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DETERMINATION OF PLATINUM BINDING BASES BY ENZYMATIC DIGESTION

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The reaction mixtures of dinucleotides ($G_{3'p5'}G$ and $G_{3'p5'}U$) with platinum complexes ($[Pt(NH_3)_3Cl]Cl$ and cis-$Pt(NH_3)_2Cl_2$) have been treated with exonuclease (snake venom phosphodiesterase and calf spleen phosphodiesterase), and the platinum binding base in dinucleotides has been determined on the basis of HPLC analysis of the enzymatic digestion products.

Studies on the interaction of antitumor active platinum complexes with DNA and its constituents are now very interesting subject in relation with the mechanism of action, and a number of techniques, involving UV, CD, NMR and Raman spectroscopy, have been applied to elucidate the binding-mode and -sites.[1-4] We have tried a new method using enzymatic digestion to expose the platinum binding base in oligonucleotides. For this purpose, it is necessary to make clear whether Pt-modified substrate is able to digest by exonuclease or not. In the present paper, we employed the reaction mixture of the platinum complexes with dinucleotides as a substrate solution, and snake venom phosphodiesterase(VPD) and calf spleen phosphodiesterase(SPD) were used for degradation of the Pt-modified substrates. These enzymes have been known to have relatively low substrate specificity. The enzymatically digested products have been identified by using HPLC with a weak cation exchange column.

Figure 1 shows the chromatogram of the reaction mixture of $[Pt(NH_3)_3Cl]Cl$ with $G_{3'p5'}G$.[5] The reaction gave two products, **I** and **II**. Treatment of the reaction mixture with VPD resulted in a complete disappearance of one of the reaction products, while the other one (**II**) was unchanged.[6] The products produced by the VPD digestion were found to be $Pt(NH_3)_3$(Guo) and 5'-GMP by comparing with the standard materials.[7] Since VPD is an enzyme that degrades oligonucleotide exonucleolytically from the 3'-OH end, **I** should be $Pt(NH_3)_3$-$G_{3'p5'}G$. On the other hand, SPD digests both **I** and **II** to produce 3'-GMP, $Pt(NH_3)_3$(Guo), Guo and $Pt(NH_3)_3$(3'-GMP).[8] Effect of the incubation time on SPD digestion showed that decrease of **I** was slightly faster than that of **II**, and that there were correlations between (a) decrease of **II** and increase of 3'-GMP and $Pt(NH_3)_3$(Guo) and between

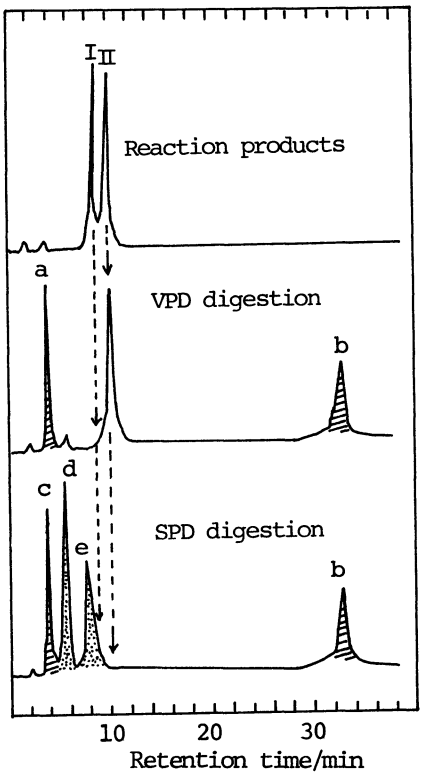


Fig. 1. High performance liquid chromatograms of the reaction mixture of $[Pt(NH_3)_3Cl]Cl$ with $G_{3'p5'}G$ and its enzymatic digestion products.

I *and* **II** --- *Reaction products*
a --- *5'-GMP*, **b** --- *$Pt(NH_3)_3$(Guo)*, **c** --- *3'-GMP*[9], **d** --- *Guo*, **e** --- *$Pt(NH_3)_3$(3'-GMP)*

Experimental Conditions of HPLC
Column ; Toyo Soda TSK-gel IEX 530 K (weak cation exchange column)
Detector ; UV at 260 nm
Eluant ; 0.1 mol dm^{-3} KH_2PO_4 (0.8 ml/min)

(b) decrease of **I** and increase of Guo and $Pt(NH_3)_3$(3'-GMP). This suggests that SPD digestion of **I** produces Guo and $Pt(NH_3)_3$(3'-GMP) and that of **II** produces 3'-GMP and $Pt(NH_3)_3$(Guo).[7] Since SPD is an enzyme that degrades oligonucleotide exonucleolytically from the 5'-OH end, it is concluded that **II** and **I** are $G_{3'p5'}G$-$Pt(NH_3)_3$ and $(NH_3)_3Pt$-$G_{3'p5'}G$, respectively. These conclusions are summarized below ;

$$(NH_3)_3Pt\text{-}G_{3'p5'}G \xrightarrow{\text{VPD}} (NH_3)_3Pt(Guo) + 5'\text{-GMP}$$

$$G_{3'p5'}G\text{-}Pt(NH_3)_3 \xrightarrow{\text{VPD}} \text{no digestion}$$

$$(NH_3)_3Pt\text{-}G_{3'p5'}G \xrightarrow{\text{SPD}} (NH_3)_3Pt(3'\text{-GMP}) + Guo$$

$$G_{3'p5'}G\text{-}Pt(NH_3)_3 \xrightarrow{\text{SPD}} (NH_3)_3Pt(Guo) + 3'\text{-GMP}$$

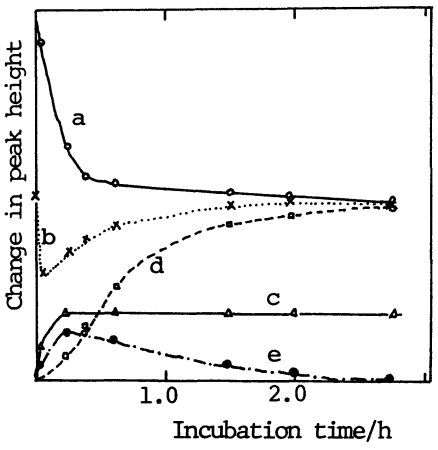
VPD cannot cut the phosphodiester bond when the platinum(II) exists in the side of the recognition site (3'-OH end), while SPD can digest such Pt-modified substrate.

An experiment was undertaken to see whether the phosphodiester bond of cis-$Pt(NH_3)_2(G_{3'p5'}G)$ can be digested by the treatment with VPD or SPD. cis-$Pt(NH_3)_2(G_{3'p5'}G)$ is the complex with an intrastrand crosslink between the adjacent guanine bases through the N(7)-N(7) sites.[10] Such an intrastrand guanine-guanine dimer appears to be a possible binding mode of cis-$Pt(NH_3)_2Cl_2$ with DNA.[1] VPD could not cut the phosphodiester bond of cis-$Pt(NH_3)_2(G_{3'p5'}G)$ even when an excess of VPD was added. The complex could not be also digested by SPD. Of course, $G_{3'p5'}G$ could be easily digested under the conditions used.

As uridine is a poor ligand for platinum(II), especially in an acidic solution, the reaction of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$ is expected to give a complex, cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$, in which only the guanine base is bound to the platinum(II). To see if such a complex is digested by VPD, the

Fig. 2. Change of enzymatic digestion products of cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$ as a function of incubation time with VPD.

a --- reaction product of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$, **b** --- $G_{3'p5'}U$ and/or 5'-UMP, **c** --- guanosine, **d** --- cis-$Pt(NH_3)_2(Guo)_2$, **e** --- enzyme-substrate complex



Experimental conditions of HPLC were the same as those in Fig. 1. The reaction mixture contained unreacted starting material, $G_{3'p5'}U$, and Guanosine(Guo) in figure arises from VPD digestion of the unplatinated $G_{3'p5'}U$. In early period of VPD digestion, unplatinated $G_{3'p5'}U$ was rapidly digested to Guo and 5'-UMP. The retention time of $G_{3'p5'}U$ was the same as that of 5'-UMP, as expected from their charge. This is why curve **b** *steeply decrease at the early period and thereafter turn to increase. $G_{3'p5'}U$ and 5'-UMP can be separated by using a strong anion exchange column. With 2 days incubation with VPD, all the peak heights bacame constant. Further addition of VPD no longer changed these peak heights. Decrease in curve* **a** *brings to a stop at about half of the original peak height. The reaction mixture of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$ may involve the complex other than cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$.*[12]

reaction mixture of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$ was treated with VPD.[11] It gave cis-$Pt(NH_3)_2(Guo)_2$ and 5'-UMP as the VPD digestion products. cis-$Pt(NH_3)_2(Guo)_2$ is the complex, in which cis-$Pt(NH_3)_2^{2+}$ is bound to guanosine through the N(7) site. Therefore, it is sure that the reaction mixture involves cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$ as the reaction product. From the VPD digestion products, cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$ is thought to be the complex, in which cis-$Pt(NH_3)_2^{2+}$ is bound to $G_{3'p5'}U$ through the N(7) site of the guanine base. Figure 2 shows the change of the VPD digestion products as a function of incubation time with VPD. The peak height of the reaction product(curve **a**) rapidly decreases along with exponential curve, while appearance of cis-$Pt(NH_3)_2(Guo)_2$ seems to have an induction period (curve **d**). The chromatograms showed a peak of the intermediate considered to be an enzyme-substrate complex (ES complex), and the retention time of the peak was found to be identical with that of VPD. These results suggest that the VPD digestion of cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$ proceeds as follows; $E + S \rightleftharpoons ES \longrightarrow E + P1 + P2$

This means that cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$ can be digested by VPD. The results are summarized below;

$$\text{cis-}Pt(NH_3)_2(G_{3'p5'}G) \xrightarrow{\text{VPD}} \text{no digestion}$$

$$\text{cis-}Pt(NH_3)_2(G_{3'p5'}U)_2 \xrightarrow{\text{VPD}} \text{cis-}Pt(NH_3)_2(Guo)_2 + 5'\text{-UMP}$$

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References

1) S. J. Lippard, Science, 218, 1075 (1982) and references therein.

2) L. G. Marzilli, "Progress in Inorganic Chemistry," ed by S. J. Lippard, Wiley, New York(1977), p. 255 and references therein.

3) J. K. Barton and S. J. Lippard, "Nucleic Acid Metal Ion Interaction," ed by T. G. Spiro, Wiley, New York(1980), p. 31 and references therein.

4) "Metal Ions in Biological Systems," ed by H. Sigel, Marcel Dekker, New York(1980), Vol. 11.

5) $[Pt(NH_3)_3Cl]Cl$, 0.45 μM, was allowed to react with stoichiometric amount of $G_{3'p5'}G$ at 37 °C for a week. $G_{3'p5'}G$ = guanylyl(3'-5')guanosine.

6) VPD digestion was performed in a buffer solution that contained 0.1 mol dm^{-3} Tris-HCl, pH 8.9, 2.0 x 10^{-3} mol dm^{-3} of $MgCl_2$, 10 μl of VPD (0.5 mg/ml, Boehringer Mannheim) in a volume of 200 μl of the reaction mixture, at 37 °C for 10 h.

7) $Pt(NH_3)_3(Guo)$ is the complex, in which $Pt(NH_3)_3^{2+}$ is bound to Guo through the N(7) site. K. Inagaki and Y. Kidani, Inorg. Chim. Acta, 46, 35 (1980); Inorg. Chim. Acta, in press.

8) SPD digestion was performed in a buffer solution that contained 0.1 mol dm^{-3} acetate, pH 6.2, 30 μl of SPD (4 units/ml, Boehringer Mannheim) in a volume of 200 μl of the reaction mixture at 37 °C for 2 d.

9) The retention time of $G_{3'p5'}G$ was the same as that of 5'-GMP and 3'-GMP. These three compounds could be separated by using a strong anion exchange column (Partisil SAX). There was almost no unreacted $G_{3'p5'}G$ in the reaction mixture.

10) J. C. Chottard, J. P. Girault, G. Chottard, and J. Mansuy, J. Am. Chem. Soc., 102, 5565 (1980); J. P. Girault, G. Chottard, J. Y. Lallemand, and J. C. Chottard, Biochemistry, 21, 1352 (1982).

11) The reaction of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$ (Pt/base = 1.0) was performed at 37 °C for 3 d in phosphate buffer, pH 4.0. $G_{3'p5'}U$ = guanylyl(3'-5')uridine. Experimental conditions for VPD digestion are the same in ref. 6 except for the incubation time.

12) The reaction of cis-$Pt(NH_3)_2Cl_2$ with $G_{3'p5'}U$ (Pt/base = 2.0) was run at 65 °C for 3 d in phosphate buffer, pH 7.4. In this case, the complex formed was the same retention time as cis-$Pt(NH_3)_2(G_{3'p5'}U)_2$, but it could not be digested by VPD.

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