATTAGG)$_2$. Of note were the NOEs between phen to the protons located in the minor groove, such as H5/6–A_4H1′ (A), H5/6–A_8H1′ (B), H5/6–T_3H1′ (C), H5/6–T_3H2″ (F). It was not possible to unambiguously assign NOE cross-peaks between phen H5/6 and the decanucleotide H4′/H5′/5″ (D) (protons located in the minor groove) due to the overlap of these resonances. Alternatively, the NOE cross-peak from H13 to major groove proton T_3Me (H) was also observed. These data suggest that $[Co(phen)_2(DPQ)]^{3+}$ intercalates into the T_3A_4/T_7A_8 region from the minor groove, with the DPQ ligand extending to the major groove. With the metal complex binding, some intra-nucleotide sequential NOEs missed or weakened, such as A_4H8–T_3Me (I) and A_4H8/T_3H2′(J).

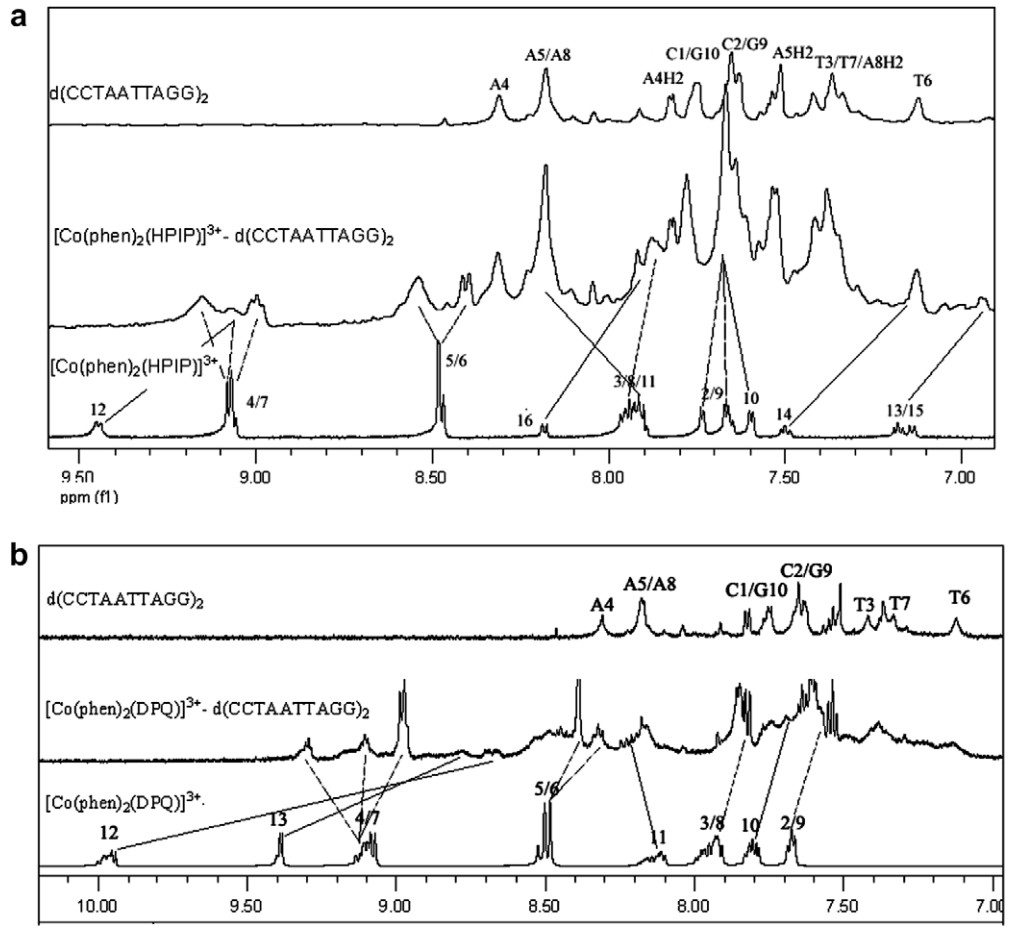

Fig. 2. 600 MHz ^{1}H NMR spectra of the complex-bounded-d(CCTAATTAGG)$_2$ at the ratio (*R*) of metal complex to duplex 1 (0.98 mM duplex) in the aromatic region.

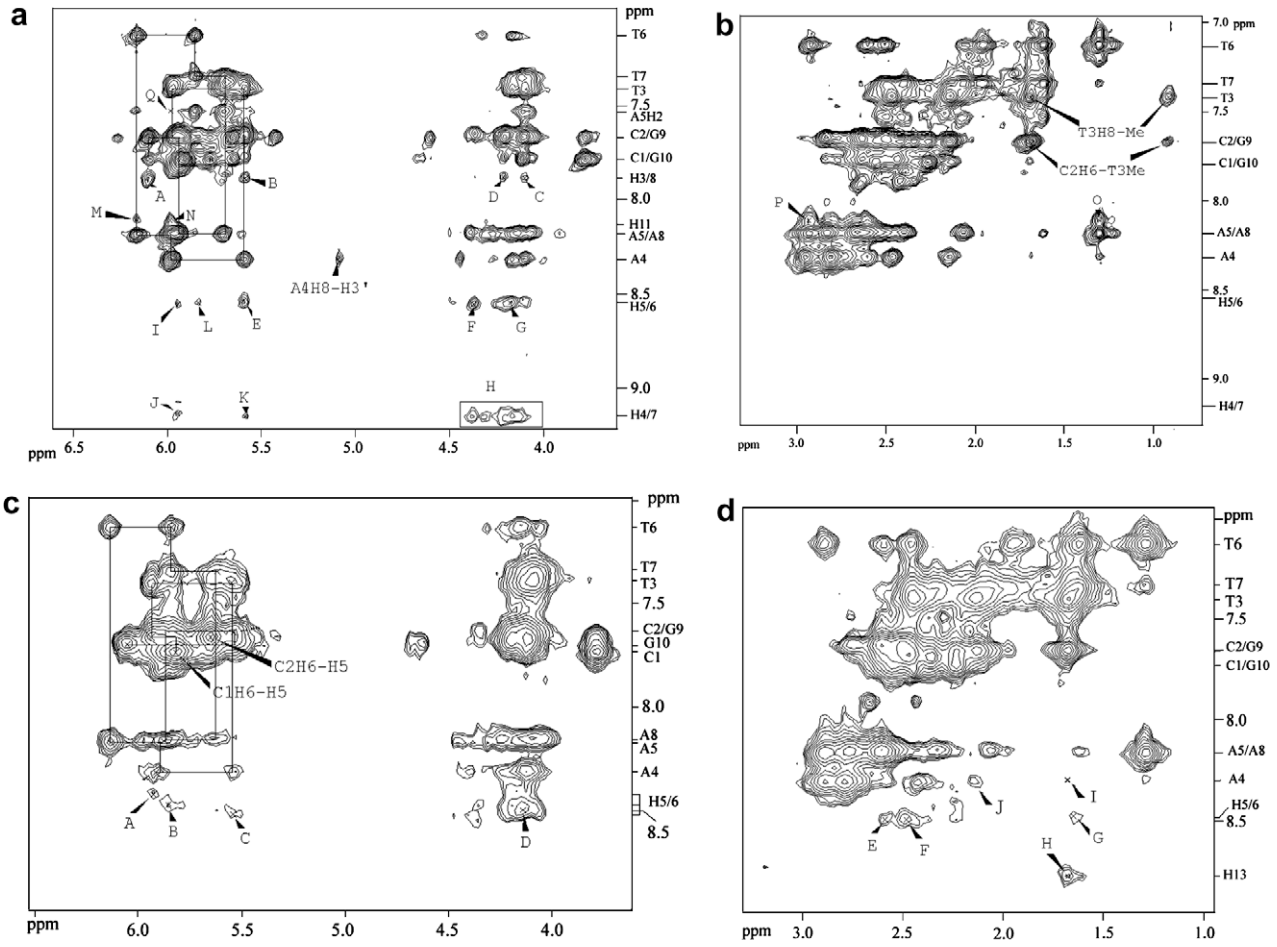

Fig. 3. Expansion of the NOESY spectrum (300 ms mixing time) of complex-bounded-d(CCTAATTAGG)$_2$ at a metal complex-to-duplex ratio of 1. (a) The aromatic to the sugar H1′ region of [Co(phen)$_2$(HPIP)]$^{3+}$-bounded-duplex; (b) the aromatic to the sugar H2′/H2″ region of [Co(phen)$_2$(HPIP)]$^{3+}$-bounded-duplex; (c) the aromatic to the sugar H1′ region of [Co(phen)$_2$(DPQ)]$^{3+}$-bounded-duplex; (d) the aromatic to the sugar H2′/H2″ region of [Co(phen)$_2$(DPQ)]$^{3+}$-bounded-duplex. The NOEs from the cobalt complex resonances to the decanucleotide are labeled. ×, Indicates the disappearance of the intrastrand sequential NOEs.

Further, to acquire the detailed binding information, a computational examination of interactions between the Co(III) complexes and the duplex was carried out by molecular modeling. The calculation results (Supplementary material Tables S5–S12) support the above conclusion.

In summary, although similar structure of the two complexes, the binding site with the same oligonucleotide is different. [Co(phen)$_2$HPIP]$^{3+}$, with HPIP, intercalated into base pairs of a normal oligonucleotide duplex at two sites C_2T_3:G_9A_8 and A_4A_5:T_7T_6, while [Co(phen)$_2$(DPQ)]$^{3+}$ intercalates into T_3A_4:T_7A_8 region from the minor groove. Maybe the difference in the binding site is derived from the width of the intercalating ligand. This result provides a theoretical basis for a rational design of a drug based on gene therapy.

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Appendix A. Supplementary material

The imino proton and sugar H1′ ^{1}H NMR spectra of the free and complex-bound-duplex; Contour plots of the COSY spectrum of free complex, TOCSY and NOESY spectra of d(CCTAATTAGG)$_2$-bounded-complex; ^{1}H NMR Chemical Shift Data for free- and d(CCTAATTAGG)$_2$-bounded-[Co(phen)$_2$(HPIP)]$^{3+}$; The NOE cross peaks derived from the interaction between the complexes and d(CCTAATTAGG)$_2$; The data on the Molecular modeling. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inoche.2009.12.012.

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