Studies on the Reaction Products of Antitumor Platinum Complex with d(ApG), d(GpA), and d(pGpA). 2′-Deoxy Sugar Is Favourable for G-N7, A-N1 Chelation

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The reaction products of dichloro[(1*R*,2*R*)-1,2-cyclohexanediamine]platinum(II), Pt(*R*,*R*-dach)Cl_2, with dinucleotides, d(ApG), d(GpA), and d(pGpA) have been separated by HPLC, and each product fractionated has been characterized by UV and CD spectral analyses. The reaction of Pt(*R*,*R*-dach)Cl_2 with d(ApG) yields Pt(*R*,*R*-dach)(d(ApG)-*N1(1),N7(2)*) (**1**, 16%), Pt(*R*,*R*-dach)(d(ApG)-*N7(1),N7(2)*) (**2**, 80%) and uncharacterized minor product (4%). The reaction with d(GpA) yielded two Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N1(2)*), (**3**, 22% and **5**, 61%), and Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N7(2)*), (**4**, 17%), but surprisingly **5** isomerizes very slowly to **3** and **4** under the subsequent incubation (half life of **5**; about 6 days at 37 °C). The reverse reaction (**3** and **4**→**5**) was not observed. The reaction with d(pGpA) also yielded two Pt(*R*,*R*-dach)(d(pGpA)-*N7(1),N1(2)*), (**3′**, 8% and **5′**, 80%) and Pt(*R*,*R*-dach)(d(pGpA)-*N7(1),N7(2)*), (**4′**, 12%). The adduct, **5′**, also isomerizes to **3′** and **4′**. The platinum adducts, **3′**, **4′**, and **5′**, are compatible with **3**, **4**, and **5**, respectively, after removal of the 5′-phosphate group upon treatment with alkaline phosphatase. A competition reaction between d(ApG) and d(GpA) for Pt(*R*,*R*-dach)Cl_2 indicates that d(ApG) was about 2 times more reactive than d(GpA). The reactivity decreases along the series; d(pGpA)>d(ApG)>d(GpA).

In the mechanism of action of antitumor platinum drugs, DNA has been considered to be the primary target.[1-3] Studies on the reactions with DNA and its constituents have indicated that the N7 of guanine base is a strongly preferred platinum binding site. The interaction between the bifunctional platinum compound and the guanine base, especially the intrastrand crosslink between two adjacent guanine bases,[4-8] appears to play an important role in cell killing.[9,10] The enzymatic digestion of platinum modified DNA has been indicated that such intrastrand crosslinked compound is the most prominent platinum adduct.[11-15] An intriguing observation is that only an intrastrand crosslinked compound between adjacent adenine and guanine base, d(ApG)-chelate, but no d(GpA)-chelate has been found after digestion in the reaction products with DNA.[12,13] The d(ApG)-chelate is a major platinum adduct next to the d(GpG)-chelate. An important question is why only the d(ApG)-chelate is found in the reaction with DNA, and this has led us to the present study.

In the previous paper,[16] we reported that the reaction of r(GpA) with *cis*-Pt$(NH_3)_2Cl_2$ gives two platinum adducts and that one of them is *cis*-Pt$(NH_3)_2$-(r(GpA)-*N7(1),N7(2)*).[17] Very recently, Chottard et al.[18] investigated *cis*-Pt$(NH_3)_2$-adducts with r(ApG) and r(GpA) by means of NMR, in which the reaction between *cis*-Pt$(NH_3)_2Cl_2$ and r(ApG) gave *cis*-Pt$(NH_3)_2$-(r(ApG)-*N7(1),N7(2)*) as a major platinum adduct (>95%). The reaction[18] of *cis*-Pt$(NH_3)_2Cl_2$ with r(GpA) yielded *cis*-Pt$(NH_3)_2$(r(GpA)-*N7(1),N7(2)*) (68%) and *cis*-Pt$(NH_3)_2$(r(GpA)-*N7(1),N1(2)*) (32%). The present paper describes about the reaction products of Pt(*R*,*R*-dach)Cl_2 with d(ApG), d(GpA), and d(pGpA) and the competition reaction between d(ApG) and d(GpA), with a common reactant Pt(*R*,*R*-dach)Cl_2. Our results are somewhat different from those obtained from the reaction[18] between *cis*-Pt$(NH_3)_2Cl_2$ and r(ApG) or r(GpA).

Experimental

The dinucleotides used were purchased from Pharmacia Co., Ltd. The platinum complexes, Pt(*R*,*R*-dach)Cl_2 and [Pt$(NH_3)_3$Cl]Cl, were prepared according to the literature.[19,20] For the purpose of characterization of the reaction products, the dinucleotide, d(ApG), d(GpA), or d(pGpA), was allowed to react with a stoichiometric amount of Pt(*R*,*R*-dach)Cl_2 in a deionized and distilled water at 37 °C for 1 day. The reaction products formed are separated and fractionated by a Hitachi 655 liquid chromatograph with Shimadzu Chromatopak C-R5A and with variable wavelength UV monitor. Fractions of 3—4 cm^3 were collected to characterize each product separated by HPLC. The parameters of the run of HPLC are as follows: Column, Cosmosil $5C_{18}$ (0.46 cm i.d.×15 cm); detector, UV at 260 nm; flow rate, 0.8 $cm^3\,min^{-1}$; mobil phase, 0.02 mol dm^{-3} phosphate buffer (pH=8.0) in 10% methanol solution. Retention time: **3**, 6.0

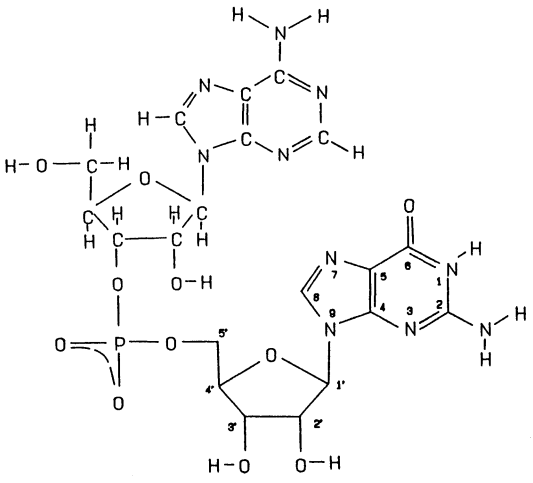

Fig. 1. Schematic structure of r(ApG) with the base and deoxyribose numbering scheme.

min; **4**, 12.2 min; **5**, 18.8 min; d(GpA), 23.2 min. For the competition reaction: Cosmosil $5C_{18}$ (0.46 cm i.d.×25 cm); Mobil phase, 0.25 mol dm^{-3} KH_2PO_4 in 9% methanol solution. Retention time: **1**, 12.2 min; **2**, 29.7 min; **3**, 12.2 min; **4**, 22.1 min; **5**, 19.8 min; **3′**, 12.0 min; **4′**, 31.1 min; **5′**, 13.5 min; d(ApG), 60.4 min; d(GpA), 62.2 min; d(pGpA), 17.4 min.

The platinum content of each product was determined by a Shimadzu AA-670G atomic absorption spectrometer. The UV and UV difference spectra were recorded on a Hitachi 557 dual-wavelength double beam spectrometer. In the measurement of UV difference spectrum, the reference cell contained a pH=5—6 solution of the each product. The CD spectrum was recorded on a JEOL J-40 spectrometer. The values are given per mole of platinum.

Results and Discussion

HPLC Analysis.[21] The reaction between Pt(*R,R*-dach)Cl_2 and d(ApG) yields two products, with a minor product (less than 4%). The two main platinum adducts are called **1** and **2**, according to their elution order. The reaction was followed at suitable time intervals by means of HPLC. The reactants are almost completely converted into **1** and **2** after 1 day reaction at 37 °C (see Figs. 8 and 9). The relative ratio of **1** (16 %) to **2** (80%) did not change throughout the reaction, suggesting parallel reaction. The Pt/base ratio (r=[Pt]/[Base], r=0.5 and 0.25) in the initial reaction solution also did not change the product ratio. The reaction of Pt(*R,R*-dach)Cl_2 with d(GpA) resulted in three platinum adducts, **3**, **4** and **5**. Figure 2 shows a time-dependence of the reaction. Relative ratios of each product to all the platinum adducts were almost constant for the first 10 hours, **3** (21—22%), **4** (16—18%) and **5** (62—59%). Afterward, the relative ratios, however, gradually change with the subsequent incubation because of an isomerization of **5** to **3** and **4**, as being mentioned later. The similar behaviour is also observed in the reaction between Pt(*R,R*-dach)Cl_2 and

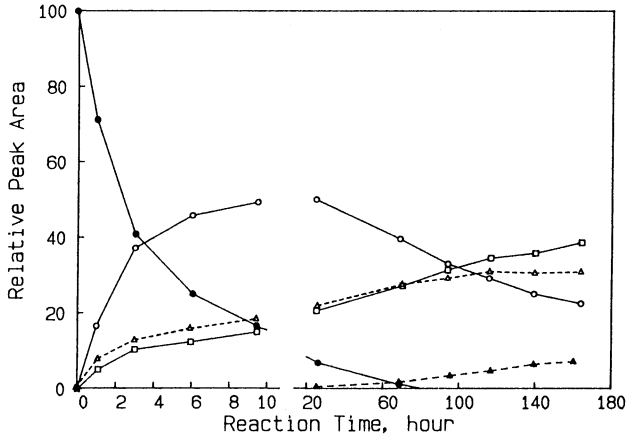

Fig. 2. Time-dependence of the reaction products of Pt(*R,R*-dach)Cl_2 with d(GpA) at 40 °C in water. (●), unreacted d(GpA); (○), Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **5**; (△), Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **3**; (▲), unknown; (□), Pt(*R,R*-dach)-(d(GpA)-*N7(1),N7(2)*), **4**.

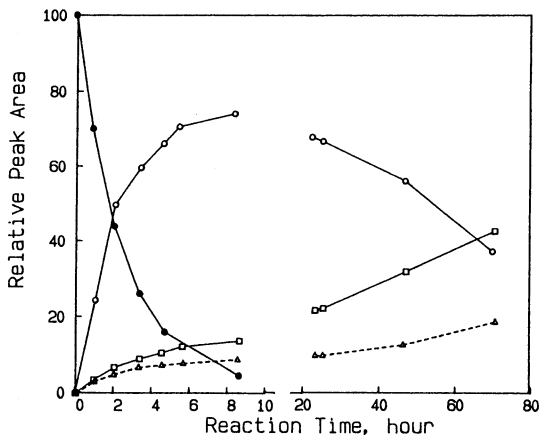

Fig. 3. Time-dependence of the reaction products of Pt(*R,R*-dach)Cl_2 with d(pGpA) at 40 °C in water. (●), unreacted d(pGpA); (○), Pt(*R,R*-dach)(d(pGpA)-*N7(1),N1(2)*), **5′**; (□), Pt(*R,R*-dach)(d(pGpA)-*N7(1),N7(2)*), **4′**: (△), Pt(*R,R*-dach)(d(pGpA)-*N7(1),-N1(2)*), **3′**.

d(pGpA). The reaction also yielded three platinum adducts, **3′** (8—9%), **4′** (10—15%), and **5′** (81—77%), in which the figures in parenthesis indicate the product ratio obtained for the first 10 hours. The reaction products, **3′**, **4′**, and **5′** were identified by HPLC after treating with alkaline phosphatase. The resulting products were completely compatible with **3**, **4**, and **5**. It is noteworthy that the product ratio of **5′** is significantly greater, compared with that of **5**, i.e. formation of **5′** largely predominates due to the presence of the 5′-phosphate group. The reaction of Pt(*R,R*-dach)Cl_2 with r(GpA) yields four platinum adducts, **8**, **9**, **10**, and **11**. In the first 10 hours reaction, the relative ratios of each product to all the platinum adducts are as follows; **8** (6%), Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*); **9** (12%), Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*); **10** (44%), Pt(*R,R*-dach)(r(GpA)-*N7(1),N7(2)*); **11** (38%), Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*). Afterward, the product ratios gradually change because **11** isomerizes to **8**, **9**, and **10**.

Characterization of Platinum Adducts. In general, the possible binding sites for platination are expected to be guanine N7 and adenine N7 and N1. The 6-NH_2 group of adenine base is also a possible platinum binding site,[22] but it would be a very weak binding site for platination because the lone pair of electrons of the 6-NH_2 group delocalizes into the π-system of the purine ring. Table 1 shows the platinum binding sites of the products obtained in this work. The platinum adducts, **1**—**5**, were fractionated from HPLC, and their platinum binding sites were determined as follows.

(1) From UV and AAS spectrophotometry, a molar extinction coefficient of 23000—25000/mol Pt was calculated at 260 nm, suggesting a presence of the 1 : 1 species. (2) The UV spectrum of **1** was in agreement with that of **3**. The UV spectral pattern of **1** and **3** was also in agreement with the calculated UV spectrum of

Table 1. Platinum Adducts Obtained from the Reaction of Pt(*R*,*R*-dach)Cl_2 with d(ApG), d(GpA), and d(pGpA)

Reactants	Reaction product	Yield/%[a]
Pt(*R*,*R*-dach)Cl_2+d(ApG)	**1**, Pt(*R*,*R*-dach)(d(ApG)-*N1(1),N7(2)*)	16
	2, Pt(*R*,*R*-dach)(d(ApG)-*N7(1),N7(2)*)	80
Pt(*R*,*R*-dach)Cl_2+d(GpA)	**3**, Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N1(2)*)	22[b]
	4, Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N7(2)*)	17[b]
	5, Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N1(2)*)	61[b]
Pt(*R*,*R*-dach)Cl_2+d(pGpA)	**3'**, Pt(*R*,*R*-dach)(d(pGpA)-*N7(1),N1(2)*)	8[b]
	4', Pt(*R*,*R*-dach)(d(pGpA)-*N7(1),N7(2)*)	12[b]
	5', Pt(*R*,*R*-dach)(d(pGpA)-*N7(1),N1(2)*)	80[b]
[Pt(NH_3)$_3$Cl]Cl+d(ApG)	**6**, Pt(NH_3)$_3$(d(ApG)-*N7(2)*)	>98
[Pt(NH_3)$_3$Cl]Cl+d(GpA)	**7**, Pt(NH_3)$_3$(d(GpA)-*N7(1)*)	>98
Pt(*R*,*R*-dach)Cl_2+r(GpA)	**8**, Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N1(2)*)	6[b]
	9, Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N1(2)*)	12[b]
	10, Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N7(2)*)	44[b]
	11, Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N1(2)*)	38[b]

a) Relative ratio of each product to all the platinum adducts.[21] b) The values obtained in the first 10 hours reaction.

Pt(NH_3)$_3$(G-*N7*) and Pt(NH_3)$_3$(A-*N1*),[16,23] suggesting platination at the *G-N7* and the *A-N1*. (3) The UV spectral pattern of **2** and **4** was in agreement with the calculated UV spectrum of Pt(NH_3)$_3$(G-*N7*) and Pt(NH_3)$_3$(A-*N7*),[16,23] suggesting platination at the *G-N7* and the *A-N7*. (4) From the pH-UV titration curve, the pK_a values at the N1 of the G base for **1**, **2**, **3**, **4**, and **5** were in the range of 8.0—8.5, a value significantly lower than the corresponding one of the unplatinated G base (pK_a ca. 9.6). Platination at the N7 of the G base results in the well-known lowering of the pKa of the N1. (5) No protonation reaction at the N7 of the G base was observed, suggesting that the N7 of the G base has already been occupied by the platinum atom. (6) The UV difference spectral change of **1**—**5** as a function of pH, — arising from the deprotonation reaction at the N1 of the G base —, agreed with that of the N7-platinated guanine derivatives.[16,23] These results ((4)—(6)) are good evidences for platination at the *G-N7*. (7) No protonation reaction at the N1 of the A base was observed for **1**, **3**, and **5**, suggesting platination at the N1 of the A base. (8) The pK_a values at the N1 of the A base of **2** and **4** were in the range of 1.7—1.9. Such decrease in the pK_a at the N1, compared with unplatinated A base (pK_a=3.6), is an evidence for platination at the N7 of the A base.[23-25] The binding sites of the platinum adducts containing r(GpA) were also determined by the same method described above.

Figures 4—6 show the CD spectra of the platinum dinucleotide adducts. The CD band in the 260 nm region is likely to arise from an interaction between the two purines. The CD spectrum of **2** is quite similar to that[18] of the structurally characterized compounds, *cis*-Pt(NH_3)$_2$(r(ApG)-*N7(1),N7(2)*) with A_{anti}, G_{anti}, which was characterized by NMR and CD spectroscopy.[18] Examination of the molecular models indicates that the interbase crosslink between the *A-N7* and *G-N7* seems to be favorable under the configuration of A_{anti} with the N-type conformer and G_{anti} with

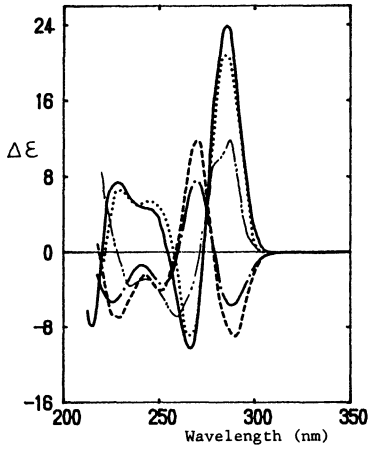

Fig. 4. Circular dichroism spectra of *G-N7*, *A-N7* platinum adducts (pH=4.6). (——), Pt(*R*,*R*-dach)(d(ApG)-*N7(1),N7(2)*), **2**; (-·-·-·-), Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N7(2)*), **4**; (------), *cis*-Pt(NH_3)$_2$(r(ApG)-*N7(1),N7(2)*);[18] (----), Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N7(2)*), **10**; (-·-·-), *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1),N7(2)*).[18]

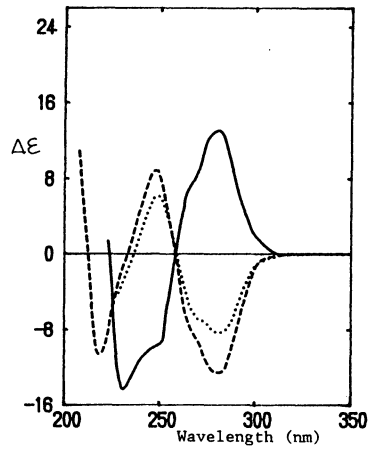

Fig. 5. Circular dichroism spectra of *G-N7*, *A-N1* platinum adducts (pH=4.6). (——), Pt(*R*,*R*-dach)(d(ApG)-*N1(1),N7(2)*), **1**; (------), Pt(*R*,*R*-dach)(d(GpA)-*N7(1),N1(2)*), **3**; (----), Pt(*R*,*R*-dach)(r(GpA)-*N7(1),N1(2)*), **9**.

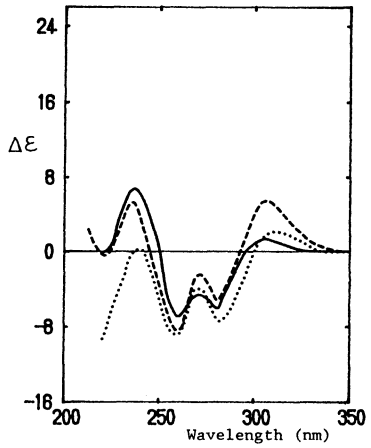

Fig. 6. Circular dichroism spectra of *G-N7*, *A-N1* platinum adducts (pH=4.6). (——), Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **5**; (----), Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*), **11**; (------), *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1),N1(2)*).[18]

the S-type conformer in the sugar moieties and that the product **2** is likely to have such sugar conformations because of the similarity of the CD spectra (see Fig. 4). Such conformation in the sugar moieties has been also found in *cis*-Pt(NH_3)$_2$(d(GpG)-*N7(1),N7(2)*).[4-7] Figure 4 also shows the CD spectra of Pt(*R,R*-dach)(r(GpA)-*N7(1),N7(2)*), (**10**), and *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1),N7(2)*).[17] The CD spectra of the both adducts are very similar to each other except for a difference in the intensity and are also similar to those of the sequence isomers, i.e. **2** and *cis*-Pt(NH_3)$_2$(r(ApG)-*N7(1),N7(2)*), except for an inversion of sign. That is, the CD spectral pattern of the adducts with platination at *G-N7* and *A-N7* have a common feature. However, the CD spectrum of **4** appears to be different from the other *G-N7* and *A-N7* adducts. This may be due to a presence of conformational isomers (probably, equilibrium mixture of syn and anti conformations).[18] In HPLC analysis, **4** shows an unusually broad peak (retention time of **4** (12.2 min) is shorter than that of **5** (18.8 min), and a half width of the peak of **4** is about 20 times larger than that of **5**.

The CD of **1** is almost mirror image of that of **3**, suggesting the reverse of the direction of the transition moments and the similarity in the overlap of the two purines (see Fig. 5). Inversion of CD bands in Fig. 5 has been also observed in the CD spectra of *cis*-Pt(NH_3)$_2$(r(GpC)-*N7(1),N3(2)*) and *cis*-Pt(NH_3)$_2$(r(CpG)-*N3(1),N7(2)*).[24] It should be stressed that the formation of the species **1** and **3** has not been found in the study of Chottard et al.[18] The species **1** and **3** are considered to be a platinum adduct with G_{anti} configuration. The CD spectral pattern of **3** is in agreement with that of *cis*-Pt(NH_3)$_2$(d(GpA)-*N7(1),N1(2)*)[27] with G_{anti} configuration which has been characterized by NMR. Figure 6 also shows CD spectra of the adducts with platination at *G-N7* and *A-N1*. They are characteristically observed in the platinum adducts with GA-sequence. The band above 300 nm, probably due to $n\rightarrow\pi^*$ electronic transition, may arises from a significant interaction between the 6-NH_2 group of the A base and O6 of the G base. The CD of **5** is also similar to that of Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*),(**11**) and *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1),N1(2)*).[18] Chottard et al. assigned *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1),N1(2)*) to the platinum adduct with G_{syn} configuration.[18] The CD spectral pattern of the Pt-adducts obtained in this work is hardly influenced by the presence and absence of the 2′-OH group of the sugar, though the CD of d(ApG) and d(GpA) are different from those of r(ApG) and r(GpA).[28]

Isomerization of 5. In the reaction of Pt(*R,R*-dach)Cl_2 with d(GpA), the relative ratios of each product for all the platinum adducts were almost constant at least for the first 10 hours. But the subsequent incubation results in a decrease of **5** and an increase of **3** and **4**. The platinum adduct, **5**, is not a monodentate intermediate but a chelate compound which platinum bound to *G-N7* and *A-N1* as described above. In order to make this point much clear, we performed further two experiments. (1) The reaction solution of Pt(*R,R*-dach)Cl_2 with d(GpA) was treated with snake venom phosphodiesterase.[14-16] The resulting solution gave dG and 5′-dAMP as an enzymatic digestion products, but **3**, **4**, and **5** were not digested at all by the enzyme. The dG and 5′-dAMP come from the enzymatic digestion of the unreacted d(GpA). This strongly suggests that **5** is a chelate compound.[29,30] The enzymatic digestion of Pt(NH_3)$_3$(d(GpA)-*N7(1)*) gave Pt(NH_3)$_3$(dG-*N7*) and 5′-dAMP under the same conditions. (2) The reaction of [Pt(R,R-dach)(OH_2)$_2$]$^{2+}$ with d(GpA) gave the platinum adducts, **3**—**5**, via a monodentate intermediate, probably Pt(*R,R*-dach)(OH_2)(d(GpA)-*N7(1)*).

Isomerization of **5** was followed by means of HPLC. The solutions containing each **1**, **2**, **3**, **4**, and **5**, which were fractionated from HPLC, was incubated at 37°C. The product **5** was slowly converted into **3** and **4** (see Fig. 7), but the reverse reaction was not observed. This suggests that **3** and **4** are a thermodynamically more stable species and that **5** is likely to be a kinetically preferred species. The product **3** is further converted into unknown compound under further incubation. The half life of **5** was about 6 days at 37 °C. Addition of halogen ions (Cl^-, Br^-, and I^-) to the solution of **5** promotes the isomerization reaction. The more detail study is now under investigation.[31] Conversion between **1** and **2** was not observed.

Competition Reaction. In order to examine a relative reactivity of Pt(*R,R*-dach)Cl_2 with d(ApG) and d(GpA), a competition experiment was carried out. Stoichiometric amounts of d(ApG) and d(GpA) were incubated with half equivalent of Pt(*R,R*-dach)Cl_2 at 37 °C. Figure 8 shows a change of the unreacted dinucleotides and the products as a function of time. The reaction appeared to be complete within 1 day. Quan-

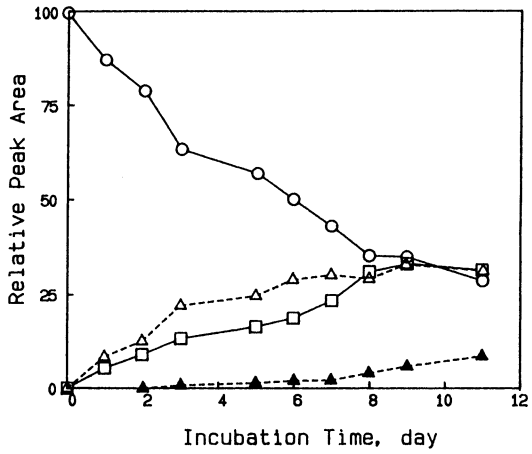

Fig. 7. Isomerization of Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **5**, to Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **3**, and Pt(*R,R*-dach)-(d(GpA)-*N7(1),-N7(2)*), **4**, at 37°C (pH=4.6). (○), Pt(*R,R*-dach)-(d(GpA)-*N7(1),N1(2)*), **5**; (△), Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*), **3**; (□), Pt(*R,R*-dach)(d(GpA)-*N7(1),-N7(2)*), **4**; (▲), unknown compound, which forms from **5** via **3**.

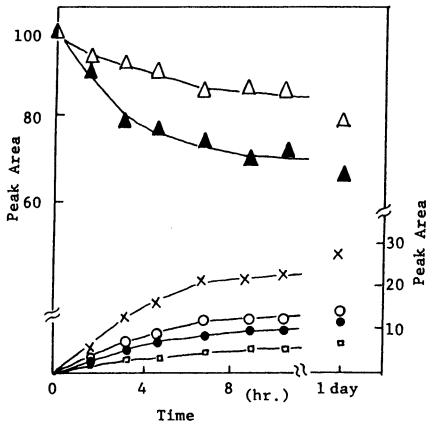

Fig. 8. Competition reaction of Pt(*R,R*-dach)Cl_2 with an equimolar mixture of d(ApG) and d(GpA) at 37°C (in water), r=0.25. (△), unreacted d(GpA); (▲), unreacted d(ApG); (×), **2**; (○), **5**; (●), **1**+**3**; (□), **4**.

titation result of the unreacted dinucleotides showed that d(ApG) is more reactive for platination than d(GpA) by a factor of about 1.7—1.9. The result is also supported by the quantitation result of the reaction products. The reaction between bifunctional platinum compound and d(ApG) or d(GpA) occurs via a two step mechanism.[32,33] The first step corresponds to formation of the 1 : 1 intermediate, and the second step to formation of the interbase crosslinked compound. In order to make the first step reaction clear, the reaction between monofunctional platinum complex, [Pt-$(NH_3)_3$Cl]Cl, and d(ApG) or d(GpA) was examined. The reaction product was exclusively Pt$(NH_3)_3$(d-(ApG)-*N7(2)*), (**6**), and Pt$(NH_3)_3$(d(GpA)-*N7(1)*), (**7**). The result agrees well with other studies.[34-36] Quantitation of the products in the competition reaction

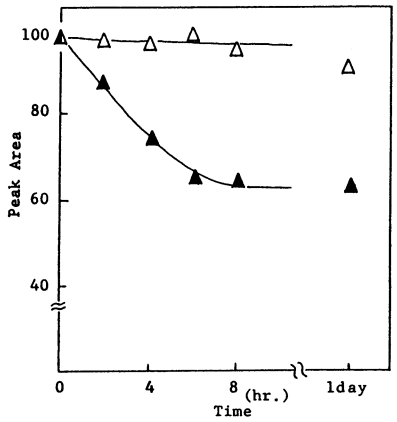

Fig. 9. Competition reaction of Pt(*R,R*-dach)Cl_2 with an equimolar mixture of d(ApG) and d(pGpA) at 37°C (in water), r=0.25. (△), unreacted d(ApG); (▲), unreacted d(pGpA).

indicated that formation of **6** is 2 times easier than that of **7**. This strongly suggests that the 1 : 1 intermediate formed in the reaction of Pt(*R,R*-dach)Cl_2 with d(ApG) or d(GpA) is the compound which platinum bound to the N7 of the G base, and that a preference in the reactivity is governed by a degree of formation of the 1 : 1 intermediate. The preference in the relative reactivity of d(ApG), compared with d(GpA), can be explained by an effect of the 5′-connected phosphodiester group, as being reported by Reedijk et al.[34] and Chottard et al.[18] i.e. interaction between the aquated platinum species and phosphodiester group on the 5′ side. To confirm the idea, the competition reaction between d(pGpA) and d(ApG) for Pt(*R,R*-dach)Cl_2 was also examined. As can be seen in Fig. 9, the reaction of Pt(*R,R*-dach)Cl_2 with d(pGpA) is much more reactive than that with d(ApG), i.e. the reactivity is greatly promoted by the presence of the 5′-phosphate group. This implies that a charge interaction, in addition to hydrogen bonding interaction, between the aquated platinum species and the 5′-phosphate group contributed to the reaction preference. It has been known that the reaction rate of *cis*-Pt$(NH_3)_2(OH_2)_2$ with 5′-dGMP is faster than that with 3′-dGMP.[37,38]

Concluding Remarks

The reaction between Pt(*R,R*-dach)Cl_2 and d(ApG) resulted in Pt(*R,R*-dach)(d(ApG)-*N7(1),N7(2)*) and Pt(*R,R*-dach)(d(ApG)-*N1(1),N7(2)*), i.e. **2** and **1**. The former product, **2**, contains 80% of the reaction products. Formation of the latter product has not been found in the reaction[18] between *cis*-Pt$(NH_3)_2Cl_2$ and r(ApG). The reaction of Pt(*R,R*-dach)Cl_2 with d(GpA) resulted in Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*),(**3** and **5**) and Pt(*R,R*-dach)(d(GpA)-*N7(1),N7(2)*), (**4**). The platinum adduct, **5**, isomerizes very slowly to **3** and **4**. The reaction between Pt(*R,R*-dach)Cl_2 and r(GpA) yields Pt(*R,R*-dach)(r(GpA)-*N7(1),N7(2)*), (**10**), and Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*), (**8**, **9**, and **11**), and

11 also isomerizes to **8**, **9**, and **10**. In the initial process of the reactions (up to 10 hours), the Pt(*R,R*-dach)(d(GpA)-*N7(1),N1(2)*) adducts contains more than 80% of all the reaction products, while the product ratio of the Pt(*R,R*-dach)(r(GpA)-*N7(1),N1(2)*) adducts is less than 60%. The interesting difference seems to arise from the difference at the C2′ position of the sugar (2′-deoxy and 2′-oxy). The absence of the 2′-OH group of the sugar seems to be favourable to the binding at the *A-N1*.

The two purine bases in **5** appears to have the same sugar orientations as *cis*-Pt(NH_3)$_2$(r(GpA)-*N7(1), N1(2)*)[18] because of the similarity of the both CD spectra. Examination of molecular model indicates that when the 1 : 1 intermediate has G_{syn} configuration with the N-type conformer, subsequent chelation is very likely to occur at the N1 of the A base. In general, the reaction between platinum complex and nucleic acid base appears to be controlled by kinetic rather than thermodynamic in character. The relative preference of **5** to **3** and **4** may originate from such a kinetic reason. Although the product **5** is a kinetically preferred species, it is thermodynamically unstable species. In the reaction of Pt(*R,R*-dach)Cl_2 with d(pGpA), formation of **5′** is much more preferred, compared with the case of the reaction with d(GpA). The presence of 5′-phosphate group may allows an increase of G_{syn} configuration, probably in the 1 : 1 intermediate.

In the reaction of antitumor platinum complex with DNA, formation of the platinum adduct,**5**, seems to be unfavorable because it requires large conformational changes of the sugar moiety. Moreover, the N1 of adenine base is involved in base pairing in double stranded DNA. This may be one of the reason why the d(GpA)-chelate was not found after the enzymatic digestion of the platinum modified DNA.

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