Rapid, Catalytic Hydrolysis of Methionine-Containing Dipeptides by a Dinuclear Palladium(II) Complex Having Thiolate Bridging Ligands

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The dinuclear complex $(Me_4N)_2[Pd_2(\mu\text{-SPh})_2Cl_4]$ is solvolyzed upon reaction with $AgClO_4 \cdot H_2O$ in acetone. The X-ray crystal structure of $(Ph_4As)_2[Pd_2(\mu\text{-SPh})_2Cl_4]$ showed that this dinuclear complex is bridged by two thiolate ligands with Cl^- ions occupying terminal sites on the palladium(II) atoms. The dinuclear solvolyzed species $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$, in which sol is H_2O or acetone, binds to methionine side chains in AcMet-X, where X is Gly, Ala, Leu, Phe, or Val. It then catalyzes hydrolysis of the amide bond involving the carboxyl group of methionine. No prior activation of the amide bond is required for hydrolysis. Dipeptides with regular amide bonds are hydrolyzed in nonaqueous solvents, under mild conditions. The reactions were followed by ^1H NMR spectroscopy. Turnover was achieved with the following N-acetylated dipeptides: AcMet-Gly, AcMet-Val, AcMet-Phe, and AcMet-Ala. One equivalent of $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$ cleaves 6−14 equiv of dipeptide. The turnover number depends on the steric bulk of the leaving amino acid. Hydrolysis kinetics were studied for AcMet-Gly, AcMet-Ala, AcMet-Val, AcMet-Phe, and AcMet-Leu. The reaction proceeds very rapidly, with a half-life of less than 7 min for AcMet-Ala at 50 °C. The half-lives at 40 °C for most of the dipeptides are shorter than 30 min. Because the rate of the reaction also depends on the volume of the leaving amino acid, the catalyst is potentially sequence-selective. The effects of temperature on the hydrolysis of AcMet-Ala were also studied. This study is a step toward the use of transition-metal complexes as reagents for the hydrolysis of lipophillic peptides and proteins.

Introduction

The half-life for the hydrolysis of amide bonds is very long at room temperature and pH 7. For AcGly-Gly it is 500 years, and for Gly-Gly, 350 years.[1] Because uncatalyzed hydrolysis of peptides is extremely slow, artificial cleavage methods are needed in analytical biochemistry and molecular biology for studies of unnatural proteins, sequencing of large or blocked proteins, analysis of protein domains, studies of protein association, and synthesis of new drugs, among other tasks. Enzymes are commonly used as cleavage agents.[2] Despite their catalytic power and sequence selectivity, proteinases have some disadvantages as cleavage reagents. For example, the cleavage at numerous sites obtained with enzymes yields relatively short peptide chains.[2] Because the substrate proteins usually are denatured before proteolytic digestion, the cleavage pattern often reveals little about their structure and function.

The chemical agents in widespread use for peptide cleavage cannot approach the selectivity and activity of enzymes, but they can potentially be very reactive and useful. Transition metal complexes are beginning to be applied to hydrolytic cleavage of unactivated amide bonds.[3−22] Several Co(III) complexes coordinate to peptides and facilitate hydrolysis, but they cleave only the N-terminal amino acid.[7] Internal and terminal amide bonds near histidine and methionine residues in peptides and proteins can be cleaved with $[Pd(H_2O)_4]^{2+}$, *cis*-$[Pd(en)(OH_2)_2]^{2+}$, $[PdCl_4]^{2-}$, and *cis*-$[Pd(OH_2)_2(dtco)]^{2+}$, eq 1.[16−21] After peptide side-chain coordination to the metal, the reaction is thought to proceed by attack at a proximate amide bond by an aqua ligand or a solvent water molecule, eq 1.[20,21]

The hydrolysis reactions promoted by metal complexes are usually stoichiometric, with a few exceptions. The most significant impediment to catalysis is that the product side chain bound to the metal atom must be displaced by new substrate for turnover to occur. The hydrolysis of histidine-containing

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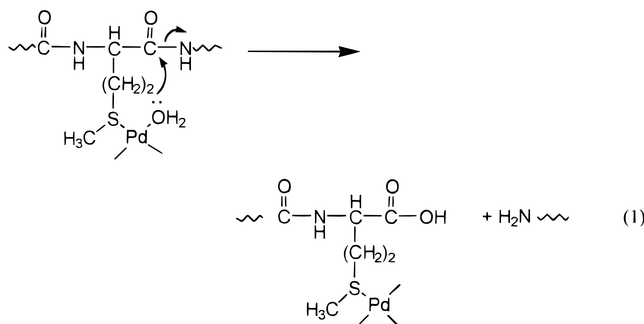

dipeptides is catalyzed by $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(OH_2)_4]^{2+}$.[18,19] The Brønsted−Lowry basicity of the imidazole group allows for breakup of the palladium(II)-histidine bond by H_3O^+ ion, which gives catalytic turnover. Because methionine side chains cannot be displaced from the coordination sphere by protonation, catalytic cleavage of methionine-containing peptides is a more difficult problem. Catalytic hydrolysis of methionine-containing dipeptides was observed for the first time in the presence of *cis*-$[Pd(en)(OH_2)_2]^{2+}$, but it is very slow for a large excess of dipeptide, with a half-life of 8 days for 10 equiv of AcMet-Gly at pH 1.0 and 40 °C.[23]

All the palladium hydrolysis reagents used to date must be applied in aqueous solution. Unfortunately, this requirement is incompatible with the cleavage of lipophilic peptides and proteins, which are insoluble or sparingly soluble in water, for example, the membrane protein rhodopsin, calsequestrin, the anion channel of red blood cell membranes, and glycoproteins.[24−27] One purpose of the work described here was to find metal complexes that would cleave these proteins.

During the hydrolysis of methionine-containing dipeptides the most active mononuclear complexes, $[Pd(OH_2)_4]^{2+}$ and *cis*-$[Pd(en)(OH_2)_2]^{2+}$, form dinuclear active species bridged by methionine side chains.[16,17,21] An important advantage of dimerization is the possibility of cooperation between metals, as was shown for hydrolysis of DNA, RNA, and their models catalyzed by polynuclear metal complexes and metalloenzymes.[28−33] These considerations suggested to us that polynuclear complexes should be good reagents for peptide hydrolysis and prompted the present research.

We chose the thiolate-bridged complex $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$, designated **1a**, for our studies.[34] An important factor in this choice of catalyst was that this binuclear complex is expected to be stable in solution. The bridging thiolate ligands in **1a** should not be displaced by methionine side chains or other donor groups in peptides and proteins. In addition, the trans effect of the thiolate sulfur atoms should allow for especially weak and potentially reversible binding of methionine to palladium(II). Indeed, we have found that this complex is an effective catalyst for rapid cleavage of methionine-containing dipeptides in nonaqueous solvents.

Experimental Procedures

Caution: *Perchlorate salts may be shock-sensitive and explosive when dried.*

Reagents and Solvents. The solvents and NMR spectroscopic standards CH_2Cl_2, diethyl ether, methanol, D_2O, acetone-d_6, TMS, and DSS were obtained from Aldrich and used as received. The complexes $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$, **1a**, and $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$, **1b**, were synthesized from $Na_2[PdCl_4]$ (Pressure Chemical Co.), PhSSPH (Aldrich), and $(Me_4N)Cl$ (Aldrich) or $(Ph_4As)Cl$ (Aldrich) by published procedures.[34] The salt $AgClO_4{\cdot}H_2O$ was obtained from Aldrich and used as received. All of the common chemicals were of reagent grade. The amino acids and peptides Ala, Gly, Leu, Phe, Met-Gly, Met-Ala, Met-Leu, and Met-Phe were obtained from Sigma. The terminal amino group of each dipeptide was acetylated by a standard procedure.[20,35]

Measurements. Routine ^1H NMR spectra, in acetone-d_6 or D_2O solution and with DSS or TMS as an internal reference, were recorded with Varian VXR 300 and Bruker XL-400 NMR spectrometers. Temperatures were kept at 40.0, 50.0, or 27.0 ± 0.2 °C. The COSY ^1H NMR spectrum of $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$, where sol is acetone or H_2O, was recorded with the Bruker XL-400 NMR spectrometer using a field gradient probe with triple-inverse geometry to improve the signal intensity.

Crystal Structure of $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ (1b). The structure was solved by Dr. Leonard Thomas of the Iowa State Molecular Structure Laboratory. Red-orange needles of $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ were grown by slow diffusion of diethyl ether into a concentrated solution of $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ in CH_2Cl_2. A crystal of the compound was mounted on a glass fiber and placed on an Enraf-Nonius CAD4 diffractometer. The unit cell constants were determined from 25 reflections in the range $5.71 < \theta < 14.10°$ found by a random search of reciprocal space. The space group $P\bar{1}$ was chosen on the basis of systematic absences and intensity statistics.

The structure was solved by direct methods, with subsequent refinements, using SHELXTL-Plus and SHELXL-93 on a Digital Equipment Corporation Microvax 3100 computer.[36] Lorentz, polarization, and semiempirical absorption corrections were applied, along with a nonlinear correction based on the decay of the standard reflections. All nonhydrogen atoms were refined anisotropically. All hydrogen atoms were refined isotropically in idealized positions.

Removal of Cl^- from $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ (1a). The complex $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ (20.5 mg, 0.025 mmol) was converted into $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$ (sol is H_2O or acetone) by treatment with an excess of $AgClO_4{\cdot}H_2O$ (64.4 mg, 0.286 mmol) in acetone-d_6 (971 μL) in the dark. The palladium complex was initially only sparingly soluble in acetone but dissolved after 3 h of stirring, giving a red-orange solution. A white precipitate of AgCl was removed by centrifugation. The resulting solution of $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$ (**2**) in D_2O was stable for a few days at 4 °C, but was prepared shortly before hydrolysis experiments. Because D_2O was later added in the hydrolysis reactions, **2** as used was a mixture of the proteated and deuterated aqua complex. The ^1H NMR chemical shifts, reported for **2** in ppm from TMS, in acetone-d_6 solution at 25 °C are as follows: 8.46 (t), 8.16 (d), 7.53 (m), 7.32 (m), 7.13 (t).

Background Cleavage of AcMet-Ala. An acetone-d_6/D_2O solution that was 7.14 mM in AcMet-Ala and 50 mM in $DClO_4$ was prepared in an NMR tube. The concentration of D_2O was approximately 1.0 M. The total volume was 700 μL. The tube was kept at 40 °C in a heating block for several weeks. The reaction was monitored periodically by ^1H NMR spectroscopy.

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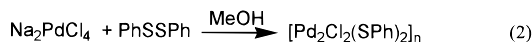
Measurement of Turnover Numbers. In an NMR tube were mixed 112 μL of a 100 mM AcMet-X solution (16 equiv, X is Gly, Ala, Val, Phe) in a 4:1 acetone-d_6 and D_2O mixture, 24.1 μL of a 30 mM $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$ solution (1 equiv) in acetone-d_6, 56 μL of a 100 mM TMS solution in acetone-d_6, and 6.0 μL of a 68% w/v solution of $DClO_4$ in D_2O. The volume was adjusted with acetone-d_6 to 800 μL, giving the following final concentrations: 14 mM peptide, 0.88 mM $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$, 90 mM $DClO_4$, and 1.5 M D_2O. The solution was kept at 50 °C, and ^1H NMR spectra were taken periodically. The concentrations of AcMet-X and X were determined on the basis of the intensities of their resonances relative to the TMS internal standard and the initial concentrations. The reaction was followed until appearance of the leaving amino acid and disappearance of the dipeptide virtually ceased.

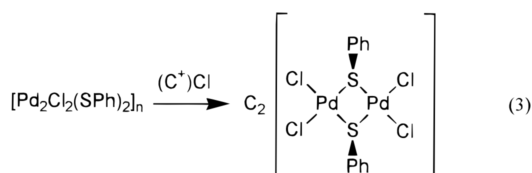
Kinetics of Hydrolysis of Dipeptides by $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$. The following solutions in acetone-d_6, D_2O, or a mixture of acetone-d_6 and D_2O were mixed in an NMR tube: 163 μL of a 30 mM solution of **2** in acetone-d_6, 49 μL of a 100 mM solution of AcMet-X (X is Gly, Ala, Phe, Val, or Leu) in 1:4 D_2O and acetone-d_6, 49 μL of a 100 mM solution of TMS, and a variable amount of $DClO_4$ in D_2O. The volume was adjusted to 700 μL with acetone-d_6. The final mixture was 7.1 mM in both the complex and the peptide. The concentration of added D_2O was 1.2 M. Acquisition of ^1H NMR spectra began as soon as possible after mixing, and 16−32 scans were taken each time. A typical kinetic plot consisted of 30 points taken over 5−7 half-lives. The concentrations of the dipeptide and of the hydrolysis products were determined, with an estimated error of ±5%, from the known initial concentrations of dipeptide and from the integrated resonances of the leaving group and the TMS internal standard. Because the proton chemical shift of HDO in acetone-d_6 varies with temperature, this resonance and that of free glycine (a product of AcMet-Gly cleavage) may overlap at temperatures above 25 °C.

Hydrolysis of AcMet-Ala by $[PdCl_4]^{2-}$. The following D_2O solutions were mixed in an NMR tube: 100 μL of 100 mM AcMet-Ala, 100 μL of 100 mM DSS, 100 μL of 100 mM $K_2[PtCl_4]$, and 400 μL of D_2O. The mixture was kept at 50 °C in a probe of an NMR spectrometer. Acquisition of spectra began as soon as possible after mixing, and 48 scans were taken each time. A typical plot consisted of about 30 points taken over 5 half-lives. The concentrations of the reactants and hydrolysis products were determined, with an estimated error of ±5%, from the known initial concentrations of the dipeptide and from the integrated resonances of the leaving group and the DSS.

Results and Discussion

Synthesis of $[Pd_2(\mu\text{-}SPh)_2Cl_4]^{2-}$. In the published procedure, $[PdCl_4]^{2-}$ is treated with PhSSPh, and the polymer $[Pd_2(\mu\text{-}SPh)_2Cl_2]_n$ is formed.[34] Reaction of Ph_4AsCl or Me_4NCl with the polymer causes fragmentation of the polymer into dinuclear complexes via displacement of the bridging Cl^- ligands, as shown in eqs 2 and 3.





Crystal Structure of $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ (1b). The crystal structure of this salt was not reported with the original synthesis. It confirms the proposed structure, which was based on elemental analysis and spectroscopic data.[34] An ORTEP drawing of the anion is shown in Figure 1. The anion has two palladium(II) ions, two bridging SPh^- ligands, and four terminal Cl^- ions. The $Pd_2S_2Cl_4$ core of the anion is essentially planar, with deviations from the plane of less that 0.10 Å for all the atoms. The phenyl rings protrude out of this plane. The idealized symmetry of the anion is C_{2v}.

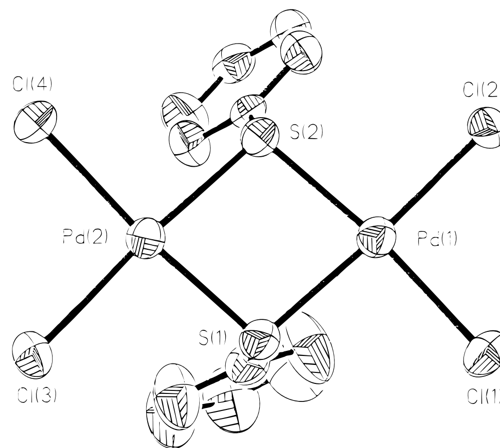


Figure 1. ORTEP representation of the dinuclear anion of $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$. The thermal ellipsoids encompass 50% of the probability. Selected bond distances (in Å): Pd(1)−Pd(2), 3.417; Pd(1)−S(1), 2.288(2); Pd(1)−S(2), 2.290(2); Pd(1)−Cl(1), 2.352(3); Pd(1)−Cl(2), 2.356(2); Pd(2)−S(1), 2.292(2); Pd(2)−S(2), 2.292(2); Pd(2)−Cl(4), 2.334(2); Pd(2)−Cl(3), 2.355. Selected bond angles (in deg): S(1)−Pd(1)−S(2), 82.67(9); S(1)−Pd(2)−S(2), 82.63(9); Pd(2)−S(1)−Pd(1), 96.59(10); Pd(1)−S(2)−Pd(2), 96.43(9). Data collection parameters: space group, $P\bar{1}$; a = 9.924(2) Å; b = 13.135(3) Å; c = 22.036(4) Å; α, 94.62(3)°; β, 91.16(3)°; γ, 103.66(3)°; V = 2779.8(10) Å^3; R = 0.0696.

The bond distances and angles give further insight into the properties of $[Pd_2(\mu\text{-}SPh)_2Cl_4]^{2-}$. The Pd−Pd distance of 3.42 Å is too long for metal−metal bonding. A typical Pd(II)−Pd(II) bond is shorter than 3.0 Å.[37] Dinuclear complexes with distances shorter than that in $(Ph_4P)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$, such as $[Pd_2(S\text{-}t\text{-}Bu)_2\{S_2CS(t\text{-}Bu)\}_2]$ (3.16 Å), lack metal−metal bonds.[38] The S−S distance of 3.02 Å also is nonbonding. As Figure 1 shows, the dinuclear complex has a rhombic structure. The nearly identical Pd−S distances of ca. 2.29 Å show that the thiolate ligands bridge symmetrically and confirm the rhombic core structure. The Pd−S and Pd−Cl distances are comparable to those in other palladium(II) complexes. The complex $[Pd_2(S\text{-}t\text{-}Bu)_2\{S_2CS(t\text{-}Bu)\}_2]$ has Pd−S distances of 2.31 and 2.32 Å.[38] The Pd−Cl distances in $[Pd_2(C_8H_{16}NS)_2Cl_2]$ are 2.37 and 2.36 Å.[39] The Pd−S−Pd (96.4 to 96.6°) and S−Pd−S (82.7 to 82.6°) angles in **1b** also show that the Pd_2S_2 core is a rhombus and not a square. The phenyl rings are fairly close to each other; the carbon atoms attached to the sulfur atoms are 4.06(2) Å apart. Weak electronic stacking interactions are common for aromatic rings, and the small tipping of the rings may be a sign of them.

Bridged dinuclear complexes of palladium(II) have been known for over forty years.[38] Many of these contain bridging halide ligands.[38] Although complexes with thiolate and thioether bridges are less common, some have been prepared. Among these are $[Pd_2(edt)_4]$, $[Pd_2(C_8H_{16}NS)_2Cl_2]$, $[Pd_2(SMe_2)_2Cl_4]$, and $[Pd_2(SC_5F_5)_6]^{2-}$.[39−42] The anion of **1b** is similar to these other complexes.

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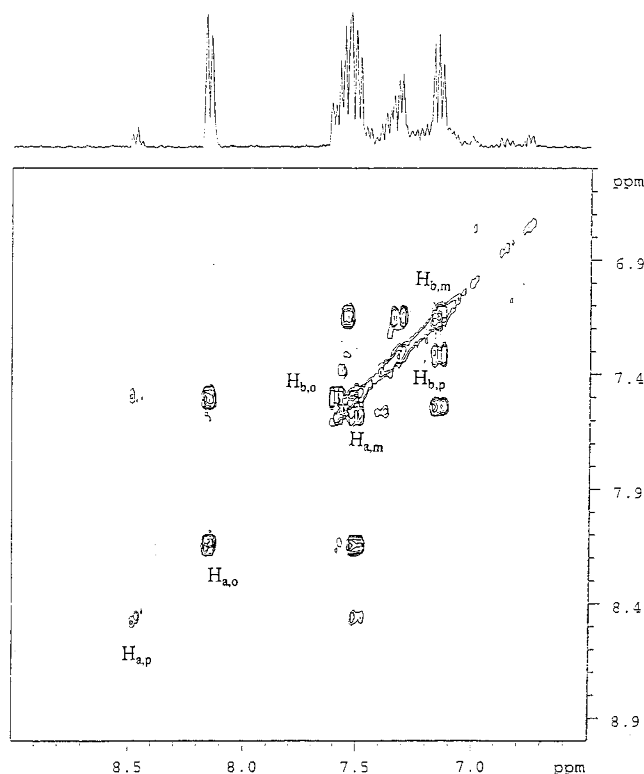
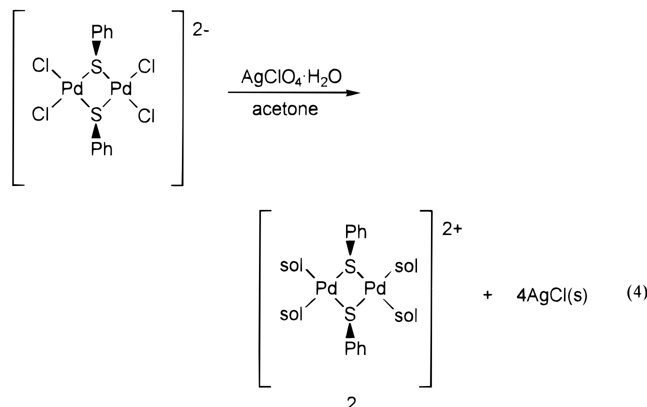


Figure 2. COSY ^{1}H NMR spectrum of the PhS^- ligands of $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$, designated **2**, in acetone-d_6 solution. Symbols $H_{a,o}$, $H_{a,m}$, and $H_{a,p}$ represent *ortho*-, *meta*-, and *para*-protons in the first ligand (a), whereas $H_{b,o}$, $H_{b,m}$, and $H_{b,p}$ represent these protons in the second ligand (b).

Unlike many palladium(II) complexes that contain terminal ligands such as thiolate, thioether, phosphine, and amine,[42-45] the complex **1b** has relatively stable bridging ligands and relatively labile terminal ligands. This combination of structural integrity and ability to bind relatively nucleophillic amino acid side chains is well suited to hydrolysis reactions. Indeed, the complex is an effective catalyst for peptide hydrolysis, as is discussed below.

Solvolysis of $[Pd_2(\mu\text{-}SPh)_2Cl_4]^{2-}$. Reaction of $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ (**1a**) with excess $AgClO_4{\cdot}H_2O$ results in displacement of the Cl^- ions by solvent molecules, as eq 4 shows. During the course of the reaction, a large amount of white solid, AgCl, precipitates from solution. The low solubility of AgCl in acetone presumably aids this reaction. Because the solvolyzed species could not, despite numerous attempts, be isolated in solid state, it was characterized in situ by NMR spectroscopy. The ^{1}H NMR spectrum of the product **2** has many resonances for the phenyl protons of the PhS^- ligands. Comparison of the ^{1}H NMR spectrum of **2** with those of PhSH and PhS^- showed that neither form of the free ligand was present in acetone solution. A ^{1}H COSY spectrum, Figure 2, was used to assign the phenyl resonances in the 7.0−8.5 ppm region to two different PhS^- ligands. The resonances at ca. 7.5 and 7.0 ppm were assigned to *meta*-protons on two separate phenyl rings, a and b, respectively. Cross-peaks showed that these protons are each coupled to two other resonances corresponding to the *ortho*- and *para*-protons of the phenyl rings. The resonances at 8.5 and 7.3 ppm were assigned to *para*-protons on the basis of their intensities relative to the resonances of the *meta*-protons. The other resonances, at 7.5 and 8.1 ppm, are assigned to the *ortho*-protons, which are coupled to the *meta*-protons.

Integration of the phenyl resonances shows that the two PhS^- ligands are present in unequal amounts. Thus, they cannot be two symmetry-inequivalent ligands in a single species. The ^{1}H COSY spectrum therefore shows that **2** consists of two separate palladium-containing species present in unequal amounts. The formation of a mixture is probably the result of substitution of Cl^- by both water and acetone ligands, designated as "sol" in eq 4 and elsewhere.



Although the 10 equiv of H_2O contributed by $AgClO_4{\cdot}H_2O$ are sufficient by amount for complete aquation of **1a**, the solvent, acetone, is much more abundant. Acetone and even poorer ligands can coordinate to metals in the presence of noncompeting anions, for example ClO_4^- or $OTeF_5^-$.[46] In the presence of water, however, competition for terminal sites on the palladium(II) atoms yields mixed species, $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$, in which solvent is both water and acetone. These mixed-ligand complexes, designated **2**, give the complicated NMR spectrum that nevertheless proved informative.

An alternative explanation for the formation of a mixture would be the incomplete removal of Cl^- ligands from complex **1a**. To check this possibility, $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ was treated for long times with large excesses of $AgClO_4{\cdot}H_2O$. Neither the time nor the excess changed the products, as judged by the NMR spectra. Evidently, the complexes **2** contain only solvent molecules, and not Cl^- ions, as terminal ligands.

Attachment of the Peptides to the Palladium(II) Atoms. In equimolar mixtures of **2** and dipeptide, the substitution reaction is complete after 1−2 min, the minimum time in which the reactants can be mixed and NMR experiment started. After this short time, the ^{1}H resonance at 2.05 ppm for the CH_3S group of free methionine is no longer observed, but peptide cleavage has not occurred to a detectable extent. Instead, a series of resonances in the region 2.20−2.80 ppm, belonging to these methyl protons, is seen for the dipeptides AcMet-X, in which X is Gly, Ala, Leu, Val, or Phe. This downfield shift is typical of coordination to palladium(II). For example, after reaction with $[Pd(OH_2)_4]^{2+}$, the CH_3S resonances for AcMet-Gly occur at 2.26−2.41 ppm.[20]

Although it was not possible to isolate the adducts of **2** with peptides, they were characterized in solution by ^{1}H NMR

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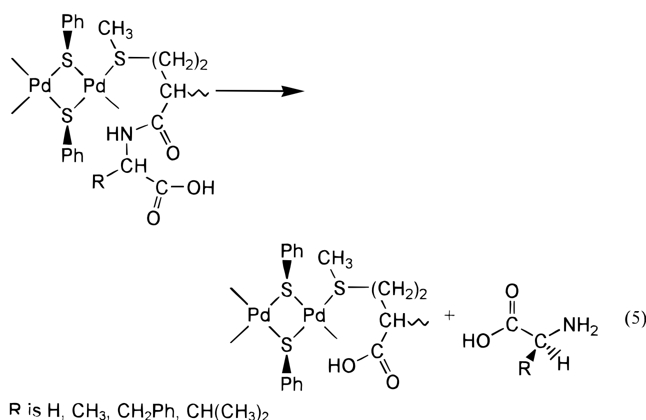
spectroscopy. The aromatic resonances of the PhS^- ligands broaden after reaction of **2** with the dipeptide, but the positions of these resonances are unchanged. This broadening suggests that more than one palladium-containing species are present after the dipeptide addition. No resonances for free PhS^- or PhSH were observed, indicating that the bridging ligand is not released. Most of the peptide resonances, those of the α-CH and side-chain groups in the C-terminal amino acid, are also broadened upon coordination to the palladium(II) catalyst. For each coordinated dipeptide, several resonances belonging to the methionine SCH_3 groups occur in the region 2.4−2.9 ppm. For example, these resonances in coordinated AcMet-Ala were observed at 2.5, 2.7, 2.8, and 2.9 ppm, and those for coordinated AcMet-Leu were found at 2.5, 2.7, and 2.8 ppm.

The chemical shifts of the methionine CH_3S groups in palladium(II) complexes are 2.20−2.40 ppm for terminal and 2.50−2.90 ppm for bridging coordination. Studies in this laboratory have found the following chemical shifts (in ppm) for other adducts of methionine with promoters of hydrolysis: *cis*-$[Pd(en)(H_2O)_2]^{2+}$, 2.37 (terminal) and 2.50 (bridging); and *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$, 2.37 (terminal) and 2.50 (bridging).[20] The chemical shifts of the new SCH_3 methionine resonances observed in this study are consistent with the presence of bridging methionine ligands.

Because the thioether group is a weaker donor than thiolate group, the methionine side chain of the dipeptides cannot displace the bridging PhS^- ligand; it displaces the terminal solvent ligands in **2**, which are labile. The methionine thioether groups possibly bridge between dinuclear units, giving a tetranuclear active form of the catalyst.

Cleavage of Peptides by Palladium(II) Complexes. Hydrolysis of AcMet-Ala in the presence of 50 mM $DClO_4$ in an acetone-d_6/D_2O solution but in the absence of palladium(II) reagents was 10% complete after 140 h. The rate constant was estimated from initial rates to be about 2×10^{-5} min^{-1}. The corresponding half-life is ca. 30 days.

After they bind to **2**, the dipeptides AcMet-X, in which X is Gly, Val, Ala, Leu, or Phe, are hydrolyzed in acetone solution, as eq 5 shows. The products of these reactions are AcMet and



the leaving amino acid X. These reactions were monitored by following the appearance of the leaving amino acid and the disappearance of the dipeptide by ^1H NMR spectroscopy; see Table 1. Because the chemical shifts depend slightly on the solvent (acetone-d_6 or D_2O), the assignments were checked by spiking the solutions with authentic samples of the amino acid at the end of the reaction. Excess $AgClO_4$ is present from the preparation of the complex **2**. However, no significant cleavage was observed in a control experiment with an acetone-d_6 solution

Table 1. Monitoring by ^1H NMR Spectroscopy of Hydrolysis of N-Acetylated Dipeptides Dissolved in a 100 mM Solution of $DClO_4$ in Acetone-d_6

substrate	resonance monitored	δ, in ppm for the substrate: free	δ, in ppm for the substrate: bound to Pd(II)	product	δ, ppm
AcMet-Gly	Gly CH_2	3.95	*a*	Gly	3.93[b]
AcMet-Ala	Ala CH_3	1.41	1.45	Ala	1.72
AcMet-Val	Val CH_3	0.95	0.99	Val	1.16
AcMet-Leu	Leu CH_3	0.94	0.95	Leu	1.13
AcMet-Phe	Phe CH	4.56	*a*	Phe	4.68

[a] The resonance was too broad for the peak position to be accurately determined. [b] Overlap of the Gly and HDO resonances at temperatures above 25 °C may complicate monitoring of this reaction.

of peptides and $AgClO_4$ during the short time required for hydrolysis in the presence of the complex **2**. Thus the Ag^+ ion is not considered a participant in the reaction.

The course of a typical reaction, the cleavage of AcMet-Ala at 50 °C, is shown in Figure 3. The very broad resonance at ca. 1.45 ppm for the alanyl CH_3 group in the coordinated AcMet-Ala disappears, and a sharp doublet for free Ala, due to the coupling to α-H, appears at 1.72 ppm. Because initial bonding of the dipeptide to **2** is rapid, the disappearance and appearance follow first-order kinetics, as shown in Figure 4. Reactions with other peptides proceed in a similar manner and were also followed by monitoring the ^1H NMR resonances of the side chain or the α-CH group in the leaving amino acid. Because of the extensive broadening upon bonding to **2**, the α-CH resonances of bound AcMet-Gly and AcMet-Phe were too weak for accurate integration, and only the appearance of glycine or phenylalanine was followed.

In all cases only the amide bond between the amino acids is hydrolyzed, not the amide bond to the acetyl group. If the latter had been hydrolyzed, acetic acid would have been formed. In none of the spectra was acetic acid detected. Regioselectivity is an important feature of hydrolysis by *cis*-$[Pd(en)(OH_2)_2]^{2+}$ and $[Pd(OH_2)_4]^{2+}$.[16−21] For example, $[Pd(OH_2)_4]^{2+}$ was shown to cleave only the Met-Ala bond of the sarcosine-containing tripeptide AcMet-Ala-Sar.[20,21] This regioselectivity is critical for the application of palladium(II) reagents to the selective hydrolysis of proteins with multiple methionine residues.[18−22]

Catalytic Turnover. Each equivalent of **2** cleaves several equivalents of dipeptide in acetone solution, as shown in Table 2. In the test for catalytic turnover, **2** was allowed to react with 16 equiv of dipeptide. Even with the relatively small amount of catalyst, the reaction was still relatively fast. Cleavage of 6−8 equiv of AcMet-Ala occurred within 24 h, and for the other dipeptides reactions were complete within several days. In 100 mM $DClO_4$ at 50 °C, turnover numbers vary from 6.4 in the case of AcMet-Phe to 13.9 in the case of AcMet-Gly.

Catalytic hydrolysis of peptides by a metal complex has been observed in only two other instances. The complex *cis*-$[Pd(en)(OH_2)_2]^{2+}$ cleaves the peptide bonds in AcHis-X, in which X is Gly, Ala, Ser, Leu, and Ile,[18,19] and in AcMet-Gly.[23] The AcMet-Gly is cleaved with a half-life of 8 days at 40 °C when the mole ration of it to the mononuclear catalyst is 10:1.[23] To our knowledge, the complex **2** is the most reactive nonenzymatic catalyst for the hydrolysis of peptides. This is only the second example of catalytic cleavage of a methionine-containing dipeptide. The turnover numbers with **2** are larger than those obtained with mononuclear complexes.[18,19,23]

The relatively high reactivity of **2** can be explained in terms of the trans effect of the bridging PhS^- ligands, which render the aqua ligands trans to them more labile than are those in

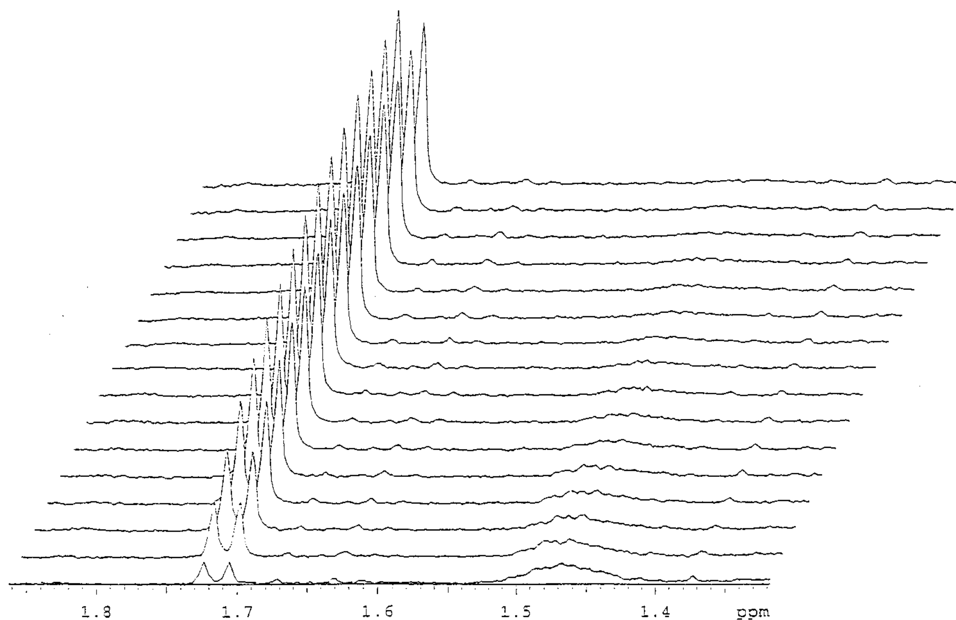


Figure 3. Proton NMR spectra of the reaction mixture of AcMet-Ala and $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$, in which sol is D_2O or $(CD_3)_2CO$, **2**, dissolved in acetone-d_6 at 50 °C. The initial concentrations of the reactants were as follows: 100 mM $DClO_4$, 7.1 mM AcMet-Ala, and 7.1 mM $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$. The alanine CH_3 resonances in bound AcMet-Ala (1.45 ppm) and free alanine (1.72 ppm) are shown. As the dipeptide is consumed, free alanine is produced. The time interval between successive spectra is 2.0 min.

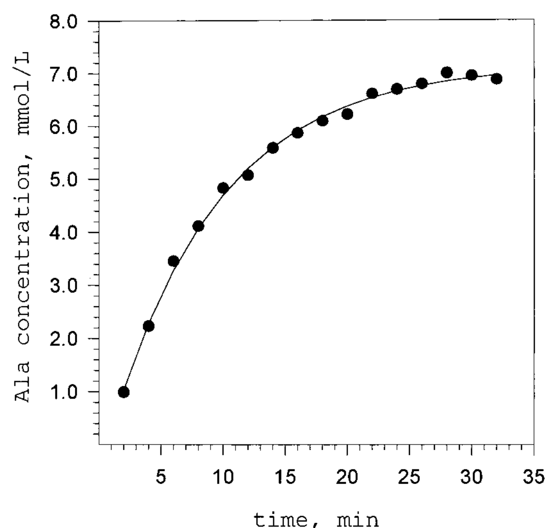


Figure 4. Plot of the alanine concentration versus time for the cleavage of AcMet-Ala by $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$, in which sol is D_2O or $(CD_3)_2CO$, **2**, dissolved in a 100 mM solution of $DClO_4$ in acetone-d_6 at 50 °C.

Table 2. Turnover Numbers for the Hydrolysis of the N-Acetylated Dipeptides by 1 Equiv of $[Pd_2(\mu\text{-SPh})_2(sol)_4](ClO_4)_2$, Dissolved in a 100 MM Solution of $DClO_4$ in Acetone-d_6

substrate	R in α-CHR	α-CHR volume, Å^3 [a]	T, °C	time, h	no. of equiv cleaved
AcMet-Gly	H	18	50	18	13.9
AcMet-Ala	CH_3	38	50	48	10.9
AcMet-Ala	CH_3	38	40	24	8.6
AcMet-Val	$(CH_3)_2CH$	76	50	66	5.9
AcMet-Phe	$C_6H_5CH_2$	106	50	65	6.4

[a] From ref 47.

cis-$[Pd(en)(OH_2)_2]^{2+}$. That hydrolysis of 10 equiv of AcMet-Gly by *cis*-$[Pd(en)(OH_2)_2]^{2+}$ is 3000 times slower ($k_{obs} = 6 \times 10^{-5}$ min^{-1}) than hydrolysis of 1 equiv is reasonable if the slow step is displacement of the AcMet product by the incoming dipeptide.[23] In **2**, where coordination of the AcMet product is labilized by the thiolates, this product is more readily displaced by the substrate, AcMet-X.

The number of equivalents of dipeptide hydrolyzed by each equivalent of **2** can be roughly correlated to the calculated volume[47] of the α-CHR fragment of the leaving amino acid. In general, the turnover is better for sterically undemanding leaving amino acids such as glycine and alanine than for bulky ones such as phenylalanine and leucine. This variation may be caused by the differences in the rates of reaction for various dipeptides, which, as discussed later, also depend on α-CHR in the leaving amino acid. To eliminate the possibility that slow reactions simply were incomplete, each reaction was run until hydrolysis became so slow that it practically ceased. We suggest that the steric bulk of a large leaving group hinders bonding of the dipeptide to the palladium(II) catalyst and makes displacement of the AcMet product difficult. Thus the differences in turnover number should depend on the ability of the incoming dipeptide AcMet-X to displace the product AcMet.

Rate of Hydrolysis. The hydrolysis reactions in equimolar mixtures of **2** and dipeptides were followed by ^{1}H NMR spectroscopy. Because the width of the resonances for the bound dipeptide precluded accurate integration, the appearance of the leaving amino acid was fit to first-order kinetics according to eq 6,

$$[X] = [X]_\infty e^{(1-k_{obs}t)} \quad (6)$$

in which t is time, k_{obs} is the rate constant, [X] is the concentration of the leaving amino acid at time t, and $[X]_\infty$ is the concentration of the leaving amino acid at the end of the reaction. Failed attempts to fit the data to the kinetics of higher order justified the fitting to eq 6. Dependence of k_{obs} on the concentration of **2** is under investigation.

As Table 3 shows, the hydrolysis reactions of AcMet-X, in which X is Gly, Ala, Leu, or Phe, are relatively fast. These

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Table 3. Rate Constants for Hydrolysis of the N-Acetylated Dipeptides by $[Pd_2(\mu\text{-}SPh)_2(sol)_4](ClO_4)_2$ Dissolved in a 100 mM Solution of $DClO_4$ in Acetone-d_6, at Various Temperatures

dipeptide	R in α-CHR	α-CHR volume, Å^3 [a]	T, °C	k_{obs}, min^{-1}
AcMet-Gly	H	18	40	0.043 ± 0.010
AcMet-Ala	CH_3	38	27	0.020 ± 0.005
AcMet-Ala	CH_3	38	40	0.052 ± 0.01
AcMet-Ala	CH_3	38	50	0.11 ± 0.05
AcMet-Val	$(CH_3)_2CH$	76	40	$(6.5 \times 10^{-3}) \pm (1 \times 10^{-3})$
AcMet-Leu	$(CH_3)_2CHCH_2$	94	40	0.016 ± 0.001
AcMet-Phe	$C_6H_5\text{-}CH_2$	106	40	$(9.0 \times 10^{-3}) \pm (6 \times 10^{-4})$

[a] From ref 47.

Table 4. Rate Constants for Hydrolysis of the N-Acetylated Dipeptides AcMet-Gly and AcMet-Ala by Three Palladium(II) Complexes

complex	leaving group	T, °C	pH	k_{obs}, min^{-1}	ref
$[Pd(H_2O)_4]^{2+}$	Ala	40	1.05	2.8×10^{-2}	21
$[Pd(H_2O)_4]^{2+}$	Ala	50	1.05	7.7×10^{-2}	21
cis-$[Pd(en)(OH_2)_2]^{2+}$	Ala	40	1.00	1.4×10^{-2}	21
$[PdCl_4]^{2-}$	Gly	40	1.14	5.1×10^{-4}	20
$[Pd_2(\mu\text{-}SPh)_2(sol)_4]^{2+}$	Ala	50	1.02	4.1×10^{-3}	this work

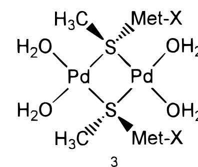
rates should make the dinuclear reagent particularly convenient for hydrolysis of peptides and proteins, cutting in half the reaction times required for *cis*-$[Pd(en)(OH_2)_2]^{2+}$ and $[Pd(OH_2)_4]^{2+}$. This may be a relatively small kinetic effect, but it is a considerable improvement from the practical point of view.

Kinetic studies show that the catalyst promotes hydrolysis over a broad range of temperatures and that the reaction rate increases with temperature. The half-life of hydrolysis of AcMet-Ala is 35, 13, and 5 min at room temperature, 40 °C, and 50 °C, respectively. Peptides in acetone solution can be cleaved overnight at near room temperature. The complex **2** accelerates the reaction by roughly a factor of 5×10^6 over the so-called background reaction at room temperature and pH 7.

The effect of the volume of the α-CHR group on the rate of hydrolysis catalyzed by **2** is shown in Table 3. The linear dependence found previously[18-21] with the mononuclear promoter *cis*-$[Pd(en)(H_2O)_2]^{2+}$ is absent in the case of **2**, but the general decrease in the rate with the increasing steric bulk of the leaving amino acid may be useful in cleaving longer peptides and proteins. When different methionine residues in a protein are followed by residues of various steric bulk, cleavage next to the small ones should be favored over cleavage next to the bulky ones. A study to test this hypothesis is in progress.

Dinuclear Metal Complexes as Hydrolysis Catalysts. Previous studies showed that the mononuclear complexes *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(H_2O)_4]^{2+}$ react with methionine-containing peptides to form the same dinuclear active species, **3**, shown below.[17,20,21] In the case of $[Pd(en)(OH_2)_2]^{2+}$, the ethylenediamine ligand is easily lost. As Table 4 shows, the preformed dinuclear complex **2** gives reaction rates kinetically comparable to those determined in our previous studies.

The dipeptide AcMet-Ala is cleaved 80 times more rapidly by **2** in acetone solution that is 1.0 M in D_2O than by $[PdCl_4]^{2-}$



in pure (55 M) D_2O solution, both at 50 °C. Because water and acetone are more labile ligands than chloride ion, **2** is more reactive than $[PdCl_4]^{2-}$ in forming dinuclear complexes with methionine as the thioether bridging ligand. This considerable difference in rates is yet another piece of evidence that dinuclear species are more effective than mononuclear complexes in promoting peptide hydrolysis.

Various hydrolytic enzymes for the cleavage of polynucleotides contain multiple metal atoms at their active sites.[28] *Bacillus cereus* phospholipase C, *Penicillium citrinium* nuclease P1, and *Escherichia coli* alkaline phosphatase contain polynuclear complexes of zinc(II), of zinc(II), and of zinc(II) and magnesium(II), respectively.[28] Dinuclear model complexes are more effective than mononuclear ones in enhancing the rate of phosphate ester hydrolysis.[48] Leucine aminopeptidase and methionine aminopeptidase have dinuclear active sites, and both metal ions seem to participate in substrate binding and catalysis.[28,49-51] While one metal ion binds the terminal amino group, activates the nucleophile, and stabilizes the transition state, the other one polarizes the carbonyl group and also helps to activate the nucleophile and stabilize the transition state.[52,53]

Metal cations in hydrolytic enzymes can play several roles.[28,29,53] They enhance the acidity of aqua ligands so that hydroxo ligands, which are more nucleophilic, become available at physiological pH values. Metal cations act as Lewis acids, binding the substrate and activating it toward nucleophillic attack, or they stabilize the emerging negative charge, usually on oxygen atoms. A single metal cation is often incapable of promoting all of these interactions, and additional cations are needed. Polynuclear catalysis has been well documented in cleavage of nucleic acids and other phosphate esters.[28,48] Similar studies with proteins and other amides are only beginning.[28,29]

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Supporting Information Available: The crystallographic information file (CIF) for the complex $(Ph_4As)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ is available. Access information is given on any current masterhead page.

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