Selective Hydrolysis of Peptides, Promoted by Palladium Aqua Complexes: Kinetic Effects of the Leaving Group, pH, and Inhibitors

Longgen Zhu[†] and Nenad M. Kostić*

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa 50011

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Abstract: This study is a step toward a method for selective internal cleavage of peptides and proteins by coordination complexes as artificial metallopeptidases. Palladium(II) aqua complexes attached to the sulfur atom of methionine in peptides promote, under relatively mild conditions, regioselective hydrolysis of the amide bond involving the carboxylic group of the methionine residue to which they are attached. This is a regular amide bond, and no prior activation for cleavage is involved. Kinetics of hydrolysis was studied with the peptides AcMet-Gly, AcMet-Ala, AcMet-Ser, AcMet-Val, AcMet-Leu, and AcMet-Ala-Ser and with the palladium(II) complexes containing aqua, hydroxo, ethylenediamine, and 1,5-dithiacyclooctane ligands. The reactions were followed by ^{1}H NMR spectroscopy and by two-dimensional thin-layer chromatography. Kinetic effects of pH, temperature, added thioethers, and an added thiol revealed the following. The mononuclear complexes that are initially added to the reaction mixture form binuclear complexes that are the active promoters. The half-lives of hydrolysis reactions are as short as 13 min at 40 °C. Most important, the rate constant for hydrolysis depends on the steric bulk of the leaving fragment in such a way that the reaction is somewhat sequence-selective.

Introduction

Various biological processes involve hydrolytic cleavage of proteins and peptides. Although the half-life for hydrolysis of the amide bond in neutral aqueous solution is ca. 7 years,[1] this reaction is very efficiently catalyzed, in a sequence-selective manner, by proteolytic enzymes.[2] Most of the chemical (non-enzymatic) reagents for amide hydrolysis are inferior to enzymes. They are noncatalytic promoters of stoichiometric reactions, they are usually applied to activated amides such as *p*-nitroanilides and strained lactams, and they require relatively harsh reaction conditions.[3-8] One promising approach to enzyme-like hydrolysis of peptides involves catalytic antibodies.[9-11]

The other promising approach involves coordination complexes of transition metals.[12-41] In fact, many proteolytic enzymes and other hydrolases contain essential metal ions at their active sites. In these studies, however, carboxylic esters are used more often than amides because the former are easier to cleave than the latter. Complexes of cobalt(III)[13] and of copper(II)[14] have been studied in some detail, and general features of the mechanisms by which they promote peptide hydrolysis are known. Because cobalt(III) complexes bind to the N-terminal amino acid residue, only *the N-terminal amide bond* in the peptide is cleaved. In regioselectivity, therefore, these complexes resemble aminopeptidases, one class of exopeptidases.

Most proteolytic enzymes, however, are endopeptidases; they cleave *internal amide bonds* in peptides. Cleavage of such bonds in hydrolytic and oxidative reactions mediated by metal complexes has only recently been achieved.[42-50] Even though the latest such

[†] Permanent address: Coordination Chemistry Institute, Nanjing University, Nanjing, PRC.

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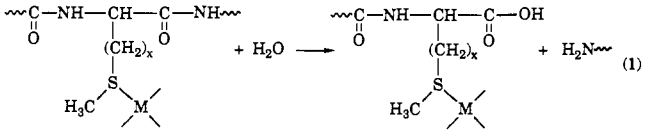
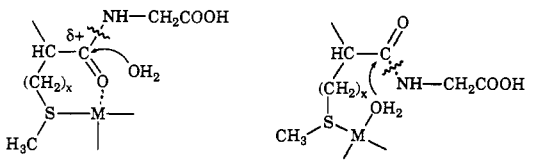
complexes are synthesized and attached to substrates in multistep procedures that require additional reagents in solution, this method holds great promise because cleavage itself is fast and specific.[42-46] Our goal is to mediate hydrolytic cleavage by metal complexes that are easily prepared and that are attached to substrates by short incubation, in situ. Unlike the fragments after an oxidative reaction (mediated, for instance, by cyanogen bromide), fragments after a hydrolytic reaction can form new amide bonds so that hydrolytic cleavage may, in principle, be followed by reattachment. This potential for reversibility makes the hydrolytic approach attractive to biochemists and molecular biologists.

Previous studies in this laboratory[51,52] showed that certain complexes of platinum(II) and palladium(II), designated M in eq 1, attached to the sulfur atom of *S*-methylcysteine ($x = 1$) in peptides promote, under relatively mild conditions, selective hydrolysis of the unactivated amide bond involving the carboxylic group of the amino acid that anchors the metal complex. This reaction is shown schematically in eq 1. Control experiments



showed that hydrolysis does not occur unless the promoter (metal complex) is attached to the substrate. There are two general mechanisms, schematically illustrated below. In one, the metal(II) atom acts as a Lewis acid and forms an S,O chelate that activates the scissile amide bond toward external attack by water; in the other, the metal(II) atom acts as a "vehicle" and internally delivers an aqua ligand to the scissile bond. The two mechanisms cannot be distinguished by kinetic methods; other evidence is required.



Reactions promoted by platinum(II) complexes have half-lives in the range of 2 days to 2 h at 40 °C.[51] We now focus our investigations on palladium(II) complexes because they undergo ligand substitution much faster than similar platinum(II) complexes. The present study furnishes additional evidence for regioselectivity of hydrolysis, provides first evidence for its possible sequence-selectivity, and further explores the dependence of the hydrolysis rate on the solution conditions. This study is a significant step toward our ultimate goal of designing an artificial inorganic endopeptidase.

Experimental Procedures

Chemicals. 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 salt $K_2[PdCl_4]$ were obtained from Aldrich Chemical Co. Anhydrous $AgClO_4$ was obtained from G. Frederick Smith Chemical Co. All common chemicals were of reagent grade. Dipeptides Met-Gly, Met-Ala, Met-Ser, Met-Val, and Met-Leu and the tripeptide Met-Ala-Ser were obtained from Sigma Chemical Co. The terminal amino group in each of the peptides was acetylated, and the complex $[Pd(H_2O)_3(OH)]^+$ was prepared and characterized, as in our previous study.[52] The complexes *cis*-$[Pd(dtco)Cl_2]$,[53] which contains 1,5-dithiacyclooctane, and *cis*-$[Pd(en)Cl_2]$[54] were prepared by published methods and converted into the corresponding diaqua complexes by treatment with 2 equiv of $AgClO_4$ at pH 2.0 according to a published method.[55] In each case, the solid AgCl was removed by filtration in the dark, and fresh stock solution of the aqua complex was used in further experiments.

Table I. ^1H NMR Chemical Shifts (δ, in ppm) in D_2O Solutions, at pH* 1.03–1.06

group	hydrolysis substrate	$[Pd(H_2O)_2(OH)(\text{substrate})]^+$ [a]	hydrolysis product
	AcMet-Gly		Gly
Gly CH_2	4.00 s	4.04 s	3.91 s
$CH_3C(O)$	2.05 s	2.07 s	
Met CH_3	2.11 s	2.47 s	
	AcMet-Ala		Ala
Ala CH_3	1.44 d	1.44 d	1.58 d
Ala CH	4.39 q	4.42 q	4.14 q
$CH_3C(O)$	2.03 s	2.07 s	
Met CH_3	2.11 s	2.45 s	
	AcMet-Ser		Ser
Ser CH			4.22 t
Ser CH_2	3.97, 3.89 qd	3.97, 3.89 qd	4.13, 4.03 qd
$CH_3C(O)$	2.04 s	2.06 s	
Met CH_3	2.11 s	2.47 s	
	AcMet-Val		Val
Val α-CH	4.28 d	4.34 d	3.98 d
Val CH_3	0.96 dd	0.96 dd	1.06 dd
$CH_3C(O)$	2.02 s	2.07 s	
Met CH_3	2.10 s	2.44 s	
	AcMet-Leu		Leu
Leu α-CH	4.43 t	4.44 t	4.08 t
Leu CH_3	0.91 dd	0.91 dd	0.97 dd
$CH_3C(O)$	2.02 s	2.09 s	
Met CH_3	2.11 s	2.47 s	
	AcMet-Ala-Ser		Ala-Ser
Ala CH_3	1.41 d	1.43 d	1.58 d
Ala CH	4.11 q		4.18 q
Ser CH_2	3.96, 3.88 qd		3.97, 3.93 qd
Ser CH			4.60 t
$CH_3C(O)$	2.03 s	2.05 s	
Met CH_3	2.11 s	2.42 s	

[a] Since the solvent is D_2O, exchangeable H atoms are deuteriated.

Measurements. Proton NMR spectra at 300 MHz of solutions in D_2O were recorded with a Varian VXR 300 spectrometer, with DSS as an internal reference. Temperature was kept constant within ±0.1 °C. The pH was measured with a Fischer 925 instrument and a Phoenix Ag/AgCl reference electrode. The uncorrected values in deuteriated solvents were designated pH*. Ultraviolet–visible spectra were recorded with an IBM 9430 spectrophotometer, which has a monochromator with two gratings.

Kinetics of Hydrolysis. Stock solutions of $[Pd(H_2O)_3(OH)]^+$, *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$, and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ had pH* values of ca. 1.0, 2.0, and 2.0, respectively. They were prepared fresh, so that formation of polymeric hydroxo-bridged complexes was suppressed. Equimolar amounts of a substrate and of an aqua complex, both dissolved in D_2O, were mixed rapidly in an NMR tube. The solution was 8.0–20.0 mM in each; in some experiments 1.0 or 0.50 equiv of an inhibitor was also added. The total volume was 600 μL. When the hydrolysis reaction was relatively slow, the pH* value was measured at the beginning and at the end of the experiment. Because the difference was always less than 0.10, pH* was measured only at the end of the experiments involving relatively fast reactions. 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. As Table I shows, resonances of the following groups allow clear monitoring of free substrates, substrate–promoter complexes, and hydrolysis products: CH_2 of Gly, CH_3 and CH

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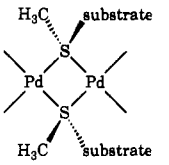
of Ala, CH of Ser, CH and CH_3 of Val, CH of Leu, and CH_3 of Ala-Ser. The error in integration of these resonances was estimated at ±5%. First-order logarithimic plots of substrate concentration or product concentration versus time were linear for 3 half-lives. The reactions were run for 6 half-lives. Typical plots consisted of 10–20 points, and correlation coefficients were 0.990–0.999.

Hydrolysis Products. Single amino acids were identified by their ^1H NMR chemical shifts and by enhancement of their resonances, without the appearance of any new resonances, upon addition of the free amino acid to the reaction mixture. The dipeptide Ala-Ser was identified not only by these two methods but also by two-dimensional chromatography of its dansyl derivative. Hydrolysis of AcMet-Ala-Ser in the presence of $[Pd(H_2O)_3(OH)]^+$, 10.0 mM each, at pH 1.0, was run for 3 h (approximately 10 half-lives) to ensure completion. To 100 μL of the product mixture was added 100 μL of a solution containing 2.5 mg of dansyl chloride (from Sigma Chemical Co.) in 1.00 mL of acetone. The pH was adjusted with triethylamine to 9.0–9.5, and the mixture was left in the dark at 40 °C for 2 h. The nylon membrane sized 7 × 7 cm was obtained from Micro Separations, Inc. A drop of ca. 1 μL of the mixture was placed in a lower left corner, 1 cm from both edges. Elution in the vertical direction was done first with a 9:1 mixture by volume of benzene and glacial acetic acid and next, after the membrane had dried, with a 20:1:1 mixture by volume of ethyl acetate, methanol, and glacial acetic acid; the second solvent was allowed to advance only to the middle of the membrane. After the membrane had dried again, elution in the horizontal direction was done with a 1.5:100 mixture by volume of 88% formic acid and water. The chromatogram was developed by illuminating the dry membrane with a long-wavelength ultraviolet lamp.

Results and Discussion

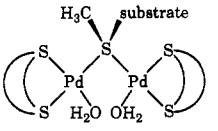
Binding of the Promoters to the Substrates. Each substrate contains a thioether group in the methionine side chain. This group, a soft Lewis base, rapidly displaces one aqua ligand in the complexes of palladium(II), a soft Lewis acid,[56-61] to form mononuclear substrate–promoter complexes of the type $[Pd(H_2O)_2(OH)(substrate)]^+$, *cis*-$[Pd(en)(H_2O)(substrate)]^{2+}$, or *cis*-$[Pd(dtco)(H_2O)(substrate)]^{2+}$ and binuclear complexes of the type $[\{Pd(H_2O)_2(OH)\}_2(\mu_2\text{-substrate})]^{2+}$, $[\{cis\text{-}[Pd(en)(H_2O)]\}_2(\mu_2\text{-substrate})]^{4+}$, or $[\{cis\text{-}[Pd(dcto)(H_2O)_2]\}_2(\mu_2\text{-substrate})]^{4+}$. Attachment of the promoter to the substrate amounts to mixing and short incubation. The net charges shown are correct for neutral substrates at pH ca. 1.0. These formulas are only nominally correct. In fact, ethylenediamine is rapidly displaced from the complexes in this acidic solution. Attachment of palladium(II) is accompanied by a prominent movement downfield of the CH_3S ^1H resonance. Its chemical shift is characteristic of the thioether coordination mode:[51,52,62-67] 2.26–2.37 ppm for terminal ligands (in mononuclear complexes) and 2.41–2.50 ppm for bridging ligands (in binuclear complexes), as shown below. The farther the proton in the substrate is from the thioether group, the less its resonance moves upon coordination, as expected. Acetylation of the terminal amino group prevents bidentate S,N coordination and formation of chelates that may be insoluble.

Our previous report[52] contains various kinetic evidence that the hydrolytically active form of the complex between palladium(II) aqua promoters and substrates is binuclear. The NMR evidence in this study corroborates this conclusion. The changes in the ^1H resonance of the CH_3S group are characteristic of the binuclear complex shown, with the unspecified ligands being H_2O and OH^- when the promoter is $[Pd(H_2O)_3(OH)]^+$ or *cis*-$[Pd(en)(H_2O)_2]^{2+}$. The bridging coordination mode mentioned above is only a detail of the complete structure shown below. There are



ample precedents for such binuclear complexes.[65-68] Since the aqua ligands in this 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 especially labile. This combination of stereochemical and kinetic properties makes binuclear complexes efficient in promoting hydrolysis. This is why the rate constants in this study are much higher than those in our first study,[51] which involved mononuclear platinum(II) complexes.

When the promoter is *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$, the doubly-bridged structure shown above would contain two bidentate dtco ligands. Unlike ethylenediamine, this dithio ether is not displaced by acid. We showed previously[52] that in the case of this promoter, the hydrolytically active complex, shown below, has only one bridge, so that there are aqua ligands cis to the substrate. Since this promoter is less efficient than the other two, we assisted the formation of the singly-bridged complex by mixing *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$ and substrates in the molar ratio of 2:1 as well as 1:1.



Regioselectivity of Hydrolysis. For any peptide and any promoter tested, the only amide bond hydrolyzed to a detectable extent is the one involving the carboxylic group of the methionine whose side chain anchors the palladium(II) promoter, as shown in eq 1. This study agrees with our previous studies,[51,52] which showed this same regioselectivity also with *S*-methylcysteine as the anchor. Since, however, methionine occurs in proteins and *S*-methylcysteine does not, this study is more relevant than the previous ones were to biochemical applications.

The hydrolysis products were identified by ^1H NMR spectroscopy in two ways—by comparison of the chemical shifts with those of pure amino acids at the same pH value and by spiking the reaction mixtures with authentic amino acids and peptides. This spiking enhanced the existing resonances and did not give rise to new ones.

The tripeptide AcMet-Ala-Ser, which contains two amide bonds "downstream" from the anchoring methionine residue, allowed a further test of regioselectivity. This peptide is convenient for two reasons: its precursor, the terminal amino group of which we acetylated, is commercially available and sufficiently soluble; and dipeptides and single amino acids, possible products of hydrolysis, are readily identified and quantitated. Both

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Table II. Hydrolysis of the Methionine–Alanine Bond in AcMet-Ala-Ser, Promoted by Palladium(II) Complexes, at pH* 1.0

promoter[a]	mol ratio promoter:subst	10^3k_{obsd}, min^{-1} 40 °C	50 °C
$[Pd(H_2O)_3(OH)]^+$	1:1	12 ± 1	38 ± 1
cis-$[Pd(dtco)(H_2O)_2]^{2+}$	1:1		6.2 ± 0.4
	2:1		12 ± 1
cis-$[Pd(en)(H_2O)_2]^{2+}$	1:1		16 ± 2

[a] Since the solvent is D_2O, exchangeable H atoms are replaced by D atoms.

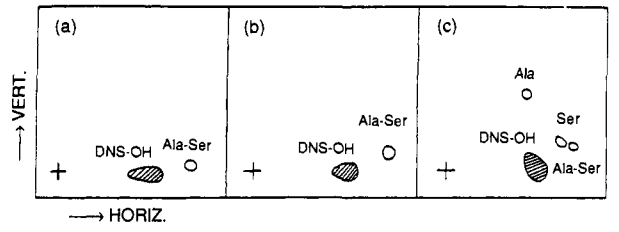

Figure 1. Two-dimensional chromatograms of the products of AcMet-Ala-Ser hydrolysis promoted by $[Pd(H_2O)_3(OH)]^+$. The starting points are marked with crosses: (a) hydrolysis products, (b) mixture of the hydrolysis products and Ala-Ser, and (c) mixture of the hydrolysis products, alanine, and serine.

Table III. Hydrolysis of the Methionine–X Bond in Dipeptides, Promoted by $[Pd(H_2O)_3(OH)]^+$,[a] at pH* 1.05

X in AcMet–X	α-CHR	V_{CHR}, Å^3	10^3k_{obsd}, min^{-1} 40 °C	50 °C
Gly	CH_2	18.2	51 ± 4	120 ± 10
Ala	$CHCH_3$	37.8	28 ± 2	77 ± 2
Ser	$CHCH_2OH$	43.7	27 ± 2	66 ± 2
Val	$CHCH(CH_3)_2$	75.5	11 ± 2	32 ± 2
Leu	$CHCH_2CH(CH_3)_2$	93.8	10 ± 2	24 ± 4

[a] See Table II, footnote a.

^{1}H NMR spectra and sensitive two-dimensional chromatograms showed that, even after prolonged incubation, only the Met–Ala bond is cleaved; see Table II and Figure 1.

The absence of cleavage of the Ala–Ser bond in the tripeptide probably can be attributed to the long distance between this bond and the palladium(II) complex anchored to the methionine side chain. A water molecule cannot be efficiently delivered by the promoter to this remote bond. A full explanation of regioselectivity of hydrolysis will require experiments with designed peptides and conformational analyses of their complexes with promoters.

Dependence of the Hydrolysis Rate Constant on the Leaving Amino Acid. The five dipeptides in Table III differ in the C-terminal amino acid, which is the leaving group in the hydrolysis reaction (eq 1). Since the strength (thermodynamic stability) and susceptibility to hydrolysis (kinetic stability) of the Met–X peptide bond should not depend significantly nor systematically on the identity of the amino acid X, the clear trends in the observed rate constants in Table III and in Figure 2 are attributed to the steric bulk of the α-CHR group in this amino acid. The steric bulk is quantitated as volume calculated on the basis of van der Waals dimensions of functional groups found in amino acids and proteins.[69] The relative volume ΔV in Figure 2 is the difference between the α-CHR volumes (in Å^3) of a given amino acid and of glycine.

The dependence of the rate constant on volume is not fortuitous because it persists at different temperatures. The bulkier the leaving amino acid, the greater the shielding of the scissile bond from the promoter and from the aqua ligand that the promoter delivers. The linear plots at both temperatures in Figure 2 have the same slope of 0.022 Å^{-3}. This slope can become appreciable

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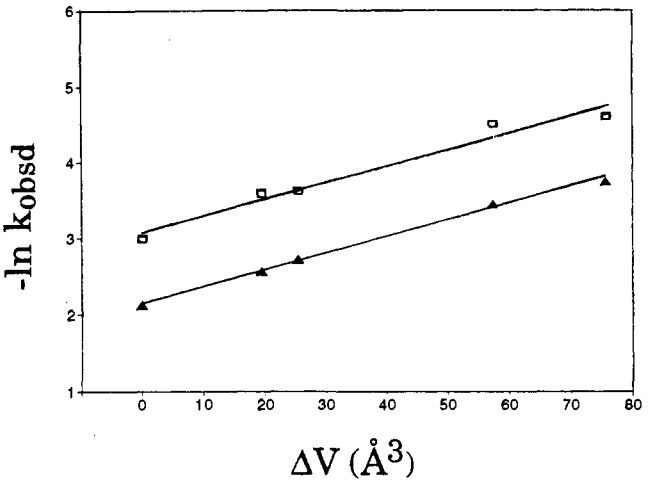


Figure 2. Dependence of the rate constant for hydrolysis of the methionine–X bond in AcMet-X on the steric bulk of the α-CHR group in amino acid X. The linear fits are -ln k_{obsd} = 2.14 + 0.022 ΔV at 40 °C (□) and -ln k_{obsd} = 3.07 + 0.022 ΔV at 50 °C (▲). $\Delta V = V_{CHR} - V_{CH_2}$.

Table IV. Effect of pH on Hydrolysis of the Methionine–Glycine Bond in AcMet-Gly, Promoted by Palladium(II) Complexes, at 50 °C

promoter[a]	mol ratio promotr:subst	pH*	10^3k_{obsd}, min^{-1}
$[Pd(H_2O)_3(OH)]^+$	1:1	1.04	150 ± 30
		1.43	69 ± 8
cis-$[Pd(dtco)(H_2O)_2]^{2+}$	2:1	1.09	11 ± 1
		1.69	7.3 ± 0.9

[a] See Table II, footnote a.

if both the promoter (which has to approach the scissile bond) and the leaving group are bulky. We will explore in the future this approach to sequence-selectivity of cleavage.

The results in Tables II and III, however, already show that the hydrolytic cleavage does not depend simply on the volume of the α-CHR group adjacent to the scissile peptide bond. The rate constant for cleavage of the Met–Ala bond in the tripeptide (Table II) is about one-half of the corresponding rate constant in the dipeptide (Table III) under nearly identical conditions. Although the palladium-promoted hydrolysis apparently becomes slower as the substrate becomes longer, our new method is applicable to proteins. We have repeatedly and consistently cleaved cytochrome *c* and myoglobin with palladium(II) complexes, but much remains to be done to optimize the efficiency and selectivity of this new method.

Dependence of the Hydrolysis Rate Constant on pH. As Table IV shows, the rate constant for hydrolysis decreases as the solution is made less acidic. This fact, however, does not mean that the peptide hydrolysis is caused simply by the acid in solution. Many control experiments, under rigorous conditions, showed that the substrates used in our studies are stable at pH* values lower than 1.0 and over time periods far longer than those needed for complete hydrolysis in the presence of metal complexes. Control experiments also showed that hydrolysis does not occur to a detectable extent in the absence of these promoters. Because of this undetectability, the rate enhancement can only be estimated on the basis of the duration of control experiments, sensitivity of ^{1}H NMR spectrometry, and rate constants for promoted reactions; this enhancement is greater than 1 × 10^4.

Since the pK_a values of the aqua ligands attached to palladium(II) fall in the general region of pH* values in Table IV,[59-61] we conclude that the decrease in the rate constants is caused by formation of hydroxo-bridged complexes, which no longer promote peptide hydrolysis. Oligomerization of metal–aqua complexes

Table V. Inhibition by Sulfur Compounds of AcMet-Gly Hydrolysis, Promoted by $[Pd(H_2O)_3(OH)]^+$,[a] at 50 °C

inhibitor	mol ratio Pd:AcMet–Gly:inhibtr	pH*	10^2k_{obsd}, min^{-1}	$\delta(CH_3S)$, ppm
AcMet	2:1:1	1.07	13.0 ± 0.3	2.29, 2.46
	2:2:I	1.08	7.1 ± 0.3	2.47
	1:1:1	1.08	2.7 ± 0.1	2.50
AcCysMe	2:1:1	1.00	13.2 ± 0.2	2.30, 2.45, 2.60
	2:2:1	1.04	5.9 ± 0.1	2.45, 2.60
	1:1:1	1.02	2.0 ± 0.1	2.48, 2.60
$S(CH_2CO_2H)_2$	2:1:1	0.88	12.6 ± 0.1	2.27, 2.45
	2:2:1	0.88	6.9 ± 0.1	2.47
	1:1:1	0.88	1.3 ± 0.4	2.50
AcCysH	2:1:1	1.07	0	2.11

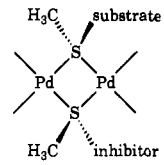
[a] See Table II, footnote a.

in solution is well known.[56] Indeed, if the promoters in Table IV are kept at pH* > 2, precipitates eventually form. We avoided these problems by maintaining pH* around 1.0.

Inhibition of Hydrolysis by Sulfur Compounds. These experiments were done for three reasons: to examine the active form of the promoter, which may be different from the complex that is initially added to the reaction mixture; to study possible inhibition of hydrolysis of multifunctional substrates such as proteins; and to investigate reactions akin to those that may interfere with hydrolysis of particular substrates in heterogeneous reaction mixtures. The sulfur-containing compounds in Table V were chosen because palladium(II) is thiophilic, because they are relevant to functional groups in proteins, and because they resemble solutes commonbly found in biological fluids and extracts. The inhibitors compete with the substrate, AcMet-Gly, for binding to the promoter. This binding is evident in the ^1H chemical shift (ca. 2.50 ppm) of the CH_3S group(s), which is characteristic for bridging coordination.[51,52,62-67]

As Table V shows, the three thioethers (*N*-acetylmethionine, *N*-acetyl-*S*-methylcysteine, and thiodiglycolic acid) behave similarly. When the molar ratio is 2:1:1, the rate constants in Table V are equal to the rate constant for the same substrate in Table III, in the absence of inhibitors. Since the promoter is present in excess over the substrate, the inhibitors do not affect the rate constant. When, however, the promoter and substrate are equimolar, the inhibitors do retard the hydrolysis reaction and this retardation increases as the inhibitor concentration is raised.

Thiol inhibitor *N*-acetylcysteine behaves differently from its thioether analog, *N*-acetyl-*S*-methylcysteine, and other inhibitors. Even when the amount of the palladium(II) complex is sufficient for equimolar binding to both the substrate and the inhibitor, ^1H NMR spectroscopy shows the substrate to remain free in solution and hydrolysis does not take place. This contrast can be attributed to different characters of the thioether and the thiolate as bridging ligands. The substrate AcMet-Gly is a thioether. When the inhibitor also is a thioether, binuclear complexes with two kinds of thioethers can form. Such a complex is shown schematically below; the unspecified ligands are aqua or hydroxo ligands. There are ample precedents for such binuclear complexes,[65-68] but we did not find precedents for complexes with mixed bridging ligands,



one a thioether and the other (from the inhibitor) a thiolate anion. Since in this latter case the substrate presumably cannot form a binuclear complex with the promoter, there is no hydrolysis. These inhibition experiments further support our conclusion that although the peptides are initially treated with mononuclear promoters, the hydrolysis is mediated by binuclear complexes between the peptides and the promoters.

Conclusion and Prospects

This is a continuation of our studies of the mechanisms by which metal complexes that are anchored to sulfur atoms in side chains of peptides promote regioselective hydrolysis of the amide bond involving the carboxylic group of the anchoring amino acid. Unlike the well-known mononuclear cobalt(III) complexes that cleave the N-terminal amide bond,[13] these binuclear palladium-(II) complexes cleave internal amide bonds. We are trying to achieve efficient cleavage in mildly acidic and neutral solutions, but the procedure at pH ca. 1.0 may already be useful. The widely-used reagents such as cyanogen bromide[70-73] and various hydrolytic enzymes[74] are applied under acidic, neutral, and basic conditions alike. There are many examples in which these and other reagents are used in 0.10 M HCl or in 70% HCOOH as solvents and above room temperature. As long as there is no nonspecific cleavage, the pH of the solution and the temperature may be adjusted for optimal reactivity. In fact, acidic solution is sometimes required to denature the protein and make the anchoring residues in them accessible to the reagent; the very stable proteins cytochrome *c*, ribonuclease, the γ-globulin and the S-peptide of ribonuclease S are cases in point.[70-73] Our preliminary successes in hydrolytic cleavage of cytochrome *c* and of myoglobin with palladium(II) aqua complexes at pH ca. 1.8 point the way toward these inorganic reagents as artificial metallopeptidases.[75] Because the hydrolysis rate constant depends on the steric bulk (that is, the identity) of the amino acid the amino group of which forms the scissible bond, there is a possibility of sequence-selective cleavage of peptides and proteins. We will try to achieve this selectivity by purposeful stereochemical design of promoter complexes.

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