Inorg. Chem. **2005**, *44*, 5141–5146

Platinum(II) Complex as an Artificial Peptidase: Selective Cleavage of Peptides and a Protein by *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Ion under Ultraviolet and Microwave Irradiation

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Received January 27, 2005

Two synthetic peptides were completely cleaved by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ (en is ethylenediamine) complex at pH 2.5 under thermal heating at 60 °C in a selective way: only the amide bonds involving the carboxylic group of the methionine residue, i.e., the Met–Z bonds (where the residue Z has a noncoordinating side chain), were hydrolyzed. Under irradiation at 300 nm, the rate constants for these cleavage reactions were approximately doubled, but side reactions occurred. Under microwave irradiation, the rate constants were increased 2–3 times at 60 °C and ca. 7 times at 100 °C, and no side reactions were detected. Microwave irradiation similarly accelerated the complete and selective cleavage of Met–Z bonds in cytochrome *c* at 60 °C in comparison with this cleavage under thermal heating, again without detected side reactions. The microwave-assisted cleavage of peptides and proteins by the platinum(II) reagent holds promise in proteomics and other biotechnological applications.

Introduction

Selective cleavage of peptides and proteins is essential in many bioanalytical and bioengeneering applications. Protein sequencing, peptide mapping,[1] folding studies,[2] protein semisynthesis,[3] and purification of fusion proteins all involve selective cleavage of peptide bonds.[4] The most desirable method of cleavage is hydrolysis of the amide group because the products of this reaction, namely, amines and carboxylic acids, can be condensed into new products or otherwise chemically modified. Amide groups, however, are extremely unreactive toward hydrolysis; the half-life for peptide hydrolysis in the pH range from 4 to 8 is several hundred years.[5]

A small number of proteolytic enzymes and synthetic reagents are available, but they do not meet all current needs. Enzymes, such as trypsin and chymotrypsin, are very effective catalysts, but they have shortcomings. Their selectivity is almost fixed and very difficult to change, they become inactive in the presence of detergents, they are incompatible with organic solvents, they digest themselves as well as the intended substrate, and they contaminate the products of substrate cleavage. Sometimes these products (peptides) are so short as to be unsuitable for chemical ligation and other applications.

Chemical reagents are less effective than enzymes and have various disadvantages.[6] The common reagent cyanogen bromide cleaves at the C-terminus of methionine residues, but irreversibly converts these residues to serine lactone, it is volatile and toxic, it is applied in very large excess over the methionine residues present in the substrate, it requires 100% formic acid or 70% trifluoroacetic acid as a solvent, and it causes various side reactions.[6] Polymers having catalytic groups, such as carboxylate, aldehyde, and imidazolyl, show some promise as artificial peptidases.[7]

Transition-metal complexes have long been known to promote cleavage of peptide bonds, but their systematic study has only recently led to practical applications.[8−19] Currently,

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10.1021/ic050137w CCC: $30.25
Published on Web 06/17/2005

palladium(II) and platinum(II) complexes, in particular [Pd(H$_2$O)$_4$]$^{2+}$, *cis*-[Pd(en)(H$_2$O)$_2$]$^{2+}$, and *cis*-[Pt(en)(H$_2$O)$_2$]$^{2+}$ (en is ethylenediamine), are the most effective inorganic reagents for protein cleavage.[14,20−26] Palladium(II) complexes have been studied in some detail. They spontaneously bind to methionine and histidine side chains and regioselectively promote hydrolytic cleavage of the second amide bond preceding this anchoring residue (in the direction of the amino terminus), that is, the X−Y bond in the X-Y-Met-Z and X-Y-His-Z sequences in which X, Y, and Z have noncoordinating side chains.[21−23,26] If Y is the proline residue, [Pd(H$_2$O)$_4$]$^{2+}$ can cleave the X−Pro bond at neutral pH.[23] The properties of the complexes can be adjusted, and they can cleave even in the presence of detergents[24] or in organic solvents.[27] Conjugates of the Pd(II) complex and β-cyclodextrin can cleave selectively the X−Pro bond in the X-Pro-Ar sequence, where Ar is an aromatic residue, at neutral pH.[26] Kinetic and stereochemical evidence suggests that palladium(II) ion, as a Lewis acid, interacts with the carbonyl oxygen, thus polarizing the scissile amide group and facilitating nucleophilic attack of solvent water at the carbon atom.[23]

The study of platinum(II) complexes has only begun, and the results are interesting and unexpected.[24,25] Similar complexes of platinum(II) and palladium(II) generally undergo similar ligand-displacement reactions, but the former reacts much more slowly than the latter.[28] Surprisingly, the regioselectivity of *cis*-[Pt(en)(H$_2$O)$_2$]$^{2+}$ completely differs from that of *cis*-[Pd(en)(H$_2$O)$_2$]$^{2+}$, stated above. The platinum(II) complex binds only to methionine side chains and promotes hydrolytic cleavage of the first amide bond following this anchoring residue (in the direction of the carboxy terminus), that is, the Met−Z bonds, where Z has a noncoordinating side chain residue.[24,25] This stark difference in regioselectivity can be attributed to the aforementioned difference between the metal ions. Because the platinum(II) complex is much more inert than its palladium(II) analogue, the ethylenediamine ligand remains coordinated to the platinum(II) ion throughout the cleavage reaction, whereas this ligand is displaced by water at the palladium(II) ion early in the reaction.

The hydrolytic cleavage of proteins by the *cis*-[Pt(en)(H$_2$O)$_2$]$^{2+}$ complex is regioselective, but the reaction takes up to 24 h for completion, depending on the substrate and the reaction conditions. Although enzymes and conventional chemical agents require similar periods of time, we sought to make the platinum(II) reagent act faster. Even a 2-fold decrease in the reaction times would be a practical improvement. Slow chemical reactions can be accelerated by energizing the reactants, creating reactive intermediates, or stabilizing the products. Besides thermal heating, there is high pressure and irradiations with light, ultrasound, and microwaves. In most of these methods, energy in different forms is supplied to the reactants.[29]

Microwave (or dielectric) heating uses the ability of some compounds to transform electromagnetic energy into heat in situ. This is emerging as a new and promising method of accelerating chemical reactions.[30,31] The effect of temperature on the reaction rates is well-known, but the effect of microwaves is not understood. Microwave irradiation can act through thermal effects or specific microwave effects. Thermal effects or dielectric heating can result from the interaction of polar molecules with the electromagnetic field. In liquids, only polar molecules selectively absorb the microwaves. Specific microwave effects are nonthermal, akin to the effects of the medium on the reaction mechanisms.[32]

In this study we explored the effects of ultraviolet light and demonstrated the effects of microwaves in accelerating selective cleavage of peptides and a protein by the *cis*-[Pt(en)(H$_2$O)$_2$]$^{2+}$ ion.

Experimental Procedures

Chemicals. The complex *cis*-[Pt(en)Cl$_2$], piperidine, and α-cyano-4-hydroxycinnamic acid were obtained from Aldrich Chemical Co. The complex *cis*-[Pt(en)(H$_2$O)$_2$]$^{2+}$ was prepared by the published procedure as a perchlorate salt.[33,34] Its concentration was determined using the published absorptivity (extinction coefficient).

Equine cytochrome *c* was obtained from Sigma Chemical Co. Trifluoroacetic acid was obtained from Alfa Aesar. Acetonitrile of HPLC grade was obtained from Fisher Scientific Co. All the Fmoc-amino acids, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazoliumhexafluorophosphate(1−) 3-oxide, 1-hydroxy-1,2,3-benzotriazole, FmocAla-Wang resin, and FmocGly-Wang resin, used in the synthesis of peptides, were purchased from Novabiochem.

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The nonapeptide AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly (AcGKAMAAPRG) and the decapeptide AcAla-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala (AcAKYGGMAARA) were synthesized by a standard manual Fmoc solid-phase procedure and purified by reversed-phase HPLC on a C18 preparative column, as described previously.[21,22] The purity, examined by analytical HPLC, was higher than 99.5%. For the nonapeptide the found and calculated masses were, respectively, 901.84 and 901.07 Da; for the decapeptide, they were 1036.57 and 1036.51 Da.

HPLC Separations. The components of the reaction mixtures were separated by a Hewlett-Packard 1100 HPLC system containing an autosampler and a multiwavelength detector set to 215, 270, and 410 nm. Absorption at 215 nm is common to all peptides and proteins, absorption at 270 nm is due to aromatic residues and the Pt(II) complex, and absorption at 410 nm is diagnostic of heme. The reversed-phase separations were done with an analytical Supelco Discovery C18 column (sized 250 × 4.6 mm, beads of 5 μm) and a preparative Vydac C18 column 218TP101522 (sized 250 × 22 mm, beads of 10 μm). The eluting solvent A was 0.10% (v/v) trifluoroacetic acid in water, and solvent B was 0.08% (v/v) trifluoroacetic acid in acetonitrile. For the reaction mixtures that involved the nonapeptide AcGKAMAAPRG, in a typical run the percentage of solvent B in the eluent was kept at 0% for 5 min after the injection of the sample, and then raised gradually to 15% over a 35 min period. For the cleavage of the decapeptide AcAKYGGMAARA, the method was the same, but the content of solvent B at 35 min was 45%. The flow rate was 1.00 mL/min in analytical runs and 10.0 mL/min in preparative runs. The size-exclusion separations were done with a Superdex peptide HR 10/30 column, having an optimal separation range from 1000 to 7000 Da. The solvent was 0.10% (v/v) trifluoroacetic acid in water, and the flow rate was 0.50 mL/min.

Mass Spectrometry. The MALDI-TOF experiments were done with a Bruker Proflex instrument. The samples were prepared by a standard dried-droplet procedure: 1.0 μL of the solution of interest was mixed with 9.0 μL of a saturated solution of the matrix (α-cyano-4-hydroxycinnamic acid) in a 2:1 (v/v) mixture of water and acetonitrile. Each spectrum consisted of 100 scans. For the sake of clarity, molecular masses are reported only for the fragments free of the Pt(en) groups, although the Pt(en)-carrying fragments were also observed in the MALDI spectra. Bradykinin and cytochrome *c* were used as external standards. The measured molecular mass of a given fragment was compared with the value calculated by PAWS software, obtained from ProteoMetrics, LLC. An excellent agreement between the measurement and calculation conclusively identifies a peptide or relatively small protein.

Ultraviolet and Microwave Irradiations. The photochemical reactions were done in a Rayonet 100 reactor, which had 16 fluorescent tubes designated 3000 Å for the experiments at 300 nm and 8 fluorescent tubes designated 3500 Å for the experiments at 350 nm. The lamps have a bandwidth of approximately 25 nm on each side of the nominal emission maximum.

The experiments involving microwave irradiation were done with a CEM (Matthews, NC) Model Discover continuous-wave microwave oven delivering 300 W and allowing continuous cooling.[35]

Study of Hydrolysis. In a typical experiment with ultraviolet irradiation, involving equimolar amounts of the Pt(II) reagent and the methionine residue in the substrate, 0.35 mL of a 60 mM solution of the nonapeptide AcGKAMAAPRG was mixed with 0.21 mL of a 100 mM stock solution of *cis*-$[Pt(en)(H_2O)_2]^{2+}$ and 1.44 mL of water. The final concentration of the peptide was 10.5 mM. The pH was adjusted by $HClO_4$ or NaOH. For a good comparison, the reaction mixture was divided into two 1.0 mL halves. One half was transferred to a quartz cuvette sized 10 × 10 × 40 mm with all walls transparent that had a rubber septum, thoroughly purged of air by bubbling with argon for 20 min, and irradiated by ultraviolet light for 1 day. The other half was kept in a 2.0 mL glass vial in the dark and heated in a dry bath (aluminum block). Samples of both halves were taken periodically; kept frozen, to quench the reaction; and analyzed by HPLC. After the reactions were completed, the pH remained within ±0.1 of the initial value. In the control experiments for possible background cleavage, the conditions were the same except that the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex was absent and the reaction was followed for much longer periods of time. The irradiated reaction mixtures had pH values of 2.0 and 2.5, and were kept at 40 and 60 °C.

For the experiments involving microwave irradiation, the stock solutions were 5.00 mM in each substrate (the nonapeptide, the decapeptide, or cytochrome *c*). In a typical experiment, involving equimolar amounts of the Pt(II) reagent and the methionine residue in the peptide, the final volume of the reaction mixture was 5.00 mL, and the final peptide concentration was 1.00 mM. The reaction mixture contained 1.00 mL of the peptide solution, 50.0 μL of a 100 mM stock solution of the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex, and 3.95 mL of water. For the experiments with cytochrome *c*, the ratio of the protein to the Pt(II) reagent was 1:5. The pH was adjusted with $HClO_4$ or NaOH. The reaction mixture was divided into two parts; a 1.0 mL portion was kept in a dry bath, and a 4.0 mL portion was irradiated by microwaves. In the control experiments for possible background cleavage, the conditions were the same, except that the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex was absent and the reaction was followed for much longer periods of time.

The progress or absence of cleavage was monitored by size-exclusion chromatography in the case of the protein substrate and by reversed-phase HPLC in the case of the peptide substrates. In all cases the separated fragments were lyophilized to dryness, redissolved, and identified by MALDI-TOF mass spectrometry. This identification method is faster and more reliable than the sequencing of terminal residues, used in our earlier studies.[20]

Determination of the Rate Constants. Because the cleavage is very slow at room temperature, at which HPLC was done, the species distribution in each chromatographic run matched that in the digest. The plots of the peak areas for the cleavage products versus time were fitted to the first-order rate law with the program SigmaPlot v. 5.0, obtained from SPSS Inc. All the kinetic plots have 5% error bars, reflecting the estimated inaccuracy in injecting the samples and integrating the peaks. Because the binding of the Pt(II) reagent to the methionine side chain is much faster than the subsequent intramolecular cleavage of the substrate, the fitting to the first-order rate law is justified. Each rate constant is the average of two consistent values, obtained by monitoring both fragments, products of the cleavage. The stated errors in the rate constants correspond to 2 standard deviations, i.e., a confidence limit greater than 95.0%. These conservative error margins are our precaution against overstating small differences.

In experiments with irradiation at 300 nm, in which the product peaks increased for approximately 3 h and then started to decrease, only the increasing part of the plot was fitted. This part corresponded to 85−90% of the cleavage reaction.

Results and Discussion

Design of the Photochemical Experiments. The interaction of the platinum(II) ion with the scissile amide group

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Table 1. Results of HPLC Separation and MALDI Mass Spectroscopic Identification of the Fragments of AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly Resulting from the Cleavage by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex under Ultraviolet Irradiation

	molecular mass (Da)		
elution time (min)	obsd	calcd	fragment
5.3	448.66	448.56	1−4
9.5	472.94	471.54	5−9

probably involves dissociation of an aqua ligand. Therefore, the ability of ultraviolet light to enhance ligand-substitution reactions at the platinum(II) atom[36,37] may be relevant to the action of the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex in cleaving peptide bonds. We considered different irradiation wavelengths. The absorption spectrum of the complex shows a maximum at 256 nm, but we did not use the light of 254 nm lest it would cause displacement of the ethylenediamine ligand. At 300 nm, phenylalanine, tyrosine, and tryptophan would be excited, since they absorb light around 280 nm. The presence of these residues might have caused side reactions that would have obscured the reaction of interest, the substrate cleavage by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex. For this reason, the methionine-containing nonapeptide, used in the experiments involving ultraviolet irradiation, lacked aromatic residues.

Cleavage of the Nonapeptide by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex under Ultraviolet Irradiation. The equimolar mixture of the nonapeptide AcGKAMAAPRG and *cis*-$[Pt(en)(H_2O)_2]^{2+}$ ions at pH 2.5 and 60 °C was irradiated at 300 nm and analyzed by HPLC. Initially, two peaks were present: that containing the intact nonapeptide, eluting at 26.2 min, and that containing the free *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex, eluting at 3.8 min. After 4 h, two new peaks, eluting at 5.3 and 9.5 min, were the only ones present in the chromatogram. These two products of cleavage were identified by MALDI mass spectrometry. Very similar HPLC and MALDI results were obtained upon irradiation at 300 nm. Table 1 shows that cleavage by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex under ultraviolet irradiation occurs on the carboxy side of the methionine residue, that is, at the first amide bond "downstream" from the anchoring residue, as shown schematically below.

AcGly-Lys-Gly-**Met**-Ala-Ala-Pro-Arg-Ala

Comparison of Ultraviolet Irradiation and Thermal Heating. Figure 1 shows a typical kinetic plot for the cleavage assisted by ultraviolet irradiation. Table 2 shows that the rate constants for the cleavage of the nonapeptide by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex were approximately 2 times higher under irradiation at 300 nm than under thermal heating, at all the pH values and temperatures examined. In other words, reaction time is approximately halved under irradiation at 300 nm.

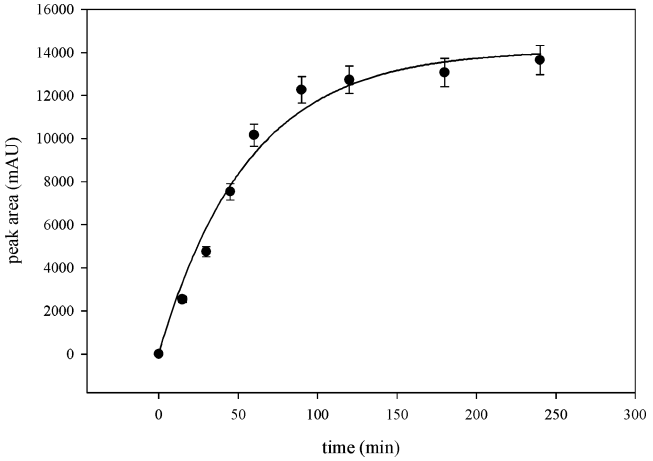

Figure 1. Kinetics of the cleavage of the nonapeptide AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex under irradiation at 300 nm at pH 2.5 and 60 °C. The appearance of the fragment 5−9 was followed by HPLC.

Table 2. Rate Constants *k* for the Cleavage of AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly by *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex under thermal heating and under ultraviolet irradiation at the wavelengths shown

		$k/10^{-3}\,min^{-1}$		
pH	*T* (°C)	thermal heating	300 nm	350 nm
2.0	40	7 ± 2	10 ± 3	9 ± 2
	60	17 ± 4	32 ± 5	nd[a]
2.5	40	5 ± 1	11 ± 2	4 ± 1
	60	13 ± 2	20 ± 4	nd[a]

[a] Not determined.

Solutions irradiated at 300 nm, however, turned from light yellow to yellow and then to brown, while the thermally heated solutions stayed light yellow. Upon prolonged irradiation, HPLC peaks for the products decreased without new peaks emerging. Brown precipitate separated upon the centrifugation of the irradiated solutions. These symptoms of side reaction persisted when irradiation at 300 nm was done at 40 °C and at a lower pH.

These symptoms were less pronounced under irradiation at 350 nm, but as Table 2 shows, the cleavage rate was similar to that under thermal heating. Although the photochemical method proved to be somewhat successful, we looked for other means of accelerating the cleavage reaction.

Effects of Microwave Irradiation on the Reaction Rates. Reactions of palladium compounds in homogeneous catalysis are markedly promoted by microwaves.[38,39] Very recently the microwave method began to be applied to platinum compounds,[40−42] but it has barely been used with peptides and proteins. Hydrolysis of these polyamides is much accelerated,[43,44] but the brutal acidic conditions used make the reaction nonselective and therefore good for protein

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sequencing but not for the production of useful peptides in high yields. Selective cleavage at both carboxy termini and amino termini of aspartyl residues occurs in a weakly acidic medium. The reaction time was 3−6 times shorter under microwaves than under conventional heating, but only 90% of the other peptide bonds stayed intact under the reaction conditions.[45] Microwave-enhanced cleavage by trypsin was recently used for protein mapping, but the reaction was incomplete.[46]

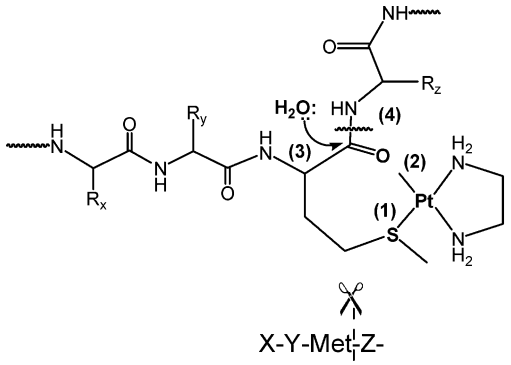
Because the thioether group is a fairly strong nucleophile for platinum(II) ion, displacement of an aqua ligand in the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex by the methionine side chain occurs within minutes in our experiments. Because the carbonyl oxygen atom is a weak nucleophile, the interaction between the methionine-anchored platinum(II) ion and the scissile amide group is slow. This step and the subsequent external attack of the solvent water at the activated amide group may, in principle, be accelerated by microwaves. We set out to test this hypothesis.

Cleavage of Oligopeptides by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex under Microwave Irradiation. Because microwave irradiation shortens reaction times and thus disfavors secondary reactions, we did the experiments at 100 °C as well as 60 °C. To maximize the microwave energy imparted to the sample, we carried out experiments with continuous cooling. We continued to use the nonapeptide AcGKAMAAPRG so that we could compare various methods for promoting the cleavage. Since the microwave irradiation should not affect the aromatic residues, we also used the decapeptide AcAKYGGMAARA. These two substrates gave consistent results. For example, an equimolar mixture of the nonapeptide and the Pt(II) reagent irradiated for 3 h at pH 2.5 and 60 °C showed only two HPLC peaks, eluting at 5.3 and 9.5 min. Evidently, the cleavage was complete. As before, both fragments were identified by MALDI mass spectrometry. As Table 1, Table S1 in the Supporting Information, and the illustration below show, the selectivity under microwave irradiation is the same as that under ultraviolet light and conventional heating.

AcAla-Lys-Tyr-Gly-Gly-**Met**-Ala-Ala-Arg-Ala

A nearly perfect match of measured and calculated molecular masses such as those in Table 1 is evidence that the fragments retain their terminal amino and carboxylic groups. Even a slight chemical modification of the fragments would have affected their molecular masses, and MALDI-TOF spectra would have shown it. Evidently, the platinum(II) reagent cleaves the substrate by a hydrolytic mechanism, as shown in Scheme 1.

Scheme 1. Proteolytic Selectivity of the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex and the Four Steps of Cleavage of Amide Bonds by Pt(II) Complexes[a]



[a] (1) Binding of Pt(II) atom to the sulfur in the methionine side chain. (2) Interaction of the Pt(II) atom with the neighboring amide group. (3) Attack of the solvent water. (4) Hydrolysis of the amide group. The unspecified ligand on the Pt(II) atom is H_2O. Amino acid residues X, Y, and Z have noncoordinating side chains.

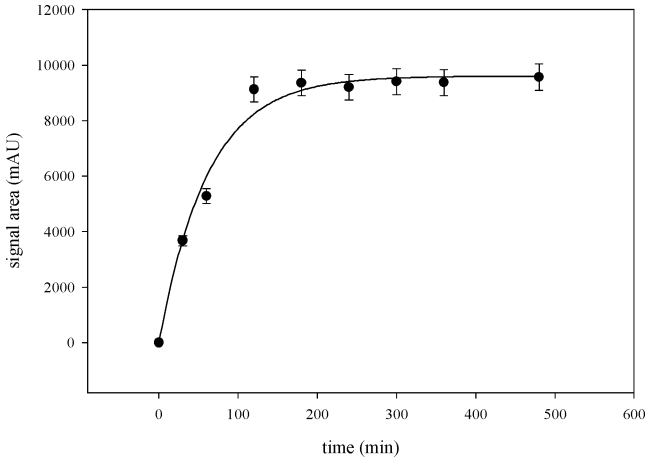


Figure 2. Kinetics of the cleavage of the nonapeptide AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex under microwave irradiation at pH 2.5 and 60 °C. The appearance of the fragment 1−4 was followed by HPLC.

Table 3. Rate Constants *k* for the Cleavage of the Nonapeptide AcGly-Lys-Ala-Met-Ala-Ala-Pro-Arg-Gly and the Decapeptide AcAla-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex at pH 2.5 under Thermal Heating and Microwave Irradiation

	$k/10^{-3}$ min^{-1}			
	nonapeptide		decapeptide	
T (°C)	thermal	microwave	thermal	microwave
60	6 ± 1	17 ± 3	2.5 ± 0.5	7 ± 1
100	80 ± 11	580 ± 35	33 ± 3	200 ± 25

Figure 2 shows that the half-life for cleavage of the nonapeptide is reduced to ca. 40 min at 60 °C. Table 3 shows that microwaves are generally 2−3 times more effective than thermal heating at 60 °C in promoting cleavage by the Pt(II) reagent and 6−7 times more effective at 100 °C. At this higher temperature the half-life for cleavage is only 3.5 min for the decapeptide and only 1.2 min for the nonapeptide. No side reactions were observed.

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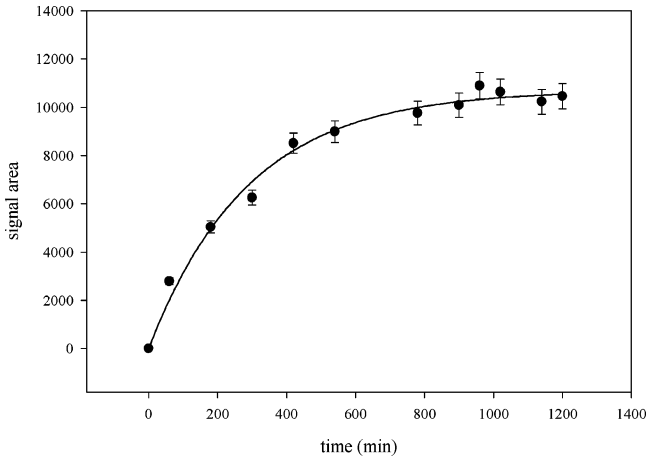
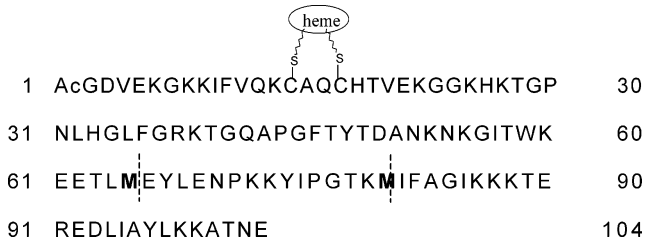


Figure 3. Kinetics of the cleavage of equine cytochrome *c* by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ complex under microwave irradiation at pH 2.5 and 60 °C. The appearance of the fragment 81−104 was followed by HPLC.

Encouraged by these results, we applied the new method to a protein.

Microwave-Promoted Cleavage of Equine Cytochrome *c* by the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ Complex. Because the protein contains multiple residues capable of binding the reagent, we added 5 equiv of the platinum(II) complex. The reaction at pH 2.5 and 60 °C was followed by size-exclusion chromatography. Intact cytochrome *c* eluted at 16.5 min. After 12 h the intact protein was absent, and three fragments were identified by MALDI mass spectrometry. Table S2, in the Supporting Information, gives evidence for selective cleavage of Met65−Glu66 and Met80−Ile81 bonds, as shown schematically below.



The growth of the peak corresponding to the fragment 81−104 at pH 2.5 and 60 °C obeyed the first-order rate law under both thermal heating and microwave irradiation; see Figure 3. The respective rate constants were $(1.4 \pm 0.4) \times 10^{-3}$ and $(3.5 \pm 0.5) \times 10^{-3}$ min^{-1}. Microwave irradiation approximately doubled the cleavage rate, as in the experiments with oligopeptides. Because the cleavage time with our reagent was comparable to that with common proteolytic enzymes, we deemed the reaction sufficiently fast for practical work and did not do experiments at 100 °C.

Conclusions and Prospects

This study confirms that the complex *cis*-$[Pt(en)(H_2O)_2]^{2+}$ promotes selective, hydrolytic cleavage of peptide bonds involving the carboxylic group of the methionine residue, i.e., the Met−Z bonds, where Z has a noncoordinating side chain.[24,25] Irradiation at 300 nm increases the rate constant approximately 2 times, but this method is impractical because of side reactions. Microwave irradiation, however, increases the rate constant as much as 7 times under conditions where side reactions are not observed. Two peptides and a protein were cleaved selectively and completely in a relatively short time. Because methionine residues are relatively rare in proteins, the products of cleavage are relatively long peptides, suitable for proteomics, semisynthesis, and other applications. Because cleavage of even large proteins gives relatively few peptides, our reagent may be useful in analyzing mixtures containing relatively many proteins. Even incomplete cleavage, achieved in yet shorter time, may be useful in proteomics applications.

Acknowledgment. This work was supported by the National Science Foundation through Grant CHE-0316868.

Supporting Information Available: Tables giving HPLC and MALDI mass spectrometry results and figures showing MALDI mass spectra and kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC050137W