Extended X-Ray Absorption Fine Structure Study on Reaction of Anti-tumor Platinum Complexes with Reduced Glutathione

Makoto OBATA,*[,a,1)] Masafumi HARADA,[b] Hiromi OHI,[c] Shiho HIROHARA,[d] Michael GOTTCHALDT,[e] and Shigenobu YANO[c]

[a] *Graduate School of Humanities and Sciences, Nara Women's University;* [b] *Faculty of Human Life and Environment, Nara Women's University; Nara 630–8506, Japan:* [c] *Endowed Research Section, Photomedical Science, Innovative Collaboration Center, Kyoto University; Kyoto 615–8520, Japan:* [d] *Graduate School of Materials Science, Nara Institute of Science and Technology; Nara 630–0192, Japan: and* [e] *Institute for Organic and Macromolecular Chemistry, Friedrich Schiller University of Jena; Humboldtstrasse 10, 07743 Jena, Germany.*

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Reactions of *cis*-diamminedichloroplatinum(II) (cisplatin) and 1,1-cyclobutanedicarboxylatodiammineplatinum(II) (carboplatin) with reduced glutathione, a tripeptide that is abundant in cells, were studied by means of X-ray absorption spectroscopy. Back-scattering amplitudes $F_i(k)$ and phase shifts $\Phi_i(k)$ were theoretically derived, and validated by applying them to calculate extended X-ray absorption fine structure (EXAFS) oscillations of cisplatin and $K_2[Pt(SCN)_4]$ in the solid state. EXAFS oscillations of reaction mixtures of cisplatin or carboplatin with reduced glutathione were fitted to the standard EXAFS equation using the $F_i(k)$ and $\Phi_i(k)$ functions to give the coordination numbers of N or O atoms ($N_{N/O}$) and of Cl or S atoms ($N_{Cl/S}$). For both cisplatin and carboplatin, the $N_{N/O}$ value decreased and the $N_{Cl/S}$ values increased monotonically as the reaction proceeded. However, the reaction rate for carboplatin was significantly slower than that for cisplatin.

Key words extended X-ray absorption fine structure; platinum complex; reduced glutathione

cis-Diamminedichloroplatinum(II), *i.e.*, cisplatin, is a potent inorganic anti-tumor drug agent,[2)] which is thought to exert its cytotoxic effect by forming intrastrand cross-links between N7 atoms of adjacent guanines of genomic DNA.[2,3)] When cisplatin is administered intravenously, however, it is exposed to various endogenous *S*-donor proteins. Platination of such proteins leads serious side effects, such as nephrotoxicity.[4)] In addition, some cisplatin-resistant tumors such as A2780cisR[5)] and SKOV-3[6)] have elevated levels of glutathione. Therefore, the interaction between anti-tumor platinum complexes and *S*-donor proteins plays an important role in drug-resistance and the appearance of side effects.[7)]

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool to investigate reactions between anti-tumor platinum complexes and S-donor proteins or peptides. Berners-Price and Kuchel studied the reactions of *cis* and *trans*-diamminedichloroplatinum(II) with reduced glutathione by means of ^{1}H, ^{13}C, ^{195}Pt and ^{15}N NMR spectroscopies using ^{15}N-enriched complexes.[8)] However, ^{15}N- or ^{195}Pt-enriched samples are expensive and hard to prepare, especially for modern sophisticated platinum complexes. For example, oxalato(1*R*,2*R*-cyclohexanediammine)platinum(II) (oxaliplatin), which is a third-generation platinum anti-tumor drug, has no replaceable NH_3 groups.

As an alternative approach, we have studied the properties of anti-tumor platinum complexes in a physiological environment by employing extended X-ray absorption fine structure (EXAFS) methodology. An EXAFS signal at the Pt L_{III} edge (11562 eV) of anti-tumor platinum complexes can be detected without any isotope-labeling. In addition, the absence of platinum ions in a biological system allows us to detect the EXAFS signal of anti-tumor platinum complexes selectively even in highly heterogeneous media, such as culture media and in cells. Analysis of the EXAFS signal using a suitable theoretical model affords direct information about coordinating species and distances, unlike NMR spectroscopy. In this communication, we present EXAFS analyses of the reactions of cisplatin and 1,1-cyclobutanedicarboxylatodiammineplatinum(II) (carboplatin) with reduced glutathione, an abundant intracellular tripeptide (Chart 1).

Back-scattering amplitudes $F_i(k)$ and phase shifts $\Phi_i(k)$ for Pt–N, Pt–Cl, Pt–O and Pt–S (i=N, Cl, O and S, respectively) were derived by means of *ab initio* self-consistent calculation using the FEFF 8.2 program.[9)] The calculated $F_i(k)$ and $\Phi_i(k)$ functions were examined for analyses of EXAFS oscillations of cisplatin and $K_2[Pt(SCN)_4]$ in the solid state. The obtained structural parameters, *i.e.*, interatomic distance r_i, coordination number N_i and Debye–Waller factor σ_i are listed in Table 1. In the case of cisplatin, the N_N value was slightly greater than expected, while the N_{Cl} value was in good agree-

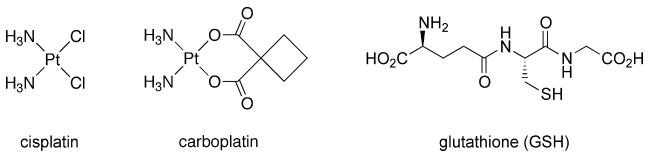

Chart 1. Structures of Anti-tumor Platinum Complexes and Reduced Glutathione

Table 1. Structural Parameters Deduced from the EXAFS of Cisplatin and $K_2[Pt(SCN)_4]$ in the Solid State[a)]

No.	Complex	Shell	N	r/Å	dE/eV	σ/Å	R/%
1	Cisplatin[b)]	Pt–N	2.3	2.04	4.2	0.076	1.2
		Pt–Cl	2.0	2.34	16.2	0.052	
2	Cisplatin[c)]	Pt–N	2.3	2.04	7.4	0.085	0.8
		Pt–Cl	1.8	2.34	17.0	0.049	
3	Cisplatin[d)]	Pt–N	2.2	2.05	11.6	0.084	0.5
		Pt–Cl	2.0	2.35	14.4	0.050	
	$K_2[Pt(SCN)_4]$	Pt–S	3.8	2.33	9.8	0.043	2.0

a) The estimated errors in the N and r values are ±0.6 and ±0.06 Å. *b*) $F_N(k)$ and $\Phi_{Cl}(k)$, and $F_N(k)$ and $\Phi_{Cl}(k)$ were used. *c*) $F_O(k)$ and $\Phi_O(k)$ were used for the Pt–N shell. *d*) $F_S(k)$ and $\Phi_S(k)$ were used for the Pt–Cl shell.

* To whom correspondence should be addressed. e-mail: mobata@yamanashi.ac.jp

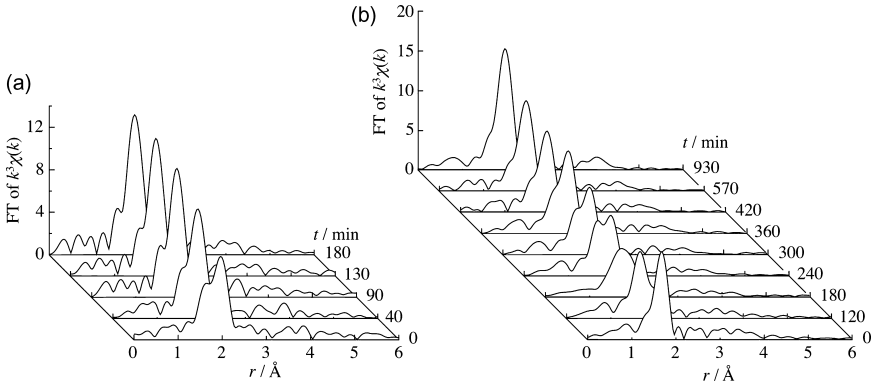

Fig. 1. Fourier Transforms of k^3-Weighted EXAFS Oscillation of the Reaction of Cisplatin (a) and Carboplatin (b) with Reduced Glutathione
Conditions: (a) $[\text{cisplatin}]_0=10$ mM, $[\text{glutathione}]_0=100$ mM, 37 °C; (b) $[\text{carboplatin}]_0=20$ mM, $[\text{glutathione}]_0=200$ mM, 37 °C.

ment with the composition. The r_i values for Pt–N and Pt–Cl were 2.04 and 2.34 Å, respectively, which also accorded well with those derived from X-ray crystallography[10] (2.00, 2.32 Å, respectively). The N_S and r_S values for $K_2[Pt(SCN)_4]$ were determined to be 3.8 and 2.33 Å, respectively, and were also close to those derived from X-ray crystallography[11] (4, 2.32 Å, respectively). Hence, we assumed an amplitude reduction factor S_0^2 of 1 in the standard EXAFS equation. In addition, analyses of the EXAFS oscillation using the $F_O(k)$, $F_S(k)$, $\Phi_O(k)$ and $\Phi_S(k)$ functions afforded essentially the same structural parameters (#2 and 3 in Table 1) as those given by the $F_N(k)$, $F_{Cl}(k)$, $\Phi_N(k)$ and $\Phi_{Cl}(k)$ functions. Therefore, we used the $F_N(k)$, $F_{Cl}(k)$, $\Phi_N(k)$ and $\Phi_{Cl}(k)$ functions for analyses of the reactions of anti-tumor platinum complexes with reduced glutathione.

Reaction of cisplatin with reduced glutathione was carried out in phosphate-buffered saline (PBS) at 37 °C. Figure 1a shows Fourier transforms of k^3-weighted Pt L_{III} EXAFS oscillations ($k^3\chi(k)$) of the reaction mixture at each reaction time. Two peaks were found at *ca.* 1.6 and 2.0 Å (before phase-shift correction) before the addition of reduced glutathione ($t=0$ min). These were assigned to back-scattering contributions of N and Cl atoms coordinated to Pt ion, respectively. As the reaction proceeds, the peak at 1.6 Å decreased, while the peak at 2.0 Å increased monotonically. After 180 min, the contribution from O/N became too small to fit, then the Fourier transform showed essentially a single peak at 2.0 Å.

The reaction rate of carboplatin with glutathione was significantly slower than that for cisplatin. Therefore the concentrations of carboplatin and reduced glutathione were adjusted to be twice those in the case of cisplatin. Figure 1b shows Fourier transforms of $k^3\chi(k)$ functions of the reaction mixture at each reaction time. At $t=0$ min, only one peak was found at *ca.* 1.6 Å, and this can be attributed to back-scattering contributions from N/O ligands. After addition of reduced glutathione, a new peak appeared at *ca.* 2.0 Å, and finally (at $t=930$ min), the peak at 1.6 Å disappeared.

Each $k^3\chi(k)$ function obtained was fitted to the standard EXAFS equation using the $F_N(k)$ and $\Phi_N(k)$, and $F_{Cl}(k)$ and $\Phi_{Cl}(k)$ functions. Figure 2 shows the N_i values as a function of reaction time. The r_i, N_i and σ_i values are listed in Tables 2 and 3. The $r_{N/O}$ and $r_{Cl/S}$ values are substantially constant at *ca.* 2.0 and 2.3 Å, respectively. Debye–Waller factors σ_i also fall within a narrow range from 0.05 to 0.08 Å. The $N_{N/O}$ values, however, decreased monotonically with the progress of both reactions, while the $N_{Cl/S}$ value increased from 2.2 to 3.5 for cisplatin and from 1.1 to 4.1 for carboplatin. This indicated that all the ligands were substituted with the S atom of reduced glutathione. Coordination of S atom releases NH_3 at the *trans* position. These phenomena and kinetics are good in accordance with the results of the NMR study reported by Berners-Price and Kuchel.[8] In the case of carboplatin, the ligands NH_3 and 1,1-cyclobutanedicarboxylato anion were also replaced by the S atom of reduced glutathione, but the substitution rate was significantly slower than that of cisplatin because of the chelating effect of the 1,1-cyclobu-

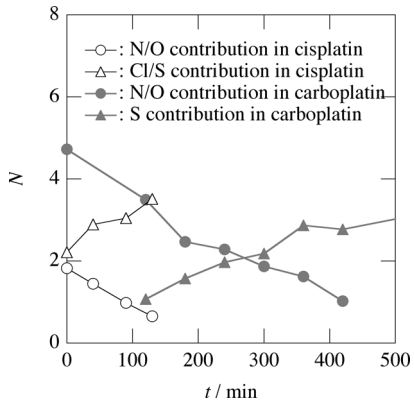

Fig. 2. The Plots of $N_{N/O}$ and $N_{Cl/S}$ Values as a Function of the Reaction Time t

Table 2. Structural Parameters Deduced from Pt L_{III} EXAFS of the Reaction Mixture of Cisplatin with Reduced Glutathione[a]

t/min	Shell	N	r/Å	dE/eV	σ/Å	R/%
0	Pt–N/O	1.8	2.01	−0.1	0.063	2.5
	Pt–Cl/S	2.2	2.32	15.0	0.059	
40	Pt–N/O	1.4	2.00	−7.1	0.080	2.4
	Pt–Cl/S	2.9	2.30	13.8	0.060	
90	Pt–N/O	1.0	1.98	−11.1	0.066	2.8
	Pt–Cl/S	3.0	2.30	12.9	0.053	
130	Pt–N/O	0.65	2.01	−9.7	0.071	1.9
	Pt–Cl/S	3.5	2.30	12.6	0.056	
180	Pt–N/O	—[b]	—[b]	—[b]	—[b]	2.5
	Pt–Cl/S	3.8	2.31	9.9	0.056	

a) $[\text{cisplatin}]_0=10$ mM; $[\text{reduced glutathione}]_0=100$ mM; PBS; 37 °C. The estimated errors in the N and r values are ±0.6 and ±0.06 Å. *b*) The back-scattering contribution from N/O was too small to fit.

Table 3. Structural Parameters Deduced from Pt L_{III} EXAFS of the Reaction Mixture of Carboplatin with Reduced Glutathione[a)]

t/min	Shell	*N*	*r*/Å	*dE*/eV	σ/Å	*R*/%
0	Pt–N/O	4.7	2.03	10.6	0.046	0.31
120	Pt–N/O	3.5	2.03	8.9	0.052	0.47
	Pt–S	1.1	2.30	14.1	0.056	
180	Pt–N/O	2.5	2.03	9.3	0.071	0.39
	Pt–S	1.6	2.31	9.8	0.071	
240	Pt–N/O	2.3	2.04	7.6	0.053	0.84
	Pt–S	2.0	2.32	13.6	0.045	
300	Pt–N/O	1.9	2.04	7.1	0.064	0.90
	Pt–S	2.2	2.32	14.0	0.046	
360	Pt–N/O	1.6	2.04	4.6	0.071	0.74
	Pt–S	2.9	2.32	13.7	0.051	
420	Pt–N/O	1.0	2.05	5.3	0.079	1.1
	Pt–S	2.8	2.32	13.0	0.050	
570	Pt–N/O	—[b)]	—[b)]	—[b)]	—[b)]	0.90
	Pt–S	3.2	2.31	11.6	0.055	
930	Pt–N/O	—[b)]	—[b)]	—[b)]	—[b)]	0.24
	Pt–S	4.1	2.32	11.2	0.052	

a) $[\text{carboplatin}]_0 = 20$ mM; $[\text{reduced glutathione}]_0 = 200$ mM; PBS; 37 °C. The estimated errors in the *N* and *r* values are ±0.6 and ±0.06 Å. *b*) The back-scattering contribution from N/O was too small to fit.

tanedicarboxylato anion.

The ultimate product of the reaction is thought to be a high-molecular-weight μ-S bridged species. In the EXAFS analysis, however, there was no significant contribution from vicinal Pt ions. This may be because of the longer distance between Pt ions and the absence of an efficient shade effect.

In conclusion, the EXAFS analyses of the reactions of cisplatin and carboplatin with reduced glutathione were performed by using standard theoretical methods without any tedious isotope-labeling. The results were good in agreement with those obtained by NMR analysis of isotope-labeled compounds. This technique should therefore be useful to examine the reactions of a variety of anti-tumor platinum complexes with endogenous *S*-donor proteins or peptides even in highly heterogeneous media, such as in culture.

Experimental

General Cisplatin and $K_2[Pt(SCN)_4]$ were prepared according to the conventional method. Carboplatin was purchased from STREM Chemicals, Inc. Reduced glutathione was purchased from Wako Pure Chemicals Inc. Dulbecco's phosphate-buffered saline (PBS) was purchased from Nacalai tesque.

Reaction of Cisplatin with Reduced Glutation Cisplatin (150 mg, 0.5 mmol) was dissolved in PBS (50 ml) by heating. After the solution had cooled, it was stirred at 37 °C. Reduced glutathione (1.53 g, 5 mmol) was added to initiate the reaction.

Reaction of Carboplatin with Reduced Glutation Carboplatin (153 mg, 0.4 mmol) was dissolved in PBS (20 ml). Reduced glutathione (1.24 g, 4 mmol) was added to initiate the reaction.

EXAFS Measurement EXAFS measurements were performed at beam lines 10B and 7C of the Photon Factory of the High Energy Acceleration Research Organization (KEK-PF), Tsukuba, Japan. The ring current was 300—450 mA, and the storage ring was operated with an electron energy of 2.5 GeV. The experiments at the Pt L_{III} edge (11562 eV) were carried out at room temperature in the transmission mode on samples in PBS solution in a glass cell (path length = 10 mm). The EXAFS oscillation ($k^3\chi(k)$) was extracted using standard procedures for preedge subtraction, data normalization, and spline removal. The obtained $k^3\chi(k)$ was Fourier transformed over the *k* range 2—16 $Å^{-1}$, using a Hanning window. After Fourier filtering and backtransforming the real space range including the Pt–O/N and Pt–Cl/S peaks (from 1 to 2.5 Å), the resulting $k^3\chi(k)$ function was used for least-squares refinements. The $k^3\chi(k)$ are theoretically given by the following equation:

$$k^3\chi(k) = S_0^2 \sum_i \left\{ \frac{k^2 N_i}{r_i^2} F_i(k) \exp(-2\sigma_i^2 k^2) \sin(2kr_i + \Phi_i(k)) \right\}$$

where S_0^2, r_i, N_i, $F_i(k)$, $\Phi_i(k)$, and σ_i represent the amplitude reduction factor, the interatomic distance, the coordination number, the back-scattering amplitude, the phase shift, and the Debye–Waller factor of the *i*-th shell, respectively, and *k* is the photoelectron wave vector defined as $k = [(2m/h^2)(E - E_0)]^{1/2}$ with the threshold energy E_0. The $F_i(k)$ and $\Phi_i(k)$ functions for the single scattering pathways were calculated by means of the FEFF 8.2 program.[9)] All calculations were performed with REX2000 ver.135 2.0.7 (Rigaku Co.).

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References and Notes

1) Present address: *Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi; 4–4–37 Takeda, Kofu 400–8510, Japan*.
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