Platinum(IV) Complexes with Dipeptide. X-ray Crystal Structure, ^{195}Pt NMR Spectra, and Their Inhibitory Glucose Metabolism Activity in *Candida albicans*

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Three dipeptide complexes of the form $K[Pt^{IV}(dipep)Cl_3]$ and two complexes of the form $K[Pt^{IV}(Hdipep)Cl_4]$ were newly prepared and isolated. The platinum(IV) complexes containing the dipeptide were obtained directly by adding KI to $H_2[PtCl_6]$ solution. The reaction using KI was rapidly completed and provided analytically pure yellow products in the form of K[Pt(dipeptide)Cl_3] for H_2digly, H_2glyα-ala, $H_2\alpha$-alagly and H_2diα-ala. The $K[Pt^{IV}$-(digly)Cl_3] complex crystallizes in the monoclinic space group $P2_1/c$ with unit cell dimensions $a = 10.540(3)$ Å, $b = 13.835(3)$ Å, $c = 8.123(3)$ Å, $\beta = 97.01(2)°$, $Z = 4$. The crystal data represented the first report of a Pt(IV) complex with a deprotonated peptide, and this complex has the rare iminol type diglycine(2−) coordinating to Pt(IV) with the bond lengths of the C2−N1 (amide) bond (1.285(13) Å). The ^{195}Pt NMR peaks of the $K[Pt^{IV}$-(dipep)Cl_3] and the $K[Pt^{IV}(Hdipep)Cl_4]$ complexes appeared at about 270 ppm and at about −130 ppm, respectively, and were predicted for a given set of ligand atoms. While the $K[Pt^{IV}(x\text{-gly})Cl_3]$ complexes, where x denotes the glycine or α-alanine moieties, were easily reduced to the corresponding platinum(II) complexes, the $K[Pt^{IV}(x$-α-ala)Cl_3] complexes were not reduced, but the Cl^- ion was substituted for OH^- ion in the reaction solution. The K[Pt(digly)Cl_3] and K[Pt(gly-L-α-ala)Cl_3] complexes inhibited the growth of *Candida albicans*, and the antifungal activities were 3- to 4-fold higher than those of cisplatin. The metabolism of glucose in *C. albicans* was strongly inhibited by K[Pt(digly)Cl_3] and K[Pt(gly-L-α-ala)Cl_3] but not by the antifungal agent fluconazole.

Introduction

There has been increasing interest in complexes of platinum(IV) with biologically important ligands because of the recent development of new anticancer drugs of platinum(IV)[1,2] and the evidence that they may react with DNA without being reduced to platinum(II).[3] There are many fewer examples of platinum(IV) amino acid or dipeptide complexes.[4−6] Conversion of the platinum(II) complexes to platinum(IV) analogues is another approach to the preparation of new antitumor platinum complexes.[7] We have reported the synthesis of the platinum(IV) complexes, which have been prepared by the oxidation of the platinum(II) complexes with H_2O_2, and their antifungal activities.[8] $[PtC1_6]^{2-}$ as the starting material is described to be unsuitable due to its kinetic inertness and/or a partial reduction to give platinum(II).[7] Recently, we reported the preparation of the platinum(II) complexes with dipeptides containing β-alanine or amino acid methyl esters.[9] The addition of an aqueous solution containing the dipeptide of β-alanine to a solution of K_2PtCl_4 has produced several species. As the pH of the solution decreased, it was kept at ca. 6 by addition of a 1 N KOH solution. More than 10 h at 60 °C was needed until the pH of the solution became nearly constant. The isolation of the desired complex from the mixture containing the green byproduct was not easy. The addition of KI to K_2PtCl_4 aqueous solution in the first step and of $AgNO_3$ in the last step was rapidly completed and provided analytically pure yellow products in the form of K[Pt(dipeptide)Cl] for glyβ-ala^{2-}, β-alagly^{2-}, and diβ-ala^{2-}. It is important to prepare the platinum(IV) complexes with oligopeptides from Pt(IV) complexes as a starting material, not by the oxidation of the corresponding Pt(II) complexes. It is also important to isolate the platinum(IV) complexes with oligopeptides in order to determine their chemical and chemotherapeutic properties. In our continuing efforts to study platinum chemistry, we are interested in the deprotonated property of the amide NH and the additivity rule of the ^{195}Pt NMR chemical shifts of the platinum complexes due to a given set of ligand atoms.[10] We report here the first synthesis of novel platinum(IV) complexes with good yield from the reaction mixture of $H_2[PtCl_6]$, KI, and oligopeptides without H_2O_2 oxidation and the antifungal activity of their complexes in vitro.

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Table 1. Crystal Data for the $K[Pt(digly)Cl_3]\cdot H_2O$ Complex

empirical formula	$PtKCl_3C_4H_8N_2O_4$
fw	488.65
crystal color, habit	pale yellow, needle
crystal dimens, mm	$0.4 \times 0.1 \times 0.10$
cryst syst	monoclinic
space group	$P2_1/n$
a, Å	10.540(2)
b, Å	13.835(3)
c, Å	8.123(3)
β, deg	97.01(2)
V, Å^3	1175.7(5)
Z	4
D_x, M gm^{-3}	2.760
D_m, M gm^{-3}	2.750
T, K	296
λ(Mo Kα), Å	0.71073
μ, cm^{-1}	130.97
θ_{max}, deg	26.47
no. of reflns measd	3114
no. of unique reflns/R_{int}	2589/0.018
no. of observations ($I > 2.00\sigma(I)$)	2485
no. of variables	160
max peak in final diff map, e Å^{-3}	1.20
min peak in final diff map, e Å^{-3}	−3.45
R	0.031
R_w	0.042
S	1.124

Experimental Section

Starting Materials. H_2PtCl_6 was purchased from Tanaka Kikinzoku, and all other reagents were purchased from Tokyo Kasei.

Data Collection. The crystal data and details of the data collection and refinement for the complexes are given in Table 1. The complex was recrystallized from water. Cell constants were determined on a Mac Science MXC18 four-circle automated diffractometer from the setting angles of 20−25 reflections. The data collection was carried out on a Mac Science MXC18 diffractometer. Intensities were measured by the $2\theta-\omega$ scan method using Mo Kα radiation ($\lambda = 0.71073$ Å). A total of 2589 independent intensities ($2\theta < 50°$) was measured for $K[Pt(digly)Cl_3]$. Of these, there are 2485 unique reflections with $I > 3.0\sigma(I)$ which were used in the solutions and refinements of the structures. Intensities were corrected for Lorentz and polarization effects, and an empirical correction based on a series of ψ scans was applied. Atomic scattering factors and anomalous dispersion effects were taken from the usual tabulation. All calculations were performed using the Crystan crystallographic software package from the Molecular Structure Corporation.

Determination of the Structures. The structure was solved by heavy-atom Patterson methods. The platinum atom was located in the initial E map, and subsequent Fourier syntheses gave the positions of the other non-hydrogen atoms. Hydrogen atoms were calculated at the ideal positions and were not refined. The non-hydrogen atoms were refined with anisotropic thermal parameters using full-matrix least-squares methods. The final refinement converged to $R = 0.031$ and $R_w = 0.042$. Final difference Fourier syntheses showed peaks at heights up to 1.94−3.55 e A^{-3}.

Preparation of the K[Pt(dipep)Cl$_3$] Complexes. $H_2[PtCl_6]\cdot 5H_2O$ (1.00 g, 2 mmol) was dissolved in 20 mL of water. To the solution was added a solution of KI (1.99 g, 12 mmol) in 20 mL of H_2O, and then the reaction mixture was heated to 70 °C for 2 min. An aqueous solution (20 mL) of H_2dipep (2 mmol) was mixed with the solution, and the heating was continued for 2 h. The pH was adjusted to about 5.5−6.5 with a drop of 1 M KOH solution every 5 min (the total volume of KOH solution was 9 mL). A 20 mL water solution of $AgNO_3$ (3.058 g, 18 mmol) was then added to the reaction mixture with stirring. After the suspension was stirred for 10 min and allowed to stand at room temperature, AgI and AgCl were filtered off. The resulting solution was then evaporated to a slurry. To this was added a water−methanol (1:4) solution, the deposited KNO_3 was removed by filtration, and the filtrate was evaporated to dryness. Repeating the removal of KNO_3 three times gave a crude yellow powder.

Isolation of the Complexes. In the digly^{2-} complex, to crude yellow crystals (1 g) was added 10 mL of cooled water, and the deposit was filtered, giving yellow pure crystals or powder. In the glyα-ala^{2-}, α-alagly^{2-}, and diα-ala^{2-} complexes, the KNO_3 was removed from the crude complex by the addition of a water−methanol (1:4, 10 mL) solution and its filtration. The solution was evaporated to dryness, and a yellow powder was obtained. The desalting was repeated three times, and the powder was placed in a vacuum desiccator for a few days. Yield: 53% for $K[Pt(digly)Cl_3]$, 86% for $K[Pt(gly\alpha\text{-}ala)Cl_3]$, 29% for $K[Pt(\alpha\text{-}alagly)Cl_3$, 87% for $K[Pt(di\alpha\text{-}ala)Cl_3]$. Anal. Calcd for $K[Pt(digly)Cl_3]\cdot H_2O$, $KPtCl_3C_4H_6N_2O_3\cdot H_2O$: C, 9.83; H, 1.65; N, 5.73. Found: C, 9.54; H, 1.49; N, 5.84. Anal. Calcd for $K[Pt(gly\alpha\text{-}ala)Cl_3]$, $KPtCl_3C_5H_8N_2O_3$: C, 12.39; H, 1.66; N, 5.78. Found: C, 12.89; H, 2.17; N, 6.36. Anal. Calcd for $K[Pt(\alpha\text{-}alagly)Cl_3]$, $KPtCl_3C_5H_8N_2O_3$: C, 12.39; H, 1.66; N, 5.78. Found: C, 11.95; H, 2.23; N, 6.05. Anal. Calcd for $K[Pt(di\alpha\text{-}ala)Cl_3]$, $KPtCl_3C_6H_{10}N_2O_3$: C, 14.45; H, 2.02; N, 5.62. Found: C, 14.22; H, 2.39; N, 5.72.

K[Pt(Hdipep)Cl$_4$]. $K[Pt(dipep)Cl_3]$ (0.200 g) was dissolved in 3 mL of water. One milliliter of 1 N HCl was then added to this solution, and the mixture was warmed at 70 °C for 10 min, evaporated to dryness using a rotary evaporator, and then placed in a desiccator containing P_2O_5 for a few days. A yellow powder was obtained. The yield was quantitative. Anal. Calcd for $K[Pt(Hdigly)Cl_4]\cdot H_2O$, $KPtCl_4C_4H_7N_2O_3\cdot H_2O$: C, 9.15; H, 1.73; N, 5.33. Found: C, 9.54; H, 1.49; N, 5.84. Anal. Calcd for $K[Pt(Hgly\alpha\text{-}ala)Cl_4]$, $KPtCl_4C_5H_9N_2O_3$: C, 11.52; H, 1.74; N, 5.38. Found: C, 11.86; H, 2.17; N, 5.86. Anal. Calcd for $K[Pt(H\alpha\text{-}alagly)Cl_4]$, $KPtCl_4C_5H_9N_2O_3$: C, 11.52; H, 1.74; N, 5.38. Found: C, 11.95; H, 2.23; N, 5.45. Anal. Calcd for $K[Pt(Hdi\alpha\text{-}ala)Cl_4]$, $KPtCl_4C_6H_{11}N_2O_3$: C, 13.47; H, 2.07; N, 5.23. Found: C, 13.86; H, 2.39; N, 5.72.

Measurements. The ^{13}C and ^{195}Pt NMR spectra were recorded using a JEOL EX-270 spectrometer. An internal lock on deuterium from the D_2O solvent was used in all measurements. The ^{195}Pt NMR spectra were referenced relative to Na_2PtCl_6 (external standard, −1622 ppm for $K_2[PtCl_4]$). The ^{13}C NMR shifts are reported relative to the methyl signal of sodium 3-(trimethylsilyl)propionate (TSP) as the internal reference. Mass spectra were run on a JEOL JMS-SX102A mass spectrometer operating in the FAB mode (xenon carrier gas) using the JMA-DA9000 DATA system. Water was used as the solvent and methanol as the matrix.

In Vitro Assay. The prepared $K[Pt(digly)Cl_3]$ and $K[Pt(gly\text{-}L\text{-}\alpha\text{-}ala)Cl_3]$ complexes were dissolved in Ca^{2+}, Mg^{2+}-free phosphate-buffered saline (PBS(−)). Cisplatin (Nippon Kayaku Co., Ltd., Japan) and fluconazole (Pfizer Pharmaceuticals, Inc.) were used as positive controls.

Fungus. *C. albicans* NIH A207 was cultured in Sabouraud's liquid medium (1% peptone, 2% glucose, 0.5% yeast extract) at 27 °C for 24 h with shaking.

Candidacidal Activity. *C. albicans* cells (1×10^3 cells/mL) were mixed with $K[Pt(digly)Cl_3]$ or $K[Pt(gly\text{-}L\text{-}\alpha\text{-}ala)Cl_3]$ or a control sample (cisplatin or fluconazole) and then incubated at 27 °C for 24 h. After incubation, the growth of the *C. albicans* cells was measured by the optical density at 620 nm as previously described.[8] In order to test the effects of $K[Pt(digly)Cl_3]$ and $K[Pt(gly\text{-}L\text{-}\alpha\text{-}ala)Cl_3]$ on the glucose metabolism in *C. albicans*. *C. albicans* suspension (5×10^7 cells/mL in 2% glucose-PBS(−), 0.1 mL) was mixed with $K[Pt(digly)Cl_3]$ or $K[Pt(gly\text{-}L\text{-}\alpha\text{-}ala)Cl_3]$ or fluconazole, and 10 μL of Alamar Blue (Alamar Biosciences, Inc., CA) was added. The mixture was incubated at 37 °C for 3 h, and the value of the difference between OD_{540} and OD_{620} was then measured.

Results and Discussion

Preparation of the Complexes. When an aqueous solution containing dipeptide was added to a solution of H_2PtCl_6 at 70 °C, the pH of the solution decreased gradually. 1 N KOH or $KHCO_3$ solution was added in order to keep the pH at ca. 6. The pH of the solution did not become constant for a long time. The reaction mixture gave very poor NMR spectral data along with bad S/N ratios, indicating the poor yield of the platinum(IV) complexes or the partial reduction of the platinum(IV). The isolation of the desired complex from the mixture containing the byproduct was not easy, and the yield was small. The

Table 2. Selected Bond Lengths (Å) and Angles (deg) for $K[Pt^{IV}(digly)Cl_3]$

Selected Bond Lengths (Å)			
Pt(1)-Cl(1)	2.359(3)	Pt(1)−Cl(2)	2.305(3)
Pt(1)−Cl(3)	2.317(3)	Pt(1)−O(2)	2.004(8)
Pt(1)−N(1)	1.973(9)	Pt(1)−N(2)	2.032(9)
N(1)−C(2)	1.283(14)	O(1)−C(2)	1.272(13)
C(1)−C(2)	1.525(16)	O(2)−C(4)	1.317(13)
O(3)−C(4)	1.209(14)	N(1)−C(3)	1.461(15)
C(4)−C(3)	1.532(17)	N(2)−C(1)	1.491(16)
Angles (deg)			
Cl(1)−Pt(1)−Cl(2)	91.4(1)	Cl(1)−Pt(1)−Cl(1)	90.2(2)
Cl(1)−Pt(1)−O(2)	97.1(3)	Cl(1)−Pt(1)−N(1)	179.3(3)
Cl(1)−Pt(1)−N(2)	95.1(3)	Cl(2)−Pt(1)−Cl(1)	178.3(2)
Cl(2)−Pt(1)−O(2)	87.5(3)	Cl(2)−Pt(1)−N(1)	88.3(3)
Cl(2)−Pt(1)−N(2)	89.5(3)	Cl(1)−Pt(1)−O(2)	91.8(3)
Cl(1)−Pt(1)−N(1)	90.1(3)	Cl(1)−Pt(1)−N(2)	90.8(3)
O(2)−Pt(1)−N(1)	83.5(4)	O(2)−Pt(1)−N(2)	167.5(4)
N(1)−Pt(1)−N(2)	84.3(4)	Pt(1)−O(2)−C(4)	114.1(7)
C(3)−N(1)−C(2)	127.8(9)	Pt(1)−N(1)−C(2)	117.2(7)
N(2)−C(1)−C(2)	113.6(10)	Pt(1)−N(1)−C(3)	114.4(7)
O(2)−C(4)−O(3)	124.3(11)	Pt(1)−N(2)−C(1)	108.7(8)
O(3)−C(4)−C(3)	118.1(10)	O(2)−C(4)−C(3)	117.6(10)
N(1)−C(3)−C(4)	109.4(10)	O(1)−C(2)−C(1)	119.9(10)
O(1)−C(2)−N(1)	124.3(10)	N(1)−C(2)−C(1)	115.7(10)

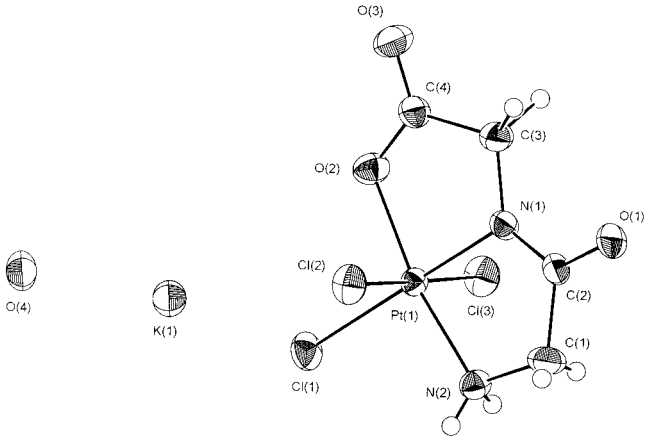

Figure 1. ORTEP drawing of the anion of $K[Pt^{IV}(digly)Cl_3]$ with 50% probability ellipsoids giving atomic numbering.

reaction using KI was completed in 2 h at 70 °C and provided analytically pure yellow products in the form of K[Pt(dipeptide)-Cl_3] for glyα-ala^{2-}, α-alagly^{2-}, and diα-ala^{2-}. In the case of the digly complex the strategy for obtaining the complexes is as follows:

$$H_2[PtCl_6] + 6KI \rightarrow [PtI_6]^{2-} + 6K^+ + 2H^+ + 6Cl^- \quad (1)$$

$$[PtI_6]^{2-} + 6K^+ + 2H^+ + 6Cl^- + H_2digly + 4KOH \rightarrow K[Pt(digly)I_3] + 3KI + 6KCl + 4H_2O \quad (2)$$

$$K[Pt(digly)I_3] + 3KI + 6KCl + 9AgNO_3 \rightarrow K[Pt(digly)Cl_3] + 9KNO_3 + 6AgI\downarrow + 3AgCl\downarrow \quad (3)$$

In eq 2, 6KCl was put in both the right side and the left side of the equation, because it became important in eq 3. In eq 3, iodide ions of $K[Pt(digly)I_3]$ in the solution containing chloride ion were substituted for chloride ions by adding an appropriate amount of Ag^+ ion, and 6AgI + 3AgCl precipitated.

Our crystal data are the first report of the Pt(IV) complex with a deprotonated peptide. The selected bond distances and bond angles for the $K[Pt(digly)Cl_3]$ complexes are listed in Table 2, and the structure of $K[Pt(digly)Cl_3]$ is shown in Figure 1. The complex anion adopts a six-coordinate geometry with oxygen (O2), amide (N1), and amine (N2) of the tridentate

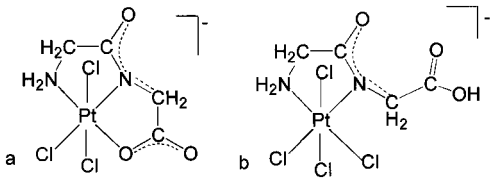

Figure 2. The structural formula of (a) $K[Pt^{IV}(digly)Cl_3]$ and (b) $K[Pt^{IV}$-$(Hdigly)Cl_4]$.

diglycine anion (digly^{2-}) and three chlorides (Cl1, Cl2, and Cl3). The platinum atom is in the center of an approximately octahedral environment. The five-membered chelate ring formed by Pt1, N2, C1, C2, and N1 has an envelope conformation in which the C1 and C2 atoms are displaced by −0.231 and −0.110 Å, respectively, on the same side of the plane defined by Pt1, N1, and N2. The dihedral angle between the plane Pt1, N1, C3 and the plane Pt1, N1, C2 is 0.8°. This indicates that the N1 nitrogen atom is also sp^2 hybridized and that the iminole structure is consistent with the bond lengths of the peptide moiety, i.e., the C2−N1 (amide) bond (1.285(13) Å) for the complex is shorter than that for the $K[Pt^{II}(digly)Cl]$ complex (1.34(1) Å). The O1−C2 (amide) bond (1.272(2) Å) for the complex is slightly longer than that for the $K[Pt^{II}(digly)Cl]$ complex (1.251(10) Å) but much shorter than an ordinary single bond (1.43 Å). It is also noted that the bond length of Pt−Cl1 is much longer than those of Pt−Cl2 and Pt−Cl3. In the FAB mass spectrum of $K[Pt^{IV}(digly)Cl_3]$, the values corresponded to the molecular weight of the $K[Pt^{IV}(digly)Cl_3]$; 437 for M − Cl^- and 473 for M species, and those of the other dipeptide complexes revealed their structure to be a monomer. The K[Pt-$(Hdipep)Cl_4$] were prepared as follows: 0.200 g of K[Pt(dipep)-Cl_3] was dissolved in 3 mL of water. HCl (1 N) was added to the solution, and the solution was warmed at 70 °C for 10 min and then evaporated to dryness. The ^{195}Pt NMR peaks appearing at about −2150 ppm for the platinum complexes containing x-gly^{2-} were assigned to those of the $K[Pt^{II}(Hdigly)Cl_2]$ and $K[Pt^{II}(H\alpha\text{-alagly})Cl_2]$ complexes as shown in Figure 2. It has been well established from the study of numerous peptide complexes that protonation occurs first at the peptide oxygen, because of its greater basicity, rather than at the peptide nitrogen.[11] We have reported the structure of $H[Pt^{II}(Hdigly)$-Cl], which has an iminole type structure between the amide N and N-terminal C atom (1.29(2) Å).[12] This result has been consistent with the protonation site being the acetyl oxygen rather than the amide nitrogen for $[Pt(NH_3)_2(Hacgly\text{-}N,O)]^+$, where Hacgly stands for acetylglycine, based on the NMR spectra.[13]

Fairlie et al. have reported amido−iminol tautomerism for the diethylenetriamine platinum(II) complexes with carboxylic acid amides or urea.[14,15] When dienPt coordinates, only N-coordination stabilizes both the amide (NH_2COR) and iminol (NH=C(OH)R) tautomers, but this stabilization depends on R; when R is $N(CH_3)_2$, the amide structure is stabilized and when R is CH_3, the iminole one is stabilized. We have prepared the Pt(II) complexes containing a dipeptide which coordinated as a tridentate or bidentate ligand. The $H[Pt^{II}(Hdigly)Cl_2]$ complex

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Table 3. ^{195}Pt NMR Chemical Shifts[a] for Platinum(II)/(IV) Complexes (ppm)

Pt(IV) complex	donor	δ_{Pt}^{obsd}	Pt(IV) complex	donor	δ_{Pt}^{obsd}	Pt(II) complex	donor	δ_{Pt}^{obsd}
K[Pt(digly)Cl_3]	Cl_3N_2O	279	K[Pt(digly)Cl$(OH)_2$][b]	ClN_2O_3	1270	K[Pt(digly)Cl[c]	ClN_2O	−1870
K[Pt(glyala)Cl_3]	Cl_3N_2O	267	K[Pt(glyala)Cl$(OH)_2$][b]	ClN_2O_3	1249	K[Pt(glyala)Cl][c]	ClN_2O	−1908
K[Pt(alagly)Cl_3]	Cl_3N_2O	247	K[Pt(alagly)Cl$(OH)_2$][b]	ClN_2O_3	1231	K[Pt(alagly)Cl][c]	ClN_2O	−1935
K[Pt(diala)Cl_3]	Cl_3N_2O	233	K[Pt(diala)Cl$(OH)_2$][b]	ClN_2O_3	1207	K[Pt(diala)Cl][c]	ClN_2O	−1957
K[Pt(Hdigly)Cl_4]	Cl_4N_2O	−115	K[Pt(Hdigly)$Cl_2(OH)_2$][b]	$Cl_2N_2O_2$	899	K[Pt(Hdigly)Cl_2][c]	Cl_2N_2	−2140
			H[Pt(Hdigly)$Cl_2(OH)_2$][b]	$Cl_2N_2O_2$	859	H[Pt(Hdigly)Cl_2][c]	Cl_2N_2	−2115
K[Pt(Hglyala)Cl_4]	Cl_4N_2	−107	K[Pt(Hglyala)$Cl_2(OH)_2$][b]	$Cl_2N_2O_2$	967	K[Pt(Hglyala)Cl_2][c]	Cl_2N_2	−2111
K[Pt(Halagly)Cl_4]	Cl_4N_2	−148	K[Pt(Halagly)$Cl_2(OH)_2$][b]	$Cl_2N_2O_2$	873	K[Pt(Halagly)Cl_2][c]	Cl_2N_2	−2185
K[Pt(Hdiala)Cl_4]	Cl_4N_2	−133 −143	K[Pt(Hdiala)$Cl_2(OH)_2$][b]	$Cl_2N_2O_2$	919	K[Pt(Hdiala)Cl_2][c]	Cl_2N_2	−2161

[a] The shifts are relative to Na_2PtCl_6 (the shift for K_2PtCl_4 is −1622 ppm). [b] Previous work (ref 8). [c] Previous work (ref 12).

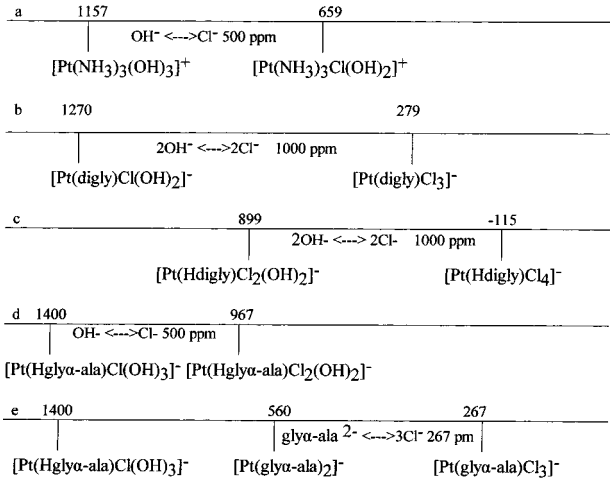

Figure 3. The relationship between the ^{195}Pt NMR chemical shift and the structure.

has an iminole in Pt−N−C bond, but the K[Pt^{II}(digly)Cl] complex does not have this type. The Pt(IV) complex obtained at this time has the same iminole structure as the H[Pt^{II}(Hdigly)Cl_2] complex, though the aqueous solution of the complexes is neutral.

The ^{195}Pt and ^{15}N NMR Spectra. Table 3 shows the ^{195}Pt NMR chemical shifts of the complexes. The peaks near 270 ppm were assigned on the basis of the data of the X-ray crystallography of the K[Pt^{IV}(digly)Cl_3]. The ^{195}Pt NMR chemical shifts of the [Pt$(NH_3)_3$Cl$(OH)_2$] and [Pt$(NH_3)_3(OH)_3$] complexes have been reported to be 659 and 1157 ppm, respectively,[17] indicating that the substitution of a Cl^- ion for one OH^- ion has caused a peak at about 500 ppm downfield shift (Figure 3a). The ^{195}Pt NMR peak of the [Pt(digly)Cl_3] complex is expected to appear near 270 ppm because this complex is the one with two chloride ions substituted for two OH^- ions of [Pt(digly)Cl $(OH)_2]^-$, of which the peak was at 1270 ppm (Figure 3b). For the same reason, the peak at −115 ppm was assigned to that of the K[Pt^{IV}(Hdigly)Cl_4] complex because this complex is the one with two Cl^- ions substituted for two OH^- ions of [Pt(Hdigly)$Cl_2(OH)_2]^-$, of which the peak was at 899 ppm (Figure 3c). The peaks at about −130 ppm in Table 3 were also assigned to those for K[Pt^{IV}(Hdipep)Cl_4] because these peaks appeared after the addition of a drop of 1 N HCl to the K[Pt^{IV}(dipep)Cl_3] aqueous solution and the peaks near 250 ppm of the K[Pt^{IV}(dipep)Cl_3] then decreased. There are two peaks at −133 ppm and −143 ppm for the K[Pt^{IV}(Hdiα-ala)Cl_4] complex, which were considered to be diastereomer complexes containing D-L-diα-ala or L-D-diα-ala. The peaks of the K[Pt^{IV}(dipep)Cl_3] and K[Pt^{IV}(Hdigly)Cl_4] complexes showed a 2000−2200 ppm downfield shift compared to those for the K[Pt^{II}(dipep)Cl] and K[Pt^{II}(Hdigly)Cl_2] complexes, respectively. In order to obtain more information about the structures of these complexes, ^{15}N digly^{2-} and ^{15}N gly^{15}Nα-ala^{2-} were used for the preparation of these complexes. The peaks near 279 ppm for the K[Pt(digly)Cl_3] complex showed a quartet with $J = 353$ and 293 Hz, and the peaks near −115 ppm for the K[Pt(Hdigly)-Cl_4] complex, $J = 425$ and 330 Hz. These two coupling constants correspond to that of the Pt−amide N and that of the Pt−amine N coordinated platinum(IV) ion, respectively. All the coupling constants of the platinum(IV) complexes are about 70% of the corresponding platinum(II) complexes.[8]

Reaction Mixture. There are several peaks more upfield than −120 ppm in the reaction mixtures containing the x-gly type peptide complexes, which are at about −2150 ppm for the complexes with digly^{2-} and α-alagly^{2-}, and more downfield peaks in the reaction mixtures containing the x-α-ala type peptide complexes, which are at about 1400 ppm for the complexes with glyα-ala^{2-} and at 560 ppm and at 1400 ppm for the complexes with diα-ala^{2-}. The ^{195}Pt NMR peaks appearing at about −2150 ppm for the platinum complexes containing x-gly were assigned to those of the K[Pt^{II}(Hdigly)-Cl_2] and K[Pt^{II}(Hα-alagly)Cl_2] complexes as shown in Table 3. The peaks near 1400 ppm are easily understood using the additivity rule of the ^{195}Pt chemical shift due to a set of ligand atoms. The ^{195}Pt chemical shift of the [Pt(Hglyα-ala)$Cl_2(OH)_2]^-$ is 967 ppm, and the substitution of one Cl^- for one OH^- causes about a 500-ppm downfield shift. The peak near 1400 ppm must be the one for the [Pt(Hglyα-ala)Cl$(OH)_3]^-$ ion (Figure 3d). The fact that the ^{195}Pt NMR peak of this complex containing ^{15}N glyα-ala^{2-} appeared as one quartet supported this structure. The small peak at 550 for the glyα-ala complex was assigned to the peak for [Pt(glyα-ala)$_2]^-$ because a substitution of three Cl^- ions for one glyα-ala^{2-} caused a 267-ppm downfield shift (K[Pt^{IV}(glyα-ala)Cl_3] and δ_{Pt} for $[PtCl_6]^{2-} = 0$ ppm). The ^{195}Pt NMR peak of this complex containing ^{15}N glyα-ala^{2-} showed multiple splitting. The platinum(IV) complexes containing the x-gly type dipeptide were easily reduced to the platinum(II) complexes in the reaction and those for x-α-ala were not reduced because the peaks of the K[Pt^{II}(digly)Cl] (δ_{Pt} =-1870 ppm) and K[Pt^{II}(α-alagly)Cl] ($\delta_{Pt} = -1935$ ppm) complexes appeared and such a peak of Pt(II) complexes containing x-α-ala did not appear. Judging from the peak height change as time passed, the reduction seemed to proceed in the order of, for example, [Pt^{IV}(Hdigly)$Cl_4]^-$ → [Pt^{II}(Hdigly)$Cl_2]^-$. In the case of the platinum(IV) complexes containing the x-α-ala type dipeptide, several peaks appeared in the chemical shift region of platinum-(IV). The yields of the K[Pt^{IV}(dipep)Cl_3] containing digly^{2-} (53%) and α-alagly^{2-} (29%) are much smaller than those for the K[Pt^{IV}(dipep)Cl_3] containing glyα-ala^{2-} (86%) and diα-ala^{2-} (87%), also suggesting that the platinum(IV) complexes contain-

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Table 4. ^{13}C NMR Chemical Shifts for Platinum(IV) Complexes (ppm)

complex	C^1H^a	C^1O^a	C^2H^a	C^2O^a		
H_2digly	43.37	169.84	46.09	179.36		
K[Pt(digly)Cl_3]	51.07	180.27	52.94	189.71		
K[Pt(Hdigly)Cl_4]	49.66	176.36	50.56	183.32		
H_2glyα-ala	43.31	169.05	54.03	182.89	19.98	
K[Pt(glyα-ala)Cl_3]	53.57	180.07 (75.15)^b	59.22	193.15 (21.90)^b	21.90	
K[Pt(Hglyα-ala)Cl_4]	50.06	177.51	57.96	181.11	18.13	
$H_2\alpha$-alagly	46.16	173.51	52.06	179.23	19.17	
K[Pt(α-alagly)Cl_3]	51.36	181.38 (70.64)^b	60.97	189.37 (23.19)^b	20.61	
H_2diα-ala	52.04	172.90	54.14	182.75	19.89	19.35
K[Pt(diα-ala)Cl_3]	59.57	181.26	61.36	193.08	21.60	20.45

^{a} $PtNH_2C^1HR^1C^1ONC^2HR^2C^2OO^-$. ^{b} The coupling constants (J_{Pt-C}) were shown in the parentheses.

ing the x-gly type dipeptide are easily reduced to the platinum(II) complexes. As stated above, the reports of amido−iminol tautomerism of the diethylenetriamine platinum(II) complexes with carboxylic acid amides or urea by Fairlie et al. also suggest that a methyl group near a coordinated amide N stabilizes the platinum complex.[14,15] Thus, the platinum(IV) complexes containing the x-α-ala type dipeptide are not easily reduced to the platinum(II) complexes because a methyl group of the x-ala stabilizes the platinum complex owing to withdrawing the electron of N^-.

The ^{13}C NMR Data. The ^{13}C NMR data are shown in Table 4. All the peaks showed downfield shifts compared with those of the free ligand. The coordination downfield shifts of ^{13}C NMR signals of the amide carbonyl carbon (C^1O) and the terminal carboxylate carbon (C^2O) of the K[Pt^{IV}(dipep)Cl_3] complexes are 77% and 72% of those of the K[Pt^{II}(dipep)Cl] complexes, respectively. The coordination downfield shifts of ^{13}C NMR signals of the C^1H and the C^2H of the K[Pt^{IV}(dipep)Cl_3] complexes are 92% and 90% of those of the K[Pt^{II}(dipep)Cl] complexes, respectively. These smaller downfield shifts might show an increase in the electron density around the carbon atom by the coordination of the two Cl^- ions in spite of the increase in the platinum valence. Because the platinum nuclei in a planar Pt(II) complex undergo a rapid chemical shift anisotropy-induced relaxation at high fields,[16] we cannot have observed satellites from coupling to ^{195}Pt in the spectra.[6] Several $^2J(^{195}Pt-^{13}C)$ of the platinum(IV) complexes were observed in the ^{13}C NMR spectra of the K[Pt(dipep)Cl_3] and K[Pt(dipep)Cl$(OH)_2$] complexes as shown in parentheses in Table 4. All the J_{Pt-C^1O} are about 70 Hz, and those of the other carbon−Pt coupling constants (J_{Pt-C^2O}, J_{Pt-C^1H}, J_{Pt-C^2H}) are 20−25 Hz. These $^2J(^{195}Pt-^{13}C)$ of the platinum(IV) complexes corresponded to those for the platinum(IV) complexes containing iminodiacetate or methyliminodiacetate, which are smaller than 45 Hz.[17] There are several peaks for K[Pt(Hα-alagly)Cl_4] and for K[Pt(Hdiα-ala)Cl_4]; therefore these complexes are not pure.

Anti-*Candida* Activity of K[Pt(digly)Cl_3] and K[Pt(gly-L-α-ala)Cl_3]. The number of *C. albicans* incubated with K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] was measured to estimate the anti-*Candida* activities. The growth of *C. albicans* cells was strongly inhibited by the addition of K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3]. The MICs of K[Pt(digly)Cl_3] (0.16 mM) and K[Pt(gly-L-α-ala)Cl_3] (0.20 mM) were 3- to 4-fold lower than those of cisplatin (0.69 mM). The MIC of fluconazole was 12.1 μM.

Effect of K[Pt(digly)Cl_3] and K[Pt(gly-L-α-ala)Cl_3] on Glucose Metabolism in *C. albicans*. Because Rabaste et al. reported that amphotericin B, an antifungal agent, inhibits glucose metabolism,[18] we measured the effect of K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] on glucose metabolism as a test of biological activity. The optimal absorption of Alamar Blue is changed by oxidation with an intracellular NAD or NADP.[19] In a preliminary experiment, we confirmed that the oxidation of Alamar Blue was induced in *C. albicans* cells stimulated with glucose but not in the cells cultured in glucose-free PBS(−) (data not shown), indicating that the concentration of NAD or NADP had increased in the cells containing glucose.[18] The oxidation caused by glucose was significantly inhibited by the addition of K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] (Figure 4). Fluconazole (121 μM) did not affect the oxidation. Acetyl-CoA is a metabolite of glucose and an essential material of fatty acids.[20] NADP is produced during fatty acid synthesis; therefore K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] might inhibit the fatty acid synthesis. Azole antifungal drugs such as fluconazole inhibited the synthesis of the fungal cell membrane. We suggested that the inhibition mechanisms of K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] might be different from that of fluconazole, because K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3 inhibited the glucose metabolism in *C. albicans* but fluconazole did not.

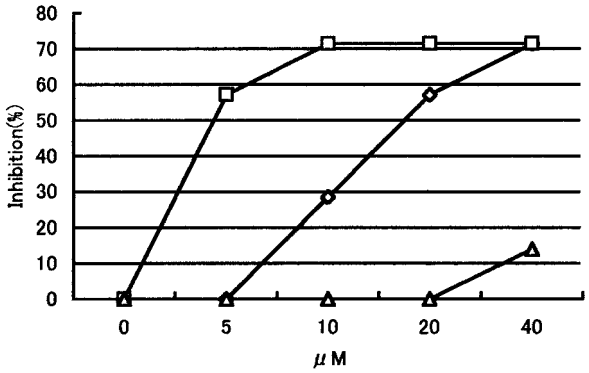

Figure 4. Effect of K[Pt(digly)Cl_3] and K[Pt(gly-L-α-ala)Cl_3] on glucose metabolism in *C. albicans*. *C. albicans* was mixed with K[Pt(digly)Cl_3] (□) or K[Pt(gly-L-α-ala)Cl_3] (○) or cisplatin (△), and Alamar Blue was then added. The mixture was incubated at 37 °C for 3 h, and the value of the difference between OD_{540} and OD_{620} was then measured. Inhibition (%) of glucose metabolism was calculated as follows. Inhibition (%) = $(A - B) \times 100/A$, where A and B are the values corresponding to the group cultured without K[Pt(digly)Cl_3] or K[Pt(gly-L-α-ala)Cl_3] and the group cultured with these samples, respectively.

Supporting Information Available: Tables giving fractional atomic coordinates, anisotropic thermal parameters, and intramolecular torsion angles for K[Pt^{IV}(digly)Cl_3]. Crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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