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Interaction of Binuclear Xylylthiolato(2,2′,2″-terpyridine)platinum(II) Complexes with DNA

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Abstract—The new binuclear platinum(II) complexes, (1,3-benzenedimethanethiolate-S)di(2,2′,2″-terpyridine)diplatinum(II) chloride tetrahydrate, **5**, and (1,4-benzenedimethanethiolate-S)di(2,2′,2″-terpyridine)diplatinum(II) chloride tetrahydrate, **6**, were synthesized in order to investigate the binding of platinum(II) complex with calf thymus DNA, which was examined by UV and CD spectroscopies. Complex **5** interacted strongly with DNA by intercalation compared to **6**.

Introduction

Over the past decade, square-planar platinum(II) complexes derived from 2,2′,2″-terpyridine have been of great interest because of intercalative interaction with DNA[1–7] and photophysical properties,[8] which are applicable to development of antitumor drugs based on intercalative mode and of supramolecule materials for sensor. For example, Lippard et al. have demonstrated by X-ray fiber diffraction study[9] that 2-hydroxyethanethiolato(2,2′,2″-terpyridine)platinum(II) complex, **1**, bound to double-stranded DNA and was distributed along the helix axis according to neighbor exclusion binding model. Wang et al. have also reported that the crystal structure of the complex between deoxycytidyl-(3′, 5′)-deoxyguanosine and **1**, in which **1** intercalated between two Watson–Crick GC base pairs of deoxycytidyl-(3′, 5′)-deoxyguanosine (Fig. 1).[10]

In order to explore the intercalative binding with DNA, we prepared two dinuclear platinum(II) complexes formed by connecting the terpyridine platinum(II) moiety with 1,3- or 1,4-benzenedimethanethiol group, (1,3-benzenedimethanethiolate-S)di(2,2′,2″-terpyridine)diplatinum(II) chloride tetrahydrate, **5** and (1,4-benzenedimethanethiolate-S)di(2,2′,2″-terpyridine)diplatinum(II) chloride tetrahydrate, **6**.

Here, we report the synthesis of **5** and **6** and their binding behavior with calf thymus DNA, which is also compared with those of mono- or dinuclear platinum(II) complexes, **1**–**4**.

With such dinuclear complexes, several examples are known:[11,12] McFadyen et al., have reported the binding behavior of DNA with mono- and dinuclear platinum(II) complexes, **2** and **4** that formed by linking two (2,2′,2″-terpyridine)platinum(II) moieties via dithioalkances. Among a series of **4**, those with $n = 5$, 6, and 7 function

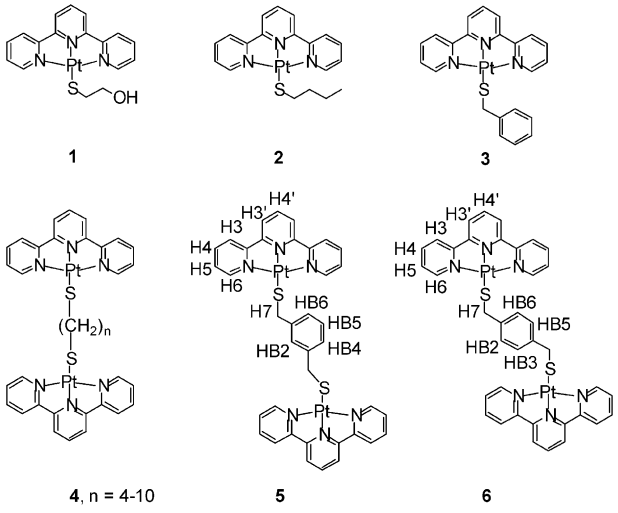

Figure 1. Structures of the platinum(II) complexes.

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as bisintercalators whereas those with $n=8$ and 10 bind in a mixed mono-functional/bifunctional mode. Our platinum complexes, **5** and **6**, are considered as the rigid models of **4** where the conformation between the two (2,2′,2″-terpyridine)platinum(II) complex is regulated by the xylyl group.

Dinuclear Pt(II) complexes, **5** and **6**, therefore, can be expected to have a unique function (i.e., the type and strength of interaction with DNA) different from **4** as well as mononuclear one.

Results and Discussion

The reaction of 2 equimolar amounts of [(2,2′,2″-terpyridine)PtCl]Cl·2H_2O and an equimolar amount of 1,3- or 1,4-benzenedimethanethiol in DMF-water (1:2 v/v) (adjusted to pH 6 with 0.1 M NaOH) at 50 °C for 2–18 h under argon gave the corresponding dinuclear platinum(II) complexes, **5** and **6**, in ca. 60% yield. Characterization of the platinum(II) complexes was done with ^{1}H NMR spectroscopy, FAB-mass, and elemental analysis.[13,14] The H6 protons of **5** and **6** in CD_3OD at 30 °C resonate at 9.14 ppm as a doublet coupled with H5 with satellite peaks due to ^{195}Pt (33%, $I=1/2$) couple. The coupling with ^{195}Pt is also observed in the methylene protons (H7) of the xylyl group. Their coupling constants, $^4J_{H6\text{-}Pt}^{195}$ and $^4J_{H7\text{-}Pt}^{195}$, are 42 and 40 Hz, respectively.

The absorption spectra of **1**, **5** and **6** in water are shown in Figure 2. Apparent molar extinction coefficients of **5** and **6** are almost double that of **1**. The absorption bands at 300–350 nm and the weaker bands at 490 nm for **5** and 495 nm for **6** are assigned to π–π* and thiolate-to-metal (LMCT) transitions.[15] The absorption spectrum of **6** in water showed the dependence on the concentration in the range of 1.0×10^{-5} M to 1.0×10^{-4} M: the apparent molecular extinction coefficient at 320 nm decreased with increasing the concentration of platinum (II) complex (Fig. 3). Complex **5** did not show significant concentration dependence.

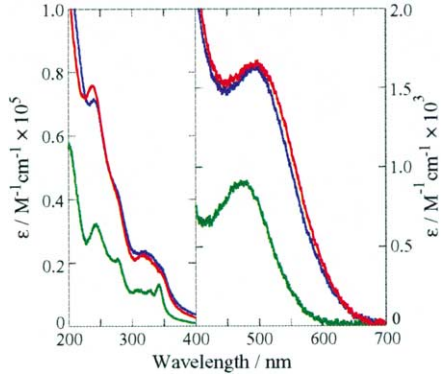

Figure 2. Absorption spectra of **1** (green), **5** (blue), and **6** (red) in water: UV (left) and visible (right) regions.

$$[\text{monomer}] + [\text{monomer}] \overset{K_D}{\rightleftharpoons} [\text{dimer}]$$

Scheme 1.

Hypochromism observed in **6** suggested that an equilibrium between monomers and a dimer exists in solution as shown in Scheme 1. According to eq 1, we estimated the dimerization constant, K_D to be 7.0×10^2 M^{-1}, which is ca. 9–16 times lower than those reported for the mononuclear platinum(II) complexes **1** (7.0×10^3 M^{-1}),[16] **2** (1.02×10^4 M^{-1}),[12] and **3** (6.3×10^3 M^{-1}).[17] It is interesting to note that the dimerization constant of **6** is ca. 6–9 times lower than those of **4** (4.4×10^3 M^{-1} for $n=4$ and 6.2×10^4 M^{-1} for $n=6$),[11] suggesting that this decrease in the dimerization constant reflects the decrease in the degree of freedom around the xylyl group compared to the methylene chain in **4**.

$$\sqrt{\frac{\varepsilon_M-\varepsilon_{obs}}{C_t}}=\sqrt{\frac{2K_D}{\Delta\varepsilon}}\times\{\Delta\varepsilon-(\varepsilon_M-\varepsilon_{obs})\} \quad (1)$$

where ε_{obs}, ε_M, and ε_D are the molar extinction coefficients of the solvent, monomer and dimer, $\Delta\varepsilon$ is $\varepsilon_M-\varepsilon_D$, and C_t is the total concentration of platinum(II) complex.

Binding of platinum(II) complexes, **5** and **6**, with calf thymus DNA was studied by absorption and CD spectroscopies. The absorption and CD spectral changes of calf thymus DNA (64 μM)[18] with increasing the concentration of platinum(II) complexes in 1 mM phosphate buffer (pH 6.8) containing 3 mM NaCl are shown in Figure 4, where each absorption spectrum is derived by subtracting the absorption spectrum of platinum(II) complex in the absence of DNA from the observed spectrum upon addition of DNA because the absorption of platinum(II) complex is considerably strong in the region investigated. When the concentration increased from 0 to 7.75 μM, the intensity at 260 nm decreased and then was attained a constant value at 7.75 μM. The absorption spectrum of **6** is similar to that of **1**.

Under the same conditions, the CD spectrum of **1** has positive signals at 260–280 nm and negative signals at 230–255 nm. When the concentration increased, the positive signals at 270–280 nm are shifted to shorter wavelength whereas the negative features at 245–250 nm

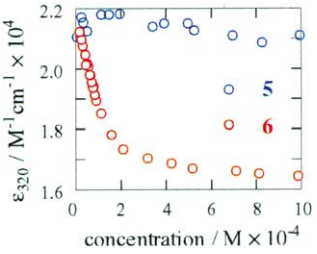

Figure 3. Change in absorption of **5** and **6** with variation of concentration.

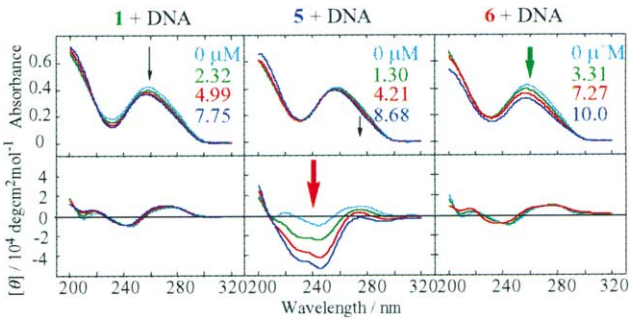

Figure 4. Absorption and CD spectra of **1**, **5**, and **6** with calf thymus DNA. The absorption spectra were recorded in 1 mM sodium phosphate (pH 6.8) containing 3 mM NaCl; the DNA concentration was 64 μM. The CD spectra were recorded in 1 mM sodium phosphate (pH 6.8) containing 3 mM NaCl.

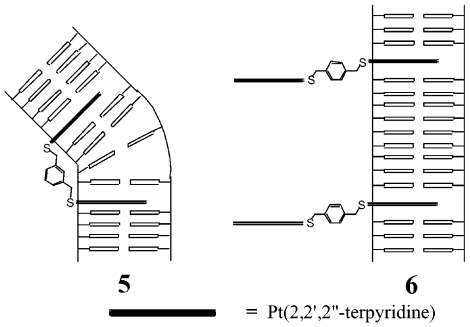

Figure 5. Proposed binding mode of **5** or **6** with calf thymus DNA.

are shifted to longer wavelength but no change in intensity was observed. The CD spectrum of **6** is also similar to that of **1** as well as absorption spectra.

However, the CD spectra of **5** differ from those of **1** and **6**: with increase in the concentration of platinum(II) complex, a new negative Cotton effect was appeared at 285 nm and the intensity of the negative Cotton effect at 245 nm increased and their signals had a tendency to saturate.

As judged on the results of CD spectra, it can be assumed that the structure of DNA has not been deformed by addition of **1** or **6** to DNA.

There is one possibility in this abnormality of CD spectra: There is no report that a large negative CD signal at 250 nm appears by stacking or by addition of *cis*-platin to DNA so far.[19] Ivanov et al., have reported that B typed DNA transforms to A typed or C typed DNA by the change of the hydration state of DNA and have also shown the CD spectra of the three typed DNA.[20] The CD spectra of **5** are similar to that of C typed DNA.[20] In **5**, the motion of the structure of DNA is restricted by the xylyl group and two Pt(2,2′,2″-terpyridine) moieties are directed to bind with DNA from one side by virtue of being 1,3-substituted phenyl center, resulting in the distortion of the structure of DNA compared to those of **1** and **6** which functions as monointercalator as shown in Figure 5. Moreover, the result of the DNA binding with **6** suggested that the substitution of the methylene chain in **4** with the xylyl group enforced the rigidity of complex to change from bisfunctional to monofunctional DNA binding.

Table 1. Increasing in melting temperature (T_m) of calf thymus DNA upon interaction with the Pt(II) complexes **3**, **5**, and **6**[a]

Complex	T_m[a]
3	75.0
5	82.5
6	78.0

[a]Calf thymus DNA = 78 μM, complex = 7.8 μM in 1 mM phosphate buffer (pH 6.8) containing 3 mM NaCl. The measurement of T_m is carried out by absorption spectroscopy and changes in absorbance were monitored at 260 nm. T_m of calf thymus DNA is 72 °C at the above conditions.

The effect of platium(II) complexes on the melting temperature (T_m) of calf thymus DNA in 1 mM phosphate buffer (pH 6.8) at containing 3 mM NaCl was investigated and the typical results are listed in Table 1.

Lippard et al. have reported that T_m of **1** increased by 3.4 °C compared to unreacted calf thymus DNA.[16] T_m is found to increase from 72 °C for unreacted calf thymus DNA to 75 for **3** and 78 °C for **6**. In **5**, T_m is 82.5 °C and a 10.5 °C increase in T_m indicates that **5** interacts strongly with DNA by intercalation, suggesting stabilization of the DNA structure. Addition of platinum(II) complex to calf thymus DNA resulted in a significant increase in the melting temperature of DNA and these results are consistent with an intercalative mode of binding.[21]

In conclusion, dinuclear platinum(II) complexes containing xylylthiolate and 2,2′,2″-terpyridine, **5** and **6**, have been prepared and characterized and their absorption and CD properties with calf thymus DNA have been investigated. 1,3-substituted **5** interacted strongly with calf thymus DNA by intercalation compared to 1,4-substituted **6**. These observations will shed light on development of new drugs including platinum(II) complexes based on intercalation with DNA and structural modification of DNA with intercalating molecule.

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13. **5**: Anal. calcd for $C_{38}H_{38}O_4N_6S_2Cl_2Pt_2$: C, 39.07; H, 3.27; N, 7.10. Found: C, 39.29; H, 3.32; N, 7.03. ^{1}H NMR (60% CD_3OD–D_2O): δ 3.58 (s, 4H, H7), 6.81 (t, 1H, HB5), 6.95 (d, 2H, HB4, HB6), 7.66 (s, 1H, HB2), 7.83 (t, 4H, H5), 8.30 (d, 4H, H3), 8.33 (t, 4H, H3′), 8.39 (t, 4H, H4), 8.43 (d, 2H, H4′), 9.14 (d, 4H, H6). $^4J_{H6}$-$^{195}{}_{Pt}$ = 42 Hz and $^4J_{H7}$-$^{195}{}_{Pt}$ = 40 Hz. FAB-MS *m*/*z* 1059.
14. **6**: Anal. calcd for $C_{38}H_{38}O_4N_6S_2Cl_2Pt_2$: C, 39.07; H, 3.27; N, 7.10. Found: C, 38.97; H, 3.99; N, 6.90. ^{1}H NMR (60% CD_3OD-D_2O): δ 3.59 (s, 4H, H7), 7.12 (s, 4H, HB2, HB3, HB5, and HB6), 7.76 (t, 4H, H5), 8.32 (d, 4H, H3), 8.37 (t, 4H, H4), 8.40 (t, 4H, H3′), 8.49 (d, 2H, H4′), 9.14 (d, 4H, H6). $^4J_{H6}$-$^{195}{}_{Pt}$ = 42 Hz and $^4J_{H7}$-$^{195}{}_{Pt}$ = 40 Hz. FAB-MS *m*/*z* 1059.
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