CHEMISTRY LETTERS, pp. 233-236, 1986.

INTERACTION OF DNA WITH ANTITUMOR ACTIVE PLATINUM COMPLEXES. PLATINUM COMPLEXES INVOLVING MESO DIAMINE LIGAND GAVE TWO Pt-d(GpG) ADDUCTS

Kenji INAGAKI,* Chikako NINOMIYA, and Yoshinori KIDANI
Faculty of Pharmaceutical Sciences, Nagoya City University,
Tanabe-dori, Mizuho-ku, Nagoya 467

Reaction products of Pt(meso-diamine)$^{2+}$ involving bulky substituted group with DNA were investigated by using enzymatic digestion technique. Main Pt-adducts were complexes with an intrastrand crosslink between two adjacent guanine bases through N7, N7. Stereoselectivity arised from steric hindrance was observed.

Many investigations suggest that bifunctional attack of antitumor active Pt(II) complexes to DNA is responsible for its biological activities.[1] Recent investigations indicate that a crosslink between two adjacent guanine bases in the same strand of DNA is a main event in the bifunctional interaction.[2] Our laboratory has utilized the Pt(II) complexes containing chiral diamine ligand in developing the second generation platinum drug, and we have been found some interesting results during the investigations.[3,4] Antitumor activity of the Pt(II) complexes is varied not only by the leaving group but also by the steric structure of the non-leaving group, e.g., the Pt(II) complexes prepared from 1,2-cyclohexanediamine(abbreviation; dach) isomers(1R,2R-, 1S,2S-, and 1R,2S-dach) showed the different antitumor activities. In the antitumor test against leukemia L-1210 and leukemia P-388, the Pt-complexes containing 1R,2R-dach indicated a slightly higer activity than those containing 1S,2S-dach, and the Pt-complexes containing 1R,2S-dach were less active and less toxic.[3] Whereas, in the case of the antitumor test against Sarcoma-180, the Pt-complexes containing 1R,2S-dach showed superior activity to those containing 1R,2R- and 1S,2S-dach.[4] It can be generally said that the Pt-complexes containing 1R,2S-dach are always less toxic.[3,4] These imply that steric structure of the non-leaving groups of the Pt(II) complexes might influence a conformation of DNA in different way (after platination to DNA). Such conformational change induced in DNA might influence replication, transcription, recombination of DNA, and recognition of a certain protein. It would seem worthwhile studing a steric effect in the reaction of DNA with several platinum complexes.

The present paper describes preferential Pt-adducts produced from the reaction of calf thymus DNA with Pt(1R,2S-dach)Cl_2, Pt(1R,3S-dach)Cl_2, and Pt(4-OH-R,S-stien)SO_4.[5] In three Pt complexes mentioned above, the cyclohexane ring and one of the 4-OH-phenyl group are almost perpendicularly oriented with respect to the platinum coordination plane. Therefore, a certain steric interaction may take place in the reaction with DNA. Since the Pt(1R,2S-dach)$^{2+}$ is asymmetric with respect to the axis that bisects the N-Pt-N

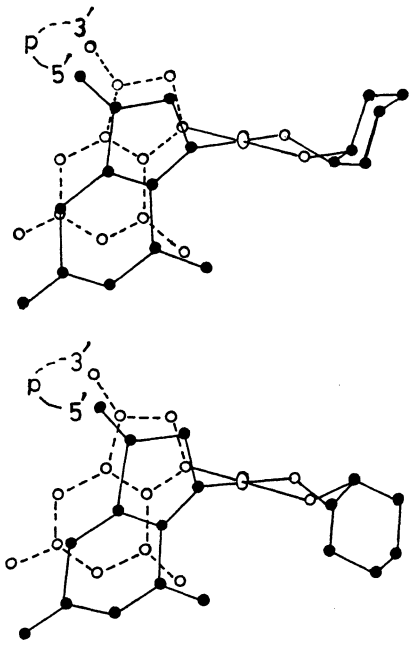

Fig. 1. Schematic structure of two Pt(1R,2S-dach)[d(GpG)], **1a** and **1b**.

angle, two Pt-adducts would be expected to be produced when Pt(1R,2S-dach)$^{2+}$ crosslinks to the N7 sites of d(GpG) with a head-to-head arrangement. This was confirmed in the previous work,[7] i.e., the reaction of Pt(1R,2S-dach)$^{2+}$ with d(GpG) gave two Pt-adducts, which were diastereomers with each other and were complexes with an interbase crosslink between two guanine bases through N7, N7, as is schematically presented in Fig. 1. Since d(GpG) is a partial structure of DNA, formation of the similar Pt-adducts would be expected in a reaction of Pt(1R,2S-dach)$^{2+}$ with DNA. Similar situation would be expected to take place in the reaction of DNA with Pt(1R,3S-dach)Cl_2 and Pt(4-OH-R,S-stien)SO_4.[8]

In buffer solution containing 0.05 M phosphate and 0.01 M NaCl, calf thymus DNA was incubated with various amounts of Pt(1R,2S-dach)Cl_2 at 37 °C for 4 d, and the treated DNA was enzymatically digested.[10] That is, a 100 µl of the treated DNA was incubated for 6 h at 37 °C in the presence of 100 units of deoxyribonuclease I and 20 mM of $MgCl_2$. After addition of 10 µl of 1 M Tris-HCl buffer, the mixture was further incubated for 12-16 h at 37 °C in the presence of 1 unit of snake venom phosphodiesterase and 3 units of alkaline phosphatase. Finally, the mixture was treated with 10 µl of calf spleen phosphodiesterase after addition of 11 µl of 1 M acetic acid (at 37 °C for 2 d). Pt(1R,3S-dach)$^{2+}$- and Pt(4-OH-meso-stien)$^{2+}$-modified DNA were treated by the same procedure ,respectively.

The HPLC study of the enzymatic digestion products indicates that Pt(diamine)[d(GpG)], which consists of two Pt-adducts as shown in Fig. 1, is a preferred Pt-adducts, i.e., the main Pt-adducts accounted for more than 75% of all the Pt-adducts at r(Pt/base)=0.055 (as measured by peak area). Since the other Pt-adducts are only minor amounts, we will restrict ourselves to a discussion of the main components. Figure 2 shows a plot of each peak area vs. r, in which chromatographic peaks are labeled subscript **a** and **b** in order of their appearance, like **1a** and **1b** in the case of Pt(1R,2S-dach)[d(GpG)].[11] In every cases, formation of Pt(diamine)[d(GpG)] compounds increases with increasing r, especially the increment at r<0.035 is large, and tends to saturate at about r=0.06. When a random distribution of the bases in calf thymus DNA is assumed, the nearest neighbor frequency of d(GpG) sequence in the DNA is about 7%. Therefore, if Pt(diamine)$^{2+}$ selectively attacks the d(GpG) sequence, formation of Pt(diamine)[d(GpG)] will attain saturation at r=0.035. Figure 2 suggests a existence of such tendency. The reaction products of Pt(R,S-dach)$^{2+}$ with a denatured DNA were also followed by the same method. Although Pt(R,S-dach)[d(GpG)] compounds, **1a** and **1b**, were still main Pt-adducts, the chromatogram also showed a increase in relative amounts of the other Pt-adducts (data not shown). Formation of Pt(R,S-dach)[d(GpG)] compounds saturated at about r = 0.02. These results suggest that the preferred platinum binding to the d(GpG) sequence in DNA is enhanced by a presence of the double stranded structure. The denatured DNA would be

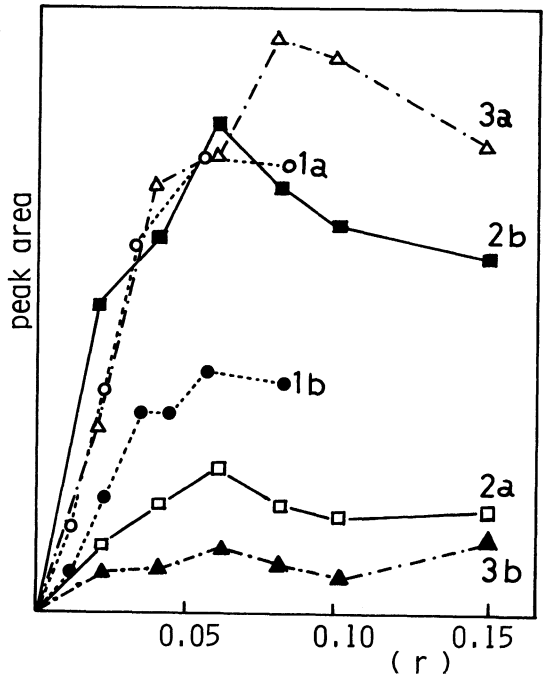

Fig. 2. Relation between peak areas of Pt(diamine)[d(GpG)] and Pt/base (r).
Pt(1R,2S-dach)[d(GpG)]; **1a, 1b**
Pt(1R,3S-dach)[d(GpG)]; **2a, 2b**
Pt(4-OH-R,S-stien)[d(GpG)]; **3a, 3b**

expected to expose several new platinum binding sites, besides those of double stranded DNA. For double stranded DNA, N3 of pyrimidines and N1 of purines are blocked by specific base paring, and as a result, a relative reactivity of platinum to N7 of guanine base is thought to be enhanced.

With comparison of three Pt(diamine)$^{2+}$ complexes, difference in the relative ratio of **a** and **b** (i.e., **1a** or **1b**/(**1a**+**1b**)) increases in the following order; **3a:3b** = 0.89:0.11 > **2a:2b** = 0.21:0.79 > **1a:1b** = 0.63:0.37. The order seems to agree with the increasing order of bulk of the axially standing group with respect to the platinum coordination plane. Whereas, in the case of the raction of Pt(diamine)$^{2+}$ with d(GpG), the two main Pt-adducts were obtained in roughly equal amounts, i.e., **3a:3b** = 0.53:0.47, **2a:2b** = 0.48:0.52 and **1a:1b** = 0.53:0.47. When DNA was allowed to react with the platinum complexes with the bulky substituted group, a certain steric selection may take place. When the platinum complexes bind to the d(GpG) sequences in DNA, the axially standing group faces either on the same side of O6 of guanine bases or the opposite side as shwon in Fig. 1. When the cyclohexane ring faces on the opposite side of O6, a steric hindrance between the cyclohexane ring and the bases will be small. Actually, the CD spectra of **Ia** and **IIb** were similar to those of Pt(ethylenediamine)[d(GpG)] and Pt(1,3-propanediamine)[d(GpG)], respectively.[7] When the axially standing group exists in the same side of the O6 atoms, the extent of the distortion of the DNA may become much greater, and it would require further changes in conformations of the base moieties adjacent to the platinum binding sites. Such conformational changes induced in DNA may be responsible in part for some of the observed changes in antitumor properties.

Using agarose gel electrophoretic technique, we examined whether or not these platinum complexes involving the bulky groups can induce some conformational changes

Table 1. Agarose gel electrophoretic data of platinum modified PM-2 DNA

	form **II**	form **III**	form **I**
Pt(1R,2S-dach)Cl_2	1.20	1.07	1.03
Pt(1R,3S-dach)Cl_2	1.23	1.08	1.03
cis-Pt$(NH_3)_2Cl_2$	1.24	1.09	1.03
Pt(1R,2R-dach)Cl_2	1.24	1.08	1.03
[Pt$(NH_3)_3$Cl]Cl	1.04	1.00	1.02
Control	1.0	1.0	1.0

The migration of the Pt-modified DNA is expressed as a ratio relative to the migration of the control DNA.
(form **I**, supercoil; form **II**, open circular; form **III**, linear)
PM-2 DNA was allowed to react with various platinum complexes for 16 h at 37°C (r = 0.1, pH 7.2), and the treated PM-2 DNAs were electrophoretically separated on 0.8 % agarose gel slab. Electrophoresis was performed in a buffer (pH 8.0) containing 40 mM Tris, 5 mM acetic acid and 1 mM EDTA for 17 h at 3.0 V/cm (staining; ethidium bromide).

in the reaction with DNA. As can be seen in Table 1, the form II PM-2 DNA, being treated with the platinum complexes, showed a marked increase in the electrophoretic mobility except for the case of $[Pt(NH_3)_3Cl]Cl$. The behavior agrees with the DNA shortening effect, observing when an open circular DNA contains local denaturation or micro loops after reaction with cis-$Pt(NH_3)_2Cl_2$.[12] However, we are not able to detect any difference in the electrophoretic mobility among the platinum complexes involving cis-configuration. Whereas, the PM-2DNA modified by the monofunctional platinum complex, $[Pt(NH_3)_3Cl]Cl$, showed a similar behavior with a control DNA. It appears that changes in the mobility mainly reflect a change of the overall structure in the DNA, being induced from the bifunctional binding of the platinum complexes involving cis-configuration to the DNA, but not reflect a minor (local) change in conformations in the vicinity of platinum binding site.

References

1) J. J. Roberts and M. P. Pera, Jr., "Molecular Aspects of Anti-Cancer Drug Action," ed by S. Neidle and M. J. Waring, The Macmillan Press Limited, London (1983), pp. 183-231.
2) J. P. Caradonna and S. J. Lippard, Dev. Oncol., 17, 15 (1984); J. P. Macquet, J. L. Butour, N. P. Johnsson, H. Razaka, B. Salles, C. Vienssen, and M. Wright, ibid., 17, 27 (1984); J. Reedijk, J. H. J. den Hartog, A. M. J. Fichtinger-Schepmann, and A. T. M. Marcelis, ibid., 17, 39 (1984).
3) Y. Kidani, K. Inagaki, and S. Tsukagoshi, Gann, 67, 923 (1976); Y. Kidani, K. Inagaki, R. Saito, and S. Tsukagoshi, J. Clin. Hematol. Oncol., 7, 197 (1977); R. J. Speer, L. M. Fall, D. P. Stewart, H. J. Ridgway, J. F. M. Hill, Y. Kidani, K. Inagaki, M. Noji, and S. Tsukagoshi, J. Clin. Hematol. Oncol., 8, 44 (1978); Y. Kidani, M. Noji, and T. Tashiro, Gann, 71, 637 (1980); M. Noji, K. Okamoto T. Tashiro, and Y. Kidani, J. Med. Chem., 24, 508 (1981).
4) Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi, and K. Kuretani, J. Med. Chem., 21, 1315 (1978).
5) Abbreviation: 1R,3S-dach = cis-1,3-cyclohexanediamine, 4-OH-R,S-stien = 1R,2S-bis(4-hydroxyphenyl)ethylenediamine. Antitumor activity of Pt(4-OH-R,S-stien)Cl_2 was lower than that of Pt(4-OH-R,R(or S,S)-stien)Cl_2.[6]
6) Master's thesis of M. Matsumoto, Nagoya City University, 1985; W. Wappes, M. Jennerwein, E. von Angerer, H. Schonenberger, J. Engel, M. Berger, and J. Wrobel, J. Med. Chem., 27, 1280, (1984).
7) K. Inagaki and Y. Kidani, Inorg. Chem., in press.
8) The cyclohexane ring of Pt(1R,3S-dach)$^{2+}$ is almost perpendicularly oriented with respect to the coordination plane.[9] One of the 4-hydroxyphenyl groups also occupies such configuration.
9) R. Saito and Y. Kidani, Bull. Chem. Soc. Jpn., 52, 57 (1979); K. Kamisawa, K. Matsumoto, S. Ooi, H. Kuroya, R. Saito, and Y. Kidani, ibid., 51, 2330 (1978).
10) K. Inagaki, K. Kasuya, and Y. Kidani, Chem. Lett., 1984, 171; K. Inagaki and Y. Kidani, Inorg. Chim. Acta, 106, 187 (1985).
11) The parameters of the run of HPLC are as follows: Column, TSK-Gel CM-2SW; Column size, 4.6x500 mm; Mobile phase, 0.05 M KH_2PO_4(pH 4.6); Flow rate, 0.7 ml/min.; Detector, UV at 260 nm. Retention time, Pt(1R,2S-dach)[d(GpG)], 32.5 min (**1a**) and 36.5 min (**1b**). Pt(1R,3S-dach)[d(GpG)], 26.7 min (**2a**) and 29.7 min (**2b**). In the case of Pt(4-OH-R,S-stien)[d(GpG)]: Column size, 4.6x250 mm; Mobile phase, 0.25 M KH_2PO_4. The other conditions are the same as above. 23.5 min (**3a**) and 27.5 min (**3b**).
12) J. Macquet and J. Butour, Biochimie, 60, 901 (1978).

(Received November 22, 1985)