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DNA binding and unwinding by self-assembled supramolecular hetero-bimetallacycles

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Abstract

Two new tetracationic hetero-bimetallacycles were prepared from a bis-pyridine amide ligand and metal (Pd and Pt) acceptors. We found that both self-assembled hetero-bimetallacycles bind and unwind supercoiled DNA as established by photophysical and gel electrophoresis analyses, respectively.

Keywords

Supramolecular; self-assembly; hetero-metallacycle; pyridine-amide ligand; DNA binding studies

The coordination-driven self-assembly of metallacyclic structures has attracted interest for a variety of promising applications.¹ Metallacycles of well-defined shape and size can be designed and synthesized using a coordination-driven directional bonding approach that combines rigid ligand-capped transition metal acceptors and mono- or multidentate donors bearing O- and N-coordinating sites. Metal units providing 90° coordination geometries, in combination with organic spacers, have been used to form cyclic architectures.² In this context, square planar Pd(II) and Pt(II) organometallic acceptors have been the most commonly used metal ions in coordination-driven self-assembly because their rigid coordination environment allows the shape of the final structures to be easily controlled.³ Organic amides bearing O- and N-coordinating sites interact with metal ions to form structurally and functionally diverse supramolecular entities.⁴ In recent years, metallasupramolecular principles have been used to design the tetracationic supramolecular architecture as a new class of synthetic agents that bind strongly and noncovalently to the major groove of DNA and cause remarkable intramolecular DNA coiling.⁵ The design of agents that can interact with DNA in a sequence-specific manner is of great importance in probing biological systems and in developing therapeutic drugs. If genes that code for DNA

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repair machinery are damaged and are no longer able to carry out their critical function, mutations can develop and accumulate, leading to diseases such as cancer.⁶ Therefore, there is a great deal of interest in metallo-insertors and intercalators that can interact intimately with DNA base pairs and can be structurally tuned to target specific DNA defects or particular DNA sequences.⁷ Sleiman⁸ reported that a self-assembled platinum molecular square binds strongly to G-quadruplex DNA and efficiently inhibits telomerase function, which suggests such complexes could be efficacious for selective antitumor therapy.

In our exploration of unique structures and properties of metallo-supramolecular complexes, a bis-pyridine amide ligand and two metal (Pd and Pt) acceptors were used to construct two hetero- bimetallacycles (HBMCs). An investigation of the binding properties of these HBMCs revealed they efficiently interact with DNA as established by photophysical and gel electrophoresis studies. To the best of our knowledge, these are the first reported metallacyclic systems constructed from a bis-pyridine amide ligand and Pd and Pt acceptors that interact with DNA.

As shown in Scheme 1, the HBMCs **4** and **5** were prepared by reaction of the H₂L (H₂L = 2,6-bis(*N*-(4-pyridyl) carbamoyl)pyridine)) ligand (**1**) with *cis*-Pd[(dppf)(OTf)₂] (**2**) or *cis*-Pt[(dppf)(OTf)₂] (**3**) [dppf = 1,1'-bis (diphenylphosphino) ferrocene; OTf = trifluoromethanesulfonate] acceptors in a [2+2] manner. NMR spectroscopy was initially used to characterize the metallacycles. The ¹H NMR spectra of **4** and **5** clearly show the formation of symmetrical species by typical resonance shifts due to metal complexation. Compared to the proton signals in H₂L, we found that upon coordination, all of the signals in both metallacycles shifted significantly (Figure S1–2). The ³¹P NMR spectrum of **4** showed one sharp peak at 34.3 ppm that was shifted upfield approximately 16.5 ppm compared to the signal of the starting acceptor due to metal coordination with the pyridines (Figure S3) The appearance of a single ³¹P signal was indicative of the presence of symmetric phosphorus nuclei. Likewise, the ³¹P NMR spectrum of **5** displayed a sharp singlet at 4.6 ppm along with ¹⁹⁵Pt satellites, indicating the formation of a single product (Figure S4).

The ESI-MS of HBMCs **4** and **5** were obtained to provide additional evidence for the formation of the metallacycles. The mass spectrum of assembly **4** showed peaks at *m/z* = 1129.0 and 703.1 corresponding to [M - 2OTf]²⁺ and [M - 3OTf]³⁺ (Figure 1a), and the mass spectrum of **5** showed [M - 2OTf]²⁺ and [M - 3OTf]³⁺ peaks at *m/z* = 1217.6 and 762.2, which were consistent with their theoretical isotopic distributions (Figure 1b).

The X-ray crystal structure of **4** clearly demonstrated that the two pyridine groups of H₂L link with two *cis*-(dppf)Pd(OTf)₂ building blocks to form the [2+2] HBMC (Figure. 2a). The ferrocenyl moieties occupied outlying sites on the partially distorted square planar Pd(II) center. Each Pd atom was coordinated with two phosphorus atoms of dppf and two nitrogen atoms from two different pyridine ligands to yield the distorted square planar geometry around the metal center.⁹ The average bond distance of Pd-N_{pyridine} was 2.09 Å, and the average Pd-P distance was 2.30 Å. The average N-Pd-N bite angle was 82.3°, indicating a slight distortion from the ideal 90° angle. For the HBMC **5**, the average bond distance of the Pt-N_{pyridine} was 2.08 Å, the average Pt-P distance was 2.28 Å, and the average N-Pt-N bite angle was 81.2°. The crystal structures of both complexes show an anion molecule captured in the cavity through hydrogen bonding interactions (Figure 2). The hydrogen bond distances between the hydrogen atoms of the aromatic group and the oxygen atoms of the triflate anion were in the range of 2.32 Å and 2.58 Å for **4** and 2.44 Å and 2.48 Å for **5**.

Because DNA is the primary pharmacological target of many metal complex antitumor compounds, the interaction between DNA and metal complexes is of paramount importance

in understanding their mechanism of action.¹⁰ To explore the DNA-binding mode of these HBMCs, we examined the interaction of **4** and **5** with supercoiled DNA by photophysical and gel electrophoresis methods. First, the potential binding ability of complexes **4** and **5** with DNA (pUC19) was studied by UV-Vis spectroscopy (Figure 3 left). The absorption peaks at 266 nm for **4** and 270 nm for **5** were attributed to an intraligand π - π^* transition. Upon addition of DNA, the above bands corresponding to complexes **4** and **5** showed significant hypochromism shifts. These results are similar to those reported previously for metallomacrocycles,¹¹ and suggest that the complexes used in this study bind to DNA by intercalation. The magnitudes of the intrinsic binding constants (K_b) were calculated to be $2.58 \times 10^5 \text{ M}^{-1}$ for **4** and $1.23 \times 10^5 \text{ M}^{-1}$ for **5**. The observed binding constant (K) values revealed that the HBMC **4** bound more efficiently to DNA than the HBMC **5**. We next investigated the binding of the complexes to DNA by a fluorescence spectroscopic method using the emission intensity of ethidium bromide (EtBr) as a probe (Figure 3 right), where binding of the complex to DNA would displace EtBr, thus decreasing its emission intensity (quenching). The ratio of the slope to the intercept obtained by plotting I_0/I vs. $[Q]$ yielded values of K_q corresponding to $3.86 \times 10^3 \text{ M}^{-1}$ for **4** and $2.14 \times 10^3 \text{ M}^{-1}$ for **5**, which indicate that the HBMC **4** had a higher quenching efficiency than **5**.

Since both complexes possessed an ability to bind with DNA, the complexes were further investigated for DNA relaxation activity. As shown in Figure 4, negatively supercoiled pUC19 DNA was treated with increasing amounts of HBMC **4** and **5**. Aliquots of the reaction mixtures were subjected to electrophoresis on 1% native agarose gels at 25 °C with 1x TAE (Tris-acetate/EDTA, pH 8.4) buffer and the voltage set at 30 V. The gels were then stained with EtBr and subsequently photographed with a UV-transilluminator. The unwinding induced by HBMCs bound to DNA was mainly dependent on the HBMC:base ratio, and complete transformation of the plasmids from the negatively supercoiled to the relaxed form could be attained for both complexes **4** and **5**, although no DNA cleavage was observed (Figure 4). These results revealed that the HBMC **5** was more efficient at unwinding DNA than HBMC **4** and that complex **5** has a high intercalation capacity. Conformational changes upon interaction of supercoiled DNA with HBMC **4** and **5** were further verified by CD experiments (Figure S7). Nearly 50% decrease in the positive bands along with shift of the bands toward higher wavelengths were noticed upon mixing supercoiled DNA with of HBMC **4** and **5**. The decrease in the positive CD band could be attributed for the structural changes in the supercoiled DNA upon interaction with HBMCs. Earlier studies had demonstrated that binding of a platinum complex with DNA induced several changes in the DNA conformation and could induce DNA deformation.¹² Therefore, the differences in DNA unwinding efficiency between the complexes may be due to their different binding affinities to DNA. The unwinding of DNA is a valuable biophysical parameter in evaluating the interaction of a complex with DNA. The unwinding of the DNA duplex is mainly caused by groove binding agents. The results herein demonstrate that both complexes **4** and **5** unwind the DNA duplex.

Control experiments established that neither the metal acceptors nor the ligand itself had relaxation activity; thus, the metallacycle complexes interacting with DNA were responsible for the observed activity (Figure S8). In addition, ³²P-radiolabelled 19-bp duplex DNA (blunt end) was used as a substrate to identify whether the complexes had any DNA cleavage activity, and the results clearly indicated that no cleavage occurred with either complex (Figure S9).

In summary, we report the preparation of two new self-assembled HBMCs, which were found to bind efficiently with duplex DNA in photophysical experiments. Based on kinetic experiments, HBMC **4** had a greater binding affinity for DNA than **5**. Gel electrophoresis studies confirmed that both HBMCs were active in the conversion of pUC19 DNA from the

negatively supercoiled form to the relaxed form under physiological conditions with no DNA cleavage activity. Further studies to determine a more detailed mechanism for DNA unwinding as well as the nature of the interactions and selectivity of these HBMCs with duplex DNA are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Calculated (top) and experimental (bottom) ESI- MS spectra of **4** (a) and **5** (b).

Figure 2. X-ray crystal structure of HBMCs **4** (a) and **5** (b) showing the encapsulation of a triflate anion through H- bonding interactions.

Figure 3.

UV/Vis absorption spectra (left) **4** (a) and **5** (b) upon incremental addition of DNA. Arrows show the absorbance changes upon increasing amount of DNA (Inset in figure: binding constant plot for the supercoiled DNA-HBMCs). Emission spectra (right) of **4** (a) and **5** (b) upon gradual addition of DNA (EB bound) (Inset in figure: Stern- volmer plot for the supercoiled DNA- HBMCs).

Figure 4.

Agarose gel electrophoresis showing the interaction of supercoiled DNA with HBMC **4** (A) and **5** (B). Plasmids at a concentration of 2×10^{-4} M were mixed with increasing concentrations of the HBMCs in 10 mM Tris-HCl (pH 7.4), incubated for 1 hr at 37 °C, and analyzed on 1% agarose gels (Lanes 1 and 11, molecular weight markers; Lane 2, control (non-modified DNA); Lanes 3–10, DNA with HBMCs concentrations of 1, 2, 3, 4, 5, 10, 15, and 20 μ M respectively). The SC and OC (open circular) indicate supercoiled and relaxed forms of plasmid DNA.

Scheme 1.
Synthesis of hetero- metallacycles **4** and **5**.