Hydrolysis of Acetyl-methionine-containing Dipeptides Promoted by Palladium(II) Complexes Containing Methionine-amino Acids as Ligands

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The [Pd(*N*,*S*-Met-a'a'H)(*N*,*S*-AcMet-aaH)]$^+$(a'a'H and aaH = amino acids) was characterized by electrospray ionization mass spectrometer(ESI-MS) and ^{1}H NMR, in which Met-a'a'H, as ligand, coordinates to Pd(II) via thioether and terminal amino group, and AcMet-aaH, as substrate, coordinates to Pd(II) via thioether and deprotonated amide nitrogen of methionine. The Met-a'a' bond in ligand is intact, the Met-aa bond in substrate, however, is activated toward hydrolysis. The difference in hydrolysis behavior between ligand and substrate may be due to a fused six-membered and five-membered ring formation via thioether, deprotonated amide nitrogen and carbonyl oxygen of methionine residue in substrate.

In our previous studies,[1-6] we examined the hydrolytic cleavage of methionine-containing peptides by palladium(II) complexes. The background of this study presented here is to explore the possibility of using the steric structure of longer peptides coordinated to Pd(II) complexes to control the hydrolytic rate and selectivity of cleavage of other peptides as substrates. To our knowledge, it is the first time to investigate that transition-metal complexes with peptides are used to promote cleavage of other peptides.

The dichloro palladium(II) dipeptide complexes *cis*-[Pd(Met-a'a'H)Cl_2], in which a'a'H is GlyH, AlaH, SerH, Ala-SerH, LysH, HisH, GluH and AspH, were used as precursors of promoters and prepared as described in literature.[7,8] The corresponding complexes with aqua ligand (actually D_2O in ^{1}H NMR measurement) were obtained by treating each of these complexes with 2 equiv. of anhydrous $AgNO_3$ in H_2O as solvent[4,5] and always prepared fresh to minimize the formation of hydroxo-bridged polynuclear complexes.[1-6]

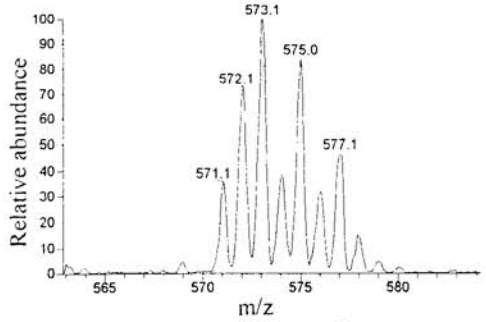


Figure 1. ESI-MS spectrum of [Pd(*N*,*S*-Met-GlyH)(*N*,*S*-AcMet-AlaH)]$^+$

As soon as mixing of the *cis*-[Pd(Met-GlyH)$(H_2O)_2$]$^{2+}$ with the equimolar amounts of acetyl methionyl alanine AcMet-AlaH, electrospray ionization mass spectrometer (ESI-MS, LCQ, Finnigan MAT) measured its molecular mass, as shown in Figure 1. The observed m/z values: 571.1, 572.1, 573.1, 575.1 and 577.1; the calculated m/z values for [Pd(*N*,*S*-Met-GlyH)(*N*,*S*-AcMet-AlaH)]$^+$ with five isotopic molecular masses of Pd(II): 571.5(Pd^{104}), 572.5(Pd^{105}), 573.5(Pd^{106}), 575.5(Pd^{108}), 577.5(Pd^{110}). In the complex, Met-GlyH coordinates to Pd(II) via thioether and terminal amino group, and AcMet-AlaH coordinates to Pd(II) via thioether and deprotonated amide nitrogen of methionine. This type of coordination was also confirmed by ^{1}H NMR. AcMet-AlaH reacts with Pd(II) accompanied by chemical schifs of SCH_3 and CH_3CO groups(δ, in ppm) moved from 2.11 and 2.03 toward downfield of 2.45 and 2.04, respectively. When *cis*-[Pd(*N*,*S*-Met-GlyH)$(H_2O)_2$]$^{2+}$ incubated with AcMet-AlaH at pH ~ 1.0 and 40 °C for 3 h, and then the mixed solution was measured by ESI-MS. Besides the existence of [Pd(*N*,*S*-Met-GlyH)(*N*,*S*-AcMet-AlaH)]$^+$, the [Pd(*N*,*S*-Met-GlyH)(*N*,*S*-AcMetH)]$^+$ was detected with m/z values: 499.9, 501.1, 502.0, 504.0 and 506.0; the calculated m/z values for the complex with five isotopic masses of Pd(II): 500.4(Pd^{104}), 501.4(Pd^{105}),502.4(Pd^{106}), 504.4(Pd^{108}), 506.4(Pd^{110}). It is of interest to indicate that the dipeptide Met-GlyH, that is a ligand in [Pd(*N*,*S*-Met-GlyH)(*N*,*S*-AcMet-AlaH)]$^+$, does not hydrolyze, the dipeptide AcMet-AlaH, that is a substrate in it, however, undergoes hydrolysis. The difference in hydrolysis behavior of Met-amino acid bond between two dipeptides is due to the Met-aa bond in acetylated dipeptide is activated.

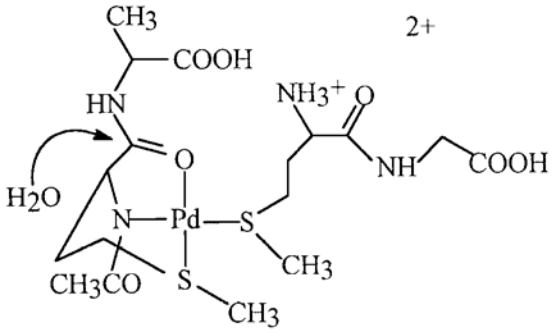
The hydrolytic reaction is easily followed by monitoring ^{1}H NMR resonance (AM500 MHz) of the leaving amino acid. The hydrolytic reactions follow the apparent first order kinetics. The kinetic data are listed in Table 1. The rate constants for cleavage, as shown in Table 1, depend to some extent on the *c*-terminal amino acid, aaH (aaH - GlyH, AlaH, ValH) in AcMet-aaH , which

Table 1. Hydrolysis of the Met-aa bond in AcMet-aaH promoted by Pd(II) aqua complexes of methionine-containing peptides at pD 0.91 ~ 1.0 and at 40 °C

promoter [Pd(Met-a'a')$(H_2O)_2$]$^{2+}$	substrate	V_{CHR},Å^3	10^3k_{obsd} min^{-1}
GlyH	AcMet-GlyH	18.2	7.76
	AcMet-AlaH	37.8	3.99
	AcMet-ValH	75.5	2.00
AlaH	AcMet-GlyH		5.48
	AcMet-AlaH		2.65
	AcMet-ValH		1.42
SerH	AcMet-AlaH		7.20
Ala-SerH	AcMet-AlaH		4.30[a]
LysH	AcMet-GlyH		0.14
GluH	AcMet-GlyH		very slow
HisH	AcMet-GlyH		very slow
AspH	AcMet-GlyH		not hydrolyzed

[a]50 °C.

is the leaving group. The steric bulk was quantitated as volume calculated from van der waals dimension between the volumes(in $Å^3$) of the α-CHR group in a given amino acid and in glycine. The bulker the leaving amino acids, the greater the shielding of the scissile bond from the palladium(II) complexes. A linear fit obtained is $-\ln k_{obsd} = 5.0 + 2.2 \times 10^{-2}\ \Delta V$ with correlation coefficient 0.986 for promoter of $[Pd(Met\text{-}GlyH)(H_2O)_2]^{2+}$, and $-\ln k_{obsd} = 5.3 + 2.2 \times 10^{-2}\ \Delta V$ with correlation coefficient 0.975 for promoter of $[Pd(Met\text{-}AlaH)(H_2O)_2]^{2+}$. The preliminary results show that the steric structure of the ligands effects greatly on hydrolytic rate. LysH, GluH, HisH and AspH in the dipeptide ligands blocked the hydrolytic reaction. The enhancement of hydrolytic rate of AcMet-AlaH promoted by $[Pd(Met\text{-}SerH)(H_2O)_2]^{2+}$, compared with $[Pd(Met\text{-}GlyH)(H_2O)_2]^{2+}$ and $[Pd(Met\text{-}AlaH)(H_2O)_2]^{2+}$ as promoters, was also caused by side chain of serine, which plays important action for cleavage of amide bond in serine-containing proteolytic enzymes.



Scheme 1.

In fact, in acid solution used in hydrolysis studies, the *N*-terminal amino group in $[Pd(N,S\text{-}Met\text{-}a'a'H)(N,S\text{-}AcMet\text{-}aaH)]^+$ is detached from Pd(II) and protonated, judging by ^1H NMR of α-CH MetH.[4,5] An active form for cleavage is proposed in Scheme 1. A fused six-membered and five-membered ring formation makes carbonyl oxygen of methionyl residue feasible to bond to Pd(II), which is observed by ESI-MS in $[Pd(Py)(S,N,O\text{-}AcMet\text{-}GlyH)]^+$, and the activated Met-aa bond in AcMet-aaH could be hydrolyzed by external attack of solvent water. The detailed mechanistic studies are undertaking.

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