Toward Artificial Metallopeptidases: Mechanisms by Which Platinum(II) and Palladium(II) Complexes Promote Selective, Fast Hydrolysis of Unactivated Amide Bonds in Peptides

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Certain platinum(II) and palladium(II) complexes attached to the sulfur atom of cysteine, *S*-methylcysteine, and methionine in peptides and in other amino-acid derivatives promote, under relatively mild conditions, selective hydrolysis of the unactivated amide bond involving the carboxylic group of the amino acid anchoring the metal complex. Kinetics of hydrolysis was studied with the substrates *N*-acetylcysteine, *N*-acetyl-*S*-methylcysteine, *N*-acetylmethionine, *N*-(2-mercaptopropionyl)glycine, leucylglycine, methionylglycine, *N*-acetylmethionylglycine, reduced glutathione, oxidized glutathione, *S*-methylglutathione, α-glutamylmethionylglycine, and γ-glutamylmethionylglycine and with platinum(II) and palladium(II) complexes containing chloro, aqua, ethylenediamine, 2,2′-bipyridine, 1,2-bis(diphenylphosphino)ethane, and 1,5-dithiacyclooctane ligands. The reactions were followed by ^{1}H NMR spectroscopy. Kinetic effects of pH, temperature, ionic strength, added chloride ions, and added thiourea are interpreted as follows. The promoter can act only if it is anchored to a side chain, and selectivity of this attachment is the main factor governing the regioselectivity of hydrolysis. Coordination of the metal atom to the deprotonated amide nitrogen atom inhibits hydrolysis, but approach of the complex to the amide oxygen atom promotes hydrolysis by two mechanisms. First, S,O chelation that activates the scissile amide bond toward external attack by water is favored in the case of platinum(II) promoters and substrates with shorter anchoring side chain. Second, without chelation, internal delivery of an aqua ligand to the scissile amide bond is favored in the case of palladium(II) promoters and substrates with longer anchoring side chain. Some promoters, among them $[PdCl_4]^{2-}$ and $[PtCl_4]^{2-}$, act as mononuclear active species. Others, most notably $[Pd(H_2O)_3(OH)]^+$, form binuclear active species. Certain substrates in the presence of this last promoter hydrolyze with half-lives of 10 min or less. This study points the way to a future method for selective cleavage of peptides and proteins by coordination complexes as artificial metallopeptidases.

Introduction

Hydrolysis of proteins and peptides has been studied more from the biochemical than from the chemical point of view. The mechanisms at the molecular level of these important reactions remain largely obscure. The half-life for hydrolysis of the amide bond in neutral aqueous solution is ca. 7 years.[1] Even with the strongest acids or bases in high concentrations, prolonged heating is necessary. Because of this extreme unreactivity,[2-4] kinetic and mechanistic studies have been done almost exclusively with amides that are variously activated by substituents, by ring strain, by forced nonplanarity, or by proximate functional groups.[5-10] Moreover, the reaction mixtures usually are heated.

Proteolytic enzymes, however, hydrolyze even unactivated amide bonds rapidly under mild conditions.[11] Although some biomimetic systems surpass chymotrypsin in the rate of stoichiometric hydrolysis of activated esters, these systems fall short of enzymes, which are catalysts and which hydrolyze unactivated amides.[12] Catalytic antibodies hold promise for enzyme-like hydrolysis of peptides because these agents are true catalysts and because they show selectivity.[13-15]

Since certain proteolytic enzymes require metal ions for activity,[16,17] hydrolysis reactions have caught the attention of inorganic chemists. In studies with metal complexes, again, carboxylic and phosphate esters are used more often than amides.[18-48] Complexes of cobalt(III)[28] and of copper(II)[32] have been studied

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more than any other, and mechanisms by which these metal ions act are known in detail. Since cobalt(III) complexes bind to the N-terminal amino-acid residue, only the N-terminal amide bond in the peptide is hydrolyzed. Although these reactions are stoichiometric, they are relevant to turnover reactions catalyzed by aminopeptidases. Recent reports of oxidative cleavage of proteins mediated by metal complexes[49-55] widen the range of inorganic methods for these biochemical operations.

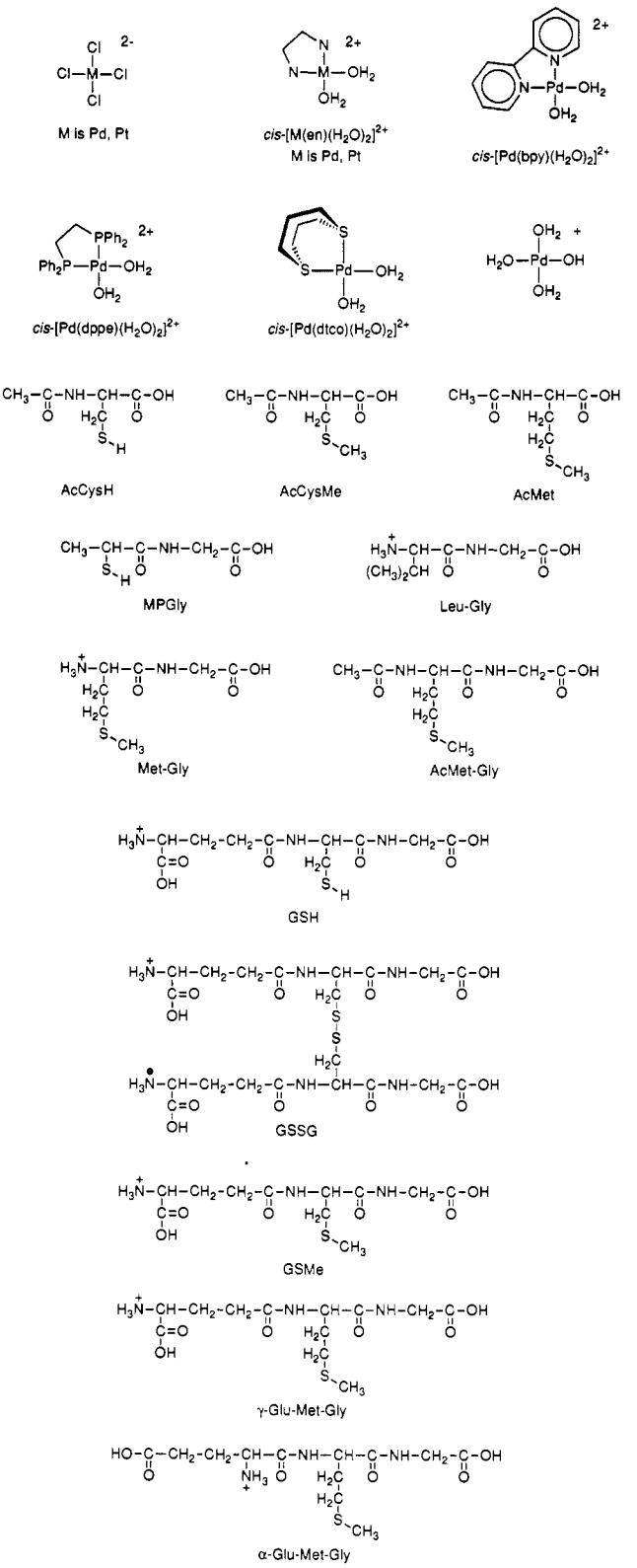
Platinum(II) complexes were used previously by others to promote hydrolysis of inorganic oligophosphates[56] and of activated phosphate esters.[57] In this laboratory certain platinum(II) complexes proved capable of promoting, under relatively mild conditions, hydrolysis of unactivated amide bonds in peptides and in other amino-acid derivatives.[58] When the substrates and platinum(II) promoters were matched so as to aid hydrolysis, the reaction half-lives fell in the range from 2 days to 2 h at 40 °C. Dependence of the rate constants on the substrate, ancillary ligand in the promoter complex, pH, ionic strength, and chloride concentration revealed several features of the mechanism.[58]

Our method differs from previous methods for peptide hydrolysis in that a thiophilic metal atom (with ancillary ligands) selectively binds to the sulfur atom of cysteine, *S*-methylcysteine, and methionine and selectively activates one of the two adjacent amide bonds. On this principle, we intend to develop metal complexes that selectively promote cleavage of peptides and proteins, not merely their shortening at the termini. In other words, we intend to mimic endopeptidases, not exopeptidases. In this study we further elucidate the mechanism by which the platinum(II) promoters act. What is more, we show that certain palladium(II) complexes promote hydrolysis with half-lives as short as several minutes. We analyze the mechanisms of hydrolysis reactions and find interesting differences between similar metal complexes as promoters and also between similar peptides as substrates. These studies are our early steps toward the ultimate goal of designing an artificial inorganic endopeptidase.

Experimental Procedures

Reagents and Solvents. Distilled water was demineralized and purified to a resistance greater than 10 MΩ-cm. The deuterium-containing compounds D_2O, $DClO_4$, and NaOD and the complexes $K_2[PdCl_4]$, $K_2[PtCl_4]$, and *cis*-$[Pt(en)Cl_2]$ were obtained from Sigma Chemical Co. Anhydrous $AgClO_4$ was obtained from G. Frederick Smith Chemical Co. All other chemicals were of reagent grade.

Chart I



Metal Complexes. The complexes tested as hydrolysis promoters are shown in Chart I. The following chloro complexes were prepared by published procedures: *cis*-$[Pd(en)Cl_2]$,[59] *cis*-$[Pd(bpy)Cl_2]$,[60] and *cis*-$[Pd(dppe)Cl_2]$.[61] The corresponding complexes with aqua (actually, D_2O) ligands were obtained by treating each of these three complexes with 2

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equiv of anhydrous $AgClO_4$ in D_2O as the solvent.[62] The complex $[Pd(H_2O)_3(OH)]ClO_4$ in solution was obtained by stirring a mixture of $K_2[PdCl_4]$ and 4 equiv of anhydrous $AgClO_4$ in D_2O for 4 h at 35 °C. The pH* (uncorrected for isotope effect) was adjusted to 1.0 by addition of $DClO_4$. The complex *cis*-$[Pd(dtco)(H_2O)_2](ClO_4)_2$, containing the ligand 1,5-dithiacyclooctane, was prepared in solution at pH* 2.0 by a published procedure.[63] In each case the solid AgCl was removed by filtration in the dark, and fresh solution of the aqua complex was used in further experiments.

The aqua complexes in solution were characterized by UV–vis and ^1H NMR spectroscopic methods. For *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$, ϵ = 260–276 M^{-1} cm^{-1} at 340–345 nm and δ_{en} = 2.63 ppm (s); the UV–vis spectrum is consistent with the recent report by others.[59] For *cis*-$[Pd(bpy)(H_2O)_2](ClO_4)_2$, ϵ = 2020 M^{-1} cm^{-1} at 310 nm and δ_{bpy} = 7.71, 8.31 ppm (both m). For *cis*-$[Pd(dppe)(H_2O)_2](ClO_4)_2$, there are no peaks in the UV–vis region; δ_{CH_2} = 2.86, 3.00 ppm (both m); $\delta_{C_6H_5}$ = 7.66, 7.84, 7.89 ppm (all m). For $[Pd(H_2O)_3(OH]ClO_4$, ϵ = 90–120 M^{-1} cm^{-1} at 390–400 nm; these values are close to those reported for the similar complex $[Pd(H_2O)_3Cl]^+$.[64]

Substrates for Hydrolysis. Amino-acid derivatives and peptides whose hydrolysis is reported here are shown in Chart I. Their charges are as expected for major species at pH 2.0. Glycine (Gly), *S*-methyl-L-cysteine (CysMe), *N*-acetyl-L-cysteine (AcCysH), *N*-(2-mercaptopropionyl)-glycine (MPGly), *N*-acetylmethionine (AcMet), methionylglycine (Met-Gly), leucylglycine (Leu-Gly), reduced glutathione (GSH), *S*-methylglutathione (GSMe), and oxidized glutathione (GSSG) were obtained from Sigma Chemical Co. The tripeptides α-Glu-Met-Gly and γ-Glu-Met-Gly were synthesized by the standard solid-state method and purified by HPLC in the Protein Facility at this university. The compound *N*-acetyl-*S*-methyl-DL-cysteine (AcCysMe) was prepared by a published method.[65] The peptide *N*-Acetylmethionylglycine (AcMet-Gly) was obtained by adding acetic anhydride (58.8 μL, 0.64 mmol) to a stirred solution of Met-Gly (128 mg, 0.64 mmol) in 1.60 mL of glacial acetic acid and stirring at ca. 35 °C for 3 h. Evaporation of the reaction mixture in vacuo at 40 °C yielded a white powder. ^1H NMR data (δ values): 2.05, s, CH_3CO; 2.11, s, CH_3S; 4.00, s, Gly CH_2.

Measurements. Proton NMR spectra at 300 MHz of solutions in D_2O were recorded with a Varian VXR300 spectrometer, with DSS as an internal reference. The sample temperature was kept constant within ±0.1 °C. Infrared spectra of mulls were recorded with an IBM98 Fourier-transform spectrometer. Photoacoustic infrared and far-infrared spectra were recorded with a Perkin-Elmer FTIR 1800 spectrometer and a MTEC 200 photoacoustic detector. A spectrum of carbon black was recorded between the sample spectra in order to normalize variations owing to the infrared source and to the spectrometer. Ultraviolet–visible spectra were recorded with an IBM 9430 spectrophotometer, whose monochromator has two gratings. The pH was measured with a Fischer 925 instrument and a Phoenix Ag/AgCl reference electrode. The uncorrected values in deuteriated solvents are designated pH*. Elemental analyses were done by Galbraith Laboratories, Inc.

Stability of Nonmetalated Substrates. Solutions of amino-acid derivatives and peptides were prepared as in the hydrolysis experiments (see below) except that the metal complexes were missing. The solvent always was D_2O, and pH* was adjusted with $DClO_4$ or NaOD. In some experiments $AgClO_4$ was added as well. The solutions were kept at 40 ± 1 °C and occasionally examined by ^1H NMR spectroscopy. Only about 10% of AcMet-Gly hydrolyzed, with liberation of glycine, over 12 days at pH* 0.70.

Attachment of Metal Complexes to Amino Acid Side Chains. Substrates and freshly prepared metal complexes in Chart I are mixed pairwise, in equimolar amounts. Reactions were followed by ^1H NMR spectroscopy. In some experiments, ternary mixtures of *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$, amino-acid derivative or peptide, and thiourea (tu) were studied. A mixture of this complex, AcMet, and thiourea in the mole ratio 1:1:2 yields the precipitate *cis*-$[Pd(dtco)(tu)_2]^{2+}$, and free AcMet remains in solution. A mixture in the mole ratio 1:1:1 contains *cis*-$[Pd(dtco)(AcMet)(tu)]^{2+}$. All of these experiments were done at pH* 0.99.

$[Pd(GS)(H_2O)]_2 \cdot H_2O$. Upon addition of 0.255 g (0.830 mmol) of reduced glutathione to an equimolar amount of *cis*-$[Pd(en)(H_2O)_2](ClO_4)_2$ in solution at pH 2.0, the color turned red, and a yellow-orange precipitate formed later. This solid was filtered out, washed with ethanol and acetone, and dried in vacuo overnight. Infrared spectrum (ν in cm^{-1}) of free GSH: 3340 and 3250, NH_3^+ and NH_2; 2524, SH; 1710, COOH; 1610, COO^- asym; 1394, COO^- sym. Infrared spectrum (ν in cm^{-1}) of $[Pd(GS)(H_2O)]_2 \cdot H_2O$: 3510, H_2O; 3244 and 3066, NH_2; 1753, COOH; 1526, NH_2; 1638, COO^- asym; 1384, COO^- sym; 564, Pd–N; 412, Pd–O; 382, Pd–S. Anal. Calcd (found) for $C_{20}H_{36}N_6O_{15}S_2Pd_2$: C, 27.38 (27.45); H, 4.14 (4.17); N, 9.58 (9.45).

Study of Hydrolysis. Equimolar amounts of a substrate and of a metal complex, both dissolved in D_2O, were mixed rapidly in an NMR tube. The solution was 50.0 mM in each, and the volume was 600 μL. Acquisition of the ^1H NMR spectra began as soon as possible, and 16 scans were taken at each time. The temperature was kept within ±0.1 °C of the nominal value. The pH* value was measured before and after the hydrolysis reaction; the difference was less than 0.10, and the final value is reported. The methylene signals of free glycine and of peptide-bound glycine were integrated with errors estimated at ±5%. Concentrations of these two forms of glycine were calculated on the basis of the signal areas and the known initial concentration of the substrate. First-order logarithmic plots of substrate concentration or of free glycine concentration versus time were linear for 3 half-lives. Slow hydrolysis reactions were followed for less than 3 half-lives. Typical plots consisted of 10–20 points, and correlation coefficients were 0.980–0.996. Enthalpies and entropies of activation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, were calculated by the Eyring equation. Their error bounds were computed by a standard method.[66]

Detection of Free Glycine. Free glycine, one of the hydrolysis products, was identified by ^1H NMR spectroscopy and by thin-layer chromatography. Addition of pure glycine to the reaction mixture enhanced the ^1H NMR signal of the hydrolysis product, and no new signal appeared.

Reactions for analysis by thin-layer chromatography were run for 6 h at 50 °C, so that hydrolysis was complete. The metal complex was precipitated out by addition of diethyldithiocarbamate, and the yellow solid was removed by filtration. The colorless filtrate was chromatographed on silica gel G with a mixture of 1-butanol, acetic acid, and water in the volume ratio 40:6:15 and developed with a solution containing 300 mg of ninhydrin in a mixture of 3 mL of glacial acetic acid and 100 mL of 1-butanol. A control solution containing glycine instead of the hydrolysis substrate was treated exactly like the reaction mixture. Both the reaction mixture and the control solution yielded a single spot with an R_f value of 0.202.

Results

Metal Complexes. The aqua complexes in Chart I are made by treatment of the corresponding chloro complexes with silver cations in acidic solution. Control experiments showed that $AgClO_4$ itself does not promote hydrolysis of substrates. On the basis of the pK_a value[67–69] for the process in eq 1, we estimate that

$$[Pd(H_2O)_4]^{2+} \rightleftarrows [Pd(H_2O)_3OH]^+ + H^+ \qquad (1)$$

the complex $[Pd(H_2O)_3OH]^+$ is the main species present at pH ca. 1.0. Kinetic studies of hydrolysis, to be discussed later, support this conclusion. Since amine, phosphine, and thioether ligands are good electron donors, other aqua complexes in Chart I are less acidic than the homoleptic aqua complex. These heteroleptic complexes exist mainly in the fully protonated form under the reaction conditions, and their formulas are written accordingly. Since aqua complexes of palladium(II) in solution form soluble oligomers upon standing, promoters for hydrolysis are always prepared fresh. Initial attachment of the promoter to the substrate amounts to displacement of a chloro or aqua ligand by the thiolate or thioether group in the side chain. This displacement is accompanied by a characteristic shift downfield of the CH_3S ^1H NMR resonance (in the case of thioethers); see Table I.

The reaction in eq 2 differs from most of the others because it yields an insoluble product with the composition [Pd(GS)-

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Table I. Effect of Coordination on ^1H NMR Chemical Shifts (δ, in ppm) of CH_3S Groups at pH* ≈ 1.0[a]

promoter[b]	active form	GSMe	AcMet-Gly
none		2.13	2.11
$[PtCl_4]^{2-}$	mononuclear	2.44	2.35
$[PdCl_4]^{2-}$	mononuclear	2.35	2.28
$[Pd(H_2O)_3(OH)]^+$	mononuclear	2.26, 2.28	2.29
	binuclear	2.41	2.47
cis-$[Pd(en)(H_2O)_2]^{2+}$	mononuclear	2.26, 2.28	2.37
	binuclear	2.44	2.50
cis-$[Pd(dtco)(H_2O)_2]^{2+}$	mononuclear		2.37
	binuclear		2.50

[a] Uncorrected for isotope effect. [b] Since the solvent is D_2O, exchangeable H atoms are deuteriated.

Table II. Hydrolysis of the Cysteine–Glycine Bond in *S*-Methylglutathione, Promoted by Complexes of Palladium(II) and Platinum(II)

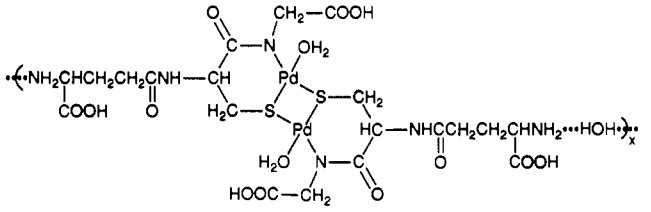
promoter[a]	pH* [b]	$10^6 k_{obsd}$, min^{-1} at 40 °C
$[PdCl_4]^{2-}$	1.20	220 ± 10
cis-$[Pd(en)(H_2O)_2]^{2+}$	0.97	300 ± 10
$[Pd(H_2O)_3(OH)]^+$	0.83	850 ● 10
cis-$[Pd(bpy)(H_2O)_2]^{2+}$	0.88	7.3 ± 0.8
$[PtCl_4]^{2-}$	1.10	2700 ● 100
cis-$[Pt(en)(H_2O)_2]^{2+}$	0.97	610 ± 40

[a] See Table I, footnote *b*. [b] See Table I, footnote *a*.

$(H_2O)_2]_2{\cdot}H_2O$. The palladium–ligand stretching vibrations in it were assigned according to previous studies.[70,71] The infrared

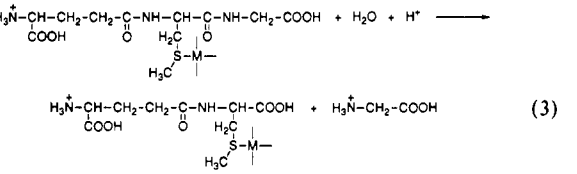
$$2GSH + 2[Pd(en)(H_2O)_2]^{2+} + H_2O \rightarrow [Pd(GS)(H_2O)_2]_2{\cdot}H_2O + 2enH_2^{2+} \quad (2)$$

spectra, elemental analysis, and insolubility in several common solvents are consistent with the following polymeric structure:



The S–H vibrational band disappears, and the Pd–S, Pd–N, and Pd–O bands appear, as glutathione is converted into the complex. Since the COOH band in the complex is shifted by only 38 cm^{-1} from its position in free glutathione, this group is not coordinated to palladium. The ^1H NMR resonance of the glycine CH_2 group is shifted upfield, to 2.74 ppm; this is a clear sign of coordination of amide group to palladium(II) via deprotonated nitrogen atom. Such coordination under the pH values and other reaction conditions used in this study is well documented.[72-74] So is the formation of stable thiolate bridges between palladium(II) atoms.[75,76]

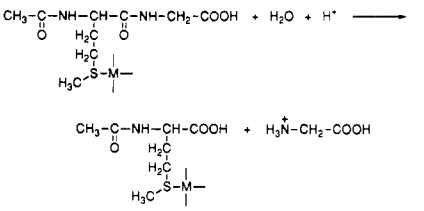
Hydrolysis of GSMe, Promoted by Pt(II) and Pd(II) Complexes. Table II shows that various complexes of divalent palladium and platinum promote selective hydrolysis of the cysteine–glycine bond (eq 3) under conditions at which free *S*-methylglutathione is completely stable for weeks. The complex *cis*-



(3)

$[Pd(dppe)(H_2O)_2]^{2+}$ produced a white precipitate with this peptide, and hydrolysis was not detected. For this substrate, platinum(II) is a better promoter than palladium(II). The fastest of these reactions, the one promoted by $[PtCl_4]^{2-}$, has a half-life of 4.3 h.

Hydrolysis of AcMet-Gly, Promoted by Pt(II) and Pd(II) Complexes. The yellow precipitate formed upon mixing of the peptide Met-Gly and $[PdCl_4]^{2-}$ is the S,N-bidentate complex.[77,78] When, however, the amino group of the methionyl residue is acetylated, the promoter complexes bind solely to the sulfur atom, and hydrolysis reactions proceed smoothly as shown in eq 4. Table



(4)

III shows that various complexes promote hydrolysis of AcMet-Gly. A typical kinetic plot is shown in Figure 1. Thiourea (tu) inhibits the hydrolysis reaction, and we studied this effect in reactions involving *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$. Kinetic results are given in Table IV. Peptide hydrolysis reactions promoted by platinum(II) complexes that we studied before had half-lives of hours and days.[58] The fastest reaction promoted by a palladium(II) complex now has a half-life of less than 30 min.

Hydrolysis of Different Substrates, Promoted by $[PdCl_4]^{2-}$. As Table V shows, the rate at which glycine is cleaved off varies considerably among different substrates. Unlike the other substrates, which contain the amide bond on the carboxylic side of the anchoring group and release glycine, AcCysMe contains only the amide bond on the amino side of the anchor and therefore releases acetic acid.

Dependence of Hydrolysis Rate on pH. In this study the effect of pH was examined with the substrates AcMet-Gly and γ-Glu-Met-Gly and with the promoter $[Pd(H_2O)_3(OH)]^+$. The former reaction is shown in eq 4, and the latter is analogous to it. The results are given in Table VI. In our previous study[58] the effect of pH was examined with the substrate *S*-methylglutathione (GSMe) and with two platinum(II) complexes as promoters. The synthetic peptide γ-Glu-Met-Gly used now is homologous to *S*-methylglutathione used before; the two substrates differ only in one methylene group in the anchoring side chain—methionine versus *S*-methylcysteine. The rate constants in this and the previous study obey the linear equations in Table VII. Although the results in Table VII do not permit comparisons of promoters in their effects on the same substrate, one generalization may tentatively be made. The reactions promoted by $[Pd(H_2O)_3(OH)]^+$, for which the slope is greater than 0.90, seem to depend on pH more than the reactions promoted by platinum(II) complexes, for which the slope is only ca. 0.10.

Dependence of Hydrolysis Rate on Ionic Strength. The reaction in eq 4 was studied in solutions containing different concentrations of the nonbinding salt $NaClO_4$. The different reaction mixtures unavoidably differed slightly in pH*. The corrected rate constants in Table VIII slightly increase as ionic strength increases, but

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(78) Theodorou, V.; Hadjiliadis, N. *Polyhedron* **1985**, *4*, 1283.

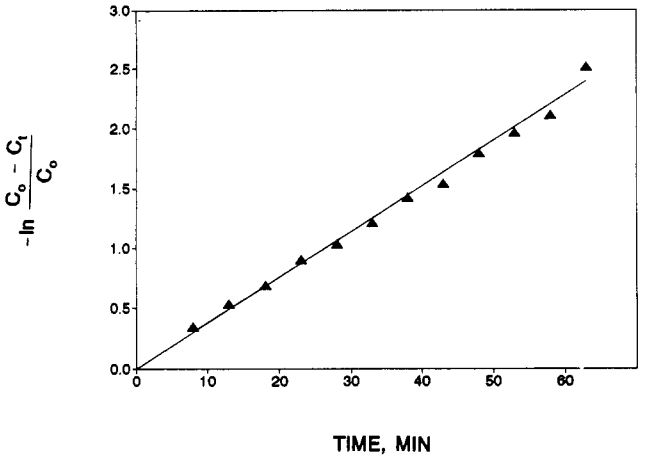


Figure 1. First-order kinetic plot for hydrolysis of the methionine–glycine amide bond in *N*-acetylmethionylglycine, promoted by *cis*-$[Pd(en)(H_2O)_2]$ (actually, by a deuteriated isotopomer of this complex) in D_2O, at pH* 1.00 (uncorrected for the isotope effect), and at 50 ± 0.1 °C.

Table III. Hydrolysis of the Methionine–Glycine Bond in *N*-Acetylmethionylglycine, Promoted by Complexes of Palladium(II) and Platinum(II)

promoter[a]	pH* [b]	$10^4 k_{obsd}$, min^{-1} at 40 °C
$[PdCl_4]^{2-}$	1.14	5.1 ± 0.1
cis-$[Pd(en)(H_2O)_2]^{2+}$	1.00	140 ± 20
$[Pd(H_2O)_3(OH)]^+$	1.07	260 ± 30
$[PtCl_4]^{2-}$	1.09	15 ± 1
cis-$[Pt(en)(H_2O)_2]^{2+}$	0.94	4.6 ± 0.6

[a] See Table I, footnote *b*. [b] See Table I, footnote *a*.

Table IV. Hydrolysis of the Methionine–Glycine Bond in *N*-Acetylmethionylglycine, Promoted by *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$ [a] in the Presence of Thiourea

mole ratio Pd:substrate:tu	pH* [b]	$10^3 k_{obsd}$, min^{-1} at 50 °C
1:1:1	0.99	0
1:0.5:0	1.09	11 ± 1
1:1:0	1.00	3.1 ± 0.5
1:0.5:0.5	0.99	0.7 ± 0.2

[a] See Table I, footnote *b*. [b] See Table I, footnote *a*.

Table V. Hydrolysis in Various Substrates, Promoted by $[PdCl_4]^{2-}$

substrate[a]	pH* [b]	$10^6 k_{obsd}$, min^{-1} at 40 °C	substrate[a]	pH* [b]	$10^6 k_{obsd}$, min^{-1} at 40 °C
GSMe	1.20	220 ± 10	GSSG	0.81	114 ± 13
GSH	0.79	12.5 ± 0.6	MPGly	0.65	*c*
AcMet-Gly	1.14	510 ± 10	AcCysMe	0.81	1.6 ± 0.2

[a] The first five substrates release glycine; the last one releases acetic acid. [b] See Table I, footnote *a*. [c] Very low.

Table VI. Effect of pH on Hydrolysis of the Methionine–Glycine Bond in Two Peptides, Promoted by $[Pd(H_2O)_3(OH)]^+$ [a]

substrate	pH* [b]	$10^2 k_{obsd}$, min^{-1} at 40 °C	substrate	pH* [b]	$10^2 k_{obsd}$, min^{-1} at 40 °C
AcMet-Gly	1.07	2.6 ± 0.3	γ-Glu-Met-Gly	1.20	1.5 ± 0.1
	1.05	2.8 ± 0.1		0.82	3.8 ± 0.1
	0.95	4.2 ± 0.1		0.49	6.9 ± 0.1
	0.88	5.0 ± 0.1			
	0.68	7.1 ± 0.3			
	0.53	8.5 ± 0.1			

[a] See Table I, footnote *b*. [b] See Table I, footnote *a*.

this small variation may still be a consequence of the change in pH* as well as in ionic strength.

Dependence of Hydrolysis Rate on Cl^- Concentration. As Table IX and Figure 2 show, the reaction in eq 4 promoted by $[Pd(H_2O)_3(OH)]^+$ is greatly inhibited by addition of NaCl. Since

Table VII. Relationships Between pH* [a] and the Rate Constant for Hydrolysis of the Amide Bond Involving Glycine in Peptides, Promoted by Palladium(II) and Platinum(II) Complexes at 40 °C

substrate	promoter[b]	log k_{obsd} =
AcMet-Gly	$[Pd(H_2O)_3(OH)]^+$	−0.53 − 0.94 pH*
γ-Glu-Met-Gly	$[Pd(H_2O)_3(OH)]^+$	−0.69 − 0.93 pH*
GSMe	$[PtCl_4]^{2-}$	−2.5 − 0.098 pH*
	trans-$[PtCl_2(H_2O)_2]$[c]	−2.3 − 0.11 pH*

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*. [c] Nominal precursor of *trans*-$[PtCl_2(GSMe)(H_2O)]^+$.[58]

Table VIII. Effect of Ionic Strength on Hydrolysis of the Methionine–Glycine Bond in *N*-Acetylmethionylglycine, Promoted by $[Pd(H_2O)_3(OH)]^+$ [a]

$NaClO_4$ concn, M	μ, M	pH* [b]	$10^2 k_{obsd}$, min^{-1} at 40 °C: exptl	$10^2 k_{obsd}$, min^{-1} at 40 °C: corr for pH* difference
0.10	0.20	0.86	6.7 ± 0.3	4.6
1.00	2.00	0.77	7.8 ± 0.2	5.6
2.00	4.00	0.67	9.3 ± 0.3	6.9
3.00	6.00	0.60	9.7 ± 0.3	8.0
4.00	8.00	0.55	10.6 ± 0.3	9.0

[a] See Table I, footnote, *b*. [b] See Table I, footnote *a*.

Table IX. Effect of Chloride Concentration on Hydrolysis of the Methionine–Glycine Bond in *N*-Acetylmethionylglycine, Promoted by 0.050 M $[Pd(H_2O)_3(OH)]^+$,[a] at Ionic Strength of 1.00 M, 40 °C, and pH* 0.84

$[Cl^-]$, M	$10^3 k_{obsd}$, min^{-1}	$[Cl^-]$, M	$10^3 k_{obsd}$, min^{-1}
0	30 ± 1	0.040	5.8 ± 1
0.010	26 ± 1	0.050	3.6 ± 0.2
0.020	18 ± 1	0.12	0.57 ± 0.05
0.030	12 ± 1	0.20	0.32 ± 0.02

[a] See Table I, footnote *b*. [b] See Table I, footnote *a*.

ionic strength was kept constant by addition of the nonbinding salt $NaClO_4$, the inhibition must be due to the chloride ion. The concentration of the promoter was 50 mM. When chloride concentration is lower than 50 mM and when it is 50 mM and greater, k_{obsd}^{-1} depends differently on it. The rate constant at the chloride concentration of 40 mM does not fit either of the plots in Figure 2.

Enthalpy and Entropy of Activation for Hydrolysis. The kinetic results are given in Table X, and the activation parameters are given in Table XI.

Discussion

Mechanisms for Amide Hydrolysis. Consideration of the substrates in Chart I reveals what structural features are required for hydrolysis. The dipeptide Leu-Gly does not hydrolyze in the presence of metal complexes because it lacks a side chain that can coordinate to the metal atom. Substrates that can form stable chelates with the intended promoter do not hydrolyze, either. For example, the dipeptide Met-Gly forms an insoluble S,N-bidentate complex with palladium(II), while its N-acetylated derivative (AcMet-Gly) hydrolyzes with loss of glycine. Similarly, the tripeptide α-Glu-Met-Gly forms an insoluble S,N,N-tridentate complex, while its isomer γ-Glu-Met-Gly hydrolyzes with loss of glycine. The amide nitrogen atom of methionine coordinates to the palladium(II) atom only when this coordination yields a tridentate chelate (in α-Glu-Met-Gly) but does not coordinate when the product would be merely a bidentate chelate (in γ-Glu-Met-Gly). This requirement for a single attachment of the promoter to the substrate is a distinctive feature of the platinum(II) and palladium(II) complexes as new reagents for cleavage of peptides. Since these two metals have high affinities for sulfur ligands, they bind selectively to side chains of cysteine (or *S*-methylcysteine) and of methionine and activate only the amide bonds adjacent to these anchors. But the anchoring side chain

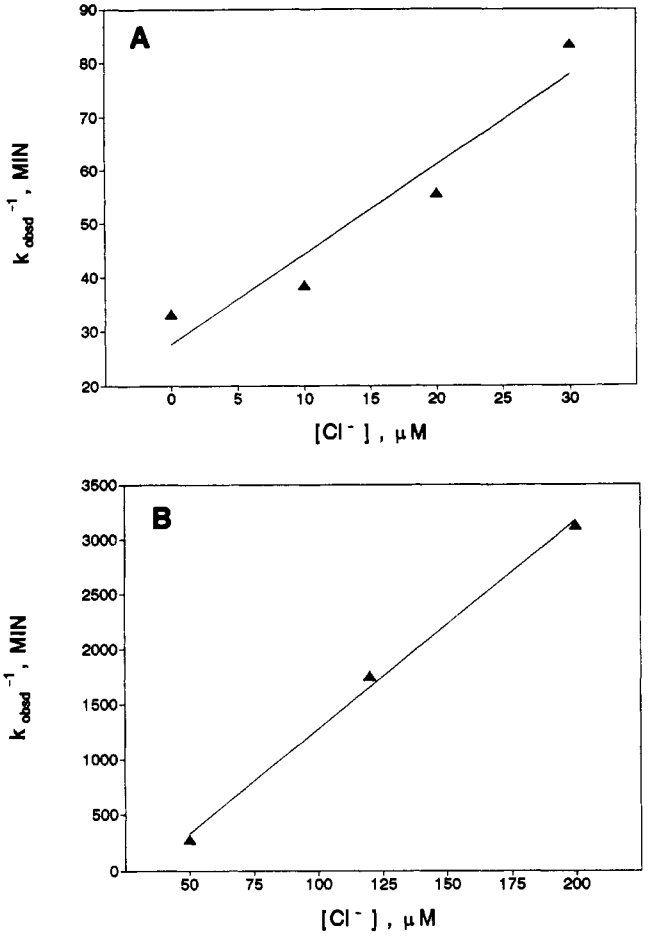


Figure 2. Effects of the chloride ion on the rate constant for hydrolysis of the methionine–glycine bond in *N*-acetylmethionylglycine, promoted by $[Pd(H_2O)_3(OH)]^+$ (actually, by a deuteriated isotopomer of this complex) in D_2O, at ionic strength of 1.00 M, at pH* 0.84 (uncorrected for the isotope effect), and at 40 ± 0.1 °C. The plots A and B differ in the ranges of chloride concentration.

Table X. Effect of Temperature on the Rate Constant for Hydrolysis of the Indicated Peptide Bonds, Promoted by Metal Complexes at pH* ≈ 1.1[a]

substrate	bond	promoter[b]	T, °C	$10^3 k_{obsd}$, min^{-1}
GSMe	Cys-Gly	$[PtCl_4]^{2-}$	40	2.68 ± 0.03
			50	6.8 ± 0.2
			60	20.50 ± 0.05
AcMet-Gly	Met-Gly	$[PdCl_4]^{2-}$	30	0.20 ± 0.01
			40	0.51 ± 0.01
			50	2.9 ± 0.1
			60	8.1 ± 0.2
		$[Pd(H_2O)_3(OH)]^+$	30	7.8 ± 0.2
			40	30 ± 1
			50	90 ± 3

[a] See Table I, footnote *a*. [b] See Table I, footnote *b*.

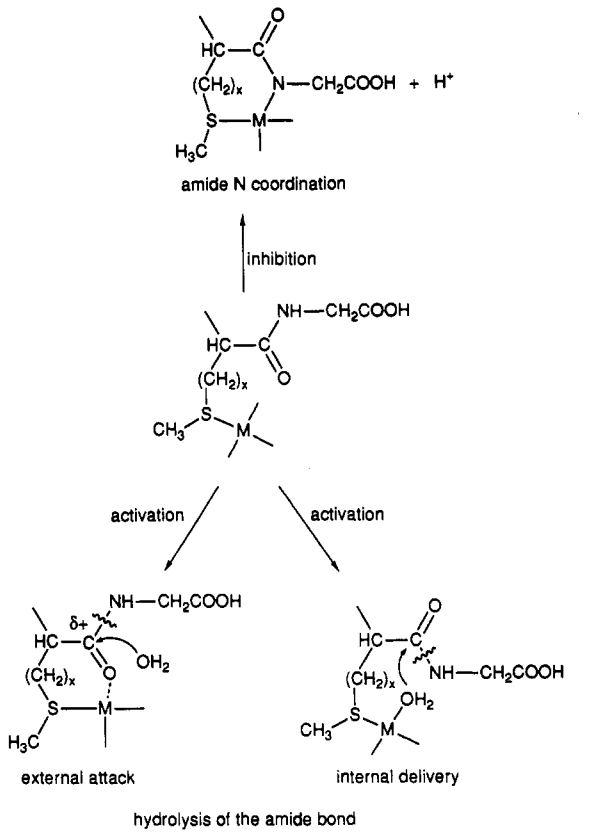
Table XI. Enthalpies and Entropies of Activation for Hydrolysis of the Amide Bond Involving the Glycine Amino Group in Peptides, Promoted by Palladium(II) and Platinum(II) Complexes

substrate	promoter[a]	ΔH^*, kJ·mol^{-1}	ΔS^*, J·mol^{-1}·K^{-1}
GSMe	$[PtCl_4]^{2-}$	85.3 ± 1.7	−56.4 ± 2.8
AcMet-Gly	$[PdCl_4]^{2-}$	105.1 ± 6.7	−4.2 ± 11.7
	$[Pd(H_2O)_3(OH)]^+$	96.3 ± 4.8	−1.2 ± 8.2

[a] See Table I, footnote *b*.

must be sufficiently long to allow approach of the metal complex to the scissile bond. As Tables II–V show, the substrate MPGly, in which the anchoring group is directly bonded to the main chain, hydrolyzes in the presence of palladium(II) complexes much slower than substrates in which the anchoring group is a part of an amino-acid side chain.

Scheme I



The interaction between the promoter and the scissile amide bond can be analyzed on the basis of the kinetic results in Tables II and III. The reactions in eqs 3 and 4 may follow two competing general pathways, shown in Scheme I. In the first pathway coordination of the peptide nitrogen atom stabilizes the amide bond involved and thus inhibits its cleavage.[22,79] In the second pathway, interaction of the metal complex with the carbonyl group can lead to cleavage by two mechanisms.[28,32] The metal atom may form a chelate with the oxygen atom, further polarizing the C=O bond and activating it toward external attack by solvent water. Alternatively, an aqua ligand attached to the metal atom may be delivered to the carbon atom internally, in an efficient intramolecular reaction. This internal delivery may proceed via a cyclic transition state and thus may share some features with the mechanism involving external attack, but our experimental evidence does not warrant such subtle distinctions. The defining feature of the two mechanisms is the origin of the water molecule—from the solvent or from the metal coordination sphere. (A third pathway, a combination of the other two, has also been proposed.[32]) The presence of an aqua ligand does not guarantee that this ligand will be internally delivered to the scissile bond; in other words, one of the (unspecified) ligands in the chelation mechanism can be a water molecule.[58] The mechanism via chelation and external attack by water may be favored when the side chain permits formation of a favorable ring. This may be the case with *S*-methylglutathione, for which $x = 1$ and the chelate ring is six-membered. Indeed, the relatively large negative entropy of activation in Table XI supports the notion that conformational freedom is diminished in the transition state for the hydrolysis of *S*-methylglutathione. The mechanism via internal delivery of an aqua ligand may be favored when the side chain permits approach of the metal–aqua fragment to the scissile bond. This may be the case with AcMet-Gly and with the α and γ isomers

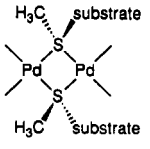
(79) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.

of Glu-Met-Gly, substrates for which $x = 2$ and for which therefore the chelate ring would be seven-membered and unfavorable. Indeed, the virtually nil entropy of activation in Table XI supports the notion that conformational freedom of these substrates is unaffected by formation of the transition state, as is characteristic of unimolecular reactions. If both mechanisms for hydrolysis in Scheme I proceed via a cyclic transition state, the observed difference in the entropy of activation might reflect the difference in the ring size caused by the difference in x. We think, however, that the difference of ca. 50 $J \cdot mol^{-1} \cdot K^{-1}$ is too large to be explained in this way.

Both platinum(II) and palladium(II) complexes selectively promote hydrolysis of the amide bond involving the carboxylic group of the anchoring amino-acid residue. Only when such an amide bond is absent, as in AcCysMe, does hydrolysis occur at the amide bond involving the amino group of the anchoring residue. For example, GSMe and AcCysMe both contain *S*-methylcysteine as an anchor. As Table V shows, the former substrate hydrolyzes ca. 140 times faster than the latter under similar conditions. Explanations of this striking regioselectivity will require conformational analysis of the putative intermediates and transition states for the mechanisms in Scheme I.

Kinetic findings in Tables II and III can be explained in terms of Scheme I. Palladium(II) is more effective than platinum(II) in assisting deprotonation of amides; the former binds to the amide nitrogen atom even at low pH values.[72,79,80] Therefore hydrolysis of GSMe (eq 3), in which the inhibition pathway is stereochemically feasible, is promoted more efficiently by platinum(II) complexes than by analogous palladium(II) complexes. Palladium(II) complexes are labile, whereas platinum(II) complexes are inert to loss of ligands (such as water). Therefore hydrolysis of AcMet-Gly (eq 4), in which the mechanism of internal delivery is stereochemically feasible, is promoted more efficiently by palladium(II) complexes than by analogous platinum(II) complexes. In summary, we propose that similar substrates differing in the length of the side chain hydrolyze by different mechanisms and that similar complexes differing in the nature of the divalent metal atom promote hydrolysis by different mechanisms. These unexpected findings rest on various evidence, which is discussed below.

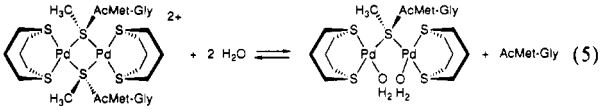
Active Form of the Promoter. Shifts downfield of the ^1H NMR signals of the substrates upon their reaction with $[PtCl_4]^{2-}$ and with $[PdCl_4]^{2-}$ are characteristic of unidentate coordination, as found in previous studies in our[58,81,82] and other[83-86] laboratories. The ^1H NMR spectra monitored during hydrolysis indicate that the main active forms of these promoters are mononuclear complexes, as shown in Scheme I; then the unspecified ligands in Scheme I are chloride ions and water molecules. When, however, the promoter is added as a preformed aqua complex, the changes in the ^1H NMR signal of the CH_3S group are characteristic of the binuclear complex shown schematically as follows:



Most of the experiments were done with $[Pd(H_2O)_3(OH)]^+$; in this case the unspecified ligands in the binuclear complex are aqua and hydroxo ligands. There are ample precedents for such binuclear complexes with thioether bridging ligands.[76,84-86] Scheme I still applies to experiments with $[Pd(H_2O)_3(OH)]^+$, except that each formula shows only half of the actual substrate–promoter complex. (In subsequent schemes, too, half of each binuclear complex will be shown.) Since the aqua ligands in the binuclear complex are positioned cis to the substrate molecules, water can be delivered to the scissile bond. Since the aqua ligands are positioned trans to the thioether ligands, palladium–oxygen bonds are labile. This combination of stereochemical and kinetic properties makes binuclear complexes more efficient than mononuclear complexes in promoting hydrolysis. In the binuclear active complex formed from $[Pd(H_2O)_3(OH)]^+$ there is at least one aqua ligand, and possibly two of them, in the cis position to each substrate (peptide), available for hydrolysis. Therefore the reactions promoted by $[Pd(H_2O)_3(OH)]^+$ are particularly fast.

Since thiourea is more nucleophilic than the methionine side chain (a thioether) toward palladium(II), mononuclear and binuclear complexes were easily prepared in solution by mixing the following three compounds in various molar proportions: *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$, AcMet or AcMet-Gly, and thiourea. The resulting complexes were detected and quantitated owing to the characteristic ^1H NMR resonances, listed in Table I, of the methylthio group in terminal and bridging positions.[86] Kinetic results are given in Table IV. When the mole ratio is 1:1:1, the complex *cis*-$[Pd(dtco)(AcMet\text{-}Gly)(tu)]^{2+}$ is formed, and hydrolysis is not observed. Evidently, an aqua ligand is necessary for hydrolysis reaction. When the mole ratio is 1:0.5:0, there are two possibilities: first, an equimolar mixture of the mononuclear complexes *cis*-$[Pd(dtco)(AcMet\text{-}Gly)(H_2O)]^{2+}$, which contains the peptide as a terminal thioether ligand, and unspent *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$; second, the binuclear complex $[Pd_2(dtco)_2(H_2O)_2(\mu\text{-}AcMet\text{-}Gly)]^{2+}$, which contains the peptide as a briding thioether ligand. Proton NMR spectra show the absence of the mononuclear complex and the presence of the binuclear one. At this mole ratio hydrolysis occurs fastest because the binuclear complex contains two aqua ligands in *cis* positions to the peptide, which are available for internal delivery to the peptide bond. When the mole ratio is 1:1:0, again there are two possibilities: *cis*-$[Pd(dtco)(AcMet\text{-}Gly)(H_2O)]^{2+}$ or $[Pd_2(dtco)_2(\mu\text{-}AcMet\text{-}Gly)_2]^{2+}$. Again, proton NMR spectra show that only the binuclear complex is present. At this mole ratio hydrolysis is ca. four times slower than at the previous ratio. This kinetic difference rules out the hypothesis that the mononuclear aqua complex *cis*-$[Pd(dtco)(AcMet\text{-}Gly)(H_2O)]^{2+}$ is responsible for hydrolysis at both mole ratios. If it were, the rate constants k_{obsd} for this unimolecular reaction would be equal. Both kinetic and spectroscopic evidence support the notion of the binuclear active complex between the substrate and the promoter.

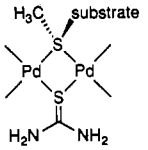
The complex $[Pd_2(dtco)_2(\mu\text{-}AcMet\text{-}Gly)_2]^{2+}$, formed when the mole ratio is 1:1:0, does not contain aqua ligands, and yet hydrolysis occurs (albeit at a reduced rate). Since proton NMR spectra show the presence of free peptide in solution, the equilibrium in eq 5 exists. Opening of the M_2S_2 rings of this type



iswell-known.[84-86] The diaqua complex in this case (at the mole ratio 1:1:0) has two options—recombination with free AcMet-Gly or hydrolysis of the coordinated AcMet-Gly. The diaqua complex in the previous case (at the mole ratio 1:0.5:0) had only the latter option. This explains the 4-fold difference between the corresponding rate constants in Table IV.

(80) Laussac, J. P.; Haran, R.; Hadjiliadis, N. *C. R. Acad. Sci. Paris, Ser. 2* **1985**, *300*, 137.
(81) Gummin, D. D.; Ratilla, E. M. A.; Kostić, N. M. *Inorg. Chem.* **1986**, *25*, 2429.
(82) Galbraith, J. A.; Menzel, K. A.; Ratilla, E. M. A.; Kostić, N. M. *Inorg. Chem.* **1987**, *26*, 2073.
(83) Norman, R. E.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1992**, *31*, 877.
(84) Goggin, P. L.; Goodfellow, R. J.; Sales, D. L.; Stokes, J.; Woodward, P. *J. Chem. Soc., Chem. Commun.* **1968**, 31.
(85) Goggin, P. L.; Goodfellow, R. J.; Reed, F. J. S. *J. Chem. Soc., Dalton Trans.* **1974**, 576.
(86) Murray, S. G.; Hartley, F. R. *Chem. Rev.* **1981**, *81*, 365.

When the mole ratio is 1:0.5:0.5, ^{1}H NMR spectroscopy shows bridging coordination of the methione side chain. The other bridging ligand is proposed to be thiourea, as in the following complex:



Although the coordination mode of thiourea is not directly evident from ^{1}H NMR spectra, it is known to act as a bridging ligand.[87] Since there are no aqua ligands in the principal form of the complex, hydrolysis reaction depends on the minor forms in which some of the ligands are aquated. Indeed, the rate constant for hydrolysis is only ca. 7% of the optimal value. These reactive, minor forms have escaped detection by ^{1}H NMR spectroscopy.

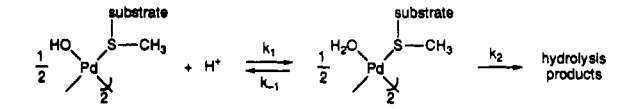
Effects of Ionic Strength on the Hydrolysis Rate. As Table VIII shows, the rate constant for hydrolysis of AcMet-Gly, for which $x = 2$, is virtually independent of ionic strength. Since Debye–Hückel theory does not strictly apply at these reaction conditions, and since pronounced effects of ionic strength are not expected for either mechanism in Scheme I, significance of this kinetic finding should not be overstated. But small effects of ionic strength may be more likely for the chelation mechanism, which involves approach of the carbonyl oxygen atom to the metal atom in the oxidation state II. Absence of these effects might therefore be taken as a weak evidence favoring the mechanism of internal delivery.

Effect of pH on the Hydrolysis Rate. As Table VII shows, hydrolysis promoted by platinum(II) complexes depends little on pH (in the relatively narrow range over which this dependence could be studied). This fact is consistent with the mechanism involving a rate-limiting chelation step, followed by external attack.[58] The shortness of the anchoring side chain ($x = 1$) in *S*-methylgluthathione favors chelation, and inertness of platinum(II) aqua complexes disfavors internal attack. But hydrolysis promoted by a palladium(II) complex depends significantly on pH (again in the relatively narrow range near the pK_a value). The observed slope is close to 1.00, the value expected on the basis of eq 6, which is derived from Scheme II. (This and subsequent

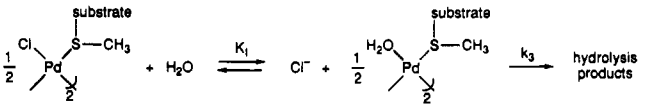
$$\log k_{obsd} = \log \frac{k_1 k_2}{k_{-1} + k_2} - \mathrm{pH} \qquad (6)$$

schemes show only half of each binuclear complex.) The agreement between the results in Table VI and the kinetic relationship in eq 6 supports the notion that coordinated water is needed for cleavage but does not prove either of the mechanisms discussed above. An aqua ligand may be a labile leaving group that facilitates chelation or a nucleophile available for intramolecular attack. The latter mechanism may be favorable because the longer side chain of methionine ($x = 2$) in AcMet-Gly may be unsuitable for chelation but suitable for delivery of a water molecule to the peptide bond. This mechanism is helped also by the lability of aqua ligands coordinated to palladium(II).

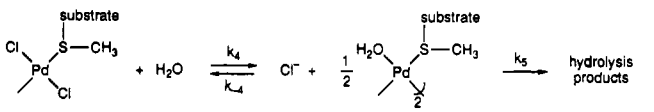
Scheme II



Scheme III



Scheme IV



Inhibition of Hydrolysis by Chloride Ion. As Table IX shows, the reaction in eq 4 is markedly inhibited by chloride ions. Different dependences of k_{obsd}^{-1} on chloride concentration obtain when this concentration is lower (Figure 2A) or higher (Figure 2B) than the concentration of the promoter, $[Pd(H_2O)_3(OH)]^+$. This inhibition can be attributed to anation of the reactive aqua ligand. At low concentration of chloride ions, this anation is partial and the binuclear complex persists, as show in Scheme III. The plot in Figure 2A fits eq 7 when $K_1 = 60$ M^{-1} and k_3

$$k_{obsd} = \frac{k_3}{1 + K_1[Cl^-]} \qquad (7)$$

$= 0.036$ min^{-1}. The reasonably good agreement between this fitted value of k_3 and the experimentally determined first entry in Table IX validates this kinetic analysis. When concentration of the chloride ion is high, anation is extensive, and the binuclear complex is cleaved into halves, as shown in Scheme IV. This cleavage is evident in the ^{1}H NMR spectra. The relationship in eq 8 is consistent with the plot in Figure 2B. Since fitting of three

$$k_{obsd} = \frac{k_4 k_5}{k_5 + k_{-4}[Cl^-]} \qquad (8)$$

microscopic rate constants would be far from unique, it was not done. But the qualitative agreement in this case, and quantitative agreement in the previous case, between the expected and found effects of chloride anion on the hydrolysis reactions supports the proposed mechanism.

Conclusion

This study points the way to a future method for nonenzymatic, selective cleavage of peptide bonds. Suitable coordination complexes, which may be termed artificial metallopeptidases, selectively bind to sulfur atoms in the side chains (of methionine and of cysteine) and selectively promote cleavage of peptide bonds adjacent to these anchoring residues. With $[Pd(H_2O)_3(OH)]^+$ as promoter, the reaction half-lives with some peptides are measured in minutes. We have recently succeeded in cleaving cytochrome *c* with a metal complex, and we are now identifying the cleavage site(s).

Acknowledgment. This work has been funded by the NSF through a PYI Award to N.M.K., Grant CHE-8858387.

(87) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; p 652.