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Hydrolysis of methionine-containing peptides in binuclear and mononuclear palladium(II) complexes

Xiaohua Chen, Xuemei Luo, Yongcheng Song, Shaozhen Zhou and Longgen Zhu*

Coordination Chemistry Institute, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, Jiangsu, 210093, P.R. China

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Abstract—The diaqua complexes of *trans*-$[Pd(py)_2Cl_2]$ (Py-pyridine), *cis*-$[Pd(edta)Cl_2]$ (edta-ethylene diamine tetraacetic acid) (complex A), *cis*-$[Pd(temed)Cl_2]$ (temed-N,N,N′,N′-tetramethylethylene diamine) and *cis*-1,2-bis(2-formylglycinebenzenesulfenyl)ethane Pd(II) dichloride (complex C) react with methionine-containing dipeptides (AcMet-aa) (aa-amino acid) via the thioether group in the methionyl side chain. The *trans*-$Pd(py)_2(H_2O)_2]^{2+}$ and *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ yield binuclear complex $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$, the diaqua complexes of complex A and complex C yield mononuclear complexes, $[PdL(AcMet\text{-aa})(H_2O)]^{2+}$ (L-edta, 1,2-bis(2-formylglycinebenzenesulfenyl)ethane). These reactions and hydrolytic cleavage of the Met-aa amide bond in the coordinated AcMet-aa are conveniently monitored by ^1H NMR spectroscopy. The *trans*-$Pd(py)_2(H_2O)_2]^{2+}$ is a very efficient promoter. The hydrolytic rate promoted by it is almost equal to that promoted by $[Pd(H_2O)_3(OH)]^+$, the fastest one up to now. Although reaction of *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ and *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ with AcMet-aa yield the same binuclear complex, $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$, the kinetic data showed that the hydrolytic rate promoted by *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ was actually controlled by temed release. The new mononuclear complexes, $[PdL(AcMet\text{-aa})(H_2O]^{2+}$, observed for the first time, are also hydrolytically active. It is of interest because they are closely associated with protein cleavage promoted by simple mononuclear Pd(II) complexes. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: palladium; hydrolysis; peptide cleavage; artificial metallopeptidase.

Selective cleavage of peptides and proteins is one of the most common tasks and the most important procedure in analytic biochemistry. Although several proteolytic enzymes are available for this purpose, its number is very limited and only trypsin is highly regioselective [1]. Furthermore, since the requirements for the traditional applications are changing today, new methods for cleavage are needed for many tasks in biochemistry and molecular biology: semisynthesis of proteins; sequencing of large or blocked proteins; structural and functional analysis of protein domain; development of new drugs, and so on. Chemical (non-enzymatic) reagents are well suited for many of these tasks. Although the need for them is growing, only one, cyanogen bromide, is available for routine use [1]. But it has several shortcomings. It is volatile and toxic, requires harsh conditions, and often produces incomplete cleavage.

Transition metal complexes are also suited for cleavage of inactivated amide bonds in peptides [2–22] and proteins [23–31]. In the early studies, complexes of cobalt(III) and copper(II) were studied more than any other. Kinetic and mechanistic studies have been done almost exclusively with amides that variously activated by substituents, by ring strain, by forced non-planarity, or by proximate functional group [2–13]. These complexes are not suitable to a practical use for analytic biochemistry, because only the N-terminal amide bond is cleaved. Practical applications, however, usually require cleavage of internal amide bonds.

In recent studies, a variety of metal complexes have been developed for direct hydrolytic or oxidative cleavage of amide bonds in peptides [14–22] and proteins [23–31]. One of them is simple palladium(II)

* Author to whom correspondence should be addressed

aqua complexes that bind to the side chains of methionine [15–19,22] and histidine [20,21] residues in short peptides followed by selectively hydrolytic cleavage of the adjacent amide group, even with turnover [21,22]. In order to further develop this new method for practical application, it is necessary to synthesize new palladium(II) complexes and investigate the action of cleavage reaction as well as understand the stereochemical requirements for cleavage. In this article, we report some palladium(II) complexes that hydrolyze Met-aa(amino acid) amide bond with different behavior. A monodentate palladium(II) complex, *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$, fast hydrolyzes Met-aa amide bond with a hydrolytic rate comparable with that promoted by $[Pd(H_2O)_3(OH)]^+$. Unlike $[Pd(H_2O)_3OH)]^+$, *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ is more stable and easy to prepare. An N,N-chelated palladium(II) complex, *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ (temed-N,N,N′,N′-tetramethyl ethylene diamine) hydrolyzes Met-aa amide bond with hydrolytic rate controlled by temedH_2^{2+} release. The action of the other two complexes, *cis*-$[Pd(edta)Cl_2]$ (edta-ethylenediaminetetraacetic acid) designated complex A and *cis*-1,2-bis(2-formylglycinebenzenesulfenyl) ethane Pd(II) dichloride designated complex C differs from their similar complexes of *cis*-$[Pd(en)Cl_2]$ (en-ethylenediamine) and *cis*-$[Pd(dtco\text{-}3\text{-}OH)Cl_2]$ (dtco-3-OH-1,5-dithiacyclooctan-3-ol). Although palladium(II) is added to the methionine-containing peptides in the form of mononuclear complexes, the complexes studied so far that actually undergo hydrolysis are binuclear complexes shown as $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$. Both NMR spectra and kinetic experiments support this conclusion. However, when the diaqua complexes of complex A and complex C were used as promoters, a new hydrolytically active mononuclear Pd(II) complex was observed for the first time. This active monomer may closely be relative to hydrolysis reaction proceeded in proteins because its steric bulk prevents formation of active binuclear complexes. This study contributes to our further understanding of the mechanism of hydrolytic cleavage of peptides and brings us a step closer to our ultimate goal, design of palladium(II) complexes as artificial metallopeptidases.

EXPERIMENTAL

Chemicals

Distilled water was demineralized. The deuterium-containing compounds D_2O, DMSO-d_6 and K_2PdCl_4 were obtained from Aldrich Chemical Co. Dipeptides Met-Gly, Met-Ala, and Met-Val were obtained from Sigma Chemical Co. The terminal amino group in each dipeptide was acetylated by a published procedure [15]. All other common chemicals were of reagent grade.

The following dichloro complexes were prepared by published procedures: *cis*-$[Pd(en)Cl_2]$ [32], $[Pd(H_2O)_3(OH)]^+$ [15], *cis*-$[Pd(temed)Cl_2]$ [32], [Pd(dien)I]I (dien-diethylenetriamine) [33].

Trans-$[Pd(py)_2Cl_2]$. 0.5 g of $PdCl_2$ was suspended in 5 ml of pyridine. The mixture was stirred and heated to 80°C. After the solid was almost dissolved, the hot solution was filtered. 50 ml of benzene was poured into the filtrate. The yellow precipitate was collected, washed with benzene and dried *in vacuo*. Yield 90%. Diffusion of methanol into the N,N-dimethyl formamide (DMF) solution of *trans*-$[Pd(py)_2Cl_2]$ in six days produced the yellow single crystals. Its crystal data were reported in literature [34].

Cis-$[Pd(edta)Cl_2]\cdot 5H_2O$. It was prepared by mixing dihydrogen disodium ethylenediaminetetraacetate (Na_2H_2EDTA) with K_2PdCl_4 in water and crystallized from concentrated hydrochloric acid. The bright yellow crystals of *cis*-$[Pd(edta)Cl_2]\cdot 5H_2O$ was obtained. Its crystal data were determined [35].

1,2-bis(2-formylglycinebenzenesulfenyl)ethane Pd(II)dichloride complex designated complex C. It was synthesized according to standard method as described in our previous paper [36]. PhCOOH → 2-HSPh COOH → (2-SPhCOOH)CH_2CH_2(2-SPhCOOH) → (2-SPhCONHCH_2COOH)CH_2CH_2(2-SPhCONH CH_2COOH) → complex C. Molecular structure of the complex C was determined [36].

The corresponding diaqua complexes were obtained by stirring the precursors with 2.0 equivalents of $AgNO_3$ in a solution of pH* 2 for 4 h at 35°C and removal of AgCl by centrifugation, all in the dark. Since the solvent was D_2O the ligand were actually D_2O, but the formulas will be written simply with H_2O. The aqua complexes were always prepared fresh and used as solutions. For complex C, because of low solubility of corresponding diaqua complex, it was treated with DMSO-d_6 instead and designated complex D.

The ^1H NMR spectra of the acetylated dipeptides in D_2O showed the following principal δ values: AcMet-Gly, 2.04, s, CH_3CO; 2.11, s, CH_3S; 3.99, q, GlyCH_2; AcMet-Ala, 2.03, s, CH_3CO; 2.11, s, CH_3S; 1.44, d, AlaCH_3; 4.39, q, AlaCH; AcMet-Val, 2.03, s, CH_3CO; 2.11, s, CH_3S; 4.27, d, ValCH; 0.96, dd, ValCH_3. Stringent control experiments showed that these peptides are stable for long time in solution of pH $\leqslant$ 1.0 and ruled out simple acid catalysis of the peptides hydrolysis [15,16]. Acidic solutions are needed to suppress formation of oligomeric palladium(II) complexes with hydroxo bridges [15,16].

Measurements

Proton NMR spectra at 500 MHz of D_2O solutions, containing sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal reference, were recorded with an AM500 spectrometer. The sample temperature was kept at 40 ± 0.5°C. The pH was measured with an Orion 901 instrument and a phoenix Ag-AgCl

reference electrode. The uncorrected values in D_2O solutions are designated pH*.

Kinetics of hydrolysis

Stock solutions of all palladium(II) complexes were prepared fresh in D_2O or DMSO-d_6. The concentration of these Pd(II) complexes was 100 mM. Equimolar amounts of AcMet-aa(aa-Gly, Ala, Val) and of the Pd(II) complexes were mixed rapidly in an NMR tube, so that the final concentration of each was 10.0–20.0 mM. The total volume was 600 μl. pH* was adjusted with 1.0 M $HClO_4$ in D_2O solution, and was measured at beginning and the end of the reaction. The difference between two measurements is always less than 0.1 pH*. Acquisition of the ^1H NMR spectra began as soon as possible, and 16 or 32 scans were taken for each time. The hydrolysis products were monitored by enhancements of the ^1H NMR resonance of free amino acids. The concentrations of the hydrolysis products (Ct and C∞) were determined on the basis of resonance area; the estimated error is ±5%. First-order plots of -ln[(C∞-Ct)/C∞] versus time contained 13–20 points and were linear over at least three half-lives, with correlation coefficients of 0.994–0.999. The observed rate constants, k_{obsd}, are the slopes of these plots. The reactions were run for six half-lives.

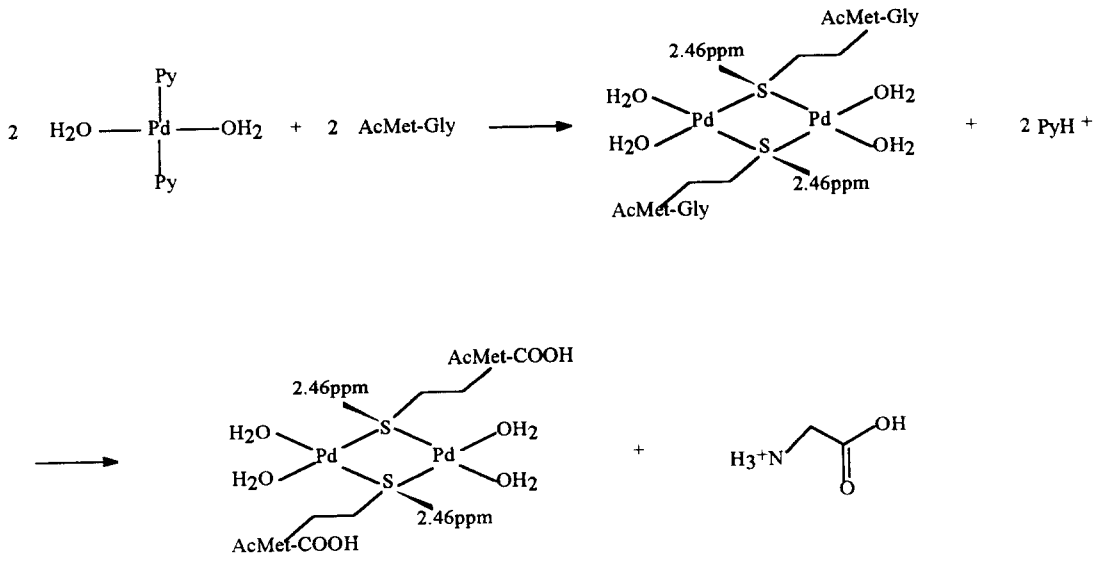
RESULTS AND DISCUSSION

Hydrolysis of peptides promoted by trans-$[Pd(py)_2(H_2O)_2]^{2+}$

We reasonably assume that the replacement of the two chloride anions in *trans*-$[Pd(py)_2Cl_2]$ by two aqua ligands would not alter the structure. As scheme 1 shows, after mixing the *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ with AcMet-Gly, free pyH^+ can be quickly detected by ^1H NMR. This release is favored by the labilizing *trans* effect of the py (compared to H_2O) and thioether and by the acid in solution. The resonance of SCH_3 of AcMet-Gly at 2.46 ppm shows that the active form is a binuclear complex [15,16,18,22]. Hydrolysis of AcMet-Gly and s-methylglutathione (GSMe) promoted by $[Pd(H_2O)_3(OH)]^+$, *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ is listed in Table 1. for hydrolysis of AcMet-Gly, these three complexes have the same active from, μ_2-s-bridged binuclear complex, that effectively promotes the hydrolysis of peptides in it. However, compared with en, pyridine is a unidentate, weak ligand and more rapidly detached after mixing with the substrate. Therefore, *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ is a more efficient promoter. The hydrolytic rate promoted by it is almost equal to that promoted by $[Pd(H_2O)_3(OH)]^+$, the fastest one up to now. The great advantage of the *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ is that it is easy to prepare and stable for stocking. As shown from Table 1, the hydrolysis of s-

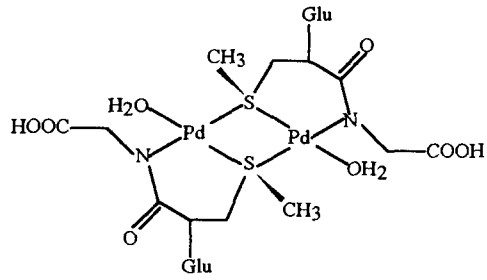
Table 1. Hydrolysis of AcMet-Gly and GSMe promoted by palladium(II) complexes at 40 C

Promoter	Substrate	pH*	k_{obsd} 10^4, min^{-1}
$[Pd(H_2O)_3(OH)]^+$	AcMet-Gly	1.07	360
trans-$[Pd(py)_2(H_2O)_2]^{2+}$	AcMet-Gly	1.05	330
cis-$[Pd(en)(H_2O)_2]^{2+}$	AcMet-Gly	1.05	199
$[Pd(H_2O)_3(OH)]^+$	GSMe	0.95	8.9
trans-$[Pd(py)_2(H_2O)_2]^{2+}$	GSMe	0.94	7.8
cis-$[Pd(en)(H_2O)_2]^{2+}$	GSMe	1.00	3.0



Scheme 1.

methylglutathione is much slower (40–60 times slower) than that of AcMet-Gly. Previous studies showed that s-methylglutathione reacts with Pd(II) complexes to form a binuclear complex as shown below [17].



Palladium(II) can effectively induce deprotonation of the amide nitrogen by binding to it [33]. When palladium(II) is already anchored to the side chain, this process is especially favorable due to six-membered chelating ring formation. The estimated pK_a for this reaction is ca 2, and displacement was observed even in solution with pH < 2.0 [37–42]. The coordination of the amide nitrogen to Pd(II) will stabilize the amide bond [15], and suppress the coordination of the carbonyl oxygen to Pd(II). This type of coordination enables to polarize the carbonyl group and activate it toward external attack by solvent water. Because, in this case, coordination of the amide nitrogen is predominant, the hydrolysis of GSMe is very slow. While, as far as AcMet-Gly is concerned, the longer side chain makes the above two types of coordination less likely because they form a seven-membered chelate ring. The aqua ligands attached to palladium(II) and *cis* to the substrates can be delivered to the carbonyl group internally. Therefore, the hydrolysis of AcMet-Gly is fast.

Dependence of hydrolysis rate constant on ligand substitution reaction

The structures of *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ are similar. They are both N,N-chelated Palladium(II) complexes. Their difference lies in that four hydrogen atoms in en are replaced by four methyl groups in temed. As the two complexes mixed with substrate AcMet-aa (aa-Gly, Ala, Val), the sulfur atom in the side chain of substrate substitutes the coordinated water, and at the same time the chemical shift of CH_3S is downfield from 2.11 ppm to 2.50 ppm. The reaction is very fast, it has completed at the beginning of monitoring. A dimer $[Pd_2(\mu\text{-AcMet-aa})_2L_2]^{4+}$ (aa-Gly, Ala, Val; L-en, temed) is formed. After formation of the dimer, $[Pd_2(\mu\text{-AcMet-Gly-})_2(temed)_2]^{4+}$, the signal of free glycine at 3.90 ppm increased. ^1H NMR also showed another two single peaks appeared at 2.62 ppm and 2.99 ppm that are assigned CH_3 and CH_2 in temed. It is clear that the release of free glycine is accompanied by the detachment of temed ligand. Therefore, we can simultaneously determine the observed rate constant of peptide hydrolysis, designated k^h_{obsd} and observed rate constant of substitution reaction of termed by water, designated k^s_{obsd}. The results are listed in Table 2. The k^s_{obsd} of en release is not given in the Table because enH_2^{2+} release in $[Pd_2(\mu_2\text{-AcMet-aa})_2(en)_2]^{4+}$ was too fast to monitor and completed only in 20 min. To compare the rates of ligand release more carefully, we have done some kinetic study on the reaction of the promoters with AcCysMe(acetyl-s-methylcysteine) at 25°C and pH* 1.52. The k^s_{obsd} values are as follows: $10^3K^s_{obsd}$ of $temedH_2^{2+}$ release is 3.56 min^{-1} and $10^3k^s_{obsd}$ of enH_2^{2+} release is 77.1 min^{-1}, 20-fold faster. Comparing the k^h_{obsd} with the k^s_{obsd} in $[Pd_2(\mu\text{-AcMet-aa})_2(temed)_2]^{4+}$, it revealed for the first time that the hydrolysis rate of dipeptides in the binuclear Pd(II) complex is governed by the temed-substitution reaction. The relationship between k^s_{obsd} and k^h_{obsd} can be explained by a consecutive kinetic equation as follows:

$$[Pd_2(\mu\text{-AcMet-aa})_2L_2]^{4+} + 4H_3^+O \xrightarrow{k^s} [Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+} + 2H_2L^{2+} \quad (1)$$

$$[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+} + 2H_3^+O \xrightarrow{k^h} [Pd_2(\mu\text{-AcMetH})_2(H_2O)_4]^{4+} + 2Haa^+ \quad (2)$$

$$[H^+aa]_t = 2Co[1-(k^h/(k^h-k^s))\exp(-k^st) + (k^s/(k^h-k^s))\exp(-k^ht)]$$

Where Co is the initial concentration of the $[Pd_2(\mu\text{-AcMet-aa})_2L_2]^{4+}$. Because pH* values measured at the beginning and at the end of reaction were always less than 0.1 pH*, we assumed that the concentration of H^+ was unchanged during the reaction. As seen from the equations, the hydrolysis reaction of peptides promoted by palladium(II) complexes proceeds stepwise. First step is detachment of ligand from Pd(II) caused by *trans* effect of thioether and by the acid in solution. Second step is the aqua ligands that *cis*-positioned to the substrate can be delivered to the carbonyl group internally, resulting in scissile of the amide bond. When $k^h \gg k^s$, that means eqn (1) is rate determining step, the hydrolysis rate is governed by detachment of the ligand. If $k^s \gg k^h$, that means eqn (2) is rate determining step, the hydrolysis rate is determined by k^h. When the ligand is temed, as shown in Table 2, the k^s_{obsd} and k^h_{obsd} are well comparable each other. In other words, in this case, the hydrolysis of peptides is governed by detachment of temed ligand and obeys the first order kinetic equation, its k_{obsd} is equal to that of ligand release. When ligand is en or py, because the detachment of en or py is fast, the hydrolysis rate of peptides is mostly governed by eqn (2).

Table 2. The k^{h}_{obsd} of dipeptides hydrolysis and the k^{s}_{obsd} of ligand release in $[Pd_2(\mu_2\text{-Ac-Met-aa})_2L_2]^{4+}$ (L-temed, en) complexes at 40 C and pH* 0.93–1.04

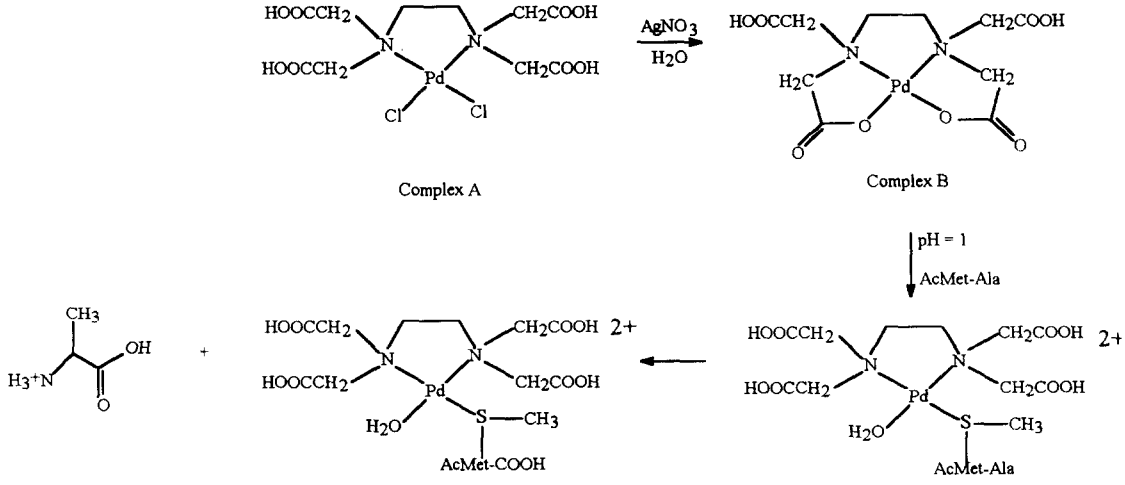
Promoter	Substrate	$10^3\ k^{s}_{obsd}$, min^{-1}	$10^3\ k^{h}_{obsd}$, min^{-1}
cis-$[Pd(temed)(H_2O)_2]^{2+}$	AcMet-Gly	7.20	6.30
	AcMet-Ala	5.90	5.10
	AcMet-Val	4.30	3.86
cis-$[Pd(en)(H_2O)_2]^{2+}$	AcMet-Gly		19.9
	AcMet-Ala		10.4
	AcMet-Val		4.6

Hydrolysis of peptides in mononuclear palladium(II) *complexes*

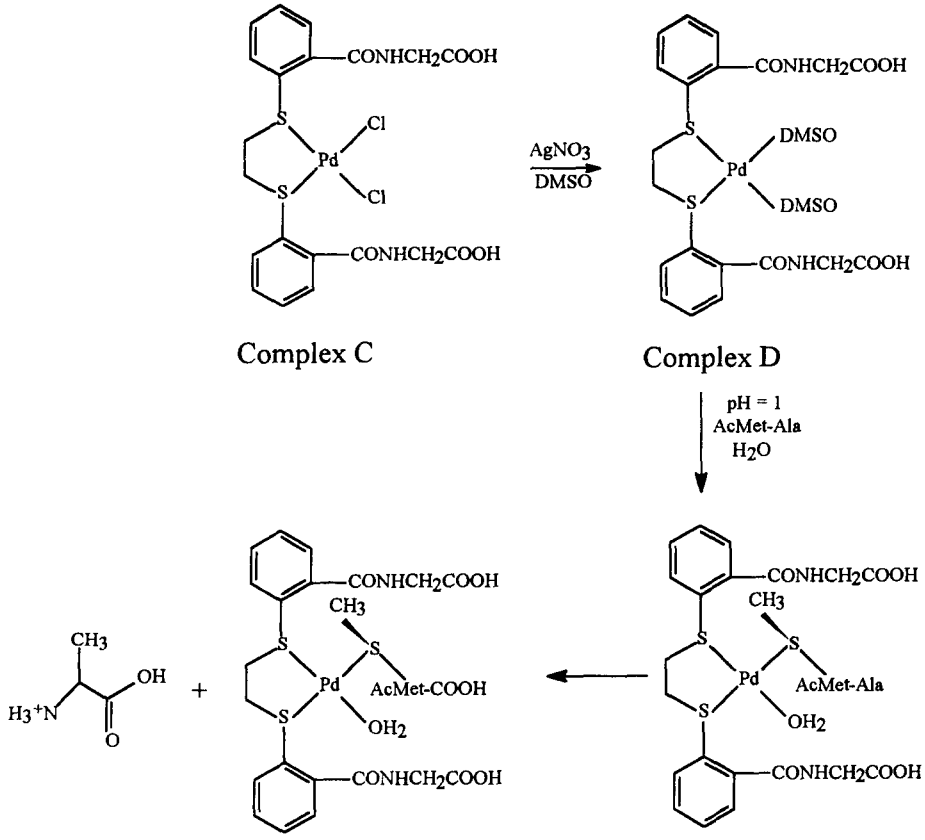
The chloride ions in complex A and complex C were removed by $AgNO_3$ in D_2O and DMSO-d_6, respectively. As schemes 2 and 3 show, complex B and complex D were formed as promoters used in hydrolysis reaction. The CH_3S resonance of the free AcMet-aa (aa-amino acid) appears at δ 2.11. The CH_3S resonances of terminal and double-bridging thioether ligands fall in two nonoverlapping intervals, 2.26–2.41 and 2.44–2.65 ppm, respectively [15–18,22]. Initial attachment of the promoter to the substrate amounts to displacement of a solvent ligand by the thioether group in the side chain. The displacement is accompanied by migration of chemical shift of the CH_3S toward downfield. Results of mixing the complex B or complex D with the dipeptides AcMet-aa (aa-Ala, Val) are shown in Table 3. Previous studies, such as *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ etc as promoters, contained various kinetic evidences that the hydrolytically active form of substrate-containing complex is binuclear complex in spite of starting promoter is monomer or dimer [15–18,22]. However, when mixing of complex B or complex D with AcMet-aa, as Table 3 shows, only one ^1H NMR resonance of SCH_3 appeared at 2.34 or 2.35 ppm that is characteristic of the mononuclear complexes (2.41 ppm in mononuclear complex $[Pd(dien)(AcMet\text{-}aa)]^{2+}$). Although complex B is a N,N-chelated palladium(II) complex, it differs from *cis*-$[Pd(en)(H_2O)_2]^{2+}$. When *cis*-$[Pd(en)(H_2O)_2]^{2+}$ attached to substrate, the thioether group coordinates to palladium(II) and a μ_2-thioether bridged dimer is formed accompanied by the detachment of the en ligand. However, when complex B reacts with substrate, because of steric bulk and weak protonation of

Table 3. Effect of coordination of AcMet-aa to palladium(II) on chemical shift (δ, ppm) of CH_3S group at pH* = 1

Promoter	AcMet-Ala	AcMet-Val
none	2.11	2.11
complex B	2.34	2.34
complex D	2.35	2.35
$[Pd(dien)(H_2O)]^{2+}$	2.41	2.41



Scheme 2.



Scheme 3.

nitrogen atom of edta, a mononuclear complex that contained edta ligand and one substrate as terminal ligand was formed. The *trans* effect of thioether group in this monomer is not enough to detach two nitrogen atoms in edta without their protonation. Complex D is similar with *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ in that they are both s,s-chelated complexes, but complex D is more bulky and prevents the formation of binuclear complex with substrate, and a mononuclear complex is formed instead, with no free glycine detected. The hydrolysis of substrates in the monomers was monitored by ^1H NMR. The observed rate constants, k_{obsd}, are listed in Table 4. As seen from the Table, although complex B and complex D have different coordinated atoms from ligands, their rate of peptide hydrolysis are quite similar. The k_{obsd} values also depend on the leaving group in the mononuclear complexes as they do in the binuclear complexes [16]. The rate of hydrolysis decreased with increase of the volume from analine to valine. In $[Pd(dien)(AcMet\text{-}aa)]^{2+}$ complex, we did not observe the hydrolysis reaction of AcMet-aa. These results confirm our conclusion that at least one aqua ligand is required for palladium(II) to promote peptide hydrolysis no matter in monomer or in

Table 4. Hydrolysis of the Met-aa bond in mononuclear Pd(II) complexes $[PdL(AcMet\text{-}aa)(H_2O)]^{2+}$ (L-edta, 1,2-bis(2-formylglycinebenzene sulfenyl)ethane; aa-Ala, Val) at pH* ≈ 1 and 50°

Promoter	Solvent	Substrate	$10^3 k_{obsd}$, min^{-1}
complex B	H_2O	AcMet-Ala	4.8
		AcMet-Val	2.3
complex D	100% DMSO	AcMet-aa	no reaction
	20% DMSO + 80% H_2O	AcMet-Ala	5.9
		AcMet-Val	3.4
$[Pd(dien)(H_2O)]^{2+}$	H_2O	AcMet-aa	no reaction

dimer [18]. Comparing the data in Table 1 with that in Table 4, although peptide hydrolysis in mononuclear complexes is much slower than that in binuclear complexes, it is important and of interest. Because this hydrolysis reaction in mononuclear complexes may closely relate to hydrolysis reaction proceeded in horst heart cytochrome *c* [28] and horse heart myoglobin [31] because of the steric bulk of proteins.

CONCLUSION

This study provides new evidences for understanding mechanism of the hydrolytic cleavage of peptides promoted by palladium(II) complexes. The Pd(II) complex with more labile ligand, such as pyridine, is a better promoter. The active species, $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$, was formed by substitution of py with thioether group of AcMet-aa prior to hydrolysis of AcMet-aa. Although reaction of *trans*-$Pd(py)_2(H_2O)_2]^{2+}$, *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(temed)(H_2O)_2]^{2+}$ with AcMet-aa yields the same binuclear complex, $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$, the hydrolysis of peptides in $[Pd_2(AcMet\text{-aa})_2(L)_2]^{4+}$ (L-en or temed) proceeds via a consecutive reaction: firstly detachment of the ligand en or temed, followed by cleavage of peptide in $[Pd_2(\mu\text{-AcMet-aa})_2(H_2O)_4]^{4+}$. The new mononuclear complexes reported here, $PdL(AcMet\text{-aa})(H_2O)]^{2+}$ (L-edta etc), are also hydrolytically active. It further confirmed that at least one aqua ligand *cis* to the substrate is required for observation of hydrolysis reaction of peptides promoted by Pd(II) complexes.

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