Water-Soluble Porphyrins Appending Platinum(II) Complexes as Binders for Synthetic Nucleic Acid Polymers

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Water-soluble porphyrins containing four platinum(II) complexes per molecule, [5α,10β,15α,20β-tetrakis(2-*trans*-(α,β,α,β-*trans*-Pt) and *cis*-(α,β,α,β-*cis*-Pt) [PtCl(NH_3)$_2$]*N*-2-aminophenylporphyrin)], were synthesized and characterized. The binding of synthetic nucleotide polymers (poly(dG)–poly(dC), poly(dA)–poly(dT)) to the porphyrins was examined spectrophotometrically in aqueous solution. UV–vis spectral data suggested that these porphyrins bind to the nucleic acids by coordinative and Coulomb interactions.

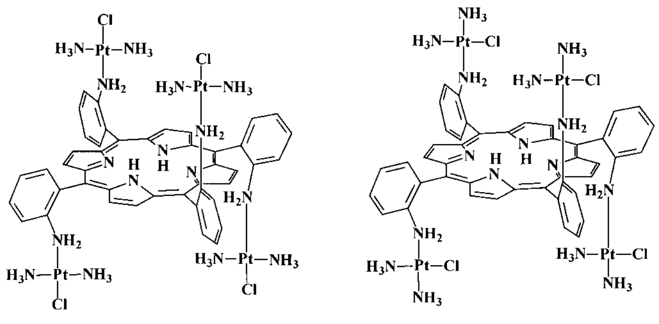
Key words porphyrin; platinum complex; nucleic acid

Cationic water-soluble porphyrins bearing quaternary ammonium salt units such as ammonium or pyridinium groups have been used for the study of porphyrin–DNA interactions,[1,2] because phosphate moieties in DNA carry negative charges. We have reported[3] the synthesis of water-soluble porphyrins appending two platinum(II) complexes and their binding properties with nucleotides and synthetic nucleic acids polymers are discussed. The binding modes between the porphyrins and synthetic nucleic acid polymers were explained based on coordinative, Coulomb, and hydrophobic interactions. In this work, to explore further the interactions in detail, we have designed and synthesized a new type of cationic water-soluble porphyrins (Fig. 1). Since the porphyrins are double-face hindered with platinum(II) complexes, fewer hydrophobic interactions with the polymers might occur. Further, the isomeric conformations of the platinum(II) complexes may show bind in a different manner to the polymers.

The synthesis of α,β,α,β-*trans*-Pt was as follows. *Trans*-[PtCl(NH_3)$_2$(*N*,*N*-dimethylformamide (DMF))]NO_3 (prepared *in situ* from 223 mg, 0.743 mmol of *trans*-[$PtCl_2$(NH_3)$_2$], 124 mg, 0.730 mmol of $AgNO_3$, and 10 ml of DMF was added to a DMF solution (10 ml) containing 5α,10β,15α,20β-tetrakis(2-aminophenyl)porphyrin (H_2TamPP)[4] (100 mg, 0.148 mmol) at −18 °C. The solution was stirred for 3 h at −18 °C and then for 3 d at room temperature.[5] The solution was then evaporated to dryness at below 35 °C.[4] The residue was dissolved in methanol (20 ml), and the solution was stred overnight at 4 °C, giving a purple solid. The solid was filtered off and then washed with diethylether (5 ml), chloroform (5 ml) and methanol (5 ml), in turn. The solid was purified by recrystallization from methanol/diethylether (1/1) and dried *in vacuo* to afford α,β,α,β-*trans*-Pt as nitrate salt (yield 252 mg, 86.1%). Nitrate salt of α,β,α,β-*cis*-Pt was prepared from H_2TamPP and *cis*-[$PtCl_2$(NH_3)$_2$] (CDDP) (yield 209 mg, 71.4%),[6] according to a procedure similar to that for α,β,α,β-*trans*-Pt.

We studied the interactions between the porphyrins and synthetic nucleic acid polymers by examining melting behavior based on UV–vis spectra. The melting temperature of poly(dA)–poly(dT) (18 μmol l^{-1} as base pair) in 20 mmol l^{-1} NaCl was 55.7 °C, whereas those of α,β,α,β-*trans*-Pt (3.5 μmol l^{-1}) and α,β,α,β-*cis*-Pt (3.5 μmol l^{-1}) were 56.2 and 57.1 °C, respectively. Thus the presence of the porphyrins did not cause significant changes in the melting temperature of poly(dA)–poly(dT), and the reannealing phenomenon was observed. These results indicate that the porphyrins bind to poly(dA)–poly(dT) by Coulomb interaction,[3] similar to the case of cationic porphyrins with quaternary ammonium groups, such as *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP).[7—9]

Figure 2 shows the UV–vis spectral changes of α,β,α,β-*trans*-Pt (3.7 μmol l^{-1}) upon addition of poly(dA)–poly(dT) (0.74—5.2 μmol l^{-1} as base pair), when the spectral changes occurred immediately after the addition. Similar results were obtained for α,β,α,β-*cis*-Pt. The spectral changes suggest the association of α,β,α,β-*trans*-Pt or α,β,α,β-*cis*-Pt with poly(dA)–poly(dT), and these were analyzed by Job's plot (Fig. 3). The results suggest that the mole ratio of α,β,α,β-*trans*-Pt to a base pair unit of poly(dA)–poly(dT) is a one-to-one complex formation, while that of α,β,α,β-*cis*-Pt is a



α, β, α, β-*trans*-Pt **α, β, α, β-*cis*-Pt**

Fig. 1. Structures of Water-Soluble Porphyrins Containing Four Platinum(II) Complexes

Charge and counterions (NO_3^-) are omitted for simplicity.

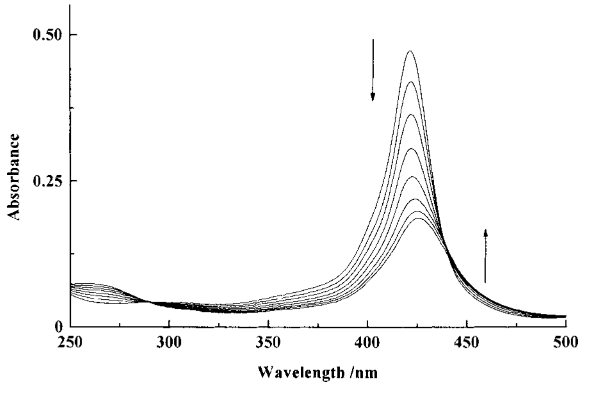


Fig. 2. UV–Vis Titration of α,β,α,β,-*trans*-Pt with Poly(dA)–Poly(dT) at 40 °C in 20 mmol l^{-1} NaCl.

The concentration of α,β,α,β-*trans*-Pt is 3.7 μmol l^{-1} and those of poly(dA)–poly(dT) are 0, 0.74, 1.5, 2.2, 3.0, 3.7, 4.5, and 5.2 μmol l^{-1} (in base pair concentration), respectively.

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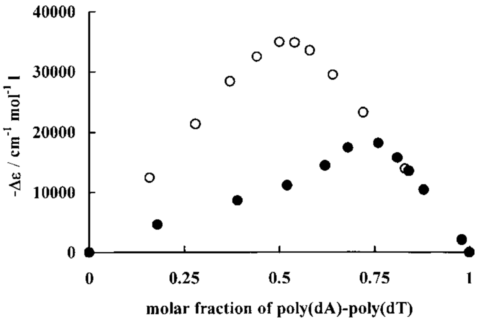

Fig. 3. Job Plots of the Spectroscopic Changes (at 420 nm) against the Molar Ratio of Poly(dA)–Poly(dT) (as Base Pair) for the Titration of *α,β,α,β-trans* (○) or *-cis* (●)-Pt with Poly(dA)–Poly(dT)

one-to-three formation. Here an obvious difference in the binding ratio between the two isomers is provided by Coulomb interaction. In general, the effect of the isomeric geometry of platinum complexes (such as $[PtCl_2(NH_3)_2]$) on the interaction between these and DNA are attributable to not Coulomb interaction but to coordination.[10] Thus the electrostatic interaction derived from the isomeric geometries seems to become a recognition factor for DNA. This must be correlated with the steric and/or electrostatic complementarity between the polymer and the platinum(II) complexes, but we could not examine the question experimentally in detail.

For the case of poly(dG)–poly(dC) with *α,β,α,β-trans*-Pt or with *α,β,α,β-cis*-Pt, the melting behavior could not be analyzed because mixing of poly(dG)–poly(dC) with the porphyrins caused a precipitate. Then, to explore the binding mode of the porphyrins to poly(dG)–poly(dC), we used guanosine monophosphate (GMP) instead of the polymer. The spectral changes in the porphyrins with GMP consisted of two steps (exemplified in Fig. 4). By adding GMP to the solution containing these porphyrins, changes occurred immediately as the first step. After the first step, gradual changes were observed over a period of 1 d as the second step. Comparisons of the spectral changes with those with poly(dA)–poly(dT) suggest that the first step will be the electrostatic association of the porphyrins with GMP. We speculate that the changes in the second step are due to the coordination of GMP to Pt in the porphyrins,[3] because a similar reaction for the binding of CDDP with GMP have been reported.[11,12] Since such binding behavior was not observed for both cases of these porphyrins with poly(dA)–poly(dT) and of porphyrins with quaternary ammonium groups such as TMPyPs,[1,3] the coordinative interaction seems to dominate over or to eclipse the Coulomb interaction.

In conclusion, two water-soluble porphyrins containing four Pt(II) complexes, *α,β,α,β-trans*-Pt and *α,β,α,β-cis*-Pt, have been synthesized, and these porphyrins have been shown to react with synthetic nucleic acid polymers. The binding behavior of the porphyrins with poly(dA)–poly(dT) suggests that these porphyrins bind to the nucleic acid by Coulomb interaction and that a difference in the ability to recognize poly(dA)–poly(dT) by these geometrical isomers occurred. For the binding with poly(dG)–poly(dC) or GMP, these porphyrins were suggested to bind to the nucleic acids by Coulomb and coordinative interactions. However, further discussion of this issue will require more detailed binding data. The study of the binding of nucleic acids to platinum(II)-appending porphyrins is in progress.

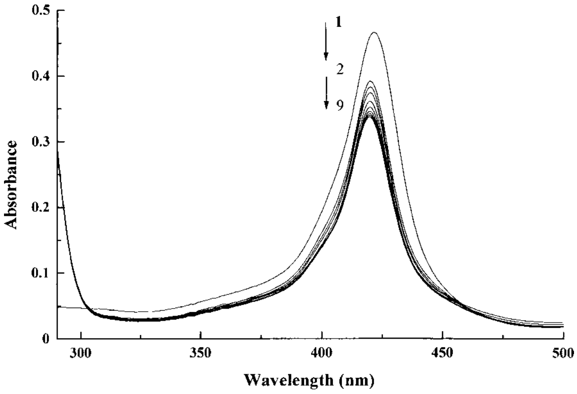

Fig. 4. Time-Dependent UV–Vis Spectra of the Reaction between *α,β,α,β-trans*-Pt (3.6 μM) and GMP (89 μM) at 40 °C in 20 mM NaCl

1 is the spectrum of *α,β,α,β-trans*-Pt; 2, 3, 4, 5, 6, 7, 8, and 9 are the spectra at 0, 1, 2, 3, 4, 5, 6, 7, and 8 h, respectively, after mixing of *α,β,α,β-trans*-Pt with GMP.

References and Notes

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6) *α,β,α,β-trans*-Pt: Purple powder, *Rf* value=0.46 ($CHCl_3$: CH_3OH : H_2O : trifluoroacetic acid=10 : 8 : 2 : 1), IR (KBr) cm^{-1} 3436—3186 ($-NH_3$ and pyrrole-NH), 1384 (NO_3), 1326 ($-NH_3$). UV–vis (H_2O) λ_{max} nm (log ε) 422 (5.11), 522 (3.99), 585 (3.63), 651 (3.28). M^+ for $C_{44}H_{58}Cl_4N_{16}Pt_4$ (FAB-MS), 1732; *m/z* 1732. ^1H-NMR (500 MHz, D_2O) δ: 7.6—8.7 (20H, m, ph-H), 9.25 (8H, br, pyrrole-H). *Anal.* Calcd for $C_{44}H_{58}Cl_4N_{12}O_{16}Pt_4 \cdot 4H_2O$: C, 25.74; H, 3.24; N, 13.64%. Found: C, 25.46; H, 3.11; N 13.65%. *α,β,α,β-cis*-Pt: Purple powder, *Rf* value=0.55 ($CHCl_3$: CH_3OH : H_2O : trifluoroacetic acid=10 : 8 : 2 : 1), IR (KBr) cm^{-1} 3432—3119 ($-NH_3$ and pyrrole-NH), 1384 (NO_3), 1302 ($-NH_3$). UV–vis (H_2O) λ_{max} nm (log ε) 424 (5.26), 522 (4.05), 585 (3.68), 648 (3.28). M^+ for $C_{44}H_{58}Cl_4N_{16}Pt_4$ (FAB-MS), 1732; *m/z* 1732. ^1H-NMR (500 MHz, D_2O) δ: 7.6—8.7, (20H, m, ph-H), 9.25 (8H, br, pyrrole-H). *Anal.* Calcd for $C_{44}H_{58}Cl_4N_{12}O_{16}Pt_4 \cdot 4H_2O$: C, 25.74; H, 3.24; N, 13.64. Found: C, 25.45; H, 3.09; N 14.13.
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