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His-Oriented Peptide Hydrolysis Promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$: a New Specific Peptide Cleavage Site

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The new specific hydrolysis of histidine-containing peptides promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$ was investigated by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance spectrometry (NMR). MS determination demonstrated that *cis*-$[Pt(en)(H_2O)_2]^{2+}$ anchors to AcGHG with the stoichiometry of either 1:1 or 2:1 (Pt/peptide), but only with 1:1 stoichoimetry to AcGHL. *Cis*-$[Pt(en)(H_2O)_2]^{2+}$ is able to promote the cleavage of the first downstream peptide bond from histidine at 60 °C and pH 2.65, and Pt-anchored peptides are the essential intermediates for the promoted hydrolysis. Moreover, the larger amount of Pt(II) complex results in higher fragmental yield and higher hydrolysis rate. In the presence of 1 equiv of Pt(II) complex, ^{1}H NMR determination confirmed the apparent first-order kinetics of the Pt(II)-promoted hydrolysis and the hydrolysis rate for AcGHG and AcGHL is 0.20 day^{-1} and 0.14 day^{-1}, respectively. Moreover, Pt(II) coordinating to histidine imidazole is the key step to form the Pt(II)-anchored peptides. The Pt(II)-activating the first His-downstream carbonyl group via synergic coordinating to His imidazole and carbonyl O atom has been proposed for the Pt(II)-promoted his-oriented peptide hydrolysis. The lower rate for AcGHL should be correlated to the steric hindrance of Leu side chain to the second Pt(II) coordinating to tripeptide. In addition, the newly confirmed specific His-oriented peptide cleavage site implies a new potential strategy for target cleavage of peptides or proteins.

Introduction

The half-life of normal peptide bonds is several hundred years at neutral pH and room temperature.[1] Selective hydrolysis of a specific peptide bond promoted by proteolytic enzymes or synthetic reagents has attracted considerable attention in the field of biochemistry and bioengineering, for their potential application in proteomics, semi-synthesis of protein, and fusion protein engineering.[2] Although nonspecific endopeptidases generate only random peptide fragments, normal proteolytic enzymes exhibit the higher efficiency and distinct specificity in promoting protein hydrolysis. However, their higher expenditure, instability, and self-cleaving behavior appeal to the artificial peptidases of high stability and fine peptide cleaving ability.[3] However, most of the reported artificial peptidases suffer from the harsh functioning conditions, lower activity, and the limited specific cleaving sites.[4] For example, cyanogen bromide (CNBr) functions in denaturing conditions and is limited to cleave the carboxyl termini of methionine residues. Therefore, developing new chemical proteases of high efficiency and specificity in mild conditions is demanded and challenging.

A variety of transition-metal complexes have been studied as artificial metalloproteases.[5] Among them, Pd(II) and Pt(II) complexes are attracting intense attention, since these complexes are able to promote the hydrolysis of peptide bonds around the specific amino acid residue via coordinating to the side chain of the specific amino acid.[6] Pd(II) complexes, such as $[Pd(H_2O)_4]^{2+}$, $[Pd(NH_3)_4]^{2+}$, and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ are able to cleave specifically the second upstream amide bond of histidine or methionine residue.[6n,o] Although Pd(II) and Pt(II) complexes display a similar coordination chemistry, Pt(II) complexes as artificial peptidases are rare, and the regioselectivity of Pt(II) complexes is

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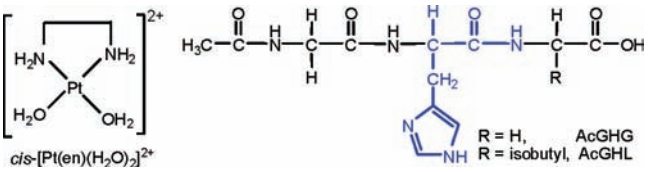
different from that of Pd(II) complexes. *Cis*-$[Pt(NH_3)_2Cl_2]$ has been reported to promote the hydrolysis of the second upstream amide bond from the Pt-anchored Met, that is, the W-X bond in segment of -W-X-Met-Y_n (W, X, Y, and Z are amino acids without distinct Pt(II) coordinating ability, $n \geq 1$).[7] The cleavage of the first downstream amide bond from Met was also reported for segment of -Met-$(Z)_n$-His- (*n*: 0, 1, 2; Z: Gly)[7c,8] and -Met-Pro-.[9] In addition, *cis*-$[Pt(en)(H_2O)_2]^{2+}$ and other several Pt(II) complexes formed by thioether bidentate, as the Met-oriented inorganic protease, have been reported also to promote selectively the cleavage of downstream Met-Z amide bonds at pH $\leq$ 2.5.[6g,h,j]

All the above Met-oriented peptide bond cleavages promoted by Pt(II) complexes are closely associated with the Pt(II) coordination to methionine residues. Although the coordination of Pt(II) to His is kinetically disfavored in moderately acidic solution, the affinity of Pt(II) to histidine is still reasonable and thermodynamically preferred.[10] Surprisingly, the peptide bond cleavage promoted by Pt(II) coordinating to histidine residue is scarcely reported. Our previous study found that incubating *cis*-$[Pt(en)(H_2O)_2]^{2+}$ with oxidized insulin B chain (pH 2.5), in which Met is absent and the two Cys are oxidized, leads to the fragments ascribed to the cleavage of His10-Leu11 peptide bond.[6p] To our knowledge, this is the first example of first His-downstream peptide bond cleavage promoted by Pt(II) complex. This new His-oriented cleavage site provides the new alternative to acquire the target peptide fragments. In this manuscript, AcGHG and AcGHL, as the simple models, were adopted to study the *cis*-$[Pt(en)(H_2O)_2]^{2+}$-promoted hydrolysis behavior of histidine-containing peptide/protein (Scheme 1). The ESI-MS and ^{1}H NMR spectrometric determination were adopted to monitor the hydrolysis process.

Results

ESI-MS Study on AcGHG Hydrolysis Promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$. For the hydrolysis of AcGHG promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$, the MS spectrum obtained after just mixing displays one intensive signal

Scheme 1. Chemical Structures of *cis*-$[Pt(en)(H_2O)_2]^{2+}$, AcGHG, and AcGHL



with *m*/*z* of 312.2 and one minor signal with *m*/*z* of 168.6 (Figure 1A). The 0.5 *m*/*z* peak space in the determined IDP (isotopic distribution pattern) of the minor one suggests that this species is doubly charged. The former peak is assigned as $[AcGHG+H]^+$, while the latter can be assigned as $[Pt(en)+2CH_3OH+H_2O]^{2+}$. The simulated IDPs for the two species given by IsoPro.30 fit well to those determined experimentally. The ionization efficiency of AcGHG is obviously higher than that of aqua Pt(II) complex in MS condition, which make the peak of AcGHG have greater relative abundance than that of *cis*-$[Pt(en)(H_2O)_2]^{2+}$, though they are of the same initial concentration. The initial MS spectrum of the incubated mixture exhibits no other distinct signals of Pt(II)-bearing species, suggesting the Pt(II) coordinating to peptide is not so efficient in acidic condition (pH 2.65). The new signals of *m*/*z* 409.6 and *m*/*z* 565.2 were found via ESI-MS monitoring after 2.5 h of incubation (60 °C, pH 2.65). The two signals are assigned respectively as $[2Pt(en)+AcGHG-2H]^{2+}$ and $[Pt(en)+AcGHG-H]^+$ (Figure 2, Table 1). The two signals indicate that Pt(II) has been coordinated to the peptide with the 1:1 or 2:1 binding stoichiometry (complex/peptide). After 5 h of incubation, two additional signals of *m*/*z* 508.2 and 330.2, which are attributed to the species of $[Pt(en)+AcGlyHis-H]^+$ and $[Pt(en)+Gly-H]^+$ (Figure 2), appeared in the MS spectrum. These two signals for Pt-anchored AcGlyHis and Gly confirm the peptide hydrolysis, and the cleavage occurs at the first downstream peptide bond from His. Moreover, the continued MS monitoring of the incubated mixture found that the two peaks increased with the incubation time until the Pt-anchored AcGHG species disappeared after 4 days of incubation (Figure 1E). This result indicates that the Pt(II)-anchoring to the AcGHG might be the origin for the sequential hydrolysis of His-Gly peptide bond, similar to the reported mechanism of metal ion-promoted hydrolysis of peptide bonds.[6m]

There were no additional signals observed even after 7 days of incubation, implying no other hydrolysis product formed in the process. Although almost all the Pt(II)-anchored AcGHG signals have disappeared, the distinct signal for $[AcGHG+H]^+$ is still the most abundant one in the MS spectrum, implying that 1 equiv of Pt(II) complex cannot lead to the complete hydrolysis of AcGHG. It is consistent with the fact that both hydrolysis fragments were bound to Pt(II). When 2 equiv of *cis*-$[Pt(en)(H_2O)_2]^{2+}$ was incubated with AcGHG, the ESI-MS spectrum obtained after 4 days showed that the peaks for Pt-anchored fragments at *m*/*z* 508.2 and 330.2 became the most abundant MS signals (Figure 3). Moreover, there was no signal for Pt-anchored AcGHG. However, incubating AcGHG at 60 °C and pH 2.65 did not lead to any new signals in the ESI-MS spectrum, except for the signal for $[AcGHG+H]^+$, even when the incubation time lasted

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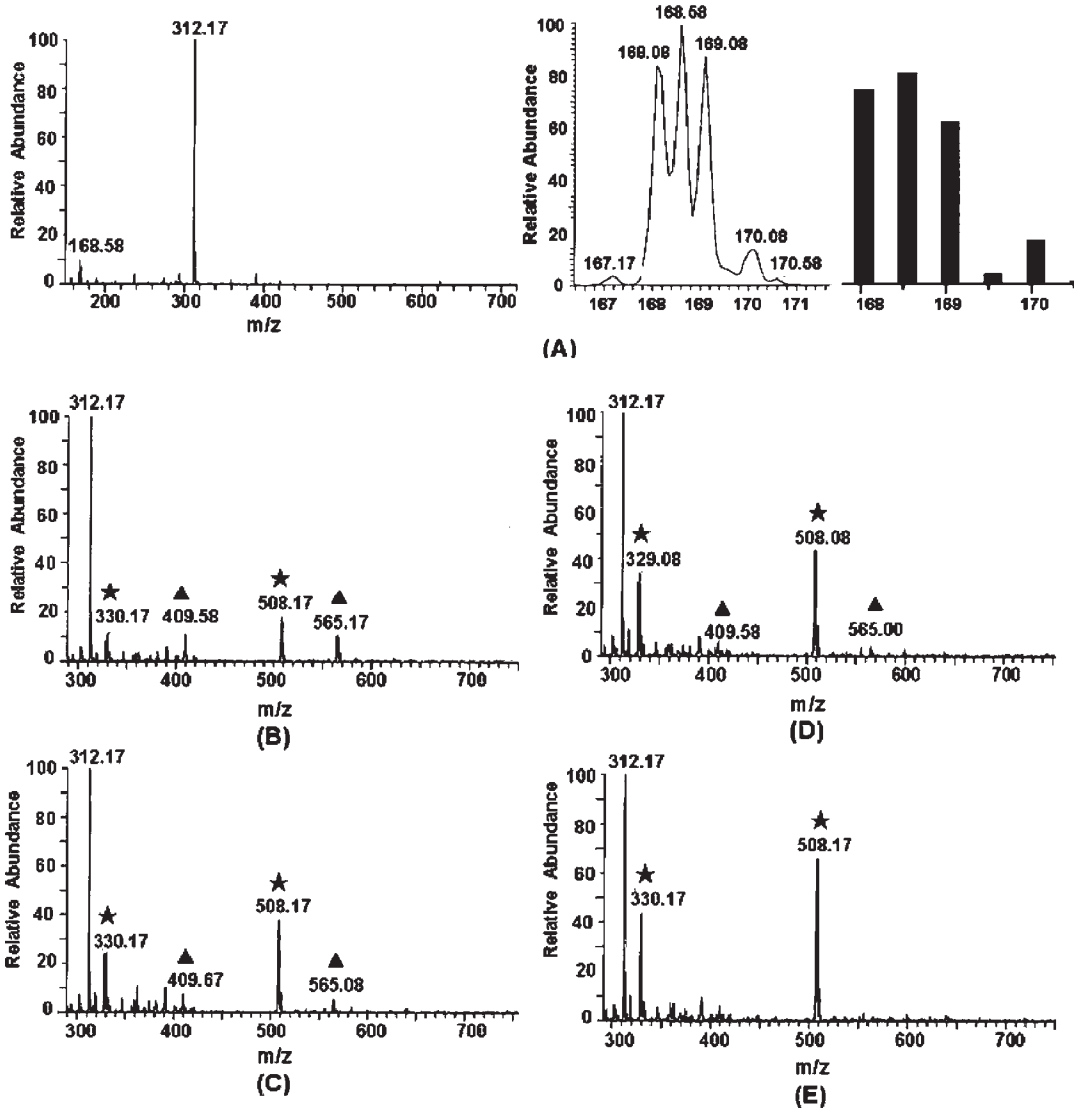


Figure 1. ESI mass spectra of incubated mixture of AcGHG and *cis*-$[Pt(en)(H_2O)_2]^{2+}$ (1:1, 60 °C, pH 2.65). (A) Spectrum obtained after just mixing (Left), and the determined and simulated IDP of $[Pt(en)+2CH_3OH+H_2O]^{2+}$ (Right); (B) spectrum obtained after 1 day of incubation; (C) spectrum obtained after 2 days of incubation; (D) spectrum obtained after 3 days of incubation; (E) spectrum obtained after 4 days of incubation. ▲, Pt-anchored AcGHG species; ★, Pt-anchored cleavage fragments.

for 7 days. The current results suggest that the observed AcGHG hydrolysis is Pt(II)-dependent and the *cis*-$[Pt(en)(H_2O)_2]^{2+}$ promote the hydrolysis of AcGHG in a catalytic mechanism of single turnover. The amount of *cis*-$[Pt(en)(H_2O)_2]^{2+}$ is essential for hydrolysis equilibrium and hydrolysis rate.

ESI-MS Study on AcGHL Hydrolysis Promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$. The hydrolysis of AcGHG promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$ suggests that this Pt(II) complex favors to cleave the first downstream peptide bond from histidine residue. Then, another histidine-containing tripeptide AcGHL was adopted as a new substrate to investigate the Pt(II)-promoted His-oriented hydrolysis. Moreover, the introduction of steric bulky Leu should be helpful to clarify the steric effect of His-oriented peptide hydrolysis. The temporal ESI-MS monitoring of the incubated mixture of AcGHL and *cis*-$[Pt(en)(H_2O)_2]^{2+}$ (1:1, pH 2.65, 60 °C) found three MS signals with *m*/*z* 390.0, 734.8, and 756.8 after just being mixed (Supporting Information, Figure S1A). The three signals can be assigned as $[AcGHL+Na]^+$, $[2AcGHL+H]^+$ and $[2AcGHL+Na]^+$. Pt-anchored AcGHL species with stoichiometric ratio of 1:1 were also observed as two minor signals with *m*/*z* of 621.0 and 638.9, which can be assigned as the signals for $[Pt(en)+AcGlyHisLeu-H]^+$ and $[Pt(en)+AcGlyHisLeu+H_2O-H]^+$. The signal with *m*/*z* 290.0 for $[Pt(en)(H_2O)(OH)]^+$ was shown separately for facilitating the comparison between the MS spectra obtained at different incubation times. However, the new peaks with *m*/*z* of 508.1, 526.0, and 385.1 assigned as cleaved fragments of $[Pt(en)+AcGlyHis-H]^+$, $[Pt(en)+AcGlyHis+H_2O-H]^+$, and $[Pt(en)+Leu-H]^+$ can only be observed after 6.5 h of incubation (Supporting Information, Figure S2; Table 2). These new signals suggest that the His-Leu bond of AcGHL has been hydrolyzed. The hydrolysis of His-Leu is obviously slower than that of His-Gly in AcGHG. One day of incubation resulted in the decrease of AcGHL signals, and the signal for Pt-anchored AcGHL species became the major species (Supporting Information, Figure S1B). However, further

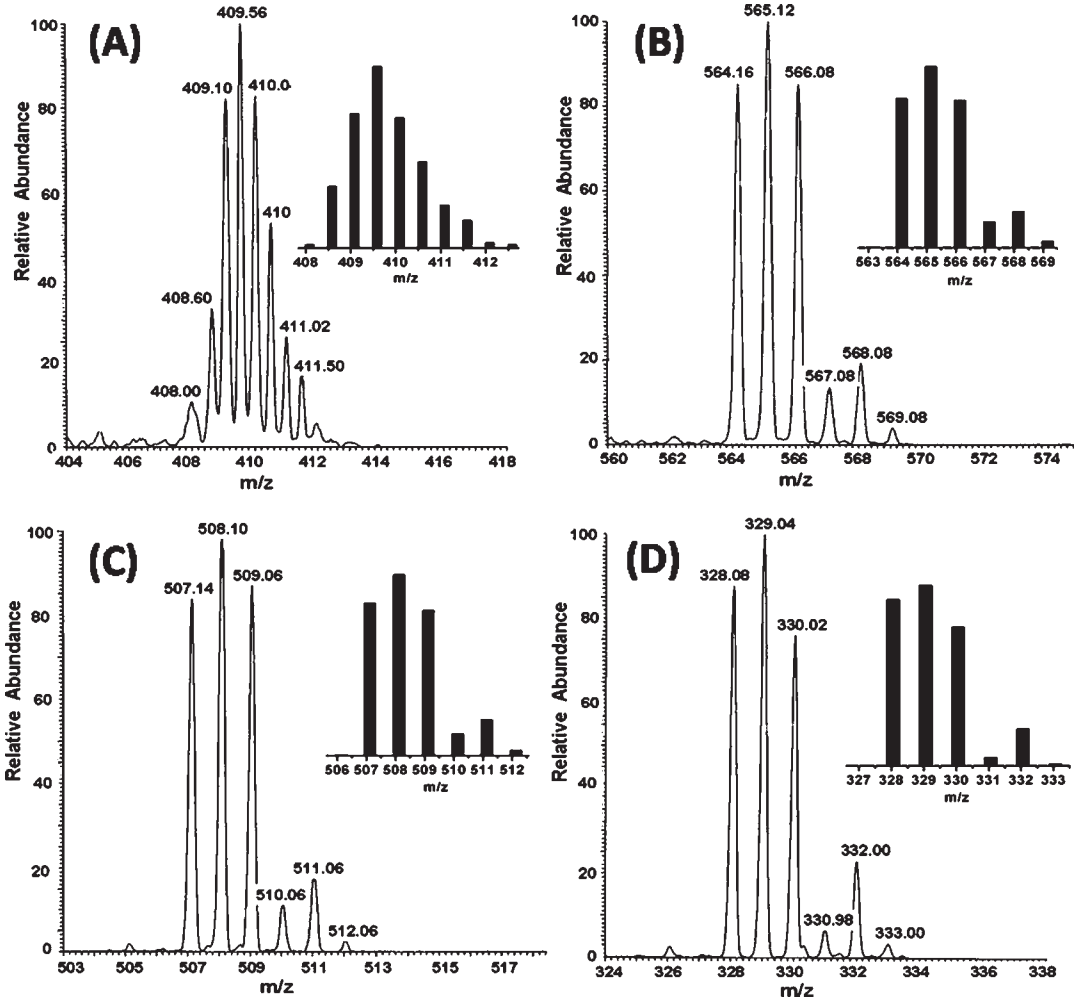


Figure 2. Zoom scan spectra and simulated isotopic distribution pattern of $[2Pt(en)+AcGHG-2H]^{2+}$ (A); $[Pt(en)+AcGHG-H]^{+}$ (B); $[Pt(en)+AcGly-His-H]^{+}$ (C); $[Pt(en)+Gly-H]^{+}$ (D). The simulated IDPs were given by IsoPro 3.0.

Table 1. Assignment of ESI-MS Signals Observed in MS Spectra Obtained during Incubating the Mixture of AcGHG and *cis*-$[Pt(en)(H_2O)_2]^{2+}$

			m/z[a]	
peak	assignment	formula	calculated	observed
(A)	$[2Pt(en)+AcGHG-2H]^{2+}$	$[C_{16}H_{31}N_9O_5Pt_2]^{2+}$	409.6	409.6
(B)	$[Pt(en)+AcGHG-H]^{+}$	$[C_{14}H_{24}N_7O_5Pt]^{+}$	565.1	565.1
(C)	$[Pt(en)+AcGlyHis-H]^{+}$	$[C_{12}H_{21}N_6O_4Pt]^{+}$	508.1	508.1
(D)	$[Pt(en)+Gly-H]^{+}$	$[C_4H_{12}N_3O_2Pt]^{+}$	329.0	329.1

[a] The data given are the highest relative abundance.

incubation made the Pt-anchored AcGHL species decrease with the incubation time, and the Pt-anchored cleaved fragments increase until the cleavage ceased upon the exhaustion of the Pt-anchored AcGHL species.

^{1}H NMR Study on the Hydrolysis of AcGHG and AcGHL. The hydrolysis of AcGHG and AcGHL has also been tracked by ^{1}H NMR in D_2O at pH 2.65 and 60 °C. Without the presence of *cis*-$[Pt(en)(H_2O)_2]^{2+}$, there is no new signal observable even when the incubation time reaches 7 days, indicating that the two tripeptides are stable in this medium. In the presence of *cis*-$[Pt(en)(H_2O)_2]^{2+}$, the two singlets for AcGHG imidazole H*a* and H*b* at 8.61 and 7.30 ppm are almost intact in the ^{1}H NMR spectrum obtained after just mixing the peptide

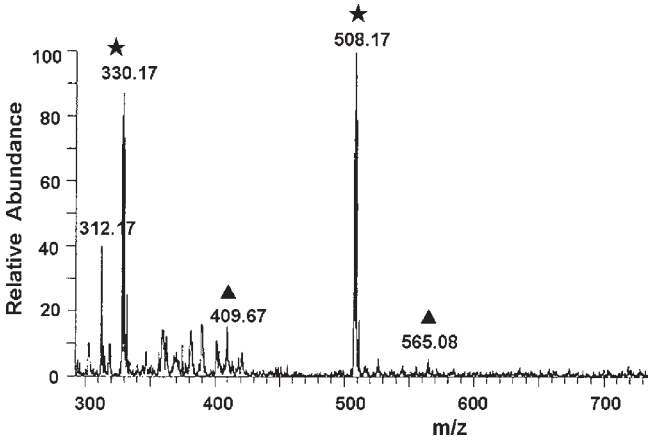


Figure 3. ESI-MS spectrum of AcGHG solution after 4 days of incubation with *cis*-$[Pt(en)(H_2O)_2]^{2+}$ in molar ratio of 1:2 at pH 2.65 and 60 °C. ▲, Pt-anchored AcGHG species; ★, Pt-anchored cleavage fragments.

and Pt(II) complex (Figure 4). The succeed incubation resulted in the minor signals of downfield shift from the two singlets, which can be ascribed to Pt(II) coordinating to His imidazole to form Pt-anchored AcGHG species. After 1 day of incubation, the increased amount of

Table 2. Assignment of ESI-MS Signals Observed in MS Spectra Obtained during Incubating the Mixture of AcGHL and *cis*-$[Pt(en)(H_2O)_2]^{2+}$

peak	assignment	formula	m/z^a calculated	m/z^a observed
(A)	$[Pt(en)+AcGlyHisLeu-H]^+$	$[C_{18}H_{32}N_7O_5Pt]^+$	621.2	621.0
(B)	$[Pt(en)+AcGlyHisLeu+H_2O-H]^+$	$[C_{18}H_{34}N_7O_6Pt]^+$	639.2	638.9
(C)	$[Pt(en)+AcGlyHis-H]^+$	$[C_{12}H_{21}N_6O_4Pt]^+$	508.1	508.1
(D)	$[Pt(en)+AcGlyHis+H_2O-H]^+$	$[C_{12}H_{23}N_6O_5Pt]^+$	526.1	526.0
(E)	$[Pt(en)+Leu-H]^+$	$[C_8H_{20}N_3O_2Pt]^+$	385.1	385.1

[a] The data given are the highest relative abundance.

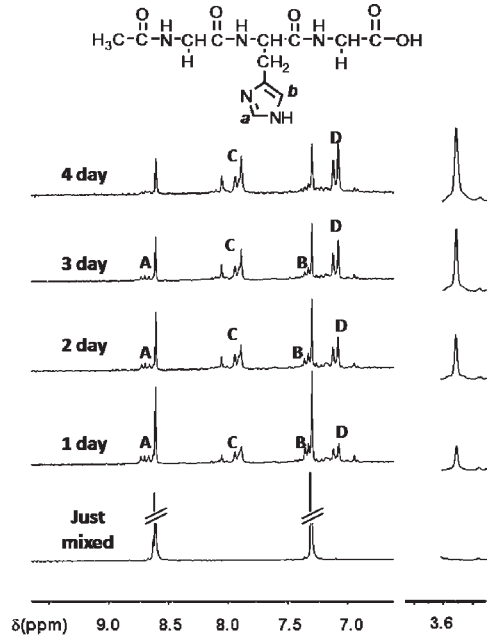


Figure 4. ^1H NMR spectra (in part) of mixed $[Pt(en)(H_2O)_2]^{2+}$ and AcGHG (molar ratio = 1:1) obtained during the incubation at 60 °C and pH 2.65. The newly appearing minor signals during the incubation were defined as four groups, groups **A** (8.66−8.73 ppm) and **B** (7.33−7.36 ppm) are the signals of H*a* and H*b* in Pt-anchored intermediates; groups **C** (7.95−8.06 ppm) and **D** (7.08−7.13 ppm) are the signals of H*a* and H*b* in Pt-anchored His-containing fragments.

Pt-anchored intermediates made the two signal groups (**A**: 8.73−8.66 ppm; **B**: 7.36−7.33 ppm) become obvious. Moreover, two new signal groups **C** and **D** appeared, respectively, at 8.06−7.95 and 7.13−7.08 ppm. These new signals can be assigned to H*a* and H*b* in Pt-anchored AcGH fragment. Further incubation led to the decrease of groups **A** and **B** and disappeared after 4 days, while the signal groups **C** and **D** increased distinctly in the process. Incubation after 4 days did not lead to any obvious change in the NMR spectrum. All these suggest Pt(II) coordinate to imidazole N slowly upon mixing, and the signal groups **A** and **B** are the signals for H*a* and H*b* in different Pt-anchored intermediates. These intermediates decrease with the hydrolysis process accompanied by the increase of signal groups **C** and **D**, which indicate the formation of Pt-anchored His-containing fragments.

In the incubation process, a new singlet appeared at 3.34 ppm, which can be assigned as the signal of methylene proton of Pt(II)-anchored Gly. The kinetics analysis according to the formation of this Pt(II) complex discloses the apparent first-order kinetics for the hydrolysis, and the rate constant k_{obsd} is 0.20 (1:1) (Figure 6A) and

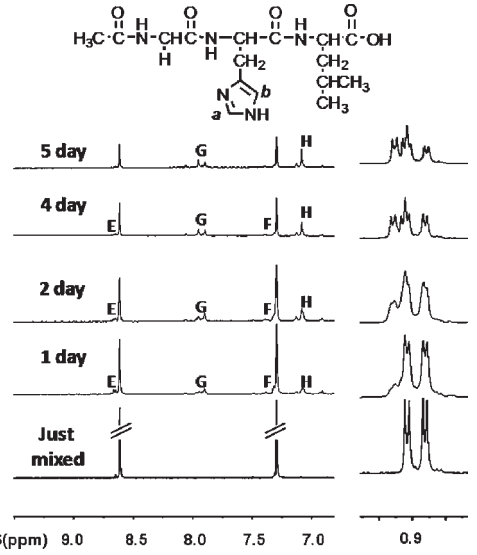


Figure 5. ^1H NMR spectra (in part) of mixed $[Pt(en)(H_2O)_2]^{2+}$ and AcGHL (molar ratio = 1:1) obtained during the incubation at 60 °C and pH 2.65. The newly appearing minor signals during the incubation were defined as four groups, groups **E** (8.65−8.69 ppm) and **F** (7.31−7.40 ppm) are the signals of H*a* and H*b* in Pt-anchored intermediates; groups **G** (7.89−8.05 ppm) and **H** (6.90−7.12 ppm) are the signals of H*a* and H*b* in Pt-anchored His-containing fragments.

0.34 day^{-1} (1:2), and the half-life $t_{1/2}$ is 3.46 and 2.03 day, respectively. The hydrolysis rate is positively correlated to the Pt(II) complex amount, more promoter leads to the higher hydrolysis rate. It was reported that Pd(II) cleaving reagents, such as *cis*-$[Pd(H_2O)_4]^{2+}$, *cis*-$[Pd(en)(H_2O)_2]^{2+}$, and *cis*-$[Pd(dtco)(H_2O)_2]^{2+}$ cleave the Met-Ala peptide bond in AcMet-Ala-Ser at pH 1.0 and 50 °C in the molar ratio of 1:1, with the cleavage rate of 54.72, 23.04, and 8.93 day^{-1}, respectively. The higher binding kinetics of Pd(II) to Met side chain than Pt(II) to histidine imidazole may be responsible for it.[6b,10] Compared with the *cis*-$[Pt(en)(H_2O)_2]^{2+}$-promoted hydrolysis rate of Cys-containing peptide GSMe (k_{obsd} = 3.02 day^{-1} at pH 2.0 and 40 °C with molar ratio 1:1),[6d] the current hydrolysis rate is obviously smaller. The low coordination kinetics of Pt(II) to His in acidic condition may be the origin.[10]

In the case of AcGHL, ^1H NMR tracking disclosed that the signals for imidazole H*a* and H*b* in AcGHL experienced a similar change process (Figure 5). There was almost no downfield shifted signal of free peptide H*a* and H*b* signals at the beginning of the incubation, although the ESI-MS spectrum obtained after just being mixed displays the MS signals for the Pt-anchored AcGHL species because of the higher sensitivity of mass spectroscopy. Initial incubation of the mixture resulted in the increase of the Pt-anchored intermediate, and the signal groups **E** and **F** as

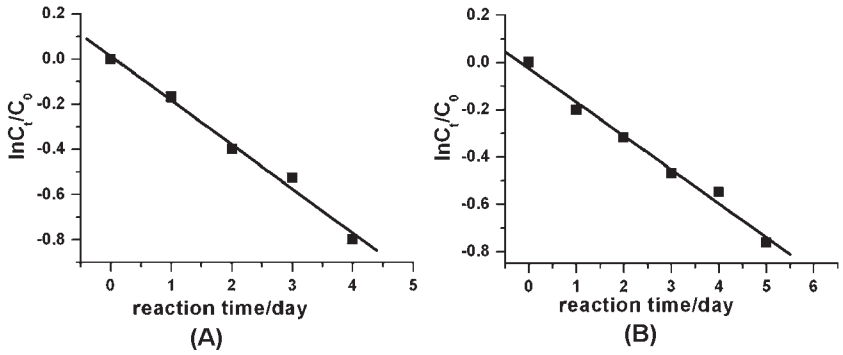


Figure 6. Apparent first-order kinetic plot for the hydrolysis of histidine-containing peptide, AcGHG (A) and AcGHL (B) at pH 2.65 and 60 °C when promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$ in molar ratio of 1:1.

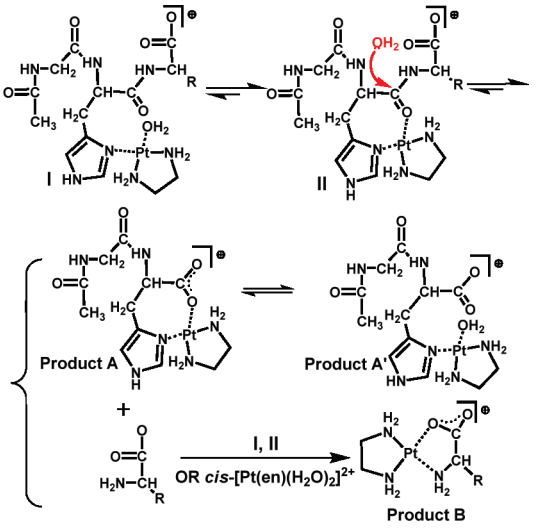
the signals for H*a* and H*b* in Pt-anchored intermediates became distinct after 1 day of incubation. The subsequent incubation led to the decrease of these signals. In fact, they are almost illegible after 5 days of incubation. The signal groups **G** and **H** as the signals for H*a* and H*b* in Pt-anchored His-containing fragments appeared also after 1 day of incubation. The two signal groups increased with the incubation time. On the other hand, two doublets at 0.91 and 0.86 ppm for protons of two Leu methyl group displayed the distinct changes during incubation. Quantitative analysis of the doublets' change demonstrates also the apparent first-order kinetics of AcGHL hydrolysis, and the k_{obsd} is 0.14 day^{-1} in the presence of 1 equiv of Pt(II) complex (Figure 6B), lower than that of AcGHG in the same condition. The result suggests that the Pt(II) promoted hydrolysis of histidine-containing peptide is reduced by the bulky side chain of Leu.

Discussion

MS and ^{1}H NMR studies confirmed that the histidine-containing tripeptides can only be hydrolyzed specifically at the first downstream peptide bond from histidine in the presence of *cis*-$[Pt(en)(H_2O)_2]^{2+}$. ESI-MS tracking disclosed that the formation of Pt-anchored peptides is the key step for the hydrolysis, and the exhaustion of the Pt(II)-anchored intermediate results in the end of hydrolysis. Moreover, more Pt(II) complex anchored on to peptide leads to more hydrolysis and higher hydrolysis rate. In addition, the formation of Pt-anchored intermediates is quicker in the case of AcGHL than in AcGHG. On the other hand, ^{1}H NMR study indicated that Pt(II)-anchoring to the peptide via coordinating to the imidazole N atom and the peptides are hydrolyzed in an apparent first-order kinetics. The hydrolysis rate of AcGHG is larger than that of AcGHL, although the Pt-anchored AcGHL intermediates are formed more quickly.

For the hydrolysis of AcGHL, the proposed mechanism was shown in Scheme 2. It is essential that His imidazole N coordinates to the Pt(II) center to form the Pt(II)-anchored intermediate **I** via replacing one coordinating water molecule, similar to the normal metal-promoted peptide hydrolysis via metal coordinating to a specific side chain. A MS signal of *m*/*z* 638.9 confirms the formation of this species. Then, the first downstream amide oxygen atom is able to coordinate to the same Pt(II) center in a synergic manner to form intermediate **II** (MS signal of *m*/*z* 621.0) via replacing the second coordinating water molecule. The carbonyl platination should enhance the electrophilicity of the amide carbon atom

Scheme 2. Proposed Hydrolysis Mechanism of AcGHL When Promoted by *cis*-$[Pt(en)(H_2O)_2]^{2+}$



and favor the subsequent nucleophilic attacking from water molecule, resulting in the hydrolysis of the His-Leu peptide bond. Carbonyl platination still favors to stabilize the tetrahedral transitional state in nucleophilic attack. This mechanism is quite similar to the carbonyl protonation-induced amide hydrolysis via an S_N2 pathway; the Pt(II) center and water molecule act as Lewis acid and nucleophilic reagent, respectively. In fact, similar mechanisms have been proposed for other Pd(II)- and Pt(II)-promoted hydrolysis of peptide bonds.[6h,m] Among the two direct hydrolysis products, the platinated AcGH (Product **A**, MS signal of *m*/*z* 508.1) can transfer to product **A′** (MS signal of *m*/*z* 526.1) via replacing the carboxyl group with a water molecule, while the cleaved Leu is able to grasp Pt(II) from intermediate **I** and **II** or *cis*-$[Pt(en)(H_2O)_2]^{2+}$ to form product **B** (MS signal of *m*/*z* 385.1), and finally stop the Pt(II)-promoted hydrolysis.

For AcGHG, it can be hydrolyzed in a similar pathway. However, the MS signal of *m*/*z* 409.6, which is assigned as diPt(II) species $[2Pt(en)+AcGHG-2H]^{2+}$, underwent the similar decrease process as that of intermediate **I** and **II** (R = H) and finally disappeared. It is suspected that the C-terminal Gly may chelate the second Pt(II) to form the dinuclear Pt(II) complex, which also undergoes hydrolysis in a similar way. In the case of AcGHL, ESI-MS determination did not find the dinuclear complex species. The bulky side chain of Leu, iso-butyl group, may induce the steric

hindrance to the second Pt(II) center, disfavoring the formation of a dinuclear intermediate. It may be the origin for the lower hydrolysis rate of AcGHL.

This study displays that the current His-oriented hydrolysis rate is much smaller than the reported Cys- and Met-oriented hydrolysis; the lower Pt(II) coordination kinetics of histidine imidazole than S atom of Cys/Met residues might be the origin,[10] considering the proposed hydrolysis mechanism. When Cys or Met coexists in the peptides, Pt(II) coordinating to Cys or Met is more efficient than that to His, which makes the Cys- or Met-oriented cleavage dominant in the hydrolysis and the His-oriented hydrolysis is almost unobservable. This may be the reason why all the previous studies demonstrate only the Cys- or Met-oriented cleavage. However, *cis*-[Pt(en)$(H_2O)_2]^{2+}$ might promote only the hydrolysis at the His-oriented site when S atom of Cys or Met was oxidized or pre-occupied by Pt(II) complexes without hydrolysis promotion ability, such as [Pt(dien)$(H_2O)]^{2+}$ (dien, diethylenetriamine).[6h,k] Since Zn^{2+} and Cu^{2+} normally display higher affinity to histidine imidazole than Pt(II), their ability to promote the His-oriented peptide bond hydrolysis cannot be excluded. However, Cu^{2+} and Zn^{2+} are the general coordinating substrates of many other groups in peptide side chain, except for the S-containing groups and imidazole; therefore, no higher specificity is expected for promoting the His-oriented hydrolysis. In fact, there is still no Cu^{2+}/Zn^{2+} promoted His-oriented peptide hydrolysis reported so far. Therefore, the newly found His-oriented peptide cleavage promoted by *cis*-[Pt(en)$(H_2O)_2]^{2+}$ implies the strategy to regulate the specific peptide cleavage site. In fact, our report about the specific His10-Leu11 cleavage found in *cis*-[Pt(en)$(H_2O)_2$]-promoted hydrolysis of oxidized insulin B chain is an example.[6p]

Conclusions

In conclusion, ESI-MS and ^{1}H NMR determination suggests that the [Pt(en)$(H_2O)_2]^{2+}$-promoted hydrolysis of His-containing tripeptide occurs selectively at the first downstream peptide bond from His residue in peptides without Met and Cys residues. Moreover, the hydrolysis kinetic and conversion rate is dependent on Pt(II) complex amount. The mechanism via the Pt(II)-coordinating to histidine imidazole and carbonyl group is proposed. Both tripeptides display the apparent first-order hydrolysis kinetics, and the hydrolysis rate for AcGHG and AcGHL is 0.20 day^{-1} and 0.14 day^{-1}, respectively. The bulk side chain of first His-downstream amino acid may decrease the hydrolysis rate via disfavoring the second Pt(II) center coordinating to tripeptide. This His-oriented hydrolysis is much slower than those Cys/Met-oriented one. Besides the new specific His-oriented peptide cleavage site, favoring to acquire the diversified target peptide fragments, the current study implies also a new strategy to alter the specific hydrolysis site from Cys/Met-oriented ones to a His-oriented site via deactivating the Cys or Met sites.

Experimental Section

Materials and General Methods. *Cis*-[Pt(en)Cl_2] was purchased from Sigma. AcGHG and AcGHL were synthesized and characterized by C-Strong Co., Ltd. in Shanghai, and their purity was determined by HPLC. Anhydrous $AgBF_4$ and 3-(trimethylsilyl)-1-propanesulfonic acid sodium (DSS) were purchased form Aldrich. All the chemicals were of the reagent grade. Routine ^{1}H NMR spectra in D_2O with DSS as internal reference were recorded by Bruker DRX 500 NMR spectrometers. The pH values were measured with a Sartorius pH instrument standardized with standard buffers of pH 4.00, 6.86, and 9.18. Electrospray ionization mass spectra (ESI-MS, positive mode) were recorded using a Finnigan LCQ ion-trap mass spectrometer, by loading 1.0 μL solution into the injection valve of the LCQ unit, and then injecting into the mobile phase solution (50% aqueous methanol). The rate for electrospray interface into the mass analyzer was of 200 $\mu L \cdot min^{-1}$. The voltage employed at the electrospray needles was 4.5 kV, and the capillary was heated to 200 °C. Zoom scan and isotopic distribution pattern (IDP) simulation (given by IsoPro 3.0) were performed for each of the major species detected.

Hydrolysis of AcGHG and AcGHL Promoted by *cis*-[Pt(en)$(H_2O)_2]^{2+}$. Doubly distilled water or D_2O was used for solution preparation. All solutions were freshly prepared prior to use. The stock solution (10.0 mM) of AcGHG and AcGHL were prepared directly by dissolving the peptide in doubly distilled H_2O. A solution of *cis*-[Pt(en)$(H_2O)_2]^{2+}$ was obtained by mixing *cis*-Pt(en)Cl_2 with 2 equiv of $AgBF_4$ followed by stirring overnight in dark at 40 °C and removing a white precipitate via centrifugation. The final concentration of *cis*-[Pt(en)$(H_2O)_2]^{2+}$ was 8.0 mM.

A typical experimental procedure for MS monitoring in water was as follows: 24 μL of 10.0 mM AcGHG or AcGHL was mixed with 30 or 60 μL of *cis*-[Pt(en)$(H_2O)_2]^{2+}$ (8.0 mM) in a molar ratio of 1:1 or 1:2 (peptide/Pt(II) complex). The pH value of the resultant mixture was around 2.65; HCl or NaOH solution (0.01 M) was used to adjust the pH value in case the pH value deviated distinctly. H_2O were then added to make the total volume of the mixed solution up to 100 μL. The mixture was incubated at 60 °C, and the MS determination was carried out immediately after mixing. The MS spectra were determined every 0.5 h in the initial 10 h, and then every day in the following 7 days of incubation.

^{1}H NMR monitoring was carried out in D_2O, and the typical procedure was as follows: 120 μL of AcGHG or AcGHL (10.0 mM) was mixed with 10 μL of DSS (10.0 mM), 150 (1:1) or 300 μL (1:2) of *cis*-[Pt(en)$(D_2O)_2]^{2+}$ (8.0 mM). The final total volume was adjusted to 600 μL with the pH being at 2.65 (pD value has been converted to pH value) by adding DCl, NaOD, or D_2O. The solutions were incubated at 60 °C and ^{1}H NMR spectra were determined at different incubation time. Control experiments without the Pt(II) complex have also been carried out similarly in D_2O solution.

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Supporting Information Available: Further details are given in Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.