Preparations and ^{13}C and ^{195}Pt NMR Spectra of Platinum(II) Peptide Complexes and Their Growth-Inhibitory Activity against Mouse Meth A Solid Tumor in Vivo

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Several dipeptide complexes of the form K[Pt(x-gly or gly-x)Cl], where x-gly or gly-x stands for a dipeptide anion (where x is glycine, alanine, valine, or leucine) moieties, were synthesized, purified, and characterized by various analytical and spectroscopic techniques (^{13}C, ^{195}Pt NMR and MS(FAB)). The complexes of the form K[Pt(x-gly)Cl] were pale brown and those of K[Pt(gly-x)Cl] were light yellow in solid form or in solution. The former was more labile than the latter in H_2O solution. Growth inhibition assays of K[Pt(alagly)Cl], K[Pt(glyala)Cl], and *cis*-diamminedichloro platinum(II) (Cisplatin) against methylcholanthrene induced Meth A fibrosarcoma (Meth A) solid tumors transplanted in BALB/c mice were measured. In mice in the group administered K[Pt(glyala)Cl] doses of 26 mg kg^{-1} d^{-1}, 28.9% of slight growth inhibition was observed. The side effects related to the decrease of body weight were mild. Cisplatin did not show any antitumor activity under the present administration conditions. The toxicities of the dipeptide complexes against normal mouse bone marrow cells were measured. All of them exhibited toxicity against bone marrow cells, but K[Pt(alagly)Cl] and K[Pt(glyala)Cl] were 1000 times less toxic than Cisplatin.

There has been a great deal of interest in the bioinorganic complexes of platinum(II) since the first report of the antitumor activities of *cis*-diamminedichloro platinum(II).[1] Many platinum complexes of nucleotides, functional amines, and amino acid esters, most of which are of the *cis*-dichloro type, have been prepared.[2—4] But in almost all cases, ammine or simple alkylamine complexes are toxic. Recently Tsubomura et al. have synthesized Cisplatin-type complexes of amino sugars which show good antitumor activity in vivo.[5] On the other hand, amino acid and several dipeptide complexes which contain sulfur atoms, for example, a methionine or cysteine residue, have been investigated because sulfur binds easily to platinum(II) and this binding might be important for biological systems.[6—9] The crystal structure of $[Pt(H_{-1}glymet)Cl]^-$ where glymet indicates glycylmethionine and H_{-1} a deprotonated N(peptide), shows that the amine nitrogen, the peptide nitrogen, and the thioether sulfur are coordinated to the metal.[10] The major difficulty in studying platinum complexes containing peptides is their kinetic inertness and, as a consequence, the difficulty in studying pure species.[11] When working in solution, the attainment of true thermodynamic equilibrium is a recurring problem. The peptide complexes of Co^{II}, Pd^{II}, Ni^{II}, and Cu^{II} [12—21] are known to be labile.

Only a few platinum(II) complexes containing di or tripeptides without sulfur atoms have been reported.[22—27] Volshtein and Motyagina have reported the preparation of *trans*-$[PtCl_2(diglyH)]_2$.[22] Mogilevkina et al. reported that the reaction of the compound with alkali gave $[Pt(diglyH)_2]$.[23] Recently, Appleton et al. reported the reactions of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ with digly and trigly.[24] Margerum et al. have shown that $[PtCl_4]^{2-}$ reacts with small oligoglycyl peptides to give complexes which have been characterized by ^{195}Pt NMR spectroscopy, with the use of ^{15}N-labeled ligands.[25] The structures differed greatly from those of the complexes of Co^{II}, Pd^{II}, Ni^{II}, Cu^{II} where in mono complexes, ML, dipeptides behave as

tridentate ligands with two 5-membered chelate rings with amino, deprotonated amide and carboxylate oxygen donors. On the other hand, carboxylate (O) is not coordinated in most of the platinum(II) complexes studied. Reactions of platinum(II) with oligopeptides have produced several species in all cases, and most of them have not crystallized,[26] except a few references with analytical data and/or X-ray crystal data.[10] ^{195}Pt NMR is useful for examining the number and the kind of Pt species in solution. We are interested in the nature of those platinum(II) complexes containing simple dipeptides, in the ability of platinum to form deprotonated N(peptide) bonds, and in carboxylate coordination of those platinum(II) complexes. In this paper, we describe the results of visible, ^{13}C and ^{195}Pt NMR and FABMS spectroscopy of the complexes containing x-gly or gly-x where x is a glycine, alanine, valine, or leucine moiety. The color and the stability of the complexes with gly-x differed greatly from those with x-gly, the former were yellow and stable, the later brown and labile.

Only neutral complexes have shown appreciable antitumor activity and the charged complexes tested have been inactive and relatively non-toxic.[27] The explanation could be related to membrane transport phenomena and/or the greater efficiency with which charged molecules are eliminated from the body. However, "Platinum Blues" which have deprotonated amide coordination are reported to be effective in antitumor activity.[28] Growth inhibition assays of K[Pt(alagly)Cl], K[Pt(glyala)Cl], and Cisplatin against Meth A solid tumors transplanted in BALB/c mice were measured.

Experimental

Starting Materials. Dipeptides except enriched dipeptides were purchased from Tokyo Kasei, and K_2PtCl_4 from Kojima Kagaku. All other reagents were of analytical grade. (^{15}N)glycyl(^{15}N)alanine and (^{15}N)alanyl(^{15}N)glycine were synthesized in our laboratory from (^{15}N)glycine (99%) and (^{15}N)alanine (99%) purchased from ISOTEC, Inc. Analytical data are as follows. Anal. Calcd for (^{15}N)gly(^{15}N)-alaH $C_4H_8N_2O_3$: C, 35.82; H, 6.01; N, 22,38%. Found: C, 35.61; H, 5.83; N, 22.44%. Calcd for (^{15}N)ala(^{15}N)glyH $C_4H_8N_2O_3$: C, 35.82; H, 6.01; N, 22,38%. Found: C, 35.61; H, 5.83; N, 22.44%.

Cisplatin was purchased from Nihon Kayaku Co., Ltd., Tokyo, Japan and was used as a positive control for antitumor assays in vivo and in vitro. Animals: Six-week old BALB/c male SPF mice were supplied by Nihon SLC, Shizuoka, Japan. Tumor cells: Meth A fibrosarcoma was used in this study and was obtained from the Cancer Cell Repository, Institute of Development, Aging and Cancer, Tohoku University, and was maintained by weekly passage (intraperitoneal inoculation) of ascites cells in mice. Alamar blue was purchased from Kanto Chemical Co., Inc., Tokyo, Japan.

Preparation of Potassium Chloro(dipeptidato)-platinate(II). To a solution of K_2PtCl_4 (1 mmol) in water was added an aqueous solution containing dipeptide (1 mmol). The mixture was heated at 60 °C with stirring. Potassium hydroxide solution (0.5 M, 1 M=1 mol dm^{-3}) was then added to bring the pH to 5.5. After about 15 h the solution was evaporated to dryness and the reaction mixture was partially dissolved in MeOH. The methanol solution was evaporated to a slurry. After 2—3 d, a small amount of potassium chloride was crystallized from the slurry. The powder left was the desired product. Yields were 33—40%. Calcd for K[Pt(digly)Cl]·H_2O, $KPtClC_4H_8N_2O_4$: C, 11.50; H, 1.93; N, 6.71%. Found: C, 11.55; H, 1.80; N, 6.63%. Calcd for K[Pt(^{15}N)gly(^{15}N)alaCl]·1.5H_2O, $KPtClC_5H_8N_2O_3$: C, 13.62; H, 2.52; N, 6.36%. Found: C, 13.53; H, 2.46; N, 6.13%. Calcd for K[Pt(^{15}N)ala(^{15}N)-glyCl]·1.5H_2O, $KPtClC_5H_8N_2O_3$: C, 13.62; H, 2.52; N, 6.36%. Found: C, 14.02; H, 2.52; N, 5.86%. Calcd for K[Pt-(glyval)Cl]·0.5H_2O, $KPtClC_7H_{12}N_2O_3$: C, 18.65; H, 2.91; N, 6.21%. Found: C, 18.89; H, 3.07; N, 6.24%. Calcd for K[Pt(valgly)Cl]·H_2O, $KPtClC_7H_{12}N_2O_3$: C, 18.28; H, 3.07; N, 6.09%. Found: C, 18.15; H, 3.12; N, 6.26%. Calcd for K[Pt(glyleu)Cl], $KPtClC_8H_{14}N_2O_3$: C, 21.08; H, 3.10; N, 6.15%. Found: C, 22.27; H, 3.72; N, 6.30%. Calcd for K[Pt-(leugly)Cl]·1.5H_2O, $KPtClC_8H_{14}N_2O_3$: C, 19.90; H, 3.55; N, 5.80%. Found: C, 19.64; H, 3.34; N, 5.78%.

Measurements. UV/vis spectra were recorded on a JASCO UVIDEC505 spectrometer. The mixture was kept in a pH stat, AT107 automatic titrater (Kyotodenshikougyou Ltd.) set to maintain pH values constant at 6 for 24 h. ^{13}C (125 MHz), ^{15}N (50.54 MHz), and ^{195}Pt (107.1 MHz) NMR spectra were recorded on JEOL ALPHA spectrometers. All ^{13}C and ^{195}Pt NMR spectra were proton-decoupled in D_2O. ^{195}Pt spectra were externally referenced to and relative to K_2PtCl_4. Referenced to Na_2PtCl_6 in D_2O, δ_{Pt} for K_2PtCl_4 is -1622. ^{13}C spectra were obtained with an internal deuterium lock, and were referenced to sodium 3-(trimethylsilyl)propansulfonate (TSP). Mass spectra were run on a JEOL JMS-SX102A mass spectrometer operating in the FAB mode (Xenon carrier gas) and JMA-DA9000 DATA system. Water was used as the solvent and methanol as the matrix. Elemental analyses (C, H,N) carried on a Yanaco MT-3 were within ±0.4% of the calculated values.

In vivo antitumor assays of Pt complexes against Meth A solid tumor: Mice were inoculated with 1×10^7 Meth A tumor cells subcutaneously in the right groin. The solutions of samples in sterilized phosphate buffered saline (PBS(−)) were administered intravenously on days 7, 14, 21. After 26 d of tumor inoculation, the average weight of the solid tumor was determined and compared with those of the non-treated group. The inhibition ratio of tumor growth was calculated as follows:

$$\text{Inhibition ratio } (\%) = (A - B)/A \times 100,$$

where A and B represent the mean tumor weight of the non-treated group and the mean tumor weight of the treated group, respectively.

Direct cytotoxicities of K[Pt(alagly)Cl] and K[Pt(glyala)-Cl] against Meth A cells: Meth A cells cultured in RPMI 1640 (10% FBS) were used for the experiment. Meth A cells were adjusted to a concentration of 2×10^5 cells/ml in RPMI 1640 (10% FBS), and 100 μl of this suspension was added to each well of a 96-well microtest plate. A solution of K[Pt(alagly)Cl] or K[Pt(glyala)Cl] (500, 50, or 5 μg/well

in PBS(−)) was added to the Meth A cells, and the cells were cultured for 48 h. To measure the cytotoxic activities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against Meth A cells, Alamar blue (Kanto Chemical Co., Inc., Tokyo, Japan) was added 3 h before the incubation was completed. After the incubation, the absorbance at 570 nm and 600 nm was measured. The values of the absorbance at 570—600 nm were represented as the number of viable Meth A cells. A solution of Cisplatin (5000, 500, or 50 μg/well in PBS(−)) was used as a positive control.

Toxicities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against normal bone marrow cells: Bone marrow cells were collected from the thigh bone of a normal BALB/c mouse. Treatment with tris-HCl buffer was conducted to exclude red blood cells. The obtained cells were adjusted to a concentration of 1×10^6 cells/ml in RPMI 1640, and 100 μl of this suspension were added to each well of a 96-well microtest plate. A solution of K[Pt(alagly)Cl] or K[Pt(glyala)Cl] (500, 50, 5, or 0.5 μg/well in PBS(−)) was added to the bone marrow cells and the cells were cultured for 48 h. To test the cytotoxicities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against normal bone marrow cells, [methyl-^{3}H]TdR (0.5 μ Ci/well) was added 5 h before incubation was completed. After the incubation, the cultured cells were harvested and radioactivity was measured using a liquid scintillation counter (Beckman Instruments Inc., CA). A solution of Cisplatin (5000, 500, or 50 μg/well) was used as a positive control. Statistical analysis of the data was performed using Student's t-test for significant differences.

Results and Discussion

Several dipeptide complexes of the form K[Pt(dipeptide)Cl] were synthesized, purified and characterized by various analytical and spectroscopic techniques (^{13}C and ^{195}Pt NMR, and FABMS). The results are presented and discussed with the structures given in **I** (Chart 1). The optically active carbon atom of the complexes is near the amine (head) moiety of the peptide when x-gly was used and near the amide (tail) moiety of the peptide when gly-x was used. Margerum et al.[25] reported that the ^{195}Pt NMR spectra of the complexes of fully ^{15}N-substituted di, tri, and tetra glycine with K_2PtCl_4 at pH 6—7 at 25 °C showed well-resolved peaks with coupling constants between nitrogen and platinum. Pt in all the complexes bonded to N(amine) and N(peptide), but not O(carboxylate). On the other hand, Appleton et al. have reported the complex $[\{Pt(NH_3)_2\}_2(digly)]SO_4$ in which Pt is bonded to O(carboxylate), N(peptide), and N(amine).[24]

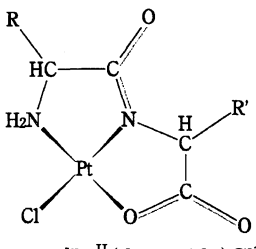

Chart 1. $[Pt^{II}(dipeptide)Cl]^-$ **I**.

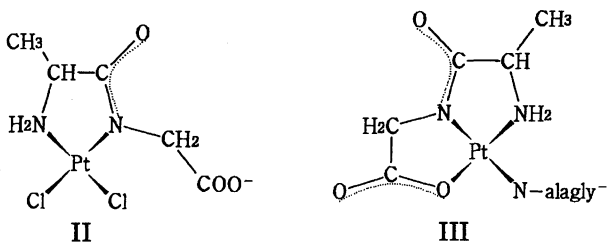

Chart 2. Proposed platinum complexes of the N_2Cl_2 (**II**) and the N_3O (**III**) type.

Reactions of K_2PtCl_4 with Dipeptides. In the course of the reaction of dipeptide with $[PtCl_4]^{2-}$, the color of the solution changed from red to yellow when gly-x was used and to pale brown when x-gly was used. The ^{195}Pt NMR spectra showed the existence of at least two species in the solution. After the solution was evaporated to dryness, the mixture was added to absolute methanol and partially dissolved. Satisfactory analytical results were obtained for the solid soluble in methanol. The complexes showed one ^{195}Pt peak (Fig. 1) and the solution of the complex with gly-x was yellow and that of x-gly pale brown as is shown in Fig. 2. The later showed broad peaks at 400 and 700 nm at high concentrations. In the FABMS spectra of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] the values 414 and 453 corresponded to the molecular weight of the K[Pt(alagly)Cl] and K[Pt(glyala)Cl]; M species, and $M+K^+$ ions.

^{13}C NMR. Table 1 shows the ^{13}C chemical shifts of the complexes prepared. When the coordinated carboxylate was part of a five-membered chelate ring, the carbon resonated in the vicinity of 190 ppm. As shown in Table 1, all the carbons of CO-2 resonated between 190 and 200 ppm. This indicates that all the carboxylates were coordinated to platinum. The ^{13}C peaks of CH-1 shift to a low field from about 8 to 11 ppm, and the amount of shift for the complexes with gly-x is larger than for x-gly, indicating that the alkyl groups compensate for the deficiency of the electron of the CH-1 carbon atoms owing to the metal coordination of the adjacent nitrogen atom. A similar trend is shown in the CH-2 carbon chemical shifts, that is, the magnitudes of the shifts for the complexes with x-gly are larger than those for gly-x.

^{195}Pt NMR Spectra of the Complexes with the ^{15}N Enriched Dipeptide. As is shown in Fig. 1, the complexes of the fully ^{15}N-substituted alanylglycine with K_2PtCl_4 show well-resolved ^{195}Pt NMR spectra, where individual coupling constants between nitrogen and platinum can be distinguished. The peak at −1924 ppm for K[Pt(glyala)Cl] is two doublets ($^1J_{Pt-N}=346$ and 507 Hz) due to coordination of ^{15}N to ^{195}Pt, which results in a 1 : 1 splitting of the resonance. Similar coupling constants ($^1J_{Pt-N}=373$ and 498 Hz) were obtained for K[Pt(alagly)Cl] with (^{15}N)alanyl(^{15}N)glycine. In this structure, the two coordinated nitrogens are chemically different, and each nitrogen splits the signal into

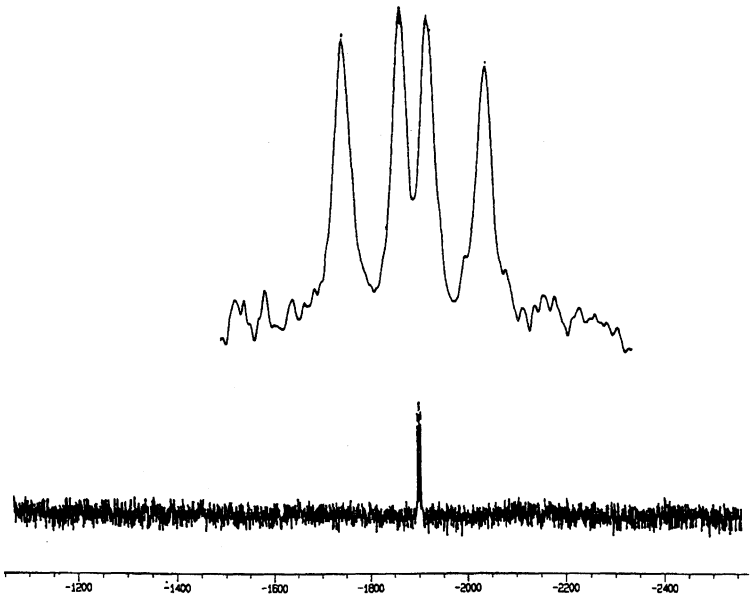

Fig. 1. ^{195}Pt NMR spectrum and splitting pattern for [Pt(alagly)Cl]$^-$, doublet of doublets with $^1J_{Pt-N}$=373 and 498 Hz, respectively.

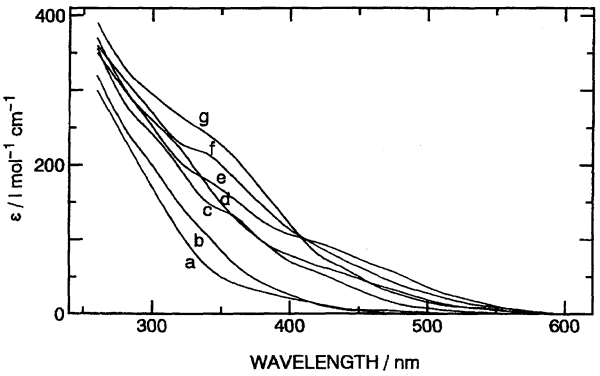

Fig. 2. UV-vis spectra of platinum(II) complexes of dipeptide, a; digly, b; glyala, c; glyval, d; glyleu, e; valgly, f; leugly, g; alagly.

a doublet with its own characteristic coupling constant. The larger coupling constant $^1J_{Pt-N}$=507 Hz for K[Pt(glyala)Cl] is attributed to the platinum-deprotonated peptide nitrogen interaction; the smaller $^1J_{Pt-N}$=346 Hz is attributed to the platinum–amine nitrogen.[26] The magnitude of the coupling constants reflects the strength of the platinum–nitrogen interactions and the degree of s character of the nitrogen bonding orbitals. The bond between the deprotonated nitrogens and platinum is stronger than the bond between the amine nitrogen and the metal center. There is little difference in the $^1J_{Pt-N}$ between [Pt(glyala)Cl]$^-$ and [Pt(alagly)Cl]$^-$.

^{195}Pt NMR Spectra. The chemical shifts of the ^{195}Pt NMR spectra of the platinum dipeptide species soluble in absolute methanol are shown in Table 2 along with those of the complexes reported. As is shown at the right of Table 2, very large chemical shifts (over a 1100 ppm range) were observed as ammonia, peptides,

Table 1. ^{13}C NMR Chemical Shifts (ppm)[a]

		CH-1[b]	CO-1	CH-2	CO-2
digly	Ligand	43.37	169.84	46.09	179.36
	Complex	51.69	183.14	53.75	193.76
glyala	Ligand	43.31	169.08	54.03	182.89
	Complex	54.29	182.99	58.86	196.86
glyval	Ligand	43.31	169.54	63.96	181.37
	Complex	54.47	182.57	68.22	194.05
glyleu	Ligand	43.31	169.36	57.06	182.81
	Complex	54.27	182.67	62.53	196.37
alagly	Ligand	52.08	173.50	46.17	179.21
	Complex	61.18	184.52	51.99	193.48
valgly	Ligand	61.84	172.20	46.07	179.07
	Complex	70.08	183.37	51.87	193.53
leugly	Ligand	55.02	173.17	46.14	179.05
	Complex	63.72	184.46	52.08	193.55

a) Shifts are relative to TSP (ppm). b) -1 and -2 in the table stand for the head moiety and the tail one.

and OH$^-$ groups replaced chlorides in $[PtCl_4]^{2-}$.[30—33] Margerum et al. have reported that the chemical shifts can be calculated from the linear relationships for Pt$(NH_3)_y(X)_{4-y}$.[29]

$$X = Cl^- \quad \delta_{Pt}{}^{calcd} = -1645 - 233y \qquad (1)$$

$$X = OH^- \quad \delta_{Pt}{}^{calcd} = -233 - 605y \qquad (2)$$

The $\delta_{Pt}{}^{obsd}$ and the $\delta_{Pt}{}^{calcd}$ did not always agree and they found systematic differences between variations in $\delta_{Pt}{}^{obsd}$ and $\delta_{Pt}{}^{calcd}$ when nitrogens from peptides rather than ammonia are coordinated and the chelate rings are formed. The first chelate ring formed by amine and deprotonated-N(peptide) donors gave $\delta_{Pt}{}^{obsd}$ values that were about 110 ppm more negative than the $\delta_{Pt}{}^{calcd}$ for two coordinated NH_3 molecules. The formation of

Table 2. ^{195}Pt NMR Chemical Shifts for Platinum(II) Complexes (ppm)[a)]

Complex	Doner	δ_{Pt}^{obsd}	Complex	Doner	δ_{Pt}^{obsd}
K[Pt(gly)Cl_2]	Cl_2NO	−1660	$[Pt(H_2O)_4]^{2+}$ [30)]	O_4	+31
K[Pt(ala)Cl_2]	Cl_2NO	−1650	$[Pt(OH)_4]^{2-}$ [31)]	O_4	−165
K[Pt(digly)Cl][b)]	ClN_2O	−1887	*cis*-$[PtCl_2(H_2O)_2]$ [32)]	Cl_2O_2	−811
K[Pt(glyala)Cl][b,c)]	ClN_2O	−1918	$[PtCl_3(H_2O)]^-$ [32)]	Cl_3O	−1185
K[Pt(glyval)Cl][b)]	ClN_2O	−1927	*cis*-$[Pt(NH_3)_2(OH)_2]$ [31)]	N_2O_2	−1572
K[Pt(glyleu)Cl][b)]	ClN_2O	−1935	*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ [24)]	N_2O_2	−1580
K[Pt(alagly)Cl][b,d)]	ClN_2O	−1946	$K_2[PtCl_4]$ [33)]	Cl_4	−1628
K[Pt(valgly)Cl][b)]	ClN_2O	−1950	$[Pt(NH_3)_3(OH)]^+$ [31)]	N_3O	−2062
K[Pt(leugly)Cl][b)]	ClN_2O	−1953	*cis*-$[Pt(NH_3)_2Cl_2]$ [31)]	Cl_2N_2	−2097
			$[Pt(NH_3)_3Cl]^+$ [31)]	ClN_3	−2354
			$[Pt(NH_3)_4]^{2+}$ [31)]	N_4	−2579

a) Shifts are relative to Na_2PtCl_6 (the shift for K_2PtCl_4 is −1922 ppm). b) This work. c) $^1J_{Pt-N}$ = 346 and 507 Hz for K[Pt(^{15}Ngly^{15}Nala)Cl]. d) $^1J_{Pt-N}$ = 373 and 498 Hz for K[Pt-(^{15}Nala^{15}Ngly)Cl].

a single chelate ring with glycine for $Pt(NH_3)_2$(gly-N, O)$^+$ had no appreciable effect on the ^{195}Pt NMR shift; δ_{Pt}^{obsd} for *cis*-$Pt(NH_3)_2$(gly-N)$(OH)^+$ were −2129 and −2126 ppm, respectively. We assume that the δ_{Pt}^{calcd} for the $Pt(N_2ClO)$ complex can be obtained from the average of Eq. 1 of $y=2$, that is for $Pt(N_2Cl_2)$ and Eq. 2 of $y=2$, that is for $Pt(N_2O_2)$. By the assumptions mentioned above, the δ_{Pt}^{calcd} for $Pt(N_2ClO)$ is expected to be $-1777-110(=-1887)$ ppm. All of the chemical shifts of the complexes obtained fall between −1880 and −1960 ppm. The δ_{Pt}^{obsd} of the complexes with x-gly are more negative than those of the complexes with gly-x for [Pt(dipeptide)Cl]$^-$. This indicates that the ligands of the type of x-gly are more basic than those of gly-x. A single chelate ring does not seriously distort the RNH_2–Pt–N– bond angle. Two linked five-membered rings of the [Pt(gly-x)Cl]$^-$ will force deviations from the ideal 90° bond angles between Pt and the cis-nitrogens to give a distorted square-planar geometry.

After heating [Pt(alagly)Cl]$^-$ at 80 °C in D_2O for 8 h, a small amount of black precipitate appeared in the NMR tube. Two new peaks appeared at δ_{Pt}^{obsd} = −2139 ppm and −2166 ppm in the ^{195}Pt NMR spectrum (Fig. 3). These chemical shifts of the new peaks indicate N_2Cl_2 or N_3O type coordination (Chart 2). The peptide ion and chloride ion released from the decomposed complex may recoordinate to the other platinum complexes as is expected in the forms of II and III, since no dimer was detected from in the FABMS spectra. The [Pt(glyala)Cl]$^-$ seemed to change slower than the [Pt(alagly)Cl]$^-$ under similar conditions.

Growth-Inhibitory Activity of the K[Pt(alagly)Cl] and K[Pt(glyala)Cl] Complexes against Mouse Meth A Solid Tumor in vivo. A delay in tumor growth was observed in K[Pt(glyala)Cl] administered mice. However, K[Pt(alagly)Cl] and Cisplatin did not show growth-inhibitory activity (Fig. 4). The inhibition of body weight was greater in Cisplatin administered mice and these mice appeared to be unhealthy. Mice administered Cisplatin had a bad coat of fur and decreases in momentum and the intake of food were also observed. However, the same phenomena were not observed in mice administered K[Pt(alagly)Cl] and K[Pt(glyala)Cl]. However, the body weight changes of mice administered K[Pt(alagly)Cl] and K[Pt(glyala)Cl] were the same as those of nontreated tumor-bearing control mice (Fig. 5).

Table 3 summarizes the results of the growth inhibition assay of Cisplatin and two conjugates against Meth A solid tumor transplanted in BALB/c mice. In mice

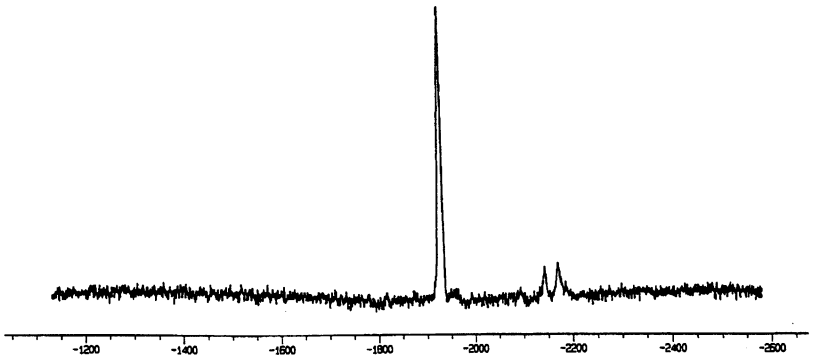

Fig. 3. ^{195}Pt NMR spectrum of the [Pt(alagly)Cl]$^-$ which was heated 80 °C for 6 h.

Table 3. Antitumor Activity of Pt Complexes against Meth A Solid Tumor Implanted in BALB/c Mice

Sample	Dose $mg\,kg^{-1}\,d^{-1}\times3$	Average tumor weight (g)	Inhibition ratio (%)	P[a)]
Tumor bearing control		10.4±0.9		
Cisplatin	10	8.9±4.5	15.0	NS
K[Pt(alagly)Cl]	13	8.7±1.5	16.9	NS
K[Pt(alagly)Cl]	26	11.0±1.8	−5.2	NS
K[Pt(glyala)Cl]	13	10.1±0.6	3.2	NS
K[Pt(glyala)Cl]	26	7.4±1.6	28.9	NS*

a) NS: Not significant. *: Vs to tumor bearing control and Cisplatin administered group.

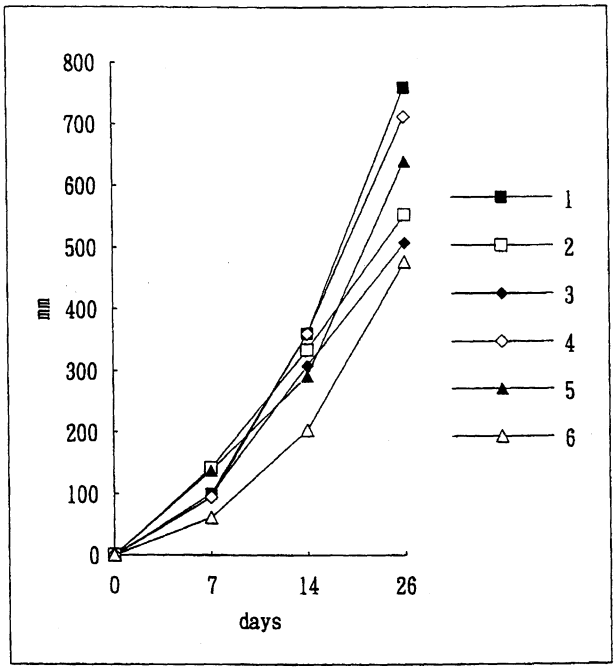

Fig. 4. Tumor growth of mice administered with $[Pt(dipeptide)Cl]^-$. 1: Tumor bearing control. 2: Cisplatin 10 $mg\,kg^{-1}\,d^{-1}\times3$. 3: $[Pt(alagly)Cl]^-$ 13 $mg\,kg^{-1}\,d^{-1}\times3$. 4: $[Pt(alagly)Cl]^-$ 26 $mg\,kg^{-1}\,d^{-1}\times3$. 5: $[Pt(glyala)Cl]^-$ 13 $mg\,kg^{-1}\,d^{-1}\times3$. 6: $[Pt(glyala)Cl]^-$ 26 $mg\,kg^{-1}\,d^{-1}\times3$.

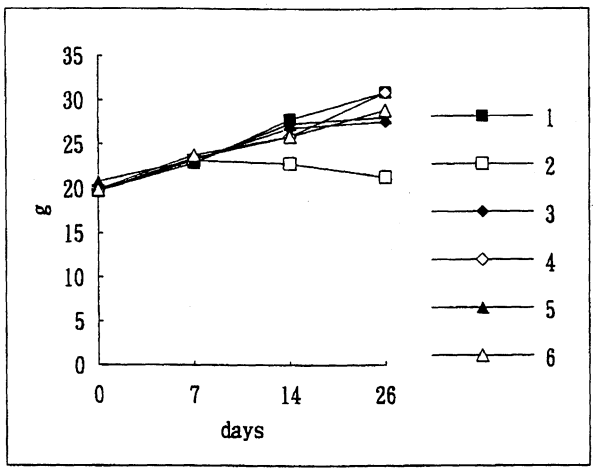

Fig. 5. Body weight changes of mice administered with $[Pt(dipeptide)Cl]^-$. 1: Tumor bearing control. 2: Cisplatin 10 $mg\,kg^{-1}\,d^{-1}\times3$. 3: $[Pt(alagly)Cl]^-$ 13 $mg\,kg^{-1}\,d^{-1}\times3$. 4: $[Pt(alagly)Cl]^-$ 26 $mg\,kg^{-1}\,d^{-1}\times3$. 5: $[Pt(glyala)Cl]^-$ 13 $mg\,kg^{-1}\,d^{-1}\times3$. 6: $[Pt(glyala)Cl]^-$ 26 $mg\,kg^{-1}\,d^{-1}\times3$.

in the group administered K[Pt(glyala)Cl] doses of 26 $mg\,kg^{-1}\,d^{-1}$, 28.9% of slight growth inhibition was observed. The above findings suggest that the side effects related to the decrease of body weight are mild. However, these two Pt conjugates did not show strong growth-inhibitory activity in Meth A inoculated mice, and Cisplatin did not show any antitumor activity under the present administration conditions.

Direct Cytotoxicities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against Meth A Cells and Normal Mouse Bone Marrow Cells. To confirm the antitumor activity, the direct cytotoxicities of K[Pt(alagly)Cl], K[Pt(glyala)Cl], and Cisplatin against Meth-A cells were measured. All of them exhibited direct cytotoxicity against Meth A cells, especially Cisplatin was 10 times more cytotoxic than the other agents (Table 4). A significant difference in the cytotoxic activity was not observed between K[Pt(alagly)Cl] and K[Pt(glyala)Cl]. To evaluate the side effects on the host immune system, the toxicities of K[Pt(alagly)Cl], K[Pt(glyala)Cl], and Cisplatin against normal mouse bone marrow cells were measured. All these compounds exhibited toxicity against normal bone marrow cells, but K[Pt(alagly)Cl] and K[Pt(glyala)Cl] were

Table 4. Direct Cytotoxicities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against Meth-A Cells[a)]

	Inhibition ratio (%)		
	Cisplatin	K[Pt(alagly)Cl]	K[Pt(glyala)Cl]
500 μg/well	ND	100	100
50 μg/well	ND	79.2	80.9
5 μg/well	82.5	7.9	9.0
500 ng/well	59.9	ND	ND
50 ng/well	13.1	ND	ND

a) ND: Not done.

Table 5. Toxicities of K[Pt(alagly)Cl] and K[Pt(glyala)Cl] against Normal Mouse Bone Marrow Cells

	Inhibition ratio (%)		
	Cisplatin	K[Pt(alagly)Cl]	K[Pt(glyala)Cl]
500 μg/well	ND	99.4	99.8
50 μg/well	ND	99.4	99.4
5 μg/well	99.8	33.8	41.7
500 ng/well	99.9	7.6	11.4
50 ng/well	91.8	ND	ND
5 ng/well	30.9	ND	ND

1000 times less toxic than Cisplatin (Table 5). No significant difference in cytotoxic activity was observed between K[Pt(alagly)Cl] and K[Pt(glyala)Cl].

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