the transesterification of ApUp, then the inability of $Zn(CR)^{2+}$ (pK_a = 8.69) or 3 (pK_a = 9.77) to form an hydroxide complex at pH 7.6 may be the reason that these complexes are poorer catalysts. Two other metal hydroxide complexes,[6,16] Cu(trpy)-$(OH)^+$ and $Co(trien)(OH)^{2+}$ (trpy = 2,2':6',2''-terpyridine; trien = triethylenetetramine), are implicated as the active catalytic species in the transesterification of RNA.

A decrease in k_{obs} with increasing [1] is found for concentrations of 1 greater than 3.29×10^{-4} M. A similar observation[12b] was reported for the cleavage of dinucleotides of RNA by $Zn(NO_3)_2$. Neither saturation in 1 nor dimerization of 1 would account for the observed kinetics. There are multiple metal binding sites on ApUp, including two phosphate esters, adenosine and, at alkaline pH, uridine. Higher order complexes between 1 and ApUp, formed by occupation of multiple binding sites at high ratios of 1:ApUp, may not readily undergo transesterification. Consistent with this proposal is the 1:1 stoichiometry of the reactive complex as determined by use of Job's method of continuous variation. The shallow curve of Figure 4 is consistent[26] with the formation of a weak complex between 1 and ApUp. At higher or lower ratios of 1 to ApUp, the rate of transesterification decreases because the concentration of the 1:1 reactive complex decreases. There are multiple binding sites on ApUp that could be occupied by 1 at high ratios of 1:ApUp; however, these studies indicate that higher order complexes undergo transesterification more slowly than does the 1:1 complex.

RNA end groups produced by metal ion catalyzed cleavage will depend on the relative rates of transesterification of the RNA strand and subsequent hydrolysis of these products. Hydrolysis of unactivated phosphate diesters is notoriously slow. However, phosphate diesters containing cyclic groups such as 2',3'-cAMP usually hydrolyze more readily than simple phosphate diesters.[27]

Zinc(II) complex 1 catalyzes the hydrolysis of 2',3'-cAMP more slowly than it catalyzes transesterification of ApUp. Because phosphate ester hydrolysis is generally promoted by metal hydroxide complexes[3,5] and at pH 7.6 a substantial proportion of 1 is present in hydroxide form,[24] $Zn([9ane]N_3(OH)^+$ may be the active catalyst in the hydrolysis of 2',3'-cAMP. Both 3',5'-cAMP[3d] and 2',3'-cAMP[16] are hydrolyzed by cobalt(III) complexes at 50 °C, but catalytic behavior is not observed.

The observed (2.3:1) 3'-AMP:2'-AMP ratio is close to the 3:1 ratio observed for hydrolysis of 2',3'-cAMP by $Zn(NO_3)_2$.[11b] In contrast, base-catalyzed hydrolysis gives close to equal amounts of adenosine 2'- and 3'-monophosphates.[28] Regioselective hydrolysis of 2',3'-cAMP by 1 may be related to the nature of the complex between 1 and 2',3'-cAMP. From molecular models, coordination of 1 to both adenosine and phosphate ester does not appear likely. In ATP hydrolysis[2a] by metal ions, the reactive complex has two metal ions and two ATPs with adenine bases stacked and metal ion coordination to an adenine and to a phosphate of different ATP molecules. Formation of a similar reactive dimeric complex of the 2',3'-cAMP complex of 1 could account for preferential formation of a single isomer of AMP. Further investigations are underway to determine the origin of regioselective hydrolysis of 2',3'-cyclic nucleotides by metal complexes.

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Selective Hydrolysis of Unactivated Peptide Bonds, Promoted by Platinum(II) Complexes Anchored to Amino Acid Side Chains†

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Certain platinum(II) complexes attached to the sulfur atom of cysteine, *S*-methylcysteine, and methionine in peptides and other amino acid derivatives promote, under relatively mild conditions, hydrolysis of unactivated amide bonds involving the platinated amino acid. Kinetics of hydrolysis was studied with the substrates *N*-acetyl-L-cysteine, *S*-methyl-L-cysteine, *N*-acetyl-*S*-methyl-DL-cysteine, *N*-(2-mercaptopropionyl)glycine, *N*-acetylmethionylglycine, leucylglycine, reduced glutathione, *S*-methylglutathione, and oxidized glutathione and with complexes of platinum(II) and platinum(IV) containing chloro, aqua, iodo, ethylenediamine, 2,2'-bipyridine, and 2,2':6',2''-terpyridine ligands. When the substrates and platinum promoters are matched so as to aid hydrolysis, the observed rate constant varies between 2.3×10^{-4} and 7.4×10^{-3} min^{-1} at 40 °C, depending on the substrate, promoter, pH, ionic strength, and chloride concentration. Unplatinated (free) substrates and substrates platinated with complexes designed to hinder hydrolysis do not hydrolyze at a detectable rate under identical conditions. The mechanism involves initial aquation of the platinum(II) complex attached to the substrate and a subsequent rate-determining step within the platinated substrate; details of the mechanism are discussed in terms of kinetic evidence and precedents. Hydrolysis is regioselective—it occurs preferably at the amide bond involving the carboxylic group of the platinated amino acid. This study may point the way toward new methods for selective, perhaps even catalytic, cleavage of peptides and proteins with metal complexes.

Introduction

Many biological processes involve hydrolysis of proteins and peptides, but relatively little is known about the mechanism of this reaction. The amide bond is extremely unreactive;[1-3] the half-life for its hydrolysis in neutral solution is ca. 9 years.[4] Hydrolytic procedures in synthetic and biochemical work call for prolonged heating and high concentration of the strongest acids or bases. Kinetic and mechanistic studies have been done almost

† This paper is dedicated to Phan To Su (1956-1990), a graduate student who ably undertook an extension of this study and in her last days still hoped to return to research.

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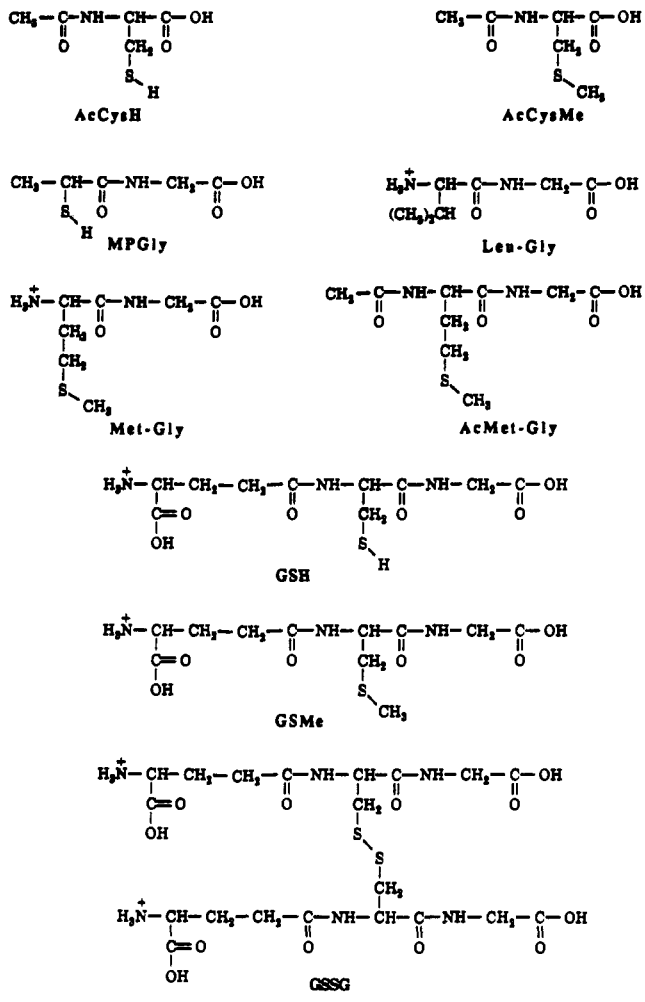
exclusively with amides that are variously activated by substituents (such as *p*-nitrophenyl), by ring strain (as in lactams), by forced nonplanarity (as in bridgehead amides), or by proximate functional groups in special positions.[5-9] In addition to this, the reaction mixtures usually are heated.

Proteolytic enzymes, however, hydrolyze even unactivated amide groups rapidly under mild conditions.[10] Biomimetic systems still fall short of this goal although some of them surpass chymotrypsin in acceleration of particular reactions. It has been pointed out[11] that these results had been obtained with activated esters, not with regular amides, and that biomimetic systems cannot turn over substrates. Oxidative cleavage of proteins mediated by metal complexes has recently been achieved.[12-17] Catalytic antibodies, which are true catalysts and which show selectivity, hold the greatest promise for enzyme-like hydrolysis of peptides.[18-20]

Because certain proteolytic enzymes require metal ions for activity,[21,22] hydrolysis reactions have caught the attention of inorganic chemists. In studies with metal complexes, too, carboxylic and phosphate esters are used more often than amides.[23-47]

Chart I



The metal usually is cobalt(III), and mechanisms of its action are known in detail.[41-47] Because its complexes bind to the N-terminal amino acid residue, only the N-terminal amide bond in the peptide is hydrolyzed. These stoichiometric reactions promoted by cobalt(III) complexes are relevant to turnover reactions catalyzed by aminopeptidases.

The present study deals with stoichiometric hydrolysis of unactivated amide bonds in peptides and other amino acid derivatives, promoted by platinum(II) complexes. This metal is convenient because its ligand-substitution reactions are slow and because ^{195}Pt NMR spectra are informative about the coordination sphere. Platinum(II) was used previously as a promoter in the hydrolysis of inorganic oligophosphates[48] and of activated phosphate esters.[49]

This work differs from the previous studies of peptide hydrolysis because the metal binds selectively to a particular side chain and activates selectively one of the two adjacent amide bonds. Both of these aspects of selectivity are examined with many complexes in which platinum(II) and platinum(IV) atoms bearing various ancillary ligands are coordinated to the amino acids and peptides shown in Chart I; the charges of these substrates are as expected for major species at pH 2.0. In the first step toward the ultimate goal of designing an artificial inorganic endopeptidase, we report on the kinetics, selectivity, and mechanism of the cleavage reaction.

Experimental Section

Chemicals. Distilled water was further purified and demineralized. Deuteriated solvents D_2O, DCl, $DClO_4$, NaOD, and *N,N*-dimethylformamide-d_7 (DMF-d_7) and complexes $K_2[PtCl_4]$, $K_2[PtCl_6]$, $Pt(en)Cl_2$, $Pt(bpy)Cl_2$, and $[Pt(trpy)Cl]Cl\cdot 2H_2O$ were obtained from Aldrich

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Chemical Co. Anhydrous $AgClO_4$, glycine (Gly), *S*-methyl-L-cysteine (CysMe), *N*-acetyl-L-cysteine (AcCysH), *N*-(2-mercaptopropionyl)-glycine (MPGly), methionylglycine (Met-Gly), leucylglycine (Leu-Gly), reduced glutathione (GSH), *S*-methylglutathione (GSMe), and oxidized glutathione (GSSG) were obtained from Sigma Chemical Co.

The complex $K_2[PtI_4]$ was prepared in situ.[50,51] A solution of $K_2[PtCl_4]$ (0.44 g, 1.0 mmol) in 25 mL of water was heated with KI (4.22 g, 25 mmol), and the color quickly changed from red to dark brown. The solution was lyophilized with a Labconco 75035 freeze-dryer and brought up in 5 mL of D_2O successively three times in order to minimize the HDO signal in the ^1H NMR spectrum. The product was verified by UV–vis spectroscopy and used without isolation.

The compound *N*-acetyl-*S*-methyl-DL-cysteine (AcCysMe) was prepared by a variation of a published method.[52] Acetic anhydride (0.74 mL, 8.0 mmol) was added to a stirred suspension of CysMe (0.68 g, 5.0 mmol) in 3.0 mL of glacial acetic acid. After 2 h of vigorous stirring, the solid dissolved. The solution was stirred for 1 h and then evaporated to dryness in vacuo. The solid residue was recrystallized from water to yield 0.59 g (66%) of a fluffy white powder, mp 153 °C. Further evaporation and recrystallization raised the overall yield to 87%, but the product was less pure; mp 151–152 °C. All the experiments were done with the first batch. Proton NMR spectrum (δ values) in D_2O: 4.53, q, CH; 2.90, m, CH_2; 2.09 s, SCH_3; 1.95, s, CH_3.

The protected peptide *N*-acetylmethionylglycine (AcMet-Gly) was prepared by adding acetic anhydride (14.7 μL, 0.16 mmol) to a stirred solution of Met-Gly (32.0 mg, 0.16 mmol) in 0.40 mL of glacial acetic acid and stirring for 3 h. The mixture was evaporated to dryness in vacuo, the resulting oil was dissolved in water, and the aqueous solution was evaporated to dryness in vacuo. The product was a white powder (27.0 mg, 70%). Proton NMR spectrum (δ values) in D_2O: 4.47, dd, CH; 4.00, s, Gly CH_2; 2.60, m, CH_2; 2.09, s, SCH_3; 2.03, s, $CH_3C(O)$.

NMR Spectroscopy. The ^1H spectra at 300 and 200 MHz were recorded with Bruker WM300 and WM200 spectrometers equipped with variable-temperature probes and with a Nicolet NT 300 spectrometer; the residual HDO signal at 4.74 ppm was used as an internal reference. The ^{13}C spectra of solutions in D_2O were recorded with the Bruker WM200 spectrometer at 50.3 MHz and with a Varian VXR300 spectrometer at 75.4 MHz; the dioxane signal at 66.5 ppm was used as an internal or external reference. The ^1H-decoupled ^{13}C spectra were acquired in 5000 scans, and multiplicity of the signals was preserved with CW decoupling. The ^{195}Pt spectra were recorded in 10-mm tubes, with the Bruker WM200 spectrometer at 42.9 MHz; a solution of $K_2[PtCl_4]$ in D_2O, with HCl added to prevent aquation, was an external reference and the chemical shift standard (0 ppm).

Other Measurements. Infrared spectra were recorded with an IBM IR98 Fourier-transform spectrometer. The samples usually were mulls; aqueous solutions were held in a 0.10-mL cell made of BaF_2. Ultraviolet–visible spectra were recorded with an IBM 9430 spectrophotometer, whose monochromator has two gratings. The pH was measured with a Fischer 805MP instrument and a Phoenix Ag/AgCl reference electrode; the uncorrected values for deuteriated solvents are designated pH*. The chloride concentration was measured with a specific electrode obtained from Fisher Scientific Co. and a double-junction Ag/AgCl reference electrode containing KNO_3 as the outer electrolyte. Voltage was measured with a Heathkit IM2215 voltmeter and registered with a Shimadzu CR3A recorder; the baseline drifted by +3 mV over 36 h. The standard solutions of KCl in $HClO_4$, at pH 2.0, spanned a range of chloride concentrations from 4.0 μM to 0.100 M; the plot of voltage versus concentration had a linear-regression coefficient of 0.986.

Stability of Nonplatinated Amide-Containing Ligands. Solutions of amino acid derivatives and peptides were prepared as in the hydrolysis experiments (see below) except that the platinum complexes were missing. The solvent always was D_2O. All of the following compounds were examined in both acidic ($DClO_4$) and basic ($DClO_4$ and an excess of NaOD) solutions: AcCysH, GSMe, AcCysMe, GSH, AcMet-Gly, Leu-Gly, MPGly, and GSSG. The first two compounds were examined also in neutral (equimolar $DClO_4$ and NaOD) solutions; AcCysMe, GSMe, and GSH were examined also in DCl solutions and in acidic solutions (either $DClO_4$ or DCl) that were nearly saturated with KCl. A solution 0.15 M in both GSMe and $AgClO_4$ was prepared in 0.20 M $DClO_4$, and pH* was adjusted to 2.0 by addition of NaOD. The solutions were kept at 40 ± 1 °C for at least 1 month and examined occasionally by ^1H NMR spectroscopy.

Reactions of $[PtCl_4]^{2-}$ with Amino Acid Derivatives and Peptides. All experiments were done in NMR tubes, and the solvent was D_2O. To a stirred solution of an amino acid derivative or a peptide in D_2O or in 0.20 M $DClO_4$ was added, in the dark, an equivalent amount of $K_2[PtCl_4]$ in D_2O, the pH* was adjusted with 0.40 M NaOD or with 3.0 M $DClO_4$, and the volume was adjusted with D_2O. The pH* value changed by less than 0.10 unit upon addition of $K_2[PtCl_4]$ to the ligand and upon final dilution of the solution. The final concentrations of the platinum complexes were between 80 and 170 mM. The ligands AcMet-Gly, GSH, and GSSG were initially dissolved in D_2O; AcCysH, AcCysMe, MPGly, and GSMe were initially dissolved in 0.20 M $DClO_4$. The ligand GSSG was heated with 1 and 2 equiv of $K_2[PtCl_4]$. In all cases, the solutions were kept in the dark, at 40 ± 1 °C, and recording of NMR spectra began 1 h after mixing of the reactants. The ^1H NMR data (δ values) follow. $[PtCl_3(AcCys)]^{2-}$: 4.55, partially buried dd, CH; 2.71, s, CH_3; 1.96, m, CH_2. $[PtCl_3(AcCysMe)]^-$: 5.05, dd, CH; 3.18 and 2.95, both dd, CH_2; 2.38, s, SCH_3; 2.06, s, $CH_3C(O)$. $[PtCl_3(AcMet\text{-}Gly)]^-$: 4.13, dd, Met CH; 3.58, s, Gly CH_2; ca. 2.24, m, Met CH_2; 1.92, s, $^3J_{Pt-H}$ = 42 Hz, Met SCH_3; 1.60, s, $CH_3C(O)$. $[PtCl_3(MPGly)]^{2-}$: broad signals, unresolved multiplets. $[PtCl_3(GSMe)]$: 4.51, dd, Cys CH; 4.04, t, Glu CH; 3.96, s, Gly CH_2; 2.92 and 2.82, both dd, Cys CH_2; 2.54, m, Glu $CH_2C(O)$; 2.18, m, Glu $CHCH_2$; 2.06, s, SCH_3. $[PtCl_3(GS)]$: broad signals, unresolved multiplets. $[PtCl_3(GSSG)]^+$: 4.57, partially buried dd, Cys CH; 3.99, buried t, Glu CH; 3.96, s, Gly CH_2; 3.22 and 2.95, both dd, Cys CH_2; 2.54, p, Glu $CH_2C(O)$; 2.16, m, Glu $CHCH_2$.

Aquation of the Cl^- Ligand. All experiments were done in the dark. Solutions containing $[PtCl_3(GS)]^-$ and $[PtCl_3(GSMe)]$ were each treated with 2 equiv of $AgClO_4$ (as a 2.3 M solution in D_2O). The mixtures were stirred, and AgCl was removed by centrifugation. The main products, *trans*-$[PtCl_2(H_2O)(GS)]$ and *trans*-$[PtCl_2(H_2O)(GSMe)]^+$, were studied in situ.

Reactions of Other Pt(II) and Pt(IV) Complexes with GSH and GSMe. Except when noted otherwise, the solvent was D_2O; its pH* was adjusted with dilute $DClO_4$ or NaOD. The GSH concentration was adjusted to 0.20 M and its pH* to 7.0, and the GSMe concentration was adjusted to 0.25 M and its pH* to 2.0, by lyophilizing a solution containing a known amount of either peptide and dissolving each residue up to a known volume of solution. The peptide and an equimolar amount of the platinum complex were mixed rapidly in the NMR tube. The solutions were kept at 40 ± 1 °C, and the complexes were studied in situ by ^1H, ^{13}C, and ^{195}Pt NMR spectroscopy. The platinum complexes in acidic and basic solutions were monitored by ^1H NMR spectroscopy for 1 month.

Upon addition of [Pt(trpy)Cl]Cl (200 μL of a 0.20 M solution) to GSH (200 μL of a 0.20 M solution), the color changed from orange to deep red in ca. 5 min. ^1H NMR spectrum (δ values) of [Pt(trpy)(GS)] at pH* 7.0: 8.76, d, trpy H^6; 8.20 and 8.03, both q, trpy $H^{3,3'}$, $H^{4,4'}$; 7.63, t, trpy H^5; 4.39, t, Cys CH; 3.79, t, Glu CH; 3.58, s, Gly CH_2; 2.88 and 2.58, both dd, Cys CH_2; 2.33, m, Glu $CH_2C(O)$; 2.19, m, Glu $CHCH_2$.

Addition of GSMe (244 μL of a 0.25 M solution) to a stirred slurry of *cis*-$[Pt(en)Cl_2]$ (19.9 mg, 60 μmol) in 0.30 mL of D_2O, followed by stirring at 40 °C for 2 h, gave a clear, pale yellow solution. ^1H NMR spectrum (δ values) of *cis*-[Pt(en)Cl(GSMe)]: 3.99, s, Gly CH_2; 3.89, t, Glu CH; 3.33, m, free en; 2.71, m, Cys CH_2; 2.57, m, Glu $CHCH_2$; 2.49, s, Cys CH_3; 2.18, m, Glu $CH_2C(O)$.

A saturated solution of *cis*-$[Pt(bpy)Cl_2]$ in DMF-d_7 has a concentration of 10 mM, as determined by weighing the undissolved solid after a slurry containing the known amount of the complex was stirred long at ca. 40 °C. The peptide GSMe (40 μL of a 0.25 M solution) was added to *cis*-$[Pt(bpy)Cl_2]$ (1.0 mL of a 10 mM solution) with stirring. ^1H NMR spectrum (δ values) of *cis*-[Pt(bpy)Cl(GSMe)]: 9.65–7.92, bpy; 4.65, dd, Cys CH; 4.24, t, Glu CH; 4.04, s, Gly CH_2; 3.08, m, Cys CH_2; 2.70, m, Glu $CH_2C(O)$; 2.31, m, Glu $CHCH_2$; 2.13, s, CH_3.

A solution of $K_2[PtCl_6]$ (28.9 mg, 70 mmol) in 0.20 M $DClO_4$ was adjusted to pH* 2.0 and diluted with D_2O to 350 μL. To the resulting 0.20 M solution was added, with stirring, 290 μL of a 0.25 M solution of GSMe, and the mixture was allowed to stand for several days. ^1H NMR spectrum (δ values) of $[PtCl_5(GSMe)]$: 4.08, dd, Cys CH; 4.00, s, Gly CH_2; 3.28, m, Cys CH_2; 2.75, s, CH_3; 2.58, m, Glu $CH_2C(O)$; 2.22, m, Glu $CHCH_2$.

Upon addition of 200 μL of a 0.20 M solution of $[PtI_4]^{2-}$ to 160 μL of a 0.25 M solution of GSMe at pH* 2.0, with stirring, the color quickly changed to pale red. A small amount of red precipitate that formed was filtered off. ^1H NMR spectrum (δ values) of $[PtI_3(GSMe)]$: 4.42, t, Glu CH; 4.39, s, Gly CH_2; 3.19, m, Cys CH_2; 3.07, m, Glu $CH_2C(O)$; 3.01, s, CH_3; 2.62, m, Glu $CHCH_2$.

Study of Hydrolysis. Solutions in D_2O of amino acid derivatives and peptides and of their various complexes with platinum were prepared as described above and kept in the dark, at 40 ± 1 °C. Their NMR spectra were recorded at 40 ■ 0.5 °C. The free (nonplatinated) amide-containing compounds and those platinum complexes that hydrolyzed slowly or not at all were examined occasionally over 1 month. Those platinum complexes that hydrolyzed relatively fast were examined frequently over

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1 day; most reactions were ca. 90% complete in this time.

Each ^1H NMR spectrum consisted of eight to twelve scans. Although chemical shifts of GSMe and GSH depend on pH, in the pH* range from 0.50 to 6.0 the adjacent Glu CH and Gly CH_2 resonances did not overlap. The basic solutions of the peptide complexes were turbid and gave precipitates; because their ^1H NMR spectra became broad and unresolved, these solutions were not studied. The CH_2 groups in both the free (3.90 ppm) and the peptide-bound (3.96 ppm) glycine—these chemical shifts depend on pH*—gave singlets of approximately equal widths. These two forms of glycine were quantitated on the basis of both the signal heights and areas and the known initial concentration of the peptide complex. The estimated error in the concentrations was ±10%. First-order logarithmic plots of concentrations of the hydrolyzing peptide complexes versus time were linear for 3 half-lives. Eight to twenty data points were used per plot, and linear-regression coefficients exceeded 0.990.

The ^1H-decoupled ^{13}C NMR spectra of $[PtCl_3(GSMe)]$ at pH* 1.9 were recorded 0.5, 5, and 24 h after this complex was prepared. The ^{195}Pt NMR spectra of this complex were scanned continuously and stored every 10 min during the first hour; later spectra, each consisting of 1150 scans, were recorded every 30 min until the reaction was practically complete.

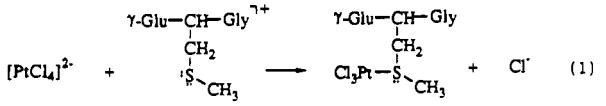
In the studies of pH effects, the pH* values of GSMe solutions in 0.20 M $DClO_4$ were adjusted with 3.0 M $DClO_4$ or with 0.40 M NaOD. Each solution was treated with an equivalent amount of $K_2[PtCl_4]$ and diluted with D_2O to the final concentration of 40 mM. The pH* changed by less than 0.10 unit upon this dilution.

The chloride concentration in one set of $[PtCl_3(GSME)]$ solutions, at pH* 1.0, was adjusted with a 3.00 M solution of NaCl, and ionic strength was brought up to 1.20 M with a 3.00 M solution of $NaClO_4$; the $[PtCl_3(GSMe)]$ concentration was 45 mM. The chloride concentration in another set of $[PtCl_3(GSMe)]^-$ solutions, now at pH* 2.9, was adjusted with NaCl as before, but ionic strength was not kept constant; the $[PtCl_3(GSMe)]^-$ concentration was 50 mM. The chloride concentration in a 1.0 mM solution of $[PtCl_3(GSMe)]$ in H_2O, at pH 2.0, without added NaCl, was monitored with a specific electrode for 60 h at room temperature. Control experiments were done with $K_2[PtCl_4]$ and with GSMe separately under identical conditions.

Solutions of $[PtCl_3(GSMe)]^-$ in dilute $DClO_4$, at pH* 2.90, were treated with 1 equivalent of a 3.00 M solution of $AgClO_4$, and ionic strength was adjusted with a 3.00 M solution of $NaClO_4$.

Results and Discussion

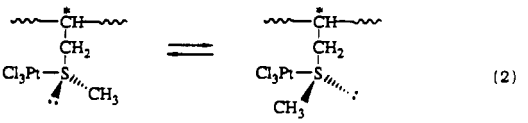
Platinum Complexes with Amino Acid Derivatives and Peptides. Because all these ligands contain ionizable groups, the charges of their complexes depend on pH. The formulas represent the major species expected on the basis of estimated pK_a values of these groups.[53] The thioether groups in AcCysMe, AcMet-Gly, and GSMe react with $[PtCl_4]^{2-}$ in the same way; the reaction with GSMe is shown in eq 1. The changes in the NMR spectra are



(1)

diagnostic of this coordination. Because such reactions of amino acid derivatives were previously studied in this laboratory,[54,55] only the reactions of the tripeptide GSMe are discussed here.

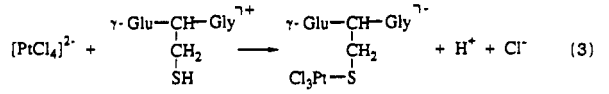
Both ^1H and ^{13}C NMR spectra show that resonances of the *S*-methylcysteine residue are the only ones significantly affected by complex formation.[56] Because the chiral carbon atoms in the peptide are configurationally rigid while the chiral sulfur atom undergoes pyramidal inversion, two diastereomers (eq 2) are



(2)

evident in NMR spectra at room temperature. Each of the three carbon atoms shown in eq 2 gives a pair of ^{13}C resonances, and the platinum atom gives a pair of ^{195}Pt resonances. The relative intensities are (1.4 ± 0.1):1.0 in each pair. Because the ^1H chemical shifts span a relatively narrow range, the ^1H resonances coalesce well below room temperature and appear as sharp singlets. Broadening and doubling of ^{13}C resonances upon binding of platinum(II) to the sulfur atom in GSMe have recently been reported.[57] Amino acids and other relatively small thioether ligands cause practically no chiral discrimination; even when the chiral carbon and sulfur atoms in them are adjacent, the two diastereomers exist in nearly equal concentrations.[54,55] The tripeptide, however, shows a small but clear discrimination of approximately 1.4:1.0. It probably arises from noncovalent long-range interactions between the platinum complex (the inverting fragment) and the polar groups in the peptide. The present work, together with previous studies in this laboratory,[54,55] shows that ^{195}Pt NMR spectroscopy is applicable to stereochemical studies of relatively complicated ligands, such as biomolecules, whose ^1H and ^{13}C NMR spectra are rich.

The (deprotonated) thiolate groups in MPGly, AcCysH, and GSH initially displace a chloride ligand in $[PtCl_4]^{2-}$, as shown for GSH in eq 3. Then dimers and oligomers form. This oli-



(3)

gomerization, which was observed previously for similar complexes,[58-61] causes broadening of the ^1H NMR resonances, but the hydrolysis reactions of interest remain clearly evident. The disulfide group in GSSG binds to $[PtCl_4]^{2-}$ as a unidentate ligand; there is a precedent for this coordination.[57]

The reaction involving $[PtI_4]^{2-}$ is analogous to the one shown in eq 3. The reactions involving *cis*-$[Pt(en)Cl_2]$, *cis*-$[Pt(bpy)Cl_2]$, and $[Pt(trpy)Cl]^+$ are analogous to those shown in eqs 1 and 3, except that the platinum atom attached to the sulfur ligand bears only one or no displaceable chloride ligand. One equivalent of silver(I) ion removes only the displaced chloride ion shown in eqs 1 and 3; additional silver(I) removes the bound chloride ligands and thus promotes aquation of the platinum complex. Because platinum(IV) complexes are inert, the substitution reaction of $[PtCl_6]^{2-}$ is slower than the reactions of the platinum(II) complexes.

Stability of the Unplatinated Amide-Containing Ligands. All the amino acid derivatives and peptides were monitored by ^1H NMR spectroscopy under conditions that were identical with, and more favorable for hydrolysis than, those under which their complexes were studied. The spectra of free peptides Leu-Gly, GSH, and GSMe show no hydrolysis of the amide bonds, i.e., no single amino acids, even over much longer times than those during which amide bonds in the platinated GSH and GSMe hydrolyze completely. Some unplatinated ligands did show minor changes, which were not investigated, but hydrolysis of peptide bonds was never observed. The tripeptide GSMe was studied particularly thoroughly, at pH* values from 0.80 to 7.0 and in the presence and absence of $AgClO_4$.

Kinetics of Peptide Hydrolysis. No reaction between $[PtCl_4]^{2-}$ and Leu-Gly and no hydrolysis of this peptide are detected in ^1H and ^{13}C NMR spectra. But addition of 1 equiv of $[PtCl_4]^{2-}$ to GSH and to GSMe causes hydrolysis of the Cys-Gly peptide bond in each tripeptide. Evidently, the platinum(II) complex must bind to a side chain (the thiolate group of Cys in GSH and the thioether group of CysMe in GSMe) in order to effect hydrolysis. When a coordinating side chain is absent, as in Leu-Gly, hydrolysis does not take place.

The complexes $[PtCl_3(GS)]^-$ and $[PtCl_3(GSMe)]$ in aqueous solution release glycine, which gives a sharp ^1H NMR signal at

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(55) Galbraith, J. A.; Menzel, K. A.; Ratilla, E. M. A.; Kostič, N. M. *Inorg. Chem.* **1987**, *26*, 2073.
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(57) Lempers, E. L. M.; Inagaki, K.; Reedijk, J. *Inorg. Chim. Acta* **1988**, *152*, 201.
(58) Sovago, I.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1981**, *43*, 425.
(59) Appleton, T. G.; Connor, J. W.; Hall, J. R.; Prenzler, P. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 382.
(60) Appleton, T. G.; Connor, J. W.; Hall, J. R.; Prenzler, P. D. *Inorg. Chem.* **1989**, *28*, 2030.
(61) Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1990**, *29*, 217.

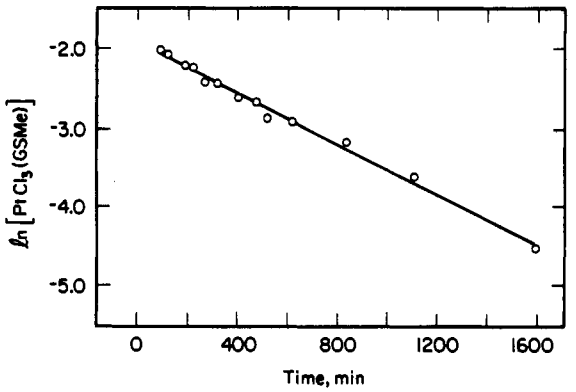
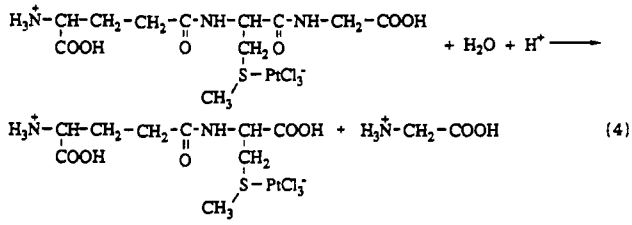


Figure 1. First-order kinetic plot for hydrolysis of the cysteine–glycine bond in the *S*-methylglutathione complex $[PtCl_3(GSMe)]$ at pH* 2.00 (uncorrected for isotope effect) and at 40 °C.

3.80 ppm and sharp ^{13}C NMR signals at 40.0 and 169.5 ppm (at pH* 2.0). All of these resonances are enhanced by addition of free glycine. After GSH and GSMe behaved similarly in several experiments, kinetic studies were continued with the latter tripeptide, whose platinum complexes do not oligomerize in solution. The reaction is shown in eq 4, and a typical kinetic plot, in Figure



(4)

1. Hydrolysis was followed until at least 90% complete. The reaction evidently is first order with respect to the platinated peptide. The rate constant is independent of the $[PtCl_3(GSMe)]$ concentration between 40 mM and 0.10 M. This, admittedly narrow, range was dictated by solubility and by NMR sensitivity.

The hydrolysis reaction is evident also in the ^{195}Pt NMR spectra. At the beginning, only the diastereomers of the tripeptide complex (the resonances at −1133 and −1162 ppm) are present. While they disappear, the diastereomers of the dipeptide complex (the resonances at −1126 and −1153 ppm) appear and, after ca. 24 h, remain as the major platinum-containing species in solution. The diastereomerism is shown in eq 2, and these spectra are quite similar to the spectra of simpler thioether complexes of platinum(II) that were studied previously in this laboratory.[54,55] Because the ^{195}Pt chemical shift greatly depends on the donor atoms, a change of less than 10 ppm during the reaction in eq 4 cannot be caused by a change in the coordination sphere of the platinum(II) atom. This change is attributed to removal of the glycine residue, which alters only the distant environment of the platinum(II) atom. In addition to these resonances, new ones at −1183 and −1520 ppm begin to appear, simultaneously, approximately 7 h after the formation of the $[PtCl_3(GSMe)]$ complex. The latter resonance is broad. Both of them are due to *trans*-$[PtCl_2(H_2O)(GSMe)]^+$, since they dominate the ^{195}Pt NMR spectrum when this aqua complex is intentionally prepared by addition of 2 equiv of silver(I) to the solution containing $[PtCl_3(GSMe)]$ and displaced chloride. Indeed, replacement by water of a chloro ligand in the coordination sphere of platinum(II) causes the ^{195}Pt resonance to move ca. 300 ppm upfield.[62,63] This rule explains the signal at −1520 ppm, but not the one at −1183 ppm.

As Table I shows, the pH value of the solution little affects the rate constant for hydrolysis, whether or not the platinum(II) atom coordinated to the peptide bears an aqua ligand. If the nucleophilic attack on the scissile amide bond were the rate-determining step in the reaction, the rate constant would markedly increase with increasing pH because hydroxide ion is a much stronger nucleophile than water. The rate not only does not increase but slightly decreases as the pH increases. Because the reaction is of first order with respect to the platinated peptide and of zeroth order with respect to the nucleophile, the rate-determining step probably is intramolecular and occurs before the attack of water or hydroxide on the amide bond.

As Table II shows, the rate constant for hydrolysis increases as the ionic strength is raised with the noncoordinating salt $NaClO_4$ but decreases as the ionic strength is raised with the coordinating salt NaCl. The former effect is small and nonspecific, but the latter one is specific and revealing about the reaction mechanism. As Table III and Figure 2 show, the rate constant

Table I. Effect of pH on the Observed Rate Constant for Hydrolysis of the Cysteine–Glycine Bond in *S*-Methylglutathione Complexes of Platinum(II) at 40 °C

pH*[a]	10^5k_{obs}, min^{-1}	pH*[a]	10^5k_{obs}, min^{-1}
	$[PtCl_3(GSMe)]^{n-}$ (n = 0, 1, 2)[b]		
0.50	270	3.90	125
1.30	220	4.85	100
2.30	180		
	trans-$[PtCl_2(H_2O)(GSMe)]^n$ (n = +, 0, −)[b]		
2.00	330	7.00	90
5.00	130		

[a] In D_2O; uncorrected for isotope effect. [b] The charge depends on the ionization state of GSMe and thus on pH.

Table II. Effect of Ionic Strength on the Observed Rate Constant for Hydrolysis of the Cysteine–Glycine Bond in the *S*-Methylglutathione Complex $[PtCl_3(GSMe)]^-$ at pH*[a] 2.90 and at 40 °C

added salt	μ, M	10^5k_{obs}, min^{-1}	added salt	μ, M	10^5k_{obs}, min^{-1}
$NaClO_4$	0.37	58	NaCl	0.32	80
	0.69	89		0.64	69
	1.10	120		1.07	44
				1.75	23

[a] In D_2O; uncorrected for isotope effect.

Table III. Effect of Chloride Concentration on the Observed Rate Constant for Hydrolysis of the Cysteine–Glycine Bond in a 0.050 M Solution of the *S*-Methylgluthione Complex $[PtCl_3(GSMe)]$ at pH*[a] 0.95, Ionic Strength 1.20 M, and at 40 °C

$[Cl^-]$, M	10^5k_{obs}, min^{-1}	$[Cl^-]$, M	10^5k_{obs}, min^{-1}
0.050	740	0.80	90
0.25	300	1.20	70
0.35	230		

[a] In D_2O; uncorrected for isotope effect.

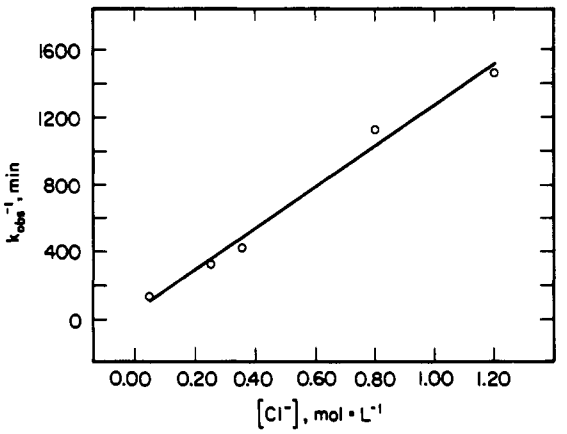


Figure 2. Effect of chloride concentration on the observed rate constant for hydrolysis of the cysteine–glycine bond in the *S*-methylglutathione complex $[PtCl_3(GSMe)]$ at pH* 1.80 (uncorrected for isotope effect), ionic strength 1.20 M (adjusted with $NaClO_4$), and at 40 °C.

(62) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247.
(63) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 673.

decreases markedly as the chloride concentration is raised at a constant ionic strength. This decrease occurs regardless of pH. Since addition of the chloride ion inhibits hydrolysis, this ion must be released before or during the rate-determining step. This release was indeed detected with a chloride-specific electrode.

Because control experiments showed that the presence of $AgClO_4$ alone does not promote hydrolysis of GSMe, this salt was used for removal of the chloride ions as AgCl. The first equivalent of silver(I) removes the free chloride ion, initially displaced from $[PtCl_4]^{2-}$ by the peptide side chain. The rate constant for hydrolysis is barely affected by this removal (see Table IV) because inhibition by an equimolar amount of chloride at low concentration is very weak. The second equivalent of silver(I) should preferentially remove a chloride ligand trans to the sulfur ligand[64] in $[PtCl_3(GSMe)]$. The rate constant for hydrolysis increases upon aquation. Because free chloride ion accumulates in solutions of both $[PtCl_3(GSMe)]$ and *trans*-$[PtCl_2(H_2O)(GSMe)]^+$, aquation of the platinum(II) complex seems to be necessary for hydrolysis of GSMe in both cases. There are two possible roles for the aqua ligand. First, it may serve as the actual nucleophile, and the platinum(II) atom may be the vehicle for its delivery to the scissile amide bond Cys–Gly. Second, it may serve as a good leaving group that facilitates coordination of the amide group to the platinum(II) atom and consequent activation of the amide bond toward nucleophilic attack. The second alternative is more likely for reasons that are discussed below.

Comparisons among various complexes show how the hydrolysis reaction depends on coordination modes and steric properties of ancillary ligands attached to the platinum(II) atom. The bulky thioether group in GSMe cannot displace the chloride ion from $[Pt(trpy)Cl]^+$ because this ligand is shielded by the proximate ortho-hydrogen atoms in the planar terpyridine,[65] but the small thiolate group in GS^- rapidly displaces the chloride ion.[66] The platinum(II) atom in the product, $[Pt(trpy)(GS)]^{2+}$, is attached to the tridentate terpyridine and the unidentate thiolate ligands. Glycine does not appear in this solution even after several weeks. The analogous complexes $[Pt(dien)(GS)]^{2+}$, $[Pt(dien)(GSMe)]^{3+}$, and $[Pt(dien)(GSSG)]^{4+}$, which contain the puckered tridentate ligand diethylenetriamine, do not hydrolyze, either.[57] The complex *cis*-$[Pt(bpy)Cl(GSMe)]^{2+}$, which contains a bulky, planar bipyridine chelate, does not hydrolyze even though it contains a displaceable chloride ligand. The analogous complex *cis*-$[Pt(en)Cl(GSMe)]^{2+}$, which contains the less bulky ethylenediamine chelate, does hydrolyze, but the reaction requires up to 20 days for completion. Aquation of this last complex dramatically accelerates the reaction—hydrolysis of *cis*-$[Pt(en)(H_2O)(GSMe)]^{3+}$ is complete in 1 day. The hydrolysis reaction evidently requires the presence of a displaceable ligand, preferably a labile one such as water, in the coordination sphere of platinum(II). Apparent hindrance of the reaction by bulky ligands indicates that the platinum complex needs to approach the scissile bond in the peptide.

Because $[PtCl_6]^{2-}$ is much more inert toward substitution, it reacts much slower than $[PtCl_4]^{2-}$ with GSMe. The product, $[PtCl_5(GSMe)]$, contains an octahedral platinum(IV) atom and hydrolyzes over approximately 1 month. An electron configuration (low-spin d^6) that hinders substitution reactions and ligands that shield the metal atom evidently hinder hydrolysis, as expected from the foregoing evidence. But this reaction occurs, albeit very slowly, probably because the Pt(IV) complex can lose ligands and because the metal in this high oxidation state is a strong Lewis acid.[67]

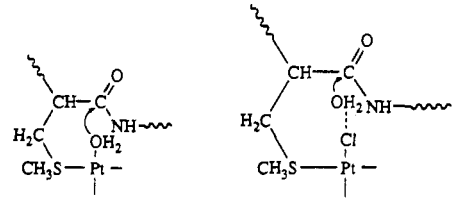
Mechanism of Peptide Hydrolysis. A partial mechanism can be drawn on the basis of the kinetic findings. A platinum complex can promote peptide hydrolysis only when it is attached to an amino acid side chain and when it contains at least one unidentate ligand such as chloride. Because this ligand must be displaced during hydrolysis, hydrolysis is inhibited by added NaCl. External attack by water or hydroxide ion (a bimolecular reaction) occurs after a rate-determining unimolecular reaction. This mechanism, involving the GSMe complex formed as in eq 1, is shown in Scheme I. The overall rate is determined by the step designated k_2. The corresponding equation (5) quantitatively accounts for the inhibition by NaCl that is shown in Figure 2.

$$k_{obs} = \frac{k_1 k_2}{k_{-1}[Cl^-] + k_2} \qquad (5)$$

The chief remaining questions concern the role of the aqua ligand and identity of the intermediate designated INT. These may be two aspects of the same issue, namely the role of the platinum complex as the promoter of the hydrolysis reaction. The reaction sequence in Scheme I is supported by direct evidence, but the role of the metal can only be surmised.

Many detailed studies of hydrolysis of amides and peptides promoted by cobalt(III) complexes[41-45] revealed two reaction paths, both involving initial attachment of the cobalt(III) atom to the N-terminal amino acid residue in a peptide. In one case, this unidentate coordination juxtaposes the carbonyl carbon atom and the water or hydroxide ligand in cis positions and thus facilitates internal nucleophilic attack. In the other case, bidentate coordination through amino and carbonyl groups activates the carbonyl carbon atom toward external attack by water or the hydroxide ion as a nucleophile. The unimolecular mechanism is more efficient than the bimolecular one. In either case, hydrolysis occurs relatively fast.

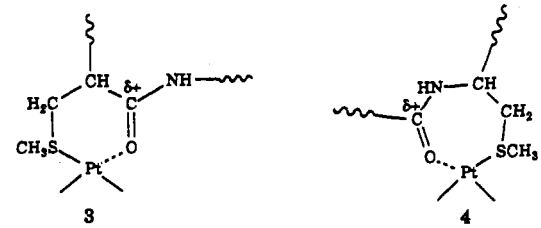
Both of these mechanisms are conceivable in the present study. Two versions of the unimolecular reaction are shown in **1** and **2**.



1 **2**

Because pK_a values of platinum(II) monoaqua complexes generally fall between 6.0 and 7.6,[68] a hydroxide ligand is an unlikely nucleophile under the acidic conditions used in this study. The water molecule may conceivably form a coordinate bond with the platinum atom, as in **1**, or a hydrogen bond with the chloro ligand, as in **2**, but this latter interaction is less likely.

In the bimolecular reaction, the amide group containing the scissile bond may coordinate to the pendant platinum(II) atom. The metal atom in this mechanism, like the proton in acid-catalyzed hydrolysis of amides,[1] acts as a Lewis acid—polarizes the carbonyl group and promotes external attack by a water molecule. Because attachment of metal ions to the nitrogen atom inhibits amide hydrolysis,[69] coordination would involve the carbonyl oxygen atom, as shown in **3** and **4**. The small decrease in the rate constant



3 **4**

for hydrolysis as pH increases, evident in Table I, may be caused by deprotonation of the nitrogen atom and its inhibitory coordination to platinum in a small fraction of the peptide complex.[70]

(64) Elding, L. I.; Gröning, Ö. *Inorg. Chem.* **1978**, *17*, 1872.
(65) Ratilla, E. M. A.; Brothers, H. M., II; Kostič, N. M. *J. Am. Chem. Soc.* **1987**, *109*, 4592.
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(67) Dixon, N. E.; Sargeson, A. M. In *Zinc Enzymes*; Spiro, T. S., Ed.; Wiley: New York, 1983; Chapter 7.
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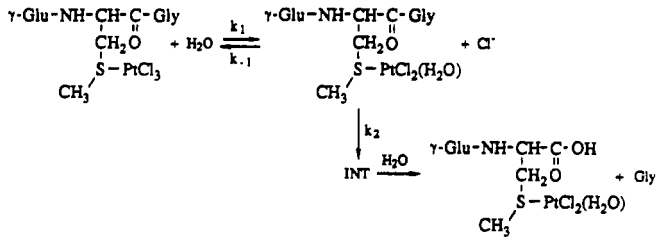
(69) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.
(70) Menabue, L.; Saladini, M.; Sola, M. *Inorg. Chem.* **1990**, *29*, 1293.

Table IV. Effect of the Aqua Ligand on the Observed Rate Constant for Hydrolysis of the Cysteine–Glycine Bond in Platinum(II) Complexes with *S*-Methylglutathione at pH* [a] 2.00 and at 40 °C

complex	equiv of free Cl^-	$10^5 k_{obs}$, min^{-1}
$[PtCl_3(GSMe)]$	1	135
$[PtCl_3(GSMe)]$	0	155
trans-$[PtCl_2(H_2O)(GSMe)]^+$	0	270
cis-$[Pt(en)Cl(GSMe)]^{2+}$	1	~10
cis-$[Pt(en)(H_2O)(GSMe)]^{3+}$	0	210

[a] In D_2O; uncorrected for isotope effect.

Scheme I

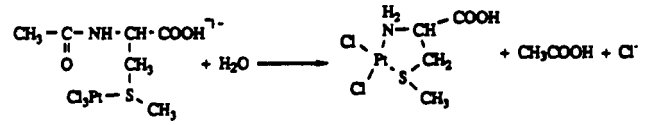


Both of these mechanisms require anchoring of the platinum complex to a side chain and entry of a water molecule into the complex. But this aqua ligand can play different roles. In the unimolecular mechanism, it is the nucleophile. In the bimolecular mechanism, it is a good leaving group, to be displaced by the carbonyl oxygen atom. Although the first aqua ligand enters trans to the sulfur ligand,[64] in a position unsuitable for chelation to the carbonyl oxygen atom, the second mechanism remains possible because further aquation, now certainly in the suitable position cis to the sulfur anchor, does occur in the hydrolysis reaction. The complex $[PtI_3(GSMe)]$ does not undergo hydrolysis, possibly because iodo ligands are too nucleophilic to be displaced by water and the carbonyl oxygen atom. The chelate intermediate designated **3** has an authentic precedent in a platinum(II) complex containing *S*-methylcysteine as a bidentate S,O ligand.[71] But the intermediate could not be detected by infrared and ^{195}Pt NMR spectroscopy during hydrolysis of $[PtCl_3(GSMe)]$. ^1H NMR spectroscopy, which is more sensitive, showed doubling of the cysteinyl CH resonance, but this is only tentative evidence of chelation. The chelate intermediate probably does not accumulate because the polarized amide group in it hydrolyzes rapidly. Its nondetection does not rule out the bimolecular mechanism.[72-74]

Selectivity of Hydrolysis. Only glycine, not glutamic acid, is formed during hydrolysis of platinated GSMe and GSH. This means that, of the two amide bonds adjacent to the anchoring residue, only the one involving its carboxylic group is cleaved, while the bond involving its amino group apparently remains intact. Unless the two amide bonds differ greatly in intrinsic hydrolytic reactivity, the selectivity in cleavage may be attributed to stereochemical factors. In the bimolecular mechanism, the six-membered chelate ring involving the carbonyl oxygen atom of the anchor itself, as in **3**, may be more favorable than the seven-membered ring involving the oxygen atom of the adjacent residue, as in **4**. Alternatively, in the unimolecular mechanism, the aqua ligand can be delivered more effectively to the former than to the latter carbonyl group.

This notion of stereochemical preference was tested by varying the position of the anchoring side chain with respect to the potentially scissile amide bond. The complex $[PtCl_3(MPGly)]^{2-}$ barely releases glycine even after 3 weeks. Although this complex oligomerizes, as do other metal complexes of MPGly,[58,75] this side reaction should not preclude hydrolysis because $[PtCl_3(GS)]^-$, which also oligomerizes, hydrolyzes as fast as the monomeric $[PtCl_3(GSMe)]$. The five-membered chelate ring involving the planar carbonyl group does not seem to form readily.

Both $[PtCl_3(AcCys)]^{2-}$ and $[PtCl_3(AcCysMe)]^-$ slowly release acetic acid; the latter reaction is shown in eq 6. The former



(6)

complex also oligomerizes, as expected. Both the S,N chelate product[76,77] and CH_3COOH are identified by ^1H NMR spectroscopy. These findings show that the amide bond involving the amino group of the anchoring residue will hydrolyze, albeit slowly, if an amide bond on the carboxy side is unavailable. The seven-membered ring shown in **4** seems to form, albeit not readily.

The side chain of methionine is longer by one methylene group than the side chains of cysteine and of *S*-methylcysteine. The effects of this greater conformational flexibility on the hydrolysis reaction were examined in experiments with Met-Gly and Ac-Met-Gly. The complex $[PtCl_3(Met\text{-}Gly)]$ does not release glycine but simply forms an S,N chelate analogous to the one shown in eq 6. The same reaction was observed previously for $[PtCl_3(Met)]^-$.[54] Acetylation of the N-terminus in Met-Gly prevents this chelation and makes available another amide bond, besides the peptide bond, for potential hydrolysis. The complex $[PtCl_3(AcMet\text{-}Gly)]^-$ does not release acetic acid but releases glycine as fast as $[PtCl_3(GSMe)]$ does. The eight-membered ring involving the acetyl oxygen atom is so unfavorable that the seven-membered ring involving the carbonyl oxygen atom becomes favorable in comparison.

To summarize, a platinum(II) complex attached to a side chain in a peptide will promote hydrolysis of an amide bond with whose carbonyl oxygen atom it can, at least in principle, form a six-membered or seven-membered chelate ring. This requirement restricts hydrolysis to amide bonds adjacent to the anchor, and hydrolysis occurs preferably on the carboxy side of it. This experimental finding remains a useful empirical rule of regioselectivity whichever mechanism is invoked to explain it.

Conclusion

This study shows that hydrolysis of unactivated amide bonds in amino acid derivatives and peptides can be promoted, under relatively mild and unrestrictive conditions, by attachment of certain platinum complexes to sulfur atoms in side chains. This attachment and subsequent regioselective hydrolysis seem to be governed by classical principles of coordination chemistry. With typical half-lives between several hours and half a day, these stoichiometric reactions may already be fast enough to be practically useful. We will examine metals other than platinum in search of faster, perhaps even catalytic, reactions; further study their mechanism; and apply the new inorganic cleavers to proteins.

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