Hydrolytic cleavage of peptides by palladium(II) complexes is enhanced as coordination of peptide nitrogen to palladium(II) is suppressed

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Abstract

We report on the kinetics and mechanism of regioselective hydrolysis of amide bonds in various dipeptides and tripeptides after these substrates react with $[Pd(H_2O)_3(OH)]^+$ and *cis*-$[Pd(en)(H_2O)_2]^{2+}$. Peptides devoid of coordinating side chains form mononuclear palladium(II) complexes and hydrolyze slowly, over weeks. Peptides containing thio ether side chains (of methionine and *S*-methylcysteine) as anchors form binuclear palladium(II) complexes and hydrolyze rapidly, with half-lives that are measured in minutes. The ethylenediamine ligand stabilizes the initial complex but is released prior to hydrolysis, so that the two mononuclear complexes form similar binuclear promoter complexes with anchoring peptides. Hydrolysis requires acidic solutions, but this reaction is not catalyzed by acid; the palladium(II) promoter is required. Binding of the sulfur-anchored palladium(II) atom to the deprotonated nitrogen atom in the amide bond inhibits hydrolysis. Methylation of the amide nitrogen atom suppresses binding of palladium(II) to it and enhances the hydrolysis rate constant as much as 300-fold.

Key words: Peptide hydrolysis; Artificial peptidase; Amide coordination; Kinetics

Introduction

Hydrolysis of proteins and peptides is important for several reasons. The great inertness of the unactivated amide bond toward hydrolysis [1] makes this reaction interesting from the chemical point of view. The need for reagents that selectively cleave proteins makes this reaction interesting also from the biochemical point of view. Proteolytic enzymes remain widely used, but two alternatives to them are beginning to emerge. One new approach involves catalytic antibodies [2–4].

The other promising alternative involves transition-metal complexes [5–8]. Complexes of cobalt(III) [5], copper(II) [6], and iron(II) [9–12] have been studied in some detail. They bind to the N-terminal amino acid residue and therefore promote cleavage of only the N-terminal amide bond. In regioselectivity, these reagents resemble aminopeptidases, one class of exopeptidases.

Most proteolytic enzymes, however, are endopeptidases; they cleave internal amide bonds in substrates. Other researchers [9–12] and we [13–15] have recently succeeded in promoting cleavage of such bonds with transition-metal complexes. After the initial work with platinum(II) [13], we found palladium(II) [14, 15] to have all the desirable properties. Its complexes are easily prepared, their attachment to substrates is achieved simply by mixing, the hydrolysis reactions have half-lives as short as 15 min, and the complexes are easily removed from the cleaved substrates. As cleavage agents, palladium(II) complexes differ from the well-known complexes of other transition metals.

Our previous kinetic and spectroscopic experiments with various substrates and with complexes containing various ligands showed the following main features of the mechanism. The promoter acts only when it is anchored to the substrate via the side chains, which contain the $(CH_2)_x$ group, of cysteine ($x=1$), *S*-methylcysteine ($x=1$), and methionine ($x=2$). Only the amide bond involving the carboxylic group of the anchoring residue is cleaved; the amide bond involving the amino group is not cleaved. The rate constant for cleavage depends on the steric bulk of the first amino acid residue in the leaving fragment (group R in Scheme 1) in a manner that should allow cleavage to be made selective to amino acid sequence [15]. These previous

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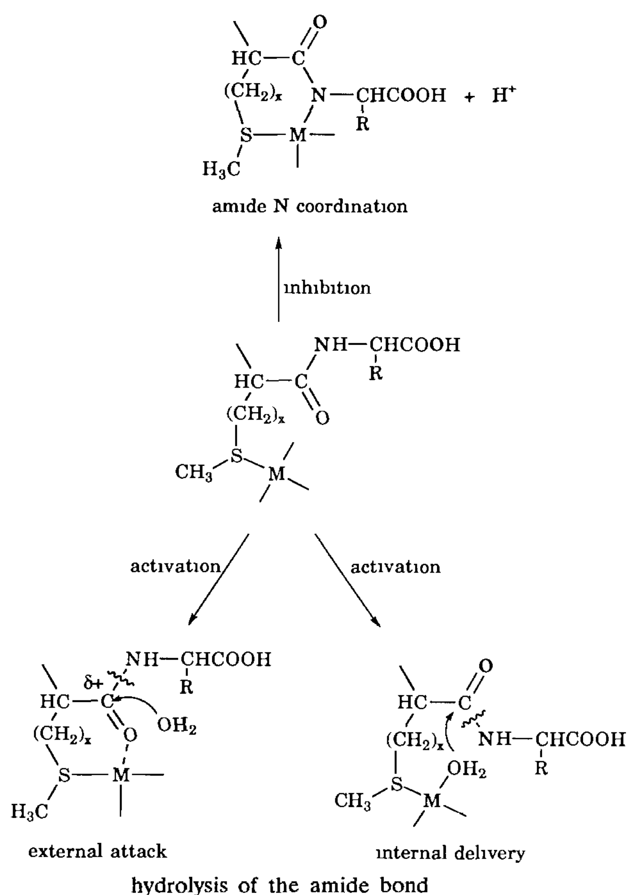


Scheme 1.

findings, taken together, are consistent with the hypothesis that hydrolysis requires interaction of the promoter with the amide carbonyl group, as shown in Scheme 1.

In this study, we test the hypothesis, also shown in Scheme 1, that interaction of the promoter with the amide nitrogen atom inhibits hydrolysis. We show that, indeed, suppression of coordination of the amide nitrogen atom to palladium(II) results in hydrolysis reactions that are faster than any that we studied before.

Experimental procedures

Chemicals

Distilled water was further demineralized and purified. The deuterium-containing compounds and $K_2[PdCl_4]$ 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 Gly–Gly, Met–Gly and Gly–Sar and the tripeptide *S*-methylglutathione (GSMe) were obtained from Sigma Chemical Co. The terminal amino group in each of the dipeptides was acetylated as in our previous study [14]. The complex *cis*-$[Pd(en)(H_2O)_2]^{2+}$ was synthesized by the published procedure [16].

The complex $[Pd(H_2O)_3(OH)]^+$ was synthesized as in our previous studies [14, 15]; this procedure is now described in some detail. The reaction vessels were wrapped up in aluminum foil, to keep the chemicals in the dark. Dissolution of 326.4 mg (1.00 mmol) of $K_2[PdCl_4]$ in 7.5 ml of D_2O and 0.50 ml of a 2.0 M $DClO_4$ required heating to 35 °C and stirring. To the clear solution were added, dropwise, 2.000 ml of a 2.000 M solution of $AgClO_4$ in D_2O. The mixture was stirred at 35 °C for 4 h and kept at 4 °C overnight. The bulk of AgCl was removed by centrifugation, and thc rest of this precipitate was removed by filtration. The yellow-brown stock solution was 0.10 M in $[Pd(H_2O)_3(OH)]^+$ and had pH* 1.0. It was kept at 4 °C and used fresh in studies of hydrolysis.

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. 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 are designated pH*. UV–Vis spectra were recorded with an IBM 9430 spectrophotometer. Mass spectra were obtained with a Kratos MS50 instrument.

AcMet–Sar

Unless otherwise indicated, the chemicals were obtained from Sigma Chemical Co. A solution containing 955 mg (5.0 mmol) of *N*-acetyl-DL-methionine, designated AcMet, and 505 mg (5.0 mmol) of triethylamine (from Eastman Kodak Co.) in 50 ml of absolute tetrahydrofuran (from Aldrich Chemical Co.) was prepared at -15 °C. To this stirred solution were added 540 mg (5.0 mmol) of ethylchloroformate and, after 15 min, 768 mg (5.0 mmol) of sarcosine ethyl ester hydrochloride, designated SarEt, and 505 mg (5.0 mmol) of triethylamine. The mixture was stirred at room temperature overnight, a white solid was filtered off, and tetrahydrofuran was evaporated under reduced pressure. The remaining yellow liquid was taken up in 50 ml of chloroform. The unspent AcMet was extracted with three 5 ml portions of a 5% aqueous solution of $(NH_4)_2CO_3$, and the unspent SarEt was extracted with three 5 ml portions of a 5% aqueous solution of acetic acid. The remaining solution in chloroform was washed with three 5 ml portions of water, and chloroform was evaporated under reduced pressure. The ethyl ester of the title dipeptide was obtained as a pale yellow oil; its yield was 1.0 g. It was dissolved in 20 ml of water at 80 °C, and to this hot solution were added 3.50 ml

of a 1.00 M aqueous solution of NaOH (equimolar with the ester), dropwise, over 1 h. The solution was cooled, brought to pH between 1 and 2 with concentrated HCl, and left overnight at 4 °C. The crystalline precipitate was filtered off, washed several times with water, and lyophilized. The yield of the title dipeptide was 0.65 g or 50%. Proton NMR spectrum (δ values): 2.04 and 1.99, both s, CH_3CO; 2.11 and 2.16, both s, Met CH_3; 2.60, m, Met CH_2; 2.98 and 3.23, both s, Sar CH_3; 4.04 and 4.26, both d, Sar CH_2. Molecular ion in the mass spectrum (*m/z* value): found, 262.0980; calc. for $C_{10}H_{18}N_2O_4S$, 262.0987.

AcCysMe–Gly and AcCysMe–Sar

Ethyl esters of these two dipeptides were obtained from 5.0 mmol of *N*-acetyl-*S*-methyl-L-cysteine, designated AcCysMe, and 5.0 mmol of glycine ethyl ester hydrochloride or of sarcosine ethyl ester hydrochloride, by the procedure described above. At room temperature AcCysMe–GlyEt is a pale yellow semi-solid, whereas AcCysMe–SarEt is a yellow liquid. Each was dissolved in 20 ml of water at 65 °C. To this solution was added an equimolar amount of a 1.00 M aqueous solution of KOH over 1 h. The solution was cooled, brought to pH between 1 and 2 with concentrated $HClO_4$, and filtered to remove $KClO_4$. The resulting solution was kept overnight at 4 °C, filtered again to remove $KClO_4$, and lyophilized to obtain a pale yellow solid. The yields of the title dipeptides were 0.50 g or 40% and 0.75 g or 60%, respectively. Proton NMR spectrum of AcCysMe–Gly (δ values): 2.07, s, CH_3CO; 2.13, s, CysMe CH_3; 4.02, s, Gly CH_2. Proton NMR spectrum of AcCysMe–Sar (δ values): 2.00 and 2.04, both s, CH_3CO; 2.11 and 2.16, both s, CysMe CH_3; 2.98 and 3.22, both s, Sar CH_3; 4.12 and 4.20, both d, Sar CH_2.

Study of hydrolysis

Stock solutions of $[Pd(H_2O)_3(OH)]^+$ and of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ in D_2O had pH* of 1.0 and 2.0, respectively. They were prepared fresh, to minimize formation of polymeric hydroxo-bridged complexes. A substrate and a palladium(II) complex were always mixed in equimolar amounts. The rest of the experimental procedure depended on the rate of hydrolysis. First, in the case of relatively fast reactions the reactants were rapidly mixed in the NMR tube to a final concentration of 5–20 mM in each; monitoring by ^1H NMR spectroscopy began as soon as possible and continued for at least three half-lives; and pH* was measured only at the end. Second, in the case of AcCysMe–Gly and of *S*-methylglutathione hydrolysis reactions were slow enough to allow measurements of pH* both after mixing in the NMR tube and at the end of hydrolysis; the difference was less than 0.10. These reactions were followed for less than three half-lives, but otherwise the procedure was as described above. Third, in the case of Gly–Gly, Gly–Sar and AcGly–Sar hydrolysis reactions were too slow for continuous monitoring by ^1H NMR spectroscopy. In these cases, the reaction mixture that was 20 mM in the substrate and in the palladium(II) complex was divided into eleven 600 μl aliquots, which were kept at 40 ± 1 °C. On eleven successive days a five-fold molar excess of a 10.0 M solution of NaI (final concentration of 0.10 M) was added to each of the eleven aliquots, and solid PdI_2 [17] was removed by centrifugation. To a 500 μl of the clear solution, transferred to an NMR tube, were added 5 μl of 2.0 M $DClO_4$, 15 μl of 0.10 M DSS and 80 μl of D_2O, and the hydrolysis products were examined by ^1H NMR spectroscopy.

The ^1H NMR spectroscopic quantitation of the reaction mixtures was the same regardless of the reaction rate. Total volume of each sample was 600 μl. Temperature was 40.0 ± 0.1 °C in most cases and 21.0 ± 0.1 °C in some cases. Quantitation was repeated at 10–20 different times, and 16 scans were recorded each time. Resonances of the CH_2 group in free glycine and in Gly–Gly and of the CH_3 group in free sarcosine were integrated with an estimated error of $\pm 5\%$. In the first two cases, when the reactions were relatively fast or relatively slow, first-order logarithmic plots of substrate concentration or of product concentration versus time contained 10–20 points and were linear for at least three half-lives, with correlation coefficients in the range 0.997–0.999. In the third case, when the reactions were very slow, second-order plots of the reciprocal value of the substrate concentration versus times were linear, with correlation coefficients in the range 0.960–0.990.

Results and discussion

Peptide group as a ligand

The peptide (or amide) group is both a very weak acid and a very weak base; typical pK_a values of the OH (protonated carbonyl) and NH groups in it are *c.* -1.0 and *c.* 15, respectively [18]. Although amides of carboxylic acids can coordinate to metal ions through either nitrogen or oxygen atoms [19], amide groups in peptides coordinate to most metal ions through the oxygen atom [18]. Certain transition-metal ions induce deprotonation of the nitrogen atom by binding to it. This process is especially favorable when the metal is already anchored to a side chain, so that a chelate ring is formed. Palladium(II) is one of the most effective transition-metal ions in displacing the proton [18]. The estimated pK_a for this reaction effected by palladium(II) is *c.* 2, and displacement was observed even in solutions with $pH < 2.0$ [18, 20–25].

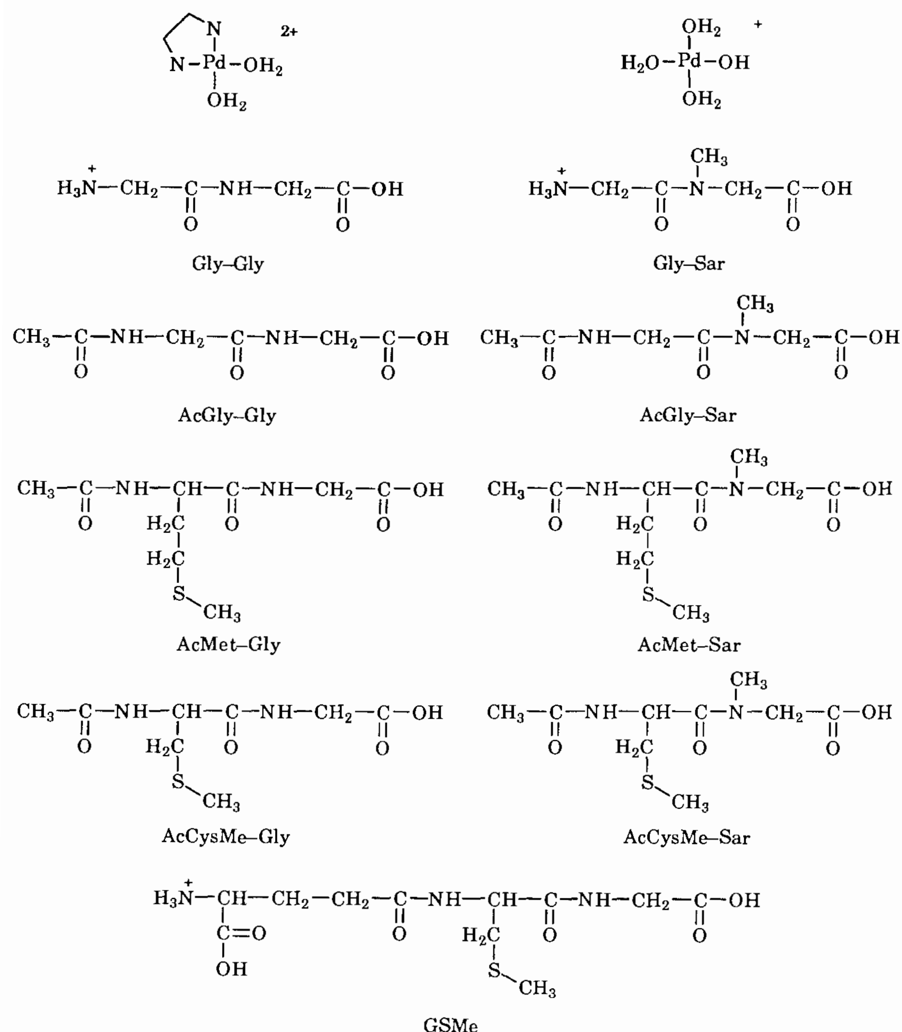


Chart 1.

As Scheme 1 shows, our palladium(II)–peptide complexes satisfy the requirements for chelation via nitrogen coordination. Unfortunately, this coordination stabilizes the C–N bond and inhibits its cleavage [18]. Since no structural study has found the peptide nitrogen atom bearing both a proton and a metal ion, deprotonation seems to be necessary (although not sufficient) [26] for nitrogen coordination to the metal. We reasoned that replacement of the hydrogen atom by a methyl group, i.e. replacement of glycine by sarcosine, should suppress the inhibitory process and thus enhance hydrolysis. In this expectation we were supported by the very recent report concerning hydrolysis of simple (non-biological) amides effected by copper(II) ions [27].

The peptides

The amino group in methionine or *S*-methylcysteine was acetylated [14], and the carboxylic group in glycine or sarcosine was esterified, prior to coupling of the carboxylic group in the former amino acid and the amino group in the latter. Our procedure is a modification of the published one [28]. The ^{1}H NMR resonances of the sarcosine-containing dipeptides are doubled, with relative intensities of *c.* 3.0:1.0 (for Gly–Sar, AcGly–Sar and AcMet–Sar) or of 2.3:1.0 (for AcCysMe–Sar) owing to the chirality of the methylated nitrogen atom. Indeed, no such doubling is observed in the ^{1}H NMR spectra of the homologous glycine-containing dipeptides.

The palladium(II) complexes (hydrolysis promoters) and the peptides (hydrolysis substrates) used in this study are shown in Chart 1. Their charges are as expected for major species present in solution at pH $\leq$ 2.0 Charges of palladium(II)–peptide complexes vary with pH and are not explicitly shown in equations and schemes. Control experiments showed no appreciable decomposition of the peptides at pH* *c.* 1.0 for days, except that AcGly–Gly yielded acetic acid. Evidently, hydrolysis of the peptide bonds does not occur to a significant extent in the absence of palladium(II) complexes.

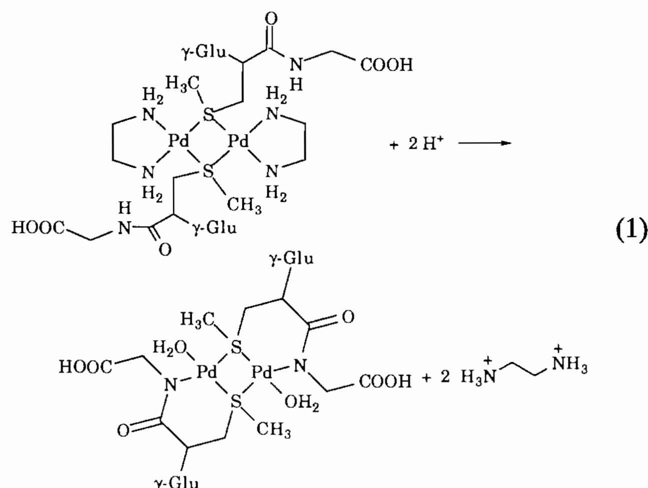
Attachment of palladium(II) complexes to peptides

Modes of palladium(II) coordination to amino acids and peptides are known [29–32]. This coordination is relevant here simply because the promoter must be attached to the substrate to effect hydrolysis, but it is the hydrolysis reaction that is the main subject of our study. Initial binding of the promoter to the substrate amounts to displacement of aqua ligands by the amide and amine nitrogen donors and carbonyl oxygen donors in Gly–Gly, Gly–Sar and AcGly–Sar and by the sulfur donor in AcMet–Gly, AcMet–Sar, AcCysMe–Gly, AcCysMe–Sar and *S*-methylglutathione (GSMe). In a mixture containing equimolar amounts of a palladium(II) promoter on the one hand and of Gly–Gly, Gly–Sar or AcGly–Sar on the other, approximately equal fractions of the dipeptide exist free and coordinated to palladium(II).

We consider Gly–Gly first. The chemical shifts (in ppm) of the CH_2 singlets at pH* 1.14 are as follows: 3.90 and 4.08 for the N-terminal and C-terminal moieties in Gly–Gly; 3.46 and 4.08 in the promoter–dipeptide complex; and 3.49 in the complex *cis*-$[Pd(H_2O)(OH)(N,O\text{-Gly})]$, prepared from $[Pd(H_2O)_3(OH)]^+$ and glycine. Because the values of 3.46 and 3.49 are close to the value of 3.47 ppm, reported for the complex *cis*-$[Pd(en)(N,O\text{-Gly})]^+$ [33, 34], we propose the conventional N,O chelation by the N-terminal glycyl moiety in the promoter–dipeptide complex. Indeed, the ^1H NMR resonance, at 4.08 ppm, of the C-terminal moiety remains unaffected by promoter binding. Structural characterization of this complex is not the goal of this study, and we follow ample precedent [29–32, 35, 36] in proposing coordination via the α-amino and carbonyl groups.

The dipeptide Gly–Sar apparently differs from Gly–Gly in binding to the proomoter. The glycyl ^1H NMR resonance is unaffected by binding, whereas the sarcosine CH_2 and CH_3 resonances move from 4.22 and 3.08 ppm, respectively, in the free dipeptide down to 4.26 and 3.16 ppm, respectively, in the promoter–dipeptide complex. The methylated amide nitrogen atom evidently remains capable of coordination to palladium(II), but this coordination is incomplete; the equimolar reaction mixture contains approximately equal amounts of free and coordinated Gly–Sar. Since ^1H NMR changes in AcGly–Sar upon promoter binding resemble changes in Gly–Sar, acetylation of the terminal amino group does not seem to affect this binding. This finding is consistent with coordination via the peptide nitrogen atom. The chemical shifts (in ppm) of *cis*-$[Pd(H_2O)(OH)(N,O\text{-Sar})]$ at pH* 1.09 are as follows: the CH_3 singlet at 2.79 and the CH_2 singlet at 3.96 in free sarcosine, respectively, correspond to the two CH_3 singlets at 2.32 and 2.46 and to the eight-line ABX pattern due to the CH_2 group at 3.14–4.02 in the complex. These results are consistent with those obtained earlier for similar sarcosine complexes of platinum(II) [37].

Initial reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ with sulfur-containing peptides is more intricate, and we studied it in some detail using *S*-methylglutathione. Displacement of the aqua ligand by the sulfur atom is fast [38], and so is formation of the binuclear complex with two thio ether ligands as bridges [39–42]. These well-known reactions produce the binuclear complex with terminal ethylenediamine ligands, which reacts further as shown in eqn. (1). This net reaction is facilitated by several



factors. Release of ethylenediamine from the initial binuclear complex is favored by the labilizing *trans* effect of the thio ether ligands [41] and by the acid in solution; free enH_2^{2+} was easily detected by a sharp ^1H NMR singlet at 3.37 ppm. Coordination of the peptide nitrogen atom in the final binuclear complex is evident in the growth of the ^1H NMR singlet of glycine at 2.74 ppm. This coordination is especially favorable because it completes a six-membered chelate ring [43–45]; the binuclear complex contains two such rings. At pH* *c.* 1.0, at which we usually do hydrolysis reactions, ethylenediamine is released within minutes of mixing the promoter and the substrate. At pH* 3.3, at which we did this particular kinetic study, this release is slow enough to be monitored. As the observed rate constants in Table 1 show, coordination of the amide nitrogen atom is relatively slow. But this unwanted reaction eventually goes to completion or nearly to completion, judging by ^1H NMR spectra. As Table 1 shows, it cannot be fully suppressed by lowering the temperature.

Dependence of hydrolysis on pH

Because the pK_a value of the aqua ligand in *cis*-$[Pd(en)(H_2O)_2]^{2+}$ is 5.6 [16], this formula correctly represents the complex in the acidic solutions used in this study. The pH dependence in Table 2, therefore, is not caused by acid–base processes involving the aqua

TABLE 1. Rate constants at different temperatures for ethylenediamine release and for coordination of the CysMe–Gly peptide nitrogen atom in the reaction between *cis*-$[Pd(en)(H_2O)_2]^{2+}$ [a] and *S*-methylglutathione (γ-Glu–CysMe–Gly) at pH* 3.3[b]

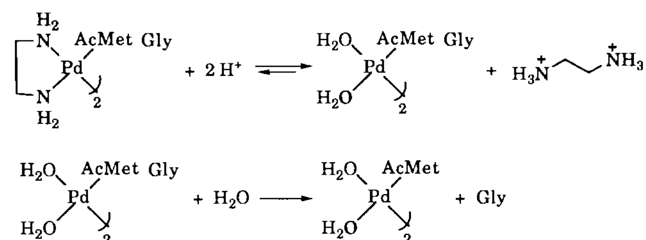
T (°C)	$10^2\ k_{obs}^{en}$ (min^{-1})	$10^3\ k_{obs}^{N}$ (min^{-1})
20	2.5±0.2	1.1±0 1
30	4.7±0.7	2.5±0 1
40	11±1	6.2±0.5
50		22±1

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

TABLE 2. Effect of pH on hydrolysis of the methionine–glycine bond in AcMet–Gly promoted by *cis*-$[Pd(en)(H_2O)_2]^{2+}$ [a]

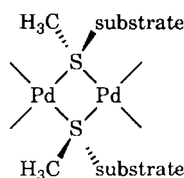
pH* [b]	$10^3\ k_{obs}$ (min^{-1}) at 50 °C
1.05	57±4
1.51	28±2
1.99	11±1
2.22	8.4±0.7
3.12	3.4±0.1

[a,b]See footnotes in Table 1.



Scheme 2.

ligand. The tabulated values satisfy the relationship log $k_{obs} = -0.60$ pH*–0.68, but the fractional slope in it defies simple interpretation. Because release of ethylenediamine, coordination of peptide nitrogen and hydrolysis of the peptide bond all depend on pH, a composite of these dependences is difficult to explain quantitatively. Qualitatively, however, there is a correlation between ethylenediamine release and peptide hydrolysis: both processes are assisted by increasing acidity. (Control experiments showed that it is not simply the acid that causes peptide hydrolysis; see above.) This correlation indicates that ethylenediamine release is required for subsequent hydrolysis, as shown in Scheme 2. If so, the promoters *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(H_2O)_3(OH)]^+$ ultimately form essentially the same active species, which effects peptide hydrolysis. Because the former complex is prepared more easily and is more stable than the latter, it has practical advantages. Our previous kinetic studies [14, 15] showed this active species to be a binuclear complex with bridging thio ether ligands, shown below. The unspecified terminal ligands in it are H_2O and OH^-.



That hydrolysis of AcMet–Gly is still observed at pH* > 3.0 probably is the consequence of the favorable structure of this substrate. A longer side chain ($x=2$) makes coordination of the glycine nitrogen atom to palladium(II) less likely because this coordination would result in a seven-membered chelate ring. A shorter side chain ($x=1$) in *S*-methylglutathione makes this coordination more likely because it produces a six-membered ring, as shown in eqn. (1). Consequently, GSMe does not hydrolyze in the presence of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ at pH* 3.30. Proton NMR spectra show only release of ethylenediamine and coordination of the peptide nitrogen in GSMe.

Need for an anchoring side chain

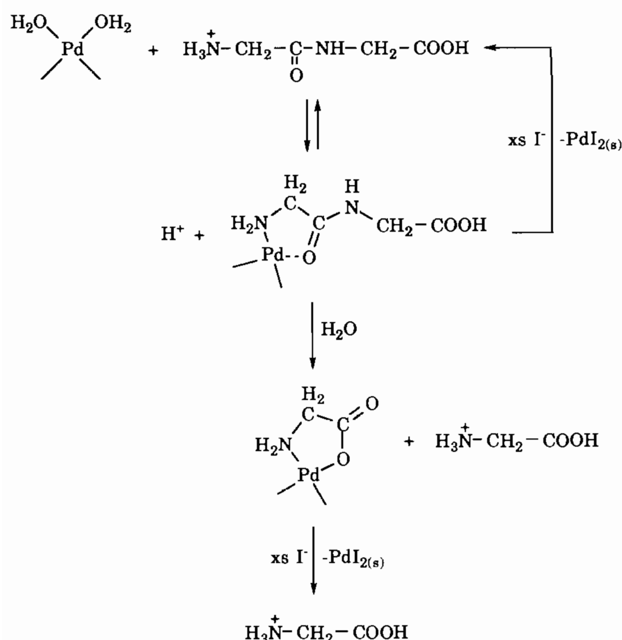
The three dipeptides listed in Table 3 (and the other substrates as well) are stable in the absence of palladium(II) complexes. Control experiments showed that less than 5% of Gly–Gly and Gly–Sar hydrolyzed at pH* *c.* 1.0 and 40 °C in 15 days. Even though the dipeptides in Table 3 lack anchoring side chains, ^1H NMR spectra showed *c.* 50% formation of the promoter–dipeptide complex, as discussed above.

Hydrolysis of Gly–Gly is shown in Scheme 3. Because the N-terminal moiety of this dipeptide and free glycine both have the ^1H NMR resonance at 3.90 ppm, the reaction was followed by monitoring the resonance, at 4.08 ppm, of the C-terminal moiety in the dipeptide. After palladium is removed as the insoluble PdI_2 – this reaction is not a part of the hydrolytic mechanism – the reaction mixture contains free Gly–Gly and free glycine, which can be quantitated by ^1H NMR spec-

TABLE 3. Hydrolysis of dipeptides that cannot effectively anchor palladium(II) promoters because they lack a suitable side chain

Promoter[a]	Substrate	pH* [b]	$10^8\ k_{obs}$ ($M^{-1}\ min^{-1}$) at 40 °C
$[Pd(H_2O)_3(OH)]^+$	AcGly–Sar	1.14	8.4±0.6
	Gly–Sar	1.17	5.8±0.5
	Gly–Gly	1.14	3.3±0.7

[a,b]See footnotes in Table 1.



Scheme 3.

troscopy. Hydrolyses of Gly–Sar and AcGly–Sar follow mechanisms analogous to that shown in Scheme 3.

The observed rate constants for disappearance of the dipeptide in Table 3 show that substrates lacking a good anchoring ligand hydrolyze very slowly, too slowly for this reaction to be followed directly by NMR spectroscopy. Very slow hydrolysis of Gly–Gly in a complex formed with *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ at pH < 1.0 has been reported without kinetic details [35]. This unreactivity is desirable. Owing to it, our palladium(II) complexes promote efficient cleavage only when they are stably anchored to the peptide or protein [14, 15].

Glycine vs. sarcosine as the leaving group

Because the five peptides in Table 4 contain a thio ether group in the side chain of *S*-methylcysteine or methionine they bind palladium(II) promoters with high affinity, and the ensuing hydrolysis is readily monitored by ^{1}H NMR spectroscopy. Every substrate hydrolyzes 1.5 to 3 times faster in the presence of $[Pd(H_2O)_3(OH)]^+$ than in the presence of *cis*-$[Pd(en)(H_2O)_2]^{2+}$. The smallness of this difference is consistent with the notion, embodied in Scheme 2 and supported by evidence discussed above, that both promoters are converted into the same active complex or into very similar active complexes.

The main conclusion from Table 4 is that sarcosine is invariably a better leaving group than glycine regardless of the promoter used. Methylation of the amide nitrogen atom suppresses (but does not abolish) the inhibition pathway in Scheme 1 and thus enhances hydrolysis. In the case of the dipeptide AcCysMe–Sar,

TABLE 4. Hydrolysis of the C-terminal peptide bond in peptides that can effectively anchor the palladium(II) promoter because they contain the thio ether side chain

Promoter[a]	Substrate	pH*[b]	$10^4\ k_{obs}$ (min^{-1}) at 40 °C
$[Pd(H_2O)_3(OH)]^+$	AcCysMe–Sar	1.27	420 ± 10[d]
	AcCysMe–Gly	1.24	5.2 ± 0.4
	γ-Glu–CysMe–Gly[c]	0.83	8.5 ± 0.1
	AcMet–Sar	1.25	1430 ± 50
	AcMet–Gly	1.25	360 ± 40
cis-$[Pd(en)(H_2O)_2]^{2+}$	AcCysMe–Sar	1.31	190 ± 10[d]
	AcCysMe–Gly	1.35	3.2 ± 0.3
	γ-Glu–CysMe–Gly[c]	0.97	3.0 ± 0.1
	AcMet–Sar	1.25	930 ± 30
	AcMet–Gly	1.25	220 ± 40

[a,b]See footnotes in Table 1. [c]*S*-methylglutathione (GSMe). [d]At 21 °C.

hydrolysis was too fast to be followed by ^{1}H NMR spectroscopy at 40 °C, and cooling to 21 °C was necessary for reliable kinetic experiments. This enhancement is about 300-fold (after the correction for the difference between 40 and 21 °C) when the side chain is shorter ($x = 1$) as in *S*-methylcysteine. The enhancement is only about four-fold when the side chain is longer ($x = 2$) as in methionine. Because propensity for peptide nitrogen coordination is greater in the former substrates than in the latter ones, suppression of this coordination enhances hydrolysis of the former more than hydrolysis of the latter.

Conclusions

Although we have already succeeded in selective cleavage of a protein with palladium(II) complexes [46], we continue to study cleavage of peptides and small organic compounds as model substrates. A good understanding of kinetics, mechanism and stereochemistry, which can be gained in studies with model systems, is necessary for a future design of coordination complexes as artificial metallopeptidases.

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