Chinese Journal of Chemistry, **2004**, *22*, 537—544 **Article**

Structure of AcMet-Gly and Its Interaction with Palladium(II) Tetraaqua Complex Studied by Electrospray Mass Spectrometry and Density Functional Theory

JIN, Chen(金晨) ZHANG, Lin(张琳) HONG, Jin(洪瑾) YANG, Gao-Sheng(杨高升)
ZHU, Long-Gen*(朱龙根)

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

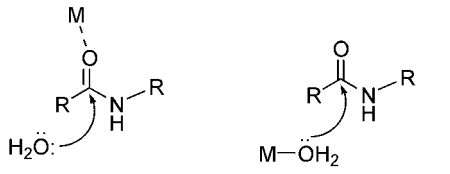
The structure of dipepide AcMet-Gly was determined by X-ray crystallographic analysis. It possesses monoclinic, space group $P2_1$ (No. 4), with cell dimensions of a=0.8571(2) nm, b=0.5871(2) nm, c=1.197(3) nm, β=99.290(10)°, V=0.5944(15) nm^3, Z=2, μ=2.74 cm^{-1}. Mononuclear chelates, described as [Pd(X)(*S*,*N*,*O*-AcMet-Gly)]$^+$, in which Pd(II) is coordinated by thioether, deprotonated amide nitrogen, carbonyl oxygen of methionine and X (AcMetGly or other ligands present in aqueous solution or in mobile phase solution), were detected 5 min after mixing AcMet-Gly with $[Pd(H_2O)_4]^{2+}$ at room temperature using electrospray ionization mass spectrometry. The geometry of $[Pd(H_2O)(S,N,O\text{-AcMet-Gly})]^+$ is optimized at density functional B3LYP/LanL2DZ level. The fused five- and six-membered chelate is responsible for cleavage of Met-Gly bond. This is the first time to provide a direct evidence for Pd(II)-mediated cleavage of dipeptides via external solvent attack.

Keywords acetylmethionylglycine, tetraaquapalladium(II) complex, X-ray structure, electrospray mass spectrometry, B3LYP density functional theory

Introduction

Palladium(II) complexes, as a promising artificial metallopeptidase, have been extensively studied for selective cleavage of methionine and histidine-containing dipeptides,[1-11] oligopeptides[12-16] and proteins.[16-20] Dipeptides AcMet-aa and AcHis-aa, in which the amino-terminus is protected by acetylation and aa is an amino acid residue, are usually cleaved at the Met-aa and His-aa bond with a modest but significant turnover.[6,7,9] In oligopeptides which contain Met or His or both, the Pd(II) complexes promote hydrolysis of the oligopeptides and the only cleavage site is the second peptide bond upstream from a methionine or a histidine residue.[12-16] This pattern of cleavage is similar to that observed in protein cleavage by Pd(II) complexes. Although a great progress has been made in the Pd(II) complex-mediated cleavage of peptide bond, little is known about the mechanism which governs the site of cleavage, even the pattern of cleavage for dipeptides and tripeptides remained puzzling and unpredictable.[13] There are two mechanisms in literatures proposed for interpretation of hydrolytic cleavage of peptide bond by transition metal complexes,[14,16,21] shown in Chart 1. A transition-metal ion either binds to amide oxygen of the scissile peptide bond, resulting in breaking the peptide bond by external attack of solvent water molecules (called as external attack mechanism), or delivers an aqua ligand to the scissile amide carbon, then cleaves it (called as internal delivery mechanism). Although the two mechanisms are kinetically distinguishable, how to distinguish them for an individual system is essential for understanding the nature of peptide bond cleavage by Pd(II) complexes and for designing new cleaving reagents.

Chart 1 Possible mechanism for a transition metal ion-promoted hydrolysis of peptide bond



Electrospray ionization mass spectrometry (ESI-MS) introduced by Fenn and his co-workers has been shown to be a powerful technique for analyzing biomolecules,[22-27] noncovalent complexes[28] and metal complexes.[29-33] Recently, the technique has been used to study the interaction of metal ions with peptides.[34-37] The ESI-MS provides a quite simple mass pattern and

* E-mail: zhulg@public1.ptt.js.cn
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allows the determination of molecular mass accurately with high sensitivity. In general, researchers endeavoring in this field have found a high correlation between the ESI-MS data and expectation from the solution phase. The strongest correlation to the solution phase is found in the stoichiometry measurement and the observed relative abundance from the ESI-MS experiments.[28,38] Therefore, the positive correlation between gas phase ESI-MS measurement and the solution phase system would be sufficient validation of the technique to elucidate compositional and structural information of the species formed in solution.

In the work present here, we report a crystal structure of AcMet-Gly and its interaction with $[Pd(H_2O)_4]^{2+}$ using electrospray ionization mass spectrometry. Many species were detected by both positive and negative ion modes, and the composition and structural information for every species are conformed by precise determination of molecular mass, zoom scan and simulation of isotope distribution pattern. A mutual feature is that all species detected contain a fused six- and five-membered ring, which is formed by coordination of Pd(II) to thioether, deprotonated amide nitrogen and carbonyl oxygen of methionine. This kind of coordination provides a clue to the mechanism by which the Met-Gly bond is cleaved by external attack of solvent water molecules.

Experimental

Doubly distilled water (dd water) was used for preparation of solutions. K_2PdCl_4 was obtained from Aldrich Chemical Co. Methionylglycine (Met-Gly) was obtained from Sigma Chemical Co. Tetraaqua palladium(II) complex, $[Pd(H_2O)_4]^{2+}$, was prepared as follows. Four equiv. of $AgBF_4$ in 1.0 mol•L^{-1} HBF_4 was mixed with one equiv. of K_2PdCl_4 in 1.0 mol•L^{-1} HBF_4, afterwards, the mixed solution was stirred at 40 ℃ for 8 h, and then the precipitate of AgCl was removed by centrifugation. The acidity of 1.0 mol•L^{-1} HBF_4 for the solution guarantees the Pd(II) aqua complex as $[Pd(H_2O)_4]^{2+}$ form present in stock solution (pK_a = 3.0).[39] The $[Pd(H_2O)_4]^{2+}$ solution was freshly prepared prior to use. The AcMet-Gly was obtained by acetylation of Met-Gly.[1,2]

Crystallography

AcMet-Gly was dissolved in dd H_2O and kept at 4 ℃ for slow evaporation. The single crystals of the AcMet-Gly suitable to X-ray crystallographic analysis were obtained. The intensity data for the dipeptide were collected on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (0.071073 nm) at room temperature. Saint program and the SADABS program were used to carry out the data integration and the empirical absorption corrections. The structure was solved by the direct method and refined on F^2 using the SHELXTL suite of program.[40] All non-hydrogen atoms were refined anisotropically by full-matrix least-squares methods and the hydrogen atoms were computed and refined isotropically using a riding model.

Electrospray ionization mass spectrometry

An LCQ electrospray ionization mass spectrometer (ESI-MS, Finnigan) was employed for molecular mass determination for the species formed by interaction of AcMet-Gly with $[Pd(H_2O)_4]^{2+}$. The sample was dissolved in water and diluted to 100 μmol•L^{-1}. A 1.0 μL of such solution was loaded into the injection valve of the LCQ unit and then injected into the mobile phase solution of 50% aqueous methanol and carried through the electrospray interface into the mass analyzer at a rate of 200 μL•min^{-1}. The applied voltage at the electrospray needle was 5 kV and the capillary was heated to 200 ℃. Both positive and negative ion mass spectra were recorded. Zoom scan was used in these experiments. The predicted isotope distribution pattern for each species was calculated using IsoPro 3.0 program.[41]

Optimization of conformation

The geometry of $[Pd(H_2O)(S,N,O\text{-AcMet-Gly})]^+$ was optimized in two steps, first constructed and optimized by the HyperChem v. 6.0 software embowering the MM^+ method of molecular mechanics, then optimized by density functional B3LYP theory in Gaussian 98 program package[42] using LanL2DZ basis set. The geometry of AcMet-Gly was constructed by determined structural data and optimized at B3LYP/D95 level for comparison of interesting bond lengths and charges on atoms in free AcMet-Gly and Pd(II)-coordinated AcMet-Gly. The RMS gradient was less than 4.184 kJ•nm^{-1}•mol^{-1} for MM^+ method and 10^{-8} a.u. of convergence for B3LYP method.

Results and discussion

Structure of AcMet-Gly

Crystallographic parameters of AcMet-Gly are given in Table 1, and selected molecular dimensions in Table 2. The molecular structure is shown in Figure 1. In crystal structure, three intermolecular hydrogen bondings are formed, N(1)—H(1A)···O(3) [N(1)—O(3) 0.2922 nm, N(1)—H(1A)—O(3) 167.0°], O(2)—H(2)···O4 [O(2)—O(4) 0.2635 nm, O(2)—H(2)—O(4) 164°] and N(2)—H(2C)···O(1) [N(2)—O(1) 0.2908 nm, N(2)—H(2C)—O(1) 136.5°]. There are three intramolecular hydrogen bondings that are N(2)—H(2C)···N(1) [N(2)—N(1) 0.2737 nm, N(2)—H(2C)—N(1) 108.9°], C(2)—H(2A)···O(3) [C(2)—O(3) 0.2798 nm, C(2)—H(2A)—O(3) 104.5°] and C(4)—H(4)···S(1) [C(4)—S(1) 0.3288 nm, C(4)—H(4)—S(1) 112.2°]. All these hydrogen bondings stabilize the conformation of the AcMet-Gly. The crystallographic data for the structure have been deposited in the Cambridge Crystallographic Data Centre with CCDC No. 206575.

Table 1 Crystal data collection and refinement parameters for AcMet-Gly

Chem. formula $C_9H_{16}N_2O_4S_1$	Space group $P2_1$ (No. 4)
F_w=248.30	T=293 K
a=0.8571(2) nm	λ=0.071073 nm
b=0.5871(2) nm	ρ_{calc}=1.387 g•cm^{-3}
c=1.197(3) nm	μ=2.74 cm^{-1}
β=99.290(10)°	$R(F_o)$=0.0424
V=0.5944(15) nm^3	$R_w(F_o)$=0.1030
Z=2	R=Σ||F_o|−|F_c||/|F_o|

Table 2 Selected bond lengths (nm) and bond angles (º) for AcMet-Gly

S(1)—C(6)	0.1794(5)	N(1)—C(4)	0.1449(5)
C(5)—C(6)	0.1524(5)	N(1)—C(7)	0.1346(5)
S(1)—C(9)	0.1783(7)	N(2)—C(3)	0.1342(5)
C(7)—C(8)	0.1488(6)	N(2)—C(2)	0.1448(5)
O(1)—C(1)	0.1198(4)	C(1)—C(2)	0.1497(5)
O(2)—C(1)	0.1306(5)	C(3)—C(4)	0.1522(5)
O(3)—C(3)	0.1216(5)	C(4)—C(5)	0.1519(5)
O(4)—C(7)	0.1231(5)		
C(6)-S(1)-C(9)	100.61(18)	O(3)-C(3)-N(2)	122.9(3)
C(4)-N(1)-C(7)	121.7(2)	N(1)-C(4)-C(3)	114.3(2)
C(2)-N(2)-C(3)	123.0(3)	C(3)-C(4)-C(5)	109.6(2)
O(1)-C(1)-O(2)	124.0(2)	N(1)-C(4)-C(5)	109.8(2)
O(1)-C(1)-C(2)	121.7(3)	C(4)-C(5)-C(6)	114.2(2)
O(2)-C(1)-C(2)	114.2(2)	S(1)-C(6)-C(5)	115.1(2)
N(2)-C(2)-C(1)	115.7(3)	O(4)-C(7)-C(8)	122.9(3)
N(2)-C(3)-C(4)	116.4(2)	N(1)-C(7)-C(8)	116.5(3)
O(3)-C(3)-C(4)	120.6(2)	O(4)-C(7)-N(1)	120.5(3)

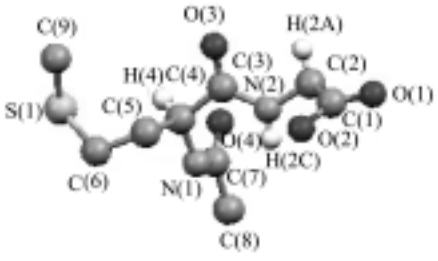


Figure 1 Structure of the AcMet-Gly with the numbering scheme. The hydrogen atoms are omitted for clarity except for the hydrogen atoms participating in intramolecular hydrogen bondings.

Interaction of AcMet-Gly with $[Pd(H_2O)_4]^{2+}$

ESI-MS was measured 5 min after mixing 20.0 mmol•L^{-1} of AcMet-Gly with $[Pd(H_2O)_4]^{2+}$ in a 1∶1 molar ratio at pH≈1.0 and room temperature. Since $[Pd(H_2O)_4]^{2+}$ complex has four labile aqua ligands that are readily substituted by many donor atoms in AcMet-Gly and ions or neutral molecules present in solution or mobile phase solution, many species would be expected. Figure 2 shows the ESI-MS spectra recorded by positive and negative ion modes. Five major species were detected and labeled. Zoom scan and simulation of isotope distribution pattern were performed for each peak in Figure 2. The results are given in Table 3 and Figure 3. The mutual feature of these species is that the Pd(II) is coordinated by thioether, deprotonated amide nitrogen and carbonyl oxygen of methionine, forming a fused six- and five-membered ring. It should be mentioned that, although electrospray mass spectrometry is a gentle method for transferring ions (positive or negative) from solution into the gas phases, when the technique is used to investigate the interaction of $[Pd(H_2O)_4]^{2+}$ with AcMetGly, the interesting questions arise. Is the dipeptide fragmented during the electrospray ionization process? How tightly must the didpeptide bind to the Pd(II) ion to be seen? ESI-MS for the AcMetGly alone showed

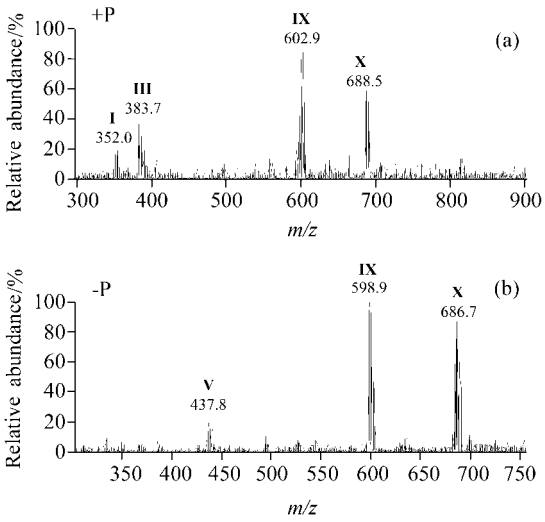


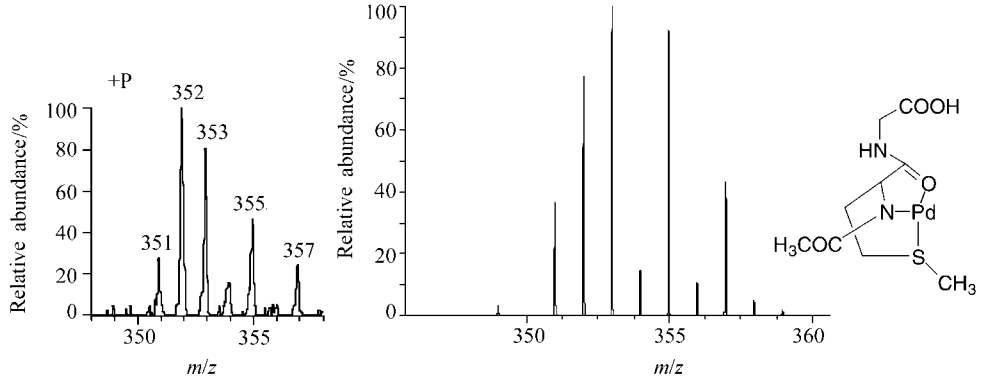
Figure 2 ESI-MS spectra measured 5 min after mixing of AcMet-Gly with $[Pd(H_2O)_4]^{2+}$ in a 1∶1 molar ratio at pH≈1.0. (a) Positive ion mass spectrum; (b) negative ion mass spectrum.

Table 3 Species detected for the mixed solution of AcMet-Gly and $[Pd(H_2O)_4]^{2+a}$

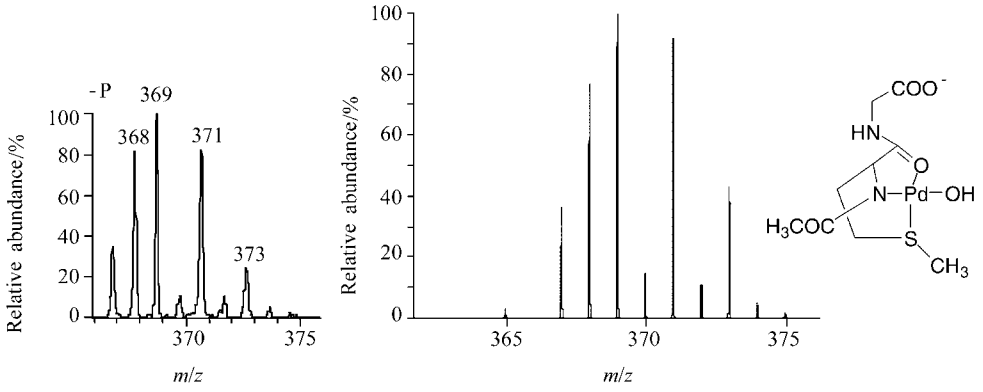
MS	Measured 5 min after mixing	Measured 10 min after incubation
Positive	**I** (352.0), **III** (383.7), **IX** (602.9), **X** (688.5)	**I** (351.9), **III** (384.8), **IV** (427.0), **VI** (504.0), **VII** (543.9), **VIII** (560.7), **IX** (602.9), **X** (688.7)
Negative	**V** (437.8), **IX** (598.9), **X** (686.7)	**II** (368.9), **IV** (424.9), **V** (438.0), **VII** (541.9), **IX** (598.9), **X** (686.7)

[a] The number in parenthesis is molecular mass for the species detected.

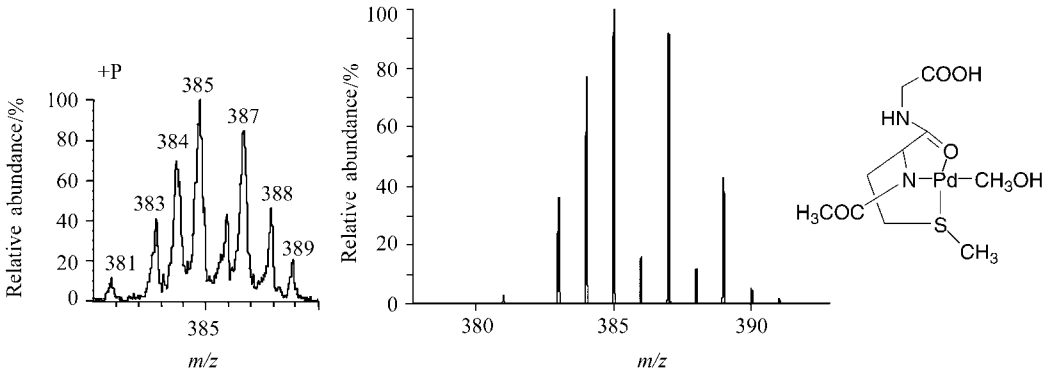
I ($C_9H_{15}N_2O_4PdS$)



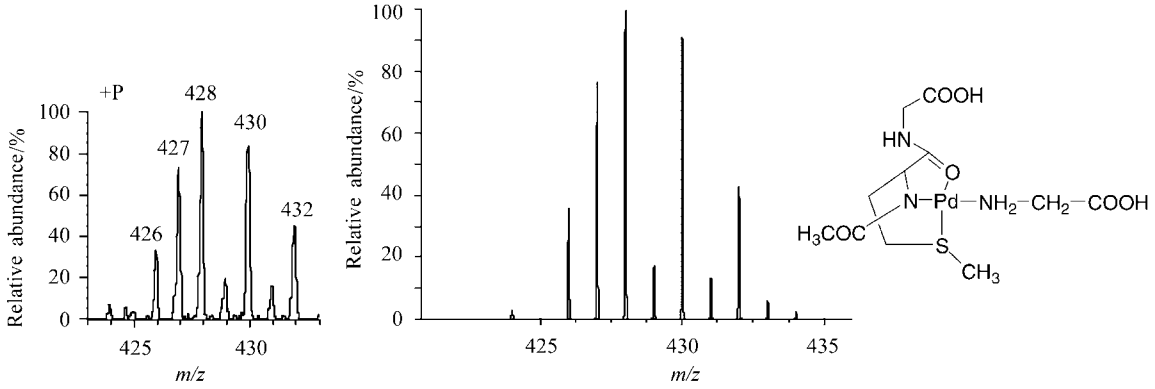
II ($C_9H_{15}N_2O_5PdS$)



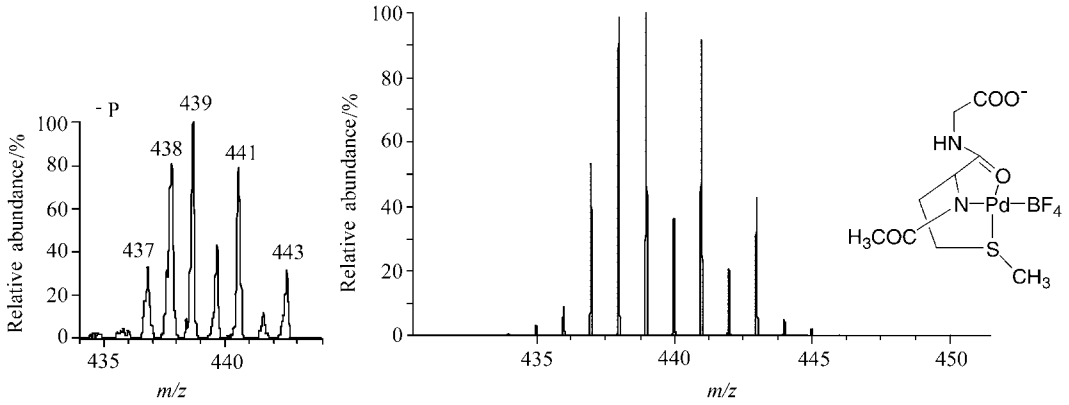
III ($C_{10}H_{19}N_2O_5PdS$)



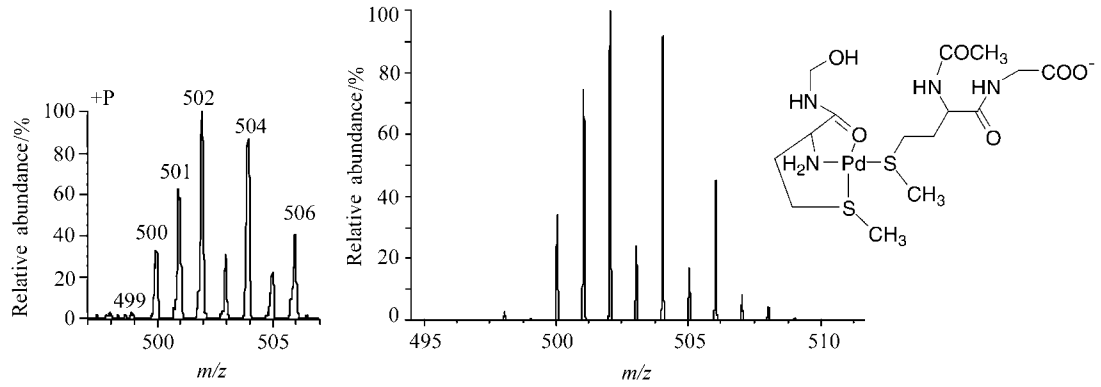
IV ($C_{11}H_{20}N_3O_6PdS$)



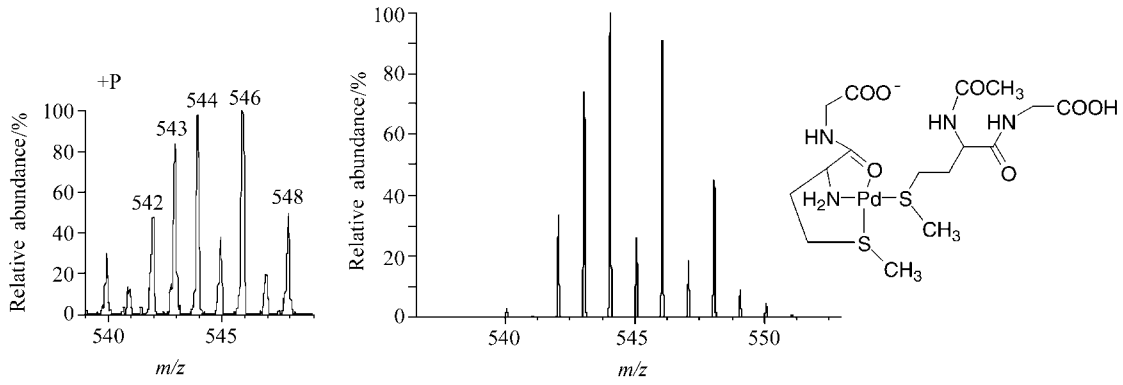
V ($C_9H_{14}BN_2O_4F_4PdS$)



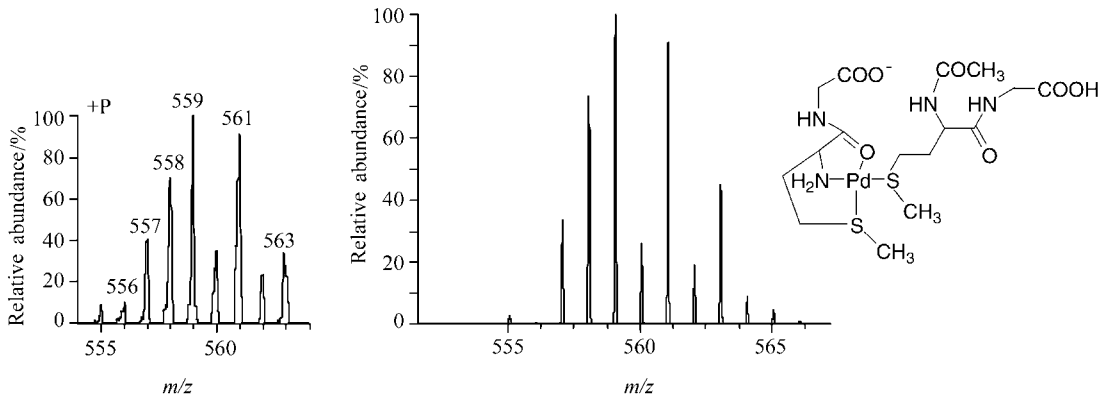
VI ($C_{14}H_{26}N_3O_6PdS_2$)



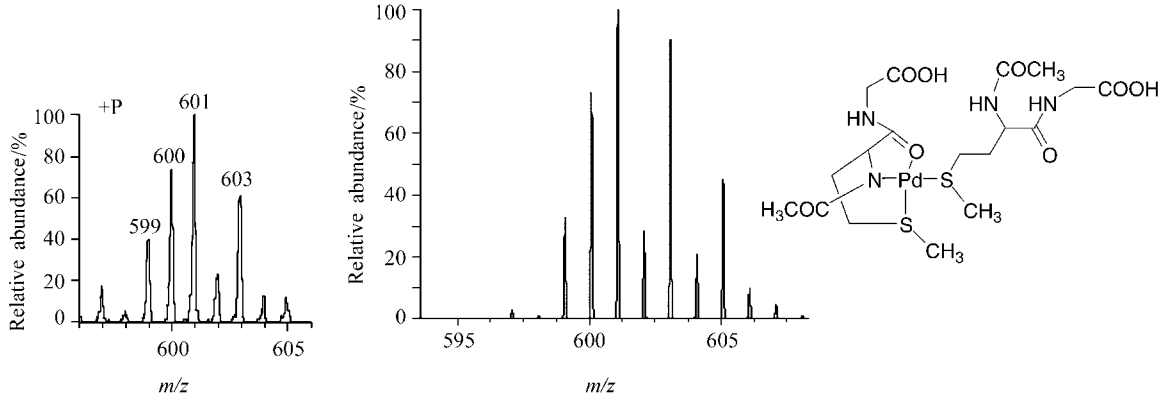
VII ($C_{16}H_{28}N_3O_7PdS_2$)



VIII ($C_{16}H_{29}N_4O_7PdS_2$)



IX ($C_{18}H_{31}N_4O_8PdS_2$)



X ($C_{18}H_{30}BF_4N_4O_8PdS_2$)

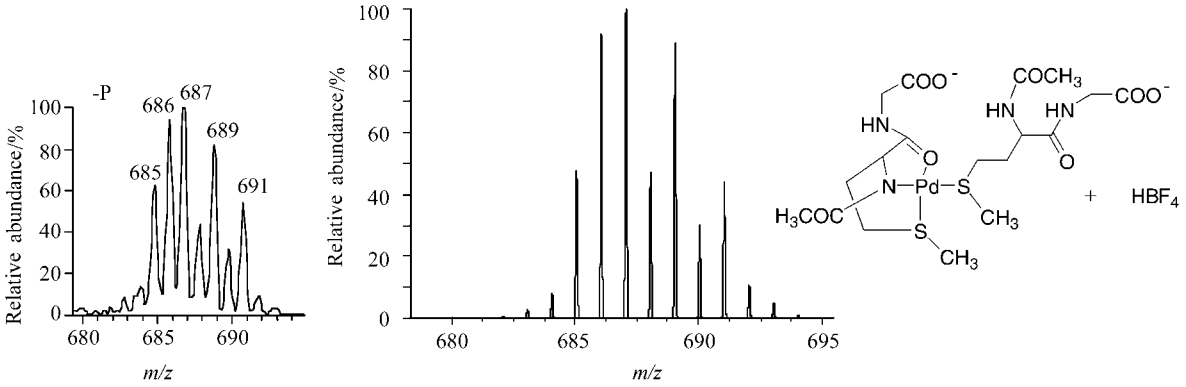


Figure 3 Zoom scan spectra, isotope distribution pattern calculated with IsoPro 3.0 program and the corresponding composition of complexes **I**—**X**.

that the fragmentation of the dipeptide is not a problem because no fragmental ion is observed. Therefore, the fragments of AcMet-Gly observed in the next experiments of Pd(II) with the dipeptide are caused by Pd(II) promotion. Previous studies already knew that when Pd(II) interacts with methionine-containing peptides, Pd(II) is preferentially anchored to the side chain of methionine.[1,2] The Pd(II)-induced deprotonation of the amide nitrogen is especially favorable when the palladium(II) is already anchored to the side chain. The estimated pK_a for this reaction is *ca.* 2, and displacement was observed even in solution with pH$<$2.0.[43-45] Although the carbonyl oxygen atom of methionine is a weak ligand atom, its coordination is facilitated by the chelate effect. In fact, this kind of coordination was proposed in the Pd(II)-mediated cleavage of cytochrome *c*[17]and confirmed by ESI-MS for investigating interaction of Pd(II) ion with tripeptide AcCys-His-Gly.[35,46] Therefore, the fused six- and five-membered chelate moiety of Pd(II) detected is consistent with that found in previous studies and a realistic moiety present in aqueous solution. The remnant coordination site of Pd(II) is occupied by sulfur atom from another dipeptide or occupied sometimes by other potential ligands present in solution, such as BF_4^-, or present in the mobile phase solution, such as CH_3OH and OH^-. The coordinated aqua ligand is undetectable in this case, as species **I** shows. Both positive and negative mass spectra in Figure 2 reveal that the dominant complexes formed are [Pd(*S*-AcMet-Gly)(*S*,*N*,*O*-AcMet-Gly)]$^+$ (**IX**) and [Pd(*S*-AcMet-Gly)(*S*,*N*,*O*-AcMet-Gly•HBF_4)]$^+$ (**X**) (see Figure 3). In **X**, BF_4^- may coordinate to Pd(II), instead of carbonyl oxygen, but, in comparison with **V**, it is reasonable to assume that chelation is kept. Therefore, in fact, the two species represent an identical Pd(II) complex in which extra AcMet-Gly is coordinated to Pd(II) through S atom of methionine. The species formed implies that some Pd(II) were lost by formation of polymetric hydroxo-bridged complex[39] in the solution or during process of ESI-MS measurement at pH≈5, though initial equimolar amount of $[Pd(H_2O)_4]^{2+}$ and AcMet-Gly was used.

Figure 4 shows the ESI-MS spectra which were recorded 10 min after incubation at 40 ℃ for 20.0 mmol•L^{-1} of AcMet-Gly mixed with $[Pd(H_2O)_4]^{2+}$ in a 1∶1 molar ratio at pH ~1.0. From the positive ion mass spectrum in Figure 4a, it is found that four new species, [Pd(Gly)(*S*,*N*,*O*-AcMet-Gly)]$^+$ (**IV**), [Pd(*S*-AcMet-Gly)(*S*,*N*,*O*-Met)]$^+$ (**VI**), [Pd(*S*-AcMet-Gly)(*S*,*N*,*O*-AcMet)]$^+$ (**VII**) and [Pd(*S*-AcMet-Gly)(*S*,*N*,*O*-MetGly)]$^+$ (**VIII**) were observed, besides **I**, **III**, **IX** and **X** which were detected in Figure 2a. In comparison with Figure 2b, three new species, **II**, **IV** and **VII**, were observed in Figure 4b. The **IV** and **VII** species were de-

tected in both positive and negative ion modes. The new species, **IV**, **VII**, **VI** and **VIII**, are associated with cleavage of AcMet-Gly. Figure 4 also confirmed that AcMet-Gly coordinates to $[Pd(H_2O)_4]^{2+}$ via thioether, deprotonated amide nitrogen and carbonyl oxygen of methionine, and cleavage of Met-Gly bond occurs by external attack of water, producing Pd(*S*-AcMet-Gly)-(*S*,*N*,*O*-AcMet)]$^+$ (**VII**) and free glycine which further coordinates to Pd(II), resulting in species **IV**. Besides, the cleavage of CH_3CO-Met bond was also observed. Two cleaved fragments, Met and Met-Gly were found in **VI** and **VIII**. This result implies that the CH_3CO-Met bond is also activated by coordination of Pd(II) to deprotonated amide nitrogen of metionine. In fact, this kind of activation of peptide bond was recently reported.[47] In the previous studies on Pd(II)-mediated hydrolysis of AcMet-Gly, the cleavage of CH_3CO-Met bond was usually neglected because a tiny amount of free acetic acid was recognized and only cleaved free glycine was monitored by ^{1}H NMR.[1,2]

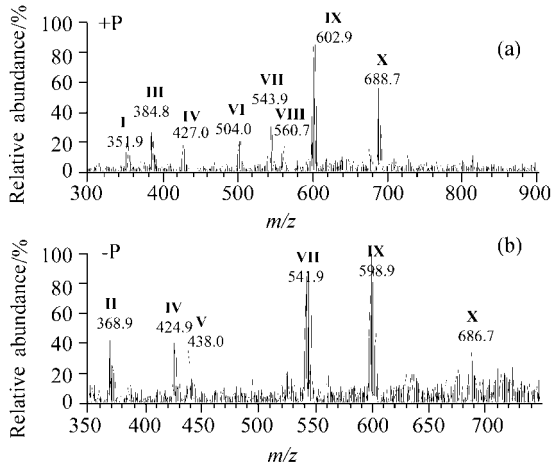


Figure 4 ESI-MS spectra measured 10 min after incubation of mixed solution of AcMet-Gly and $[Pd(H_2O)_4]^{2+}$ with a 1∶1 molar ratio at pH≈1.0. (a) Positive ion mass spectrum; (b) negative ion mass spectrum.

All ten species observed in Figures 2 and 4 can be described as [Pd(X)(*S*,*N*,*O*-Y-Met-Z)]$^+$, in which Z is Gly or OH, Y is CH_3CO or H and X is *S*-coordinated AcMet-Gly, OH^-, CH_3OH,[35] *N*-coordinated Gly, BF_4^-, or H_2O that can not be detected in ESI-MS because of its weak binding to Pd(II). This reasonable assignment means that the coordination of Pd(II) to methionine via a fused six- and five-membered ring is kept in all ten species and only the fourth coordination site can be substituted by different ligands present in aqueous solution or mobile phase solution. This chelation is responsible for the cleavage of Met-Gly and CH_3CO-Met bond. Therefore, this investigation provides a direct evidence for external attack mechanism for the cleavage of Met-Gly bond in the dipeptide promoted by Pd(II) aqua complex. We also investigated the system using ^{1}H NMR and ^{13}C NMR. Both NMR spectra are too complicate to be solved because many species present in solution, as Figure 2 shows.

For further gaining an insight into the Pd(II)-mediated cleavage of Met-Gly bond in AcMet -Gly, the geometry of the $[Pd(H_2O)(S,N,O\text{-AcMet-Gly})]^+$ was optimized at B3LYP/LanL2DZ level. The free AcMet-Gly was optimized at B3LYP/DZ level for comparison. The optimized geometry for [Pd(H_2O) (*S*,*N*,*O*-AcMet-Gly)]$^+$ is given in Figure 5. As shown in Figure 5, the Pd(II) is coordinated by sulfur, deprotonated amide nitrogen and carbonyl oxygen of methionine through fused six- and five-membered ring. The geometry parameters and charges on correlated atoms are compared for the two geometries calculated at the same level. It is found that, upon coordination of Pd(II) to carbonyl oxygen of methionine, the bond length of C(3)═O(3) is lengthened from 0.1262 to 0.1287 nm and that of C(3)—N(2) shortened from 0.1367 to 0.1348 nm and the charge on C(3) atom raised from 0.228*e* to 0.424*e*. In other words, the C═O double bond, rather than C—N bond, is weakened upon the coordination. The increased positive charge of this carbon atom and weakened C═O double bond are favorable for nucleophilic attack by solvent water to form a tetrahedral carbon intermediate, followed by breaking of Met-Gly bond. The same conclusion was obtained for mechanistic studies of Pd(II)-mediated cleavage of cytochrome *c* for the model compounds.[46] The C(7)—N(1) bond distance of 0.1389 nm in free AcMetGly is unchanged, C(7)═O(4) bond slightly lengthened from 0.1255 to 0.1262 nm upon the coordination, and the charge on C(7), O(4) and N(1) atoms is changed from 0.264*e*, −0.276*e* and −0.450*e* to 0.261*e*, −0.294*e* and −0.354*e*, respectively. Therefore, the cleavage of C(7)—N(1) bond is probably induced by lengthening the bond distance of C(7)═O(4) and by increasing the negative charge on O(4) atom. Because the variation of the two factors is not significant upon the coordination, it is expected that the C(7)═O(4) bond is less activated by Pd((II) coordination, compared to C(3)═O(3) bond. This is consistent with the experimental finding that only a tiny amount of free acetic acid was detected by ^{1}H NMR monitoring.

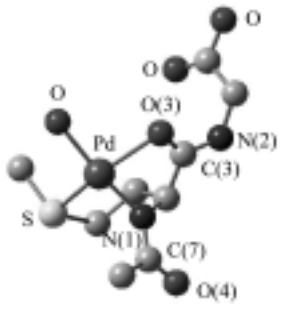


Figure 5 Optimized geometry of [Pd(H_2O)(*S*,*N*,*O*-AcMet-Gly)]$^+$ at B3LYP/LanL2DZ level. Pd—O(3), 0.228 nm; Pd—S, 0.248 nm; Pd—O(H_2O), 0.215 nm; Pd—N(1), 0.195 nm.

Conclusion

The crystal structure of the dipepide AcMet-Gly shows that its conformation is stabilized by intermolecular and intramolecular hydrogen bondings. ESI-MS spectra and geometry optimized at B3LYP/LanL2DZ level reveal that Pd(II) is coordinated by S atom, deprotonated amide N atom and carbonyl O atom of methionine to form a chelate with fused six- and five-membered ring. Due to the coordination of carbonyl O atom in the complex, the Met-Gly bond is cleaved via external attack mechanism.

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