Characterization of Isomers Which Are Produced by Reactions of (1*R*,3*S*-Cyclohexanediamine)platinum(II) with Nucleotide d(GpG)

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(Received December 18, 1992)

Synopsis. Two adducts obtained from reactions of dinucleotide (d(GpG)) with 1*R*,3*S*-cyclohexanediamineplatinum-(II) have been investigated on the basis of NMR spectroscopy. They were assigned as interbase cross-linked adducts being platinated at the N7 positions of the adjacent guanine bases. Both isomers differ with respect to the orientation of the cyclohexane ring toward the carbonyl group at the 6 position of the adjacent guanine bases. A conformational analysis of the cyclohexane ring is also described.

It has been generally accepted that DNA, especially adjacent guanine residues on DNA, is a primary target for platinum antitumor drugs.[1] Very recently, it has been found that a certain protein in cells specifically recognizes the kinks on DNA induced by a platinum drug.[2] We have also found that an antibody against Pt-(1*R*,2*R*-dach)–DNA (regarding abbreviation, see Ref. 3) has a different affinity for the various kinds of platinated DNAs.[4] Since the magnitude of antitumor activity seems to be associated with a structural change in the DNA, we investigated the structures of the platinum–dinucleotide adducts (as a model of platinated DNA).[5] The present paper describes the adducts obtained from the reaction between Pt(1*R*,3*S*-dach)Cl_2 and d(GpG).[3] Schematic structures of d(GpG) and Pt-(1*R*,3*S*-dach)Cl_2 are shown in Fig. 1.

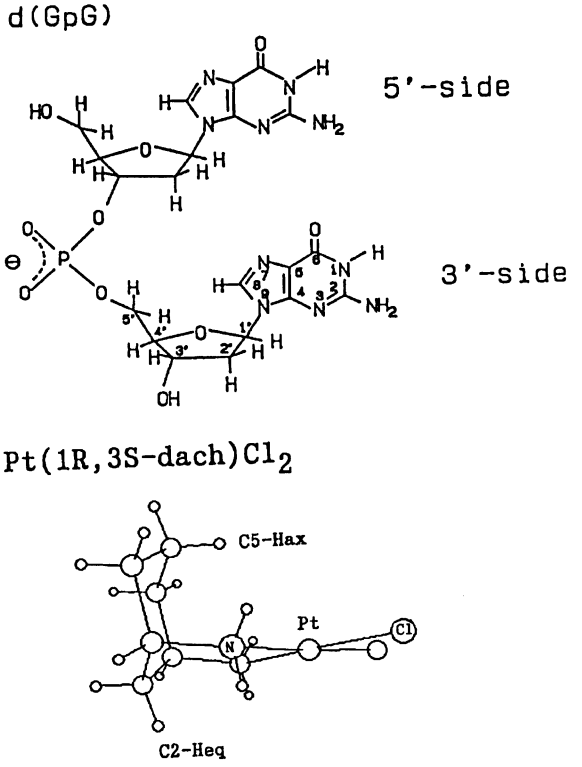

Fig. 1. Schematic structures and numbering of d-(GpG) and Pt(1*R*,3*S*-dach)Cl_2.

Experimental

Pt(1*R*,3*S*-dach)Cl_2 was prepared according to a previously described method.[6] [Pt(1*R*,3*S*-dach)$(NH_3)_2$]Cl_2 was prepared as follows. To a suspension of Pt(1*R*,3*S*-dach)Cl_2 (0.2 g) in water (15 cm^3) was added an excess amount of aqueous ammonia. The mixture was heated at 80 °C on a water bath until a colorless solution was obtained. The solution was evaporated to dryness to give [Pt(1*R*,3*S*-dach)-$(NH_3)_2$]Cl_2. The reaction of an equimolar amount of Pt-(1*R*,3*S*-dach)Cl_2 with d(GpG) gave two platinum adducts. They were separated into each adduct by using HPLC. The parameters of the run of HPLC were as follows: Column, TSK-gel IEX-530; column size, 4.6×500 mm; mobile phase, 0.05 mol dm^{-3} KH_2PO_4 (pH 4.6); flow rate 0.8 cm^3 min^{-1}; detector, UV at 260 nm.

The NMR spectra were recorded at 27 °C on a JEOL GX-400 spectrometer. Typical conditions for recording the NMR spectra include 500—1000 scans, 45° pulse width, and 32 K data points. In the case of a spectral simulation, trapezoidal window techniques were applied in order to improve the spectral resolution. NMR samples were prepared by lyophilizing each sample three times from 99.7% D_2O, and were finally dissolved in 99.95% D_2O. The pH of the NMR sample (without correction for deuterium isotope effect) was between 5 and 6.5, being measured after NMR measurements.

Results and Discussion

[Pt(1*R*,3*S*-dach)$(NH_3)_2$]Cl_2: The possible structure of [Pt(1*R*,3*S*-dach)$(NH_3)_2$]$^{2+}$ was first investigated. Table 1 and Fig. 2 show the NMR spectral data. Resonances of the cyclohexane ring protons were assigned with the aid of the 2D-COSY spectrum. The cyclohexane ring protons clearly gave separated axial and equatorial signals, indicating that the cyclohexane ring is held in a rigid conformation. A resonance at 3.06 ppm was unambiguously assigned to C1–Heq and the C3–Heq.[3] Its resolution-enhanced spectrum showed that the resonance comprised a quintet. Since [Pt-(1*R*,3*S*-dach)$(NH_3)_2$]$^{2+}$ has a symmetry plane passing through C5, Pt and the middle of the two coordinated NH_3 groups, the quintet represents experimental evidence for such a symmetry.

The resonance at 3.06 ppm was accompanied by ^{195}Pt satellite peaks, having a $^3J_{Pt-H}$ value of 71 Hz, as shown in Fig. 2. This is good evidence, indicating that the

Table 1. NMR Spectral Data of the Cyclohexane Ring Protons of [Pt(1*R*,3*S*-dach)-$(NH_3)_2]^{2+}$ and [Pt(1*R*,3*S*-dach)(d(GpG)-N7,N7)]$^{2+}$, No-1, and No-2

	Chemical shift (ppm)			Coupling constant (Hz)
[Pt(1*R*,3*S*-dach)$(NH_3)_2]^{2+}$		No-1	No-2	[Pt(1*R*,3*S*-dach)$(NH_3)_2]^{2+}$
C1–Heq/C3–Heq	3.06	2.95,2.97	2.98,3.01	$^3J_{1eq-2eq}=^3J_{3eq-2eq}=4.0$ Hz
C2–Heq	1.99	2.08	2.10	$^3J_{1eq-2ax}=^3J_{3eq-2ax}=3.2$
C2–Hax	(1.75)	(1.87)	1.93	$^3J_{1eq-6eq}=^3J_{3eq-4eq}=3.1$
C4–Heq/C6–Heq	(1.72)	(1.85)	(1.82)	$^3J_{1eq-6ax}=^3J_{3eq-4ax}=3.3$
C4–Hax/C6–Hax	(1.69)	(1.75)	(1.75)	$^2J_{2eq-2ax}=15.4$
C5–Heq	1.87	1.91	1.93	$^2J_{4eq-4ax}=^2J_{6eq-6ax}=14.2$
C5–Hax	4.03	(4.23)	(4.23)	$^2J_{5eq-5ax}=14.2$
				$^3J_{5eq-4eq}=^3J_{5eq-6eq}=2.5$
				$^3J_{5eq-4ax}=^3J_{5eq-6ax}=5.0$
				$^3J_{5ax-4ax}=^3J_{5ax-6ax}=13.5$
				$^3J_{5ax-4eq}=^3J_{5ax-6eq}=4.9$

Chemical shift in the parenthesis may have an ambiguity of ±0.02 ppm because of a severe overlap among the signals.

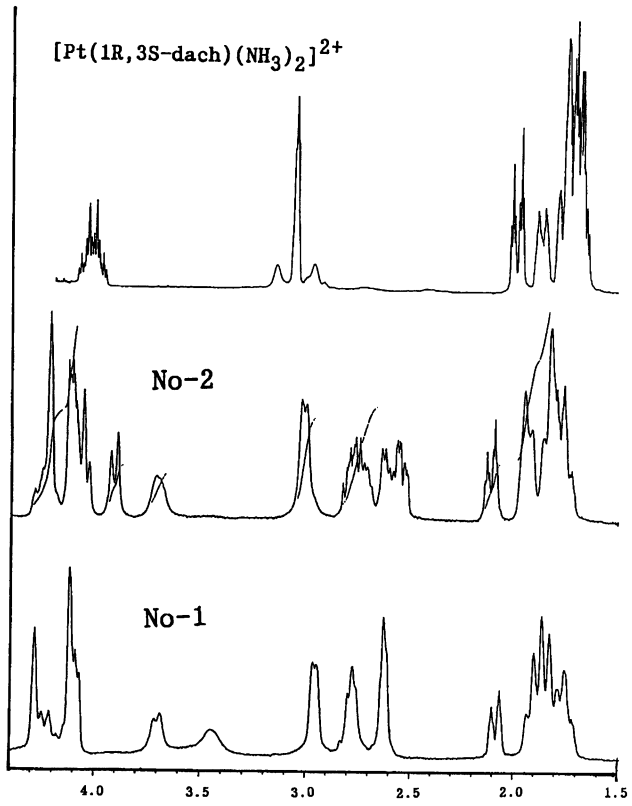

Fig. 2. 400 MHz NMR spectra of [Pt(1*R*,3*S*-dach)-$(NH_3)_2]^{2+}$ and the two isomers of [Pt(1*R*,3*S*-dach)-(d(GpG)-N7,N7)]$^{2+}$, No-2, and No-1.

chelate ring has a predominant chair conformation.[7]

It is generally found that axial protons resonate at a higher field than do equatorial protons in six-membered cyclohexane derivatives.[8] However, this is not the case for C5–Hax/Heq. That is, the resonance of C5–Hax was observed at 4.03 ppm. The molecular model indicates that the C5–Hax proton lies in the vicinity of the Pt atom only when both the chelate and the cyclohexane rings have a chair conformation (Fig. 1). It is therefore considered that the abnormally lower chemical shift is responsible for the interaction between the C5–Hax proton and the d-electron of the Pt atom. The resonance of the C5–Hax comprised four triplets. This is theoretically expected for the C5–Hax proton when the cyclohexane ring has a chair-type conformation. From the value of $^3J_{Pt-H}$, the abnormally lower chemical shift of the C5–Hax proton and the splitting pattern of C5–Hax signal, it is concluded that the cyclohexane and the chelate rings have dominant chair-type conformations. The coupling constants (Table 1) were obtained from a spectral simulation using the LAOCOON-type microcomputer program.[9] The simulated spectrum fitted in with the observed spectrum within 0.3 Hz. The coupling constant of $^3J_{5eq-4eq}$ is significantly small compared to that of $^3J_{5eq-4ax}$. In such a case, the dihedral angle of Heq–C5–C4–Heq becomes larger than 60°, and that of Heq–C5–C4–Hax should become smaller than 60°. This suggests that the cyclohexane ring of [Pt(1*R*,3*S*-dach)$(NH_3)_2]^{2+}$ has a somewhat flattened chair-type structure.

[Pt(1*R*,3*S*-dach)(d(GpG)–N7,N7)]$^{2+}$: The reaction of Pt(1*R*,3*S*-dach)Cl_2 with d(GpG) resulted in two adducts, No-1 and No-2. From the NMR spectra of No-1 and No-2, the binding ratio of Pt(1*R*,3*S*-dach)-Cl_2 to d(GpG) was found to be 1 : 1, since integration of the H1′ protons (2H) of d(GpG) was the same as that of the methine protons of the cyclohexane ring. The pK_a values obtained from the pH-titration (data not shown) indicate that both No-1 and No-2 are [Pt(1*R*,3*S*-dach)-(d(GpG)–N7,N7)]$^{2+}$, in which the Pt^{2+} is bound to the N7 site of the guanines. That is, no protonation was observed at the N7 site of the guanines, and the pK_a value at N1 of the guanines (ca. 8.5) agreed well with the value reported for the N7-platinated guanines.[5a,10] These results strongly suggest that the two adducts (No-1 and No-2) are orientational isomers which differ with respect to the positioning of the cyclohexane ring toward the coordinated d(GpG) moiety (structure

A and **B** in Fig. 3). As previously indicated, Pt(1*R*,3*S*-dach)Cl_2 has a symmetry plane which passes through Pt, C2, and C5. The binding of d(GpG) to Pt(1*R*,3*S*-dach)Cl_2 results in a lowering of such a symmetry, since d(GpG) has a chiral structure. As shown in Fig. 2, the chemical shift of C1–Heq was not the same as that of C3–Heq. This agrees well with the fact that [Pt(1*R*,3*S*-dach)(d(GpG))]$^{2+}$ no longer has a symmetry plane.

From the 2D-COSY spectra of No-1 and No-2, the resonance line of C5–Hax was observed near to 4.23±0.02 ppm. It is therefore considered that the conformation of the cyclohexane moiety is essentially the same as that of [Pt(1*R*,3*S*-dach)$(NH_3)_2$]$^{2+}$. An inspection of the molecular model shows that the C2–Heq proton of structure **B** experiences an anisotropic effect due to the carbonyl group at C(6) of the guanines. However, such an effect is not expected regarding the C2–Heq proton of structure **A**. The C2–Heq proton of No-2 resonated at a lower field, compared with that of No-1. The C1/C3–Heq protons of No-2 also resonate at a lower field. It is therefore likely that structure **B** should be assigned to No-2. On the other hand, the C5–Hax proton of structure **A** seems to be in close proximity to the carbonyl groups of the guanines. However, no downfield shift of C5–Hax was observed. In this case, the steric repulsion between the cyclohexane ring protons and the carbonyl groups seems not to be negligible. This may allow a decrease in the interaction between C5–Hax and the Pt atom; this effect allows the C5–Hax signal to move to a higher field. As a result, no net change in the chemical shift of C5–Hax can be observed. The C4/C6–Heq protons also lie near to the carbonyl groups. They resonated at a lower field in the case of No-1. These results seem to indirectly support the assignment of No-1 to structure **A**. Although a measurement of the nuclear overhauser effect (NOE) between the H8 protons of the d(GpG) moiety and the cyclohexane ring protons was tried, no NOE signal was observed. (Table 2).

The NMR spectral pattern of No-2 looks virtually the same as that of [*cis*-Pt$(NH_3)_2$(d(GpG)–N7,N7)]$^{2+}$ with an *anti–anti* configuration,[5c,11] though no detailed conformational analysis was run. A characteristic difference between No-1 and No-2 was observed for the chemical shift of the H5′/H5″ protons of the 5′G side. The H5′/H5″ protons of No-1 resonated upfield by 0.2 ppm, compared with those of No-2. Such an upfield shift may be induced by a certain conformational change, so as to reduce a steric repulsion between the carbonyl group of the guanines and the cyclohexane ring. It seems as if there is a small change in the torsion angle about the glycosyl bond (toward *high-anti*), since the chemical shifts of H5′/H5″ are influenced by an anisotropic effect of the purines.[5a] From these results, it is concluded that No-1 (structure **A**) has a sterically more distorted structure.

Table 2. Chemical Shifts of the Protons Due to the Coordinated d(GpG) Moiety

		No-2	No-1			No-2	No-1
	H8	8.37	8.31		H8	8.62	8.65
5′G	H1′	6.23	6.22	3′G	H1′	6.24	6.21
5′G	H2′	2.75	2.78	3′G	H2′	2.69	2.78
5′G	H2″	2.59	2.62	3′G	H2″	2.52	2.62
5′G	H3′	4.70	(4.8)	3′G	H3′	4.73	(4.8)
5′G	H4′	4.12	4.12	3′G	H4′	4.21	4.28
5′G	H5′	3.90	3.70	3′G	H5′	4.10	4.12
5′G	H5″	3.70	3.45	3′G	H5″	4.04	4.09

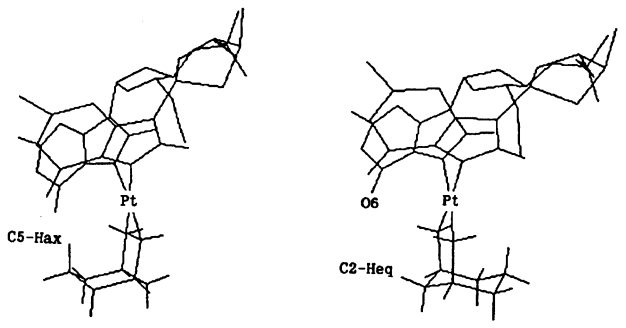

Fig. 3. Proposed structures of the two isomers, No-1 (**A**) and No-2 (**B**).

The authors thank Emeritus Professor Y. Kidani of Nagoya City University for his suggestions regarding this work. Part of this work was performed at Nagoya City University.

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3) Abbreviations: Pt(1*R*,2*R*-dach)–DNA, 1*R*,2*R*-cyclohexanediamineplatinum(II) modified DNA; Pt(1*R*,3*S*-dach)Cl_2, Dichloro-1*R*,3*S*-cyclohexanediamineplatinum(II); d(GpG), 2′-deoxyguanylyl(3′-5′)guanosine; C1–Heq, equatorial proton at position 1 of the cyclohexane ring; 3′G, 3′-guanine of d(GpG); N7, N7 site of guanine base; H5′/H5″, protons at position 5 of deoxyribose ring; ^2J, geminal coupling; ^3J, vicinal coupling; $^3J_{5eq-4ax}$, vicinal coupling between equatorial proton at position 5 and axial proton at position 4 of the cyclohexane ring.

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