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STERIC EFFECT IN BINDING OF ANTITUMOR ACTIVE PLATINUM (II) COMPLEX TO NUCLEIC ACID BASE

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Binding of Pt(1R,3S-cyclohexanediamine)$^{2+}$ to 2'-deoxyguanylyl(3'-5')guanosine, d(GpG), yielded two diastereomers, which are discriminated by using HPLC. Schematic structures of the diastereomers are proposed on the basis of UV and CD spectral studies.

KEYWORDS —— chiral metal-DNA interaction; antitumor active platinum complex; platinum complex binding mode; diastereomer; platinum guanosine complex

Many investigations suggest that DNA is a primary target of antitumor platinum drug.[1] It has recently become apparent that adjacent guanine bases on the same strand in DNA constitute a preferential platinum binding site in the reaction of cis-$Pt(NH_3)_2Cl_2$ with DNA.[2] It would be expected that chelation of cis-$Pt(NH_3)_2^{2+}$ to adjacent guanine bases leads to a kink in one strand of the double-helical structure of DNA. We have been interested in a steric effect in the interaction of antitumor platinum complexes with DNA. The present paper describes Pt-adducts formed in the reaction of 1R,3S(or 1S,3R)-cyclohexanediamine platinum(II) complex,[3] abbreviated as Pt(1,3-dach)$^{2+}$, with d(GpG).

Pt(1,3-dach)$^{2+}$ has a symmetry plane that passes through Pt, C2 and C5, and the cyclohexane ring lies roughly perpendicular to the platinum coordination plane.[4] Since d(GpG) has a chiral structure, bifunctional binding of Pt(1,3-dach)$^{2+}$ to d(GpG) would be expected to yield diastereomers, which are chemically nonequivalent.

Reaction of Pt(1,3-dach)Cl_2 with d(GpG) gave two main products, **I** and **II**, which are separated by using HPLC with a weak cation exchange column.[5] These products accounted for more than 90% of the peak area of all the products, and the peak area

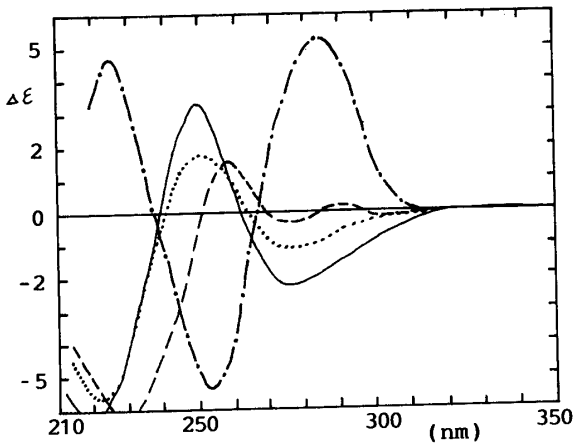

Fig. 1. Circular Dichroism Spectra of Pt(diamine)[d(GpG)] Compounds in 0.05 M KH_2PO_4 Solution (pH 4.6)

_ _ _ _ _ _ _, Pt(1,3-dach)[d(GpG)],I;
.....,, Pt(1,3-pn)[d(GpG)];
————, Pt(1,3-dach)[d(GpG)],II;
—.—.—, Pt(1,3-dach)(5′-dGMP)$_2$.

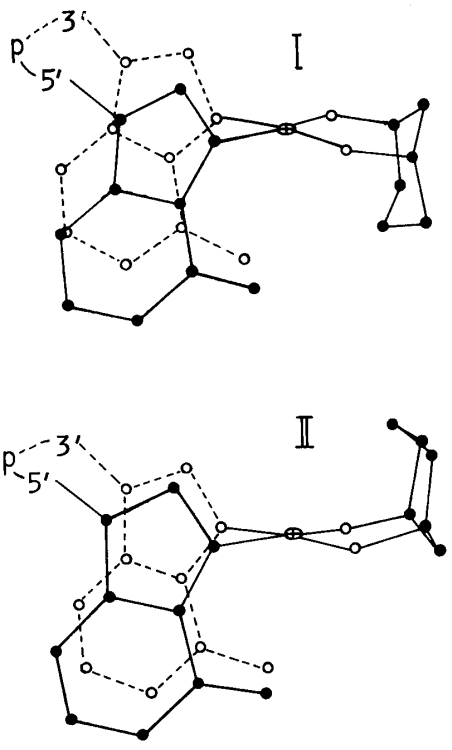

Fig. 2. Proposed Schematic Structures of Pt(1,3-dach)[d(GpG)] Compounds, I and II

of I was almost same as that of II. The products, I and II, were fractionated by HPLC and were characterized by UV and CD spectrophotometry. The platinum binding sites of I and II are found to be N7-N7 in d(GpG), as determined by the UV-pH titration curve. The pKa value of N1 is 8.4 and is good evidence for platination at the N7 position.[6,7] The binding ratio of Pt(1,3-dach)$^{2+}$ to d(GpG) was found to be 1 : 1, i.e., Pt(1,3-dach)[d(GpG)], as determined by UV and atomic absorption spectrophotometry (A molar extinction coefficient of about 24000/g-atom of platinum was calculated at λmax = 259 nm). These results strongly suggest that I and II are complexes with an interbase crosslink between two guanine bases through N7-N7. The pKa value of N1 and the UV spectrum of I were almost identical with those of II, suggesting that I and II are diastereoisomers of each other.

Figure 1 shows the CD spectra of I and II. The CD spectrum of II exhibited two negative bands at 220 nm(Δε=-6.5) and 275 nm(Δε=-2.29) and a positive band at 250 nm(Δε=4.45). It is similar to the CD spectrum of Pt(1,3-propanediamine)[d(GpG)], Pt(1,3-pn)[d(GpG)], except for its intensity. The signs of the CD bands of Pt(1,3-dach)(5'-dGMP)$_2$ are inverted compared with those of II. The CD spectrum of Pt(1,3-dach)(5'-dGMP)$_2$ is quite similar to that of the structurally characterized complex Pt(1,3-pn)(5'-dGMP)$_2$, which is a structure with a head-to-tail arrangement, as reported by Marzilli et al.[8] Therefore, it is concluded that Pt(1,3-dach)(5'-dGMP)$_2$ has a structure with a head-to-tail arrangement. On the basis of the following four

considerations, it seems reasonable to assume that **I** and **II** have a structure with a head-to-head arrangement of d(GpG) as schematically presented in Fig. 2. (1) A characteristic feature of the CD spectra of the compounds involving d(GpG) is the presence of a doublet centered at about 240 nm, which is likely to arise from an exciton interaction between the two guanine bases. The absorption in the vicinity of 240 nm is due to a $\pi \rightarrow \pi^*$ electronic transition and the direction of this transition lies approximately in the C4-C8 direction.[9] The signs of the CD bands are generally thought to be sensitive to the relative orientation of the bases. With the Pt-complexes involving d(GpG), the bands in this region are opposite in sign compared to the complexes with the head-to-tail arrangement such as Pt(1,3-pn)(5'-dGMP)$_2$ and Pt(1,3-dach)(5'-dGMP)$_2$. (2) Only one product was obtained from the reaction of Pt(1,3-pn)$^{2+}$ with d(GpG). It is a complex with an interbase crosslink between two guanine bases through N7-N7. On the other hand, the reaction of Pt(1,3-dach)$^{2+}$ with d(GpG) gave two products. Pt(1,3-pn)$^{2+}$ possesses two-fold rotation symmetry. On the other hand, the symmetry element of Pt(1,3-dach)$^{2+}$ is a plane of symmetry involving Pt, C2 and C5. Therefore, in Pt(1,3-dach)[d(GpG)], two structures would be possible as shown in Fig. 2. This agrees with the experimental results. Similarly, the reaction of Pt(1R,2S-dach)$^{2+}$ with d(GpG) would be expected to give two products, and the result obtained agreed with the prediction (data not shown). These results suggest that the formation of two products in the reaction of Pt(diamine)$^{2+}$ with d(GpG) arises from the low symmetry of the Pt(diamine)$^{2+}$ moiety. (3) If the difference in the structures of **I** and **II** comes mainly from a conformational difference of the backbone (sugar and phosphodiester moieties) such as syn <-> anti, **I** and **II** would be expected to be in equilibrium state. Such equilibrium was not observed even when they were heated to 90°C. Therefore, the conformational difference is ruled out. Chottard et al. reported that the reaction of cis-Pt(NH_3)$_2$Cl$_2$ with CpG gave an equilibrium mixture of N3(C anti)-N7(G anti) and N3(C syn)-N7(G anti) isomers and that the isomerizaion after separation of the products occurred even at room temperature.[10] (4) **I** and **II** are also main Pt-adducts in the reaction of Pt(1,3-dach)$^{2+}$ with DNA as shown below. This implies that **I** and **II** are likely to have a structure with a head-to head arrangement because DNA is a helical molecule with a head-to-head arrangement. The CD spectrum of **I** seems to be significantly different from that of **II**. In the proposed schematic structures, a bulky cyclohexane ring exists in either on the same side of the O6 or on the opposite side. When the axially standing cyclohexane ring faces the side of the O6, repulsion between the cyclohexane ring and the O6 atoms may be expected. This may cause a certain change in conformation of sugar and phosphodiester moieties. But energy barriers required for the conformational change may not be so large because the yields of **I** and **II** are almost the same. Whereas, the opposite arrangement seems to be less repulsive and this may explain why the CD spectrum of **II** is similar to that of Pt(1,3-pn)[d(GpG)].

A preliminary study of a reaction of Pt(1,3-dach)$^{2+}$ with DNA was carried out. Pt(1,3-dach)$^{2+}$ was allowed to react with calf thymus DNA at 37 C for 4 days (pH 7.3), and the reaction solution was treated with the enzymes (deoxyribonuclease I, snake venom phosphodiestease, alkaline phosphatase, and calf spleen phosphodiesterase).[6,11] An HPLC study of the enzymatic digestion products indicated that **I** and **II** are preferential Pt-adducts at low r value (r=Pt added/base, r<0.06). The relative ratio of **I** to **II** was 0.26 : 1.0, which is different from the reaction with d(GpG). That is, the formation of **I** tends to be suppressed in the reaction of Pt(1,3-dach)$^{2+}$ with DNA. The stereoselectivity presumably arises from a steric hindrance, which comes from the axially standing cyclohexane ring.

Differences in the structures of antitumor platinum complexes may give a certain kink effect in the DNA. Conformational change induced in DNA after platination may affect repair enzymes and may be related to the structure-activity relationship of antitumor platinum complexes.

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