Interaction of Palladium(II) Complexes with Sulfur-Containing Peptides Studied by Electrospray Mass Spectrometry

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Positive ion electrospray mass spectrometry has been used to investigate the interaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ (dtco-3-OH = dithiacyclooctan-3-ol), and *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ with glutathione (GSH), *s*-methylglutathione (GSMe), acetyl-*s*-methyl-cysteinylhistidylglycine (AcCysMe-His-GlyH), oxytocin, acetyl-methionine (AcMetH), and acetyl-methionylglycine (AcMet-GlyH). Reactions of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ or *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ with cysteine-containing peptides, GSH, GSMe, AcCysMe-His-GlyH, and oxytocin, form the following complexes: (1) a binuclear thiolato anion-bridged complex, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(\mu\text{-}s\text{-}GS)]_2^{2+}$; (2) mononuclear six-membered chelates involving a thioether group and the deprotonated amide nitrogen of glycine, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(S,N\text{-}GSMe)]^+$ and *cis*-$[Pd(en)(S,N\text{-}GSMe)]^+$; (3) mononuclear chelates including a thioether and a deprotonated amide nitrogen, the carbonyl oxygen of histidine and a nitrogen of imidazole, *cis*-$[Pd(en)(S,N\text{-}AcCysMe\text{-}His\text{-}GlyH)]^+$, $[Pd(S,N,N,O\text{-}AcCysMe\text{-}His\text{-}GlyH)]^+$, and $[Pd(S,N,N\text{-}AcCysMe\text{-}His\text{-}GlyH)(CH_3OH)]^+$; (4) mononuclear chelates containing disulfide and *N*-terminal amino groups, *cis*-$[Pd(en)(N,S\text{-}oxytocin)]^{2+}$ and *cis*-$[Pd(dtco\text{-}3\text{-}OH)(S,N\text{-}oxytocin)]^{2+}$. From mixing of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ or *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ or *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ with AcMetH or AcMet-GlyH, in a 1:1 mole ratio, the following complexes are observed: (1) mononuclear six-membered chelates involving the thioether and the deprotonated amide nitrogen of methionine, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(S,N\text{-}AcMetH)]^+$, *cis*-$[Pd(en)(S,N\text{-}AcMetH)]^+$, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(S,N\text{-}AcMet\text{-}GlyH)]^+$, *cis*-$[Pd(en)(S,N\text{-}AcMet\text{-}GlyH)]^+$, and *cis*-$[Pd(S,N\text{-}AcMetH)(CH_3OH)_2]^+$; (2) mononuclear chelates including the thioether, deprotonated amide nitrogen, and the carbonyl oxygen of methionine, $[Pd(py)(S,N,O\text{-}AcMetH)]^+$ and $[Pd(py)(S,N,O\text{-}AcMet\text{-}GlyH)]^+$. The finding of an interaction of Pd(II) with the carbonyl oxygen of an amide in $[Pd(S,N,N,O\text{-}AcCysMe\text{-}His\text{-}GlyH)]^+$ and $[Pd(py)(S,N,O\text{-}AcMet\text{-}GlyH)]^+$ is of interest in understanding the cleavage of the His-Gly bond in the former and the Met-Gly bond in the later. The results presented above indicate that electrospray mass spectrometry (ESMS) is a powerful tool for the analysis of reactions between palladium(II) complexes and sulfur-containing peptides. Both precise molecular masses and structural information of reaction products can be obtained. It is helpful to understand the coordination behavior of palladium(II) complexes toward sulfur-containing peptides and proteins.

Introduction

Studies of palladium(II) complexes with biologically important molecules are of considerable interest. Palladium(II) is a "soft" metal that will be suitable for formation of stable complexes with sulfur-containing amino acids, peptides and proteins. These studies were spurred by two important discoveries. First, some Pd(II) and Pt(II) complexes have carcinostatic effects and toxic side-effects.[1−4] The biochemical mechanism of Pt-induced nephrotoxicity is poorly understood. Interest in the chemistry of Pd(II) complexes also arises from the similarity in the properties of Pd(II) and Pt(II), but studies of Pd(II) complexes are more feasible because their ligand substitution rates are typically 10^5 times greater than for Pt(II).[5] Second, palladium(II) aqua complexes bind to the side chain of methionine and cysteine in peptides and proteins and catalyze regioselective hydrolysis cleavage of the adjacent peptide bond.[6−13] The two biologically important processes, nephrotoxicity and hydrolysis of sulfur-containing peptides and proteins are associated with the interaction of Pd(II) and Pt(II) complexes with sulfur-containing peptides and proteins.

Electrospray mass spectrometry (ESMS) introduced by Fenn and co-workers[14−16] has been shown to be a powerful technique for analyzing multiply charged ions, and has been applied

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primarily to large biomolecules, such as proteins.[17,18] However, the technique has not been applied to inorganic and organometallic systems systematically[19−26] and studies have just begun on the interaction of metal ions with peptides.[27]

In the present article, we report ESMS studies of the interaction of the palladium(II) complexes, *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$, and *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$, with the sulfur-containing peptides, glutathione (GSH), *s*-methylglutathione (GSMe), acetyl-*s*-methyl-cysteinyl-histidyl-glycine (Ac-CysMe-His-GlyH), oxytocin (a nonapeptide), acetyl-methionine (AcMetH), and acetyl-methionyl-glycine (AcMet-GlyH). A variety of structural information has been obtained by precise determination of molecular masses and isotope distribution patterns.

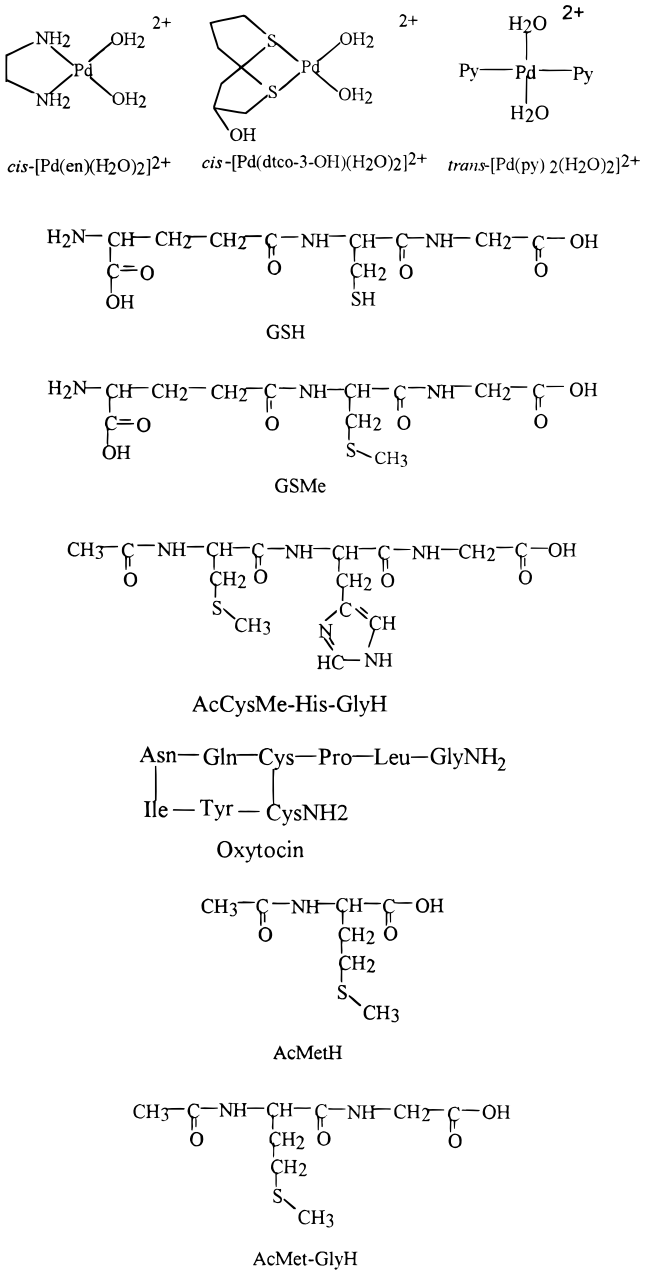
Experimental Section

Chemicals. Double-distilled water was used for preparation of solutions. $PdCl_2$ and dithiacyclooctan-3-ol were obtained from Aldrich Chemical Co. Glutathione (GSH), *s*-methyl-glutathione (GSMe), methionine, and methionylglycine (Met-Gly) were obtained from Sigma Chemical Co. Oxytocin was obtained from Fluka. All other chemicals were of reagent grade.

The following dichloro complexes were prepared and recrystallized by published procedures: *cis*-$[Pd(en)Cl_2]$ (en-ethylenediamine),[28] cis-$[Pd(dtco\text{-}3\text{-}OH)Cl_2]$ (dtco-3-OH-dithiacyclooctan-3-ol),[13,29]and *trans*-$[Pd(py)_2Cl_2]$ (py-pyridine).[11] The corresponding diaqua complexes were obtained by treating each of these complexes with 2.0 equiv of anhydrous $AgBF_4$ in H_2O and stirring for 4 h at 35 °C. The solid AgCl was removed by centrifugation in the dark. The diaqua complexes were always prepared freshly before use. The ^1H NMR spectra of these diaqua complexes in D_2O solutions gave the following principal δ values, in ppm: *cis*-$[Pd(en)(D_2O)_2]^{2+}$, 2.63 (s, CH_2); *cis*-$[Pd(dtco\text{-}3\text{-}OH)(D_2O)_2]^{2+}$, 2.20 (m, C-7$CH_2$), 2.70 (m, C-3CH), 2.90 (t, 4H, C-6 and C-8CH_2), 3.10 (m, 4H, C-2 and C-4 CH_2), and 5.10 (s, 1H, OH); *trans*-$[Pd(py)_2(D_2O)_2]^{2+}$, 7.58 (q, t, 2H, C-3 and C-5CH), 8.04 (q, C-4CH), 8.71 (t, d, 2H,C-2 and C-6CH).

The acetylmethionine (AcMetH) and acetylmethionylglycine (Ac-Met-GlyH) were obtained by the acetylation of the terminal amino group in MetH and Met-GlyH, according to a published procedure.[6] The ^1H NMR spectra of the products in D_2O gave the following principal δ values, in ppm: AcMetH, 2.04 (s, CH_3CO), 2.10 (s, CH_3S); AcMet-GlyH, 2.04 (s, CH_3CO), 2.11 (s, CH_3S), and 3.99 (q, $GlyCH_2$). On the basis of a published method,[30] the tripeptide, AcCysMe-His-GlyH was prepared in solution through the synthesis route: N^{α}-Boc-N^{τ}-tosyl-histidine → N^{α}-Boc-N^{τ}-tosyl-histidylglycine ethyl ester → N^{α}-H-N^{τ}-tosyl-histidylglycine ethyl ester → acetyl-*s*-methylcysteinyl-N^{τ}-tosyl-histidylglycine ethyl ester → acetyl-*s*-methylcysteinyl-histidylglycine. The molecular mass calculated for $C_{14}H_{22}N_5O_5S$ (AcCysMe-His-GlyH + H^+) is 372.1; the observed value by ESMS is 372.1. The molecular mass of oxytocin was measured to be 1007.5 with isotopic peaks separated by 1.0 *m*/*z* unit; calculated value of $C_{43}H_{67}N_{12}O_{12}S_2$ for *N*-terminal amino group protonated oxytocin is 1007.4. The palladium(II) diaqua complexes and peptides used in this study are listed in Chart 1.

Chart 1. Palladium(II) Aqua Complexes and Peptides



Instrumentation. Proton NMR spectra at 500 MHz of D_2O solution, containing DSS as an internal reference, were recorded with an AM500 spectrometer. An LCQ electrospray mass spectrometer (ESMS, Finnigan) was employed for molecular mass determination of peptides and their complexes with palladium(II). The sample was dissolved in water and diluted to 100 μmol L^{-1}. A 1.0 μL amount of such solution was loaded into the injection valve of the LCQ unit and then injected into the mobile phase solution (50% aqueous methanol containing 1% acetic acid), and carried through the electrospray interface into the mass analyzer at a rate of 200 μL min^{-1}. The employed voltage at the electrospray needles was 5 kV and the capilliary was heated to 200 °C. A maximum ion injection time of 200 ms along with 10 scans was set. Positive ion mass spectra were obtained. Zoom Scan was used in these experiments. The predicted isotope distribution patterns for each of complexes were calculated using the IsoPro 3.0 program. The bonding energy (eV) of sulfur 2p of the disulfide group in oxytocin

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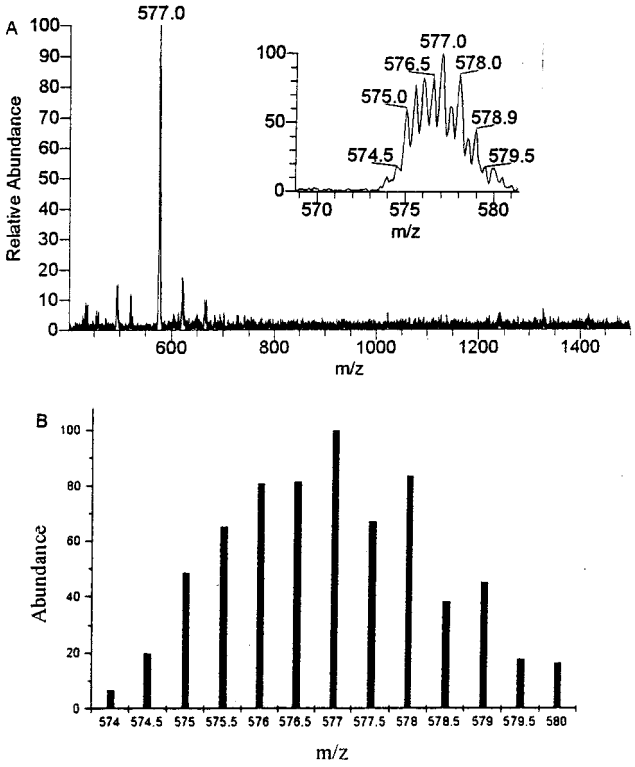


Figure 1. ESMS spectrum (A) measured 10 min after mixing of GSH with *cis*-[Pd(dtco-3-OH)$(H_2O)_2]^{2+}$. Measured *m*/*z* values with different isotope masses of Pd(II) are from 574.0 to 578.0 separated by 0.5 *m*/*z* from each other. (B) The isotope distribution pattern of $C_{32}H_{56}N_6O_{14}S_6Pd_2$ for *cis*-[Pd(dtco-3-OH)$(\mu_2$-*s*-GS$)]_2^{2+}$ was calculated with the program IsoPro 3.0.

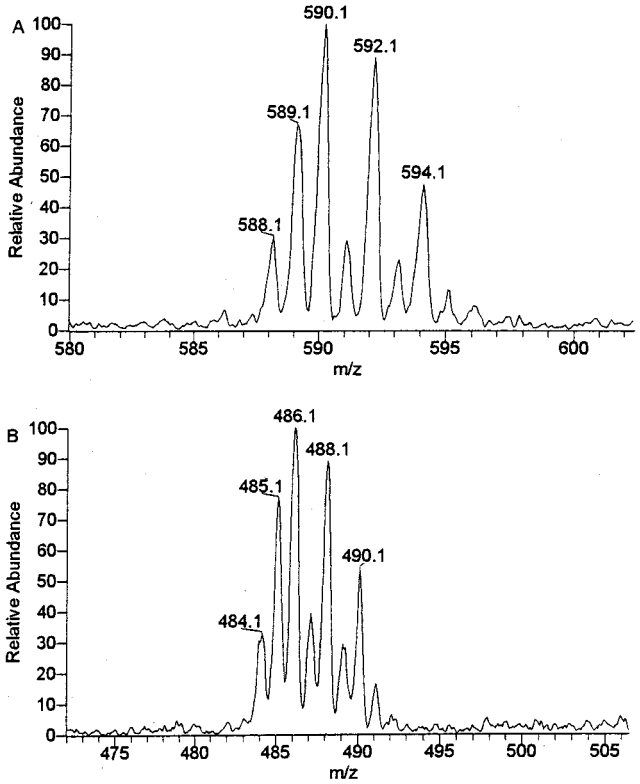
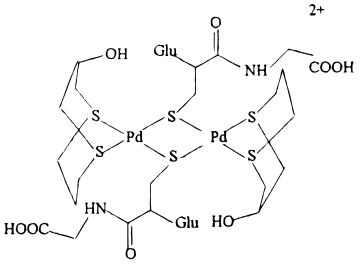


Figure 2. ESMS spectra for interaction of GSMe with *cis*-[Pd(dtco-3-OH)$(H_2O)_2]^{2+}$ (A) or with *cis*-[Pd(en)$(H_2O)_2]^{2+}$ (B), with different isotopic masses of Pd(II). (A) Measured *m*/*z* values: 588.1, 589.1, 590.1, 592.1, and 594.1. Calculated *m*/*z* values of $C_{17}H_{30}N_3O_7S_3Pd$ for *cis*-[Pd(dtco-3-OH)(*S*,*N*-GSMe)$]^+$: 588.0 (^{104}Pd). (B) Measured *m*/*z* values: 484.1, 485.1, 486.0, 488.1, 490.1. Calculated *m*/*z* values of $C_{13}H_{26}N_5O_6SPd$ for *cis*-[Pd(en) (*S*,*N*-GSMe)$]^+$: 484.1 (^{104}Pd).

and its solid complex of Pd(II) was determined by X-ray photoelectron spectroscopy (XPS, England, ESCALAB, MKII) relative to a C 1S energy of 285.0 eV. The pH was measured with an Orion 901 instrument and a Phoenix Ag−AgCl reference electrode.

Results and Discussion

Interaction of GSH and GSMe with *cis*-[Pd(dtco-3-OH)-$(H_2O)_2]^{2+}$ or *cis*-[Pd(en)$(H_2O)_2]^{2+}$. Ten minutes after of mixing GSH or GSMe with *cis*-[Pd(dtco-3-OH)$(H_2O)_2]^{2+}$ in a 1:1 mole ratio, we measured the ESMS of the mixed solution (Figures 1A and 2). As Figure 1A shows for the reaction with GSH, the isotopic peaks separated by 0.5 *m*/*z* unit are attributed to a doubly charged cationic complex. The spectrum simulation based on *cis*-[Pd(dtco-3-OH)$(\mu_2$-*s*-GS$)]_2^{2+}$, as seen in Figure 1B, fits Figure 1A very well. In this case, *cis*-[Pd(dtco-3-OH)-$(H_2O)_2]^{2+}$ reacts with GSH to form a thiolato anion-bridged binuclear complex (**1**) which is consistent with the results of



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an ^{1}H NMR study of the reactions of *cis*-diaminediaquaplatinum(II) with glutathione and amino acids containing a thiol group.[31] When *cis*-[Pd(en)$(H_2O)_2]^{2+}$ reacted with GSH a polymeric structure was formed, as pointed out in our previous study.[6] In this the en was detached due to the *trans*-effect of the thiolato anion, and Pd(II) coordinates to the thiolato anion as a bridging ligand as well, and to the amide group via a deprotonated nitrogen atom of glycine. This polymer is insoluble in common solvents making it unsuitable for ESMS determination.

Figure 2A,B shows that the reaction of *cis*-[Pd(dtco-3-OH)-$(H_2O)_2]^{2+}$ or *cis*-[Pd(en)$(H_2O)_2]^{2+}$ with GSMe is clearly different. The isotopic peaks separated by 1.0 *m*/*z* are attributed to a singly charged cationic complex. The molecular mass observed is precisely equal to that calculated for *cis*-[Pd(dtco-3-OH)(*S*,*N*-GSMe)$]^+$ and *cis*-[Pd(en)(*S*,*N*-GSMe)$]^+$ with different isotopic masses of Pd(II). This is consistent with a bidentate mononuclear complex being formed through thioether and deprotonated amide nitrogen coordination. 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 solutions with pH < 2.0.[5,32,33] Under the ESMS experimental condition, this reaction is incomplete. Free GSMe (molecular mass for GSMe + H^+ is 322.3) was detected.

These results demonstrate that GSH and GSMe show different behavior in bonding with *cis*-[Pd(dtco-3-OH)$(H_2O)_2]^{2+}$ or *cis*-[Pd(en)$(H_2O)_2]^{2+}$ complexes. GSMe could not coordinate to Pd-

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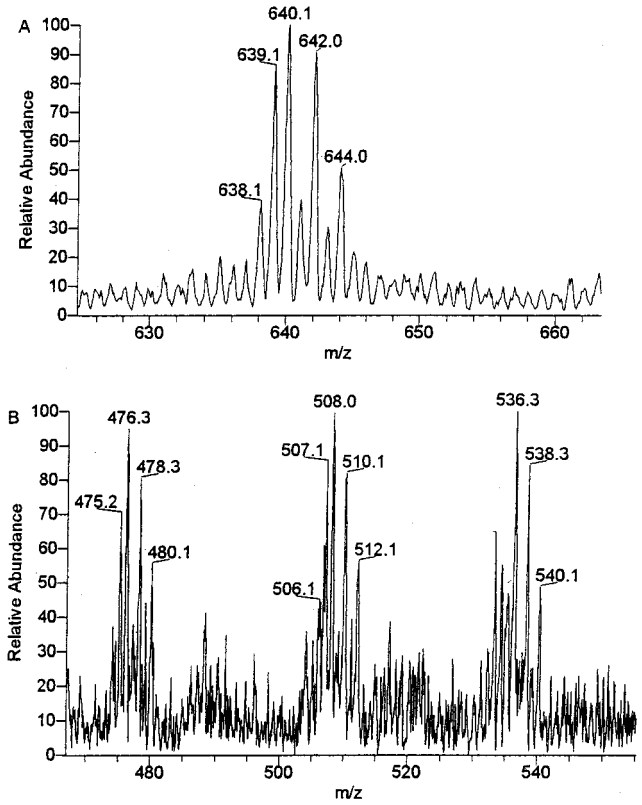
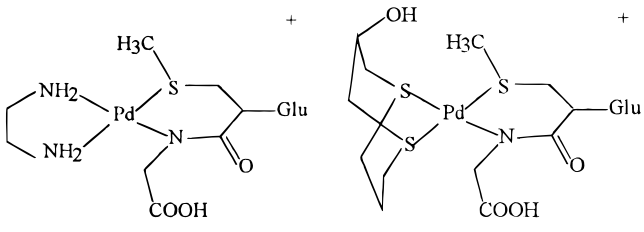


Figure 3. ESMS spectra measured 10 min after mixing of AcCysMe-His-GlyH with *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ (A) or with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (B), with different isotopic masses of Pd(II). (A) Measured *m*/*z* values: 638.1, 639.1, 640.1, 642.0, and 644.2. Calculated *m*/*z* values of $C_{20}H_{32}N_5O_6S_3Pd$ for *cis*-[Pd(dtco-3-OH)(*S*,*N*-AcCysMe-His-GlyH]$^+$: 638.1 (^{104}Pd). (B) Measured *m*/*z* values: 534.3, 535.3, 536.3, 538.3, and 540.1. Calculated *m*/*z* values of $C_{16}H_{28}N_7O_5SPd$ for *cis*-[Pd(en)(*S*,*N*-AcCysMe-His-GlyH]$^+$: 534.1 (^{104}Pd). Measured *m*/*z* values: 474.2, 475.2, 476.3, 478.3, and 480.1. Calculated *m*/*z* values of $C_{14}H_{20}N_5O_5SPd$ for [Pd(*S*,*N*,*N*,*O*-AcCysMe-His-GlyH)]$^+$: 474.0 (^{104}Pd). Measured *m*/*z* values: 506.1, 507.1, 508.0, 510.1, and 512.1. Calculated *m*/*z* values of $C_{15}H_{24}N_5O_6SPd$ for [Pd (*S*,*N*,*N*-AcCysMe-His-Gly)$(CH_3OH)]^+$: 506.1 (^{104}Pd).

(II) via a thioether-bridged linkage, instead there was a bidentate chelation through the thioether sulfur and the deprotonated amide nitrogen. There are two possible ways for the GSMe to form a bidentate chelate. One is coordination of Pd(II) to a deprotonated amide nitrogen of the cysteinyl residue, forming a five-membered ring. Another is coordination of Pd(II) to a deprotonated amide nitrogen of glycine, resulting in a six-membered ring. Based on early studies[6,28] and results described in the next subsection, it seems likely that a six-membered chelate ring was formed (**2** and **3**).



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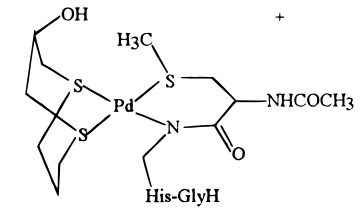
Reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ or *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ with AcCysMe-His-GlyH. A solution of *cis*-[Pd(dtco-3-OH)$(H_2O)_2]^{2+}$ or *cis*-$[Pd(en)(H_2O)_2]^{2+}$ mixed with AcCysMe-His-GlyH, in a 1:1 mole ratio and pH ∼ 1.0, was separated into two portions. One was measured by ESMS after 10 min of reaction at room temperature (Figure 3). Another one was incubated at 40 °C for 5 h and then measured by ESMS (Figure 4). As shown in Figure 3A, *cis*-[Pd(dtco-3-OH))-$(H_2O)_2]^{2+}$ reacted with AcCysMe-His-GlyH to form only *cis*-[Pd(dtco-3-OH)(*S*,*N*-AcCysMe-His-GlyH)]$^+$ in which a six-membered chelate ring was formed by the thioether and deprotonated amide nitrogen of the histidine residue (**4**). However, reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ with AcCysMe-His-GlyH formed three complexes (Figure 3B). One is *cis*-[Pd(en)-(*S*,*N*-AcCysMe-His-GlyH)]$^+$ (**5**) with coordination of the same type as that in **4**. The others are [Pd(*S*,*N*,*N*,*O*-AcCysMe-His-GlyH)]$^+$ in which the en was replaced by the imidazole nitrogen and carbonyl oxygen of the histidine residue (**6**) and [Pd (*S*,*N*,*N*-AcCysMe-His-GlyH)$(CH_3OH)]^+$ in which the coordination of the carbonyl oxygen in **6** was replaced by solvent CH_3OH (**7**). The coordination of an unprotonated amide nitrogen of histidine in **6** is ruled out because this kind of coordination is normally accompanied by deprotonation of an amide nitrogen.[5,32,33]



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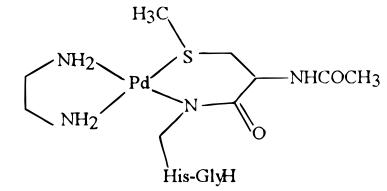

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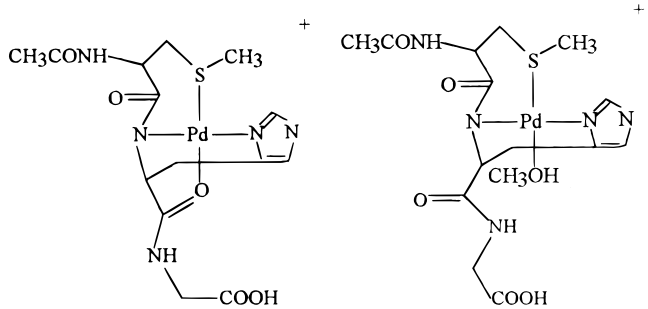


6 **7**

As Figure 4 shows, from a solution containing *cis*-[Pd(en)-$(H_2O)_2]^{2+}$ and AcCysMe-His-GlyH, after 5 h incubation at 40 °C and pH ∼ 1.0, followed by removing Pd(II) as PdI_2 by adding KI and centrifuging, we detected the dipeptide AcCysMe-HisH + H^+ (molecular mass 315.1) in solution. This is a cleavage fragment of the tripeptide AcCysMe-His-GlyH. In previous study,[12] this cleaved product was confirmed by ^{1}H NMR. The coordination of Pd(II) to the carbonyl oxygen of the histidyl residue in *cis*-[Pd(*S*,*N*,*N*,*O*-AcCysMe-His-GlyH]$^+$ (**6**) may be

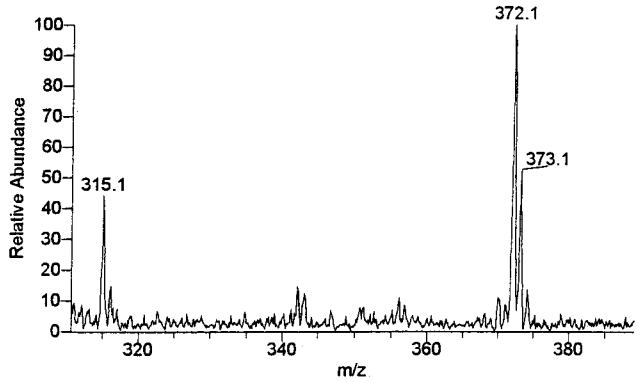


Figure 4. ESMS spectra measured after 5 h incubation of mixed solution of AcCysMe-His-GlyH with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ at 40 °C and at pH ~ 1.0, followed by removing Pd(II) as PdI_2, by adding KI and by centrifuging. Measured *m*/*z* value: 372.1. Calculated *m*/*z* value of $C_{14}H_{22}N_5O_5S$ for AcCysMe-His-GlyH + H^+: 372.1. Measured *m*/*z* values: 315.1. Calculated *m*/*z* value of $C_{12}H_{19}N_4O_4S$ for AcCysMe-HisH + H^+: 315.1.

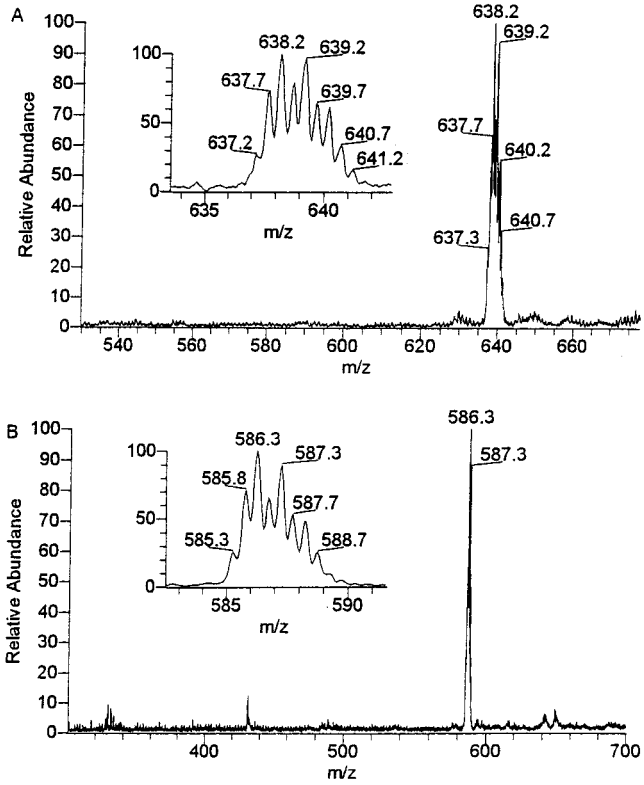


Figure 5. ESMS spectra measured 10 min after mixing of oxytocin with *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ (A) or with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (B) at room temperature. Measured *m*/*z* values: 637.2, 637.7, 638.2, 639.2 and 640.2. Calculated *m*/*z* values of $C_{49}H_{78}N_{12}O_{13}S_4Pd$ for *cis*-[Pd(dtco-3-OH)(*N*,*S*-oxytocin)]$^{2+}$: 637.2 (^{104}Pd). Measured *m*/*z* values: 585.3, 585.8, 586.3, 587.3, and 588.3. Calculated *m*/*z* values of $C_{45}H_{74}N_{14}O_{12}S_2Pd$ for *cis*-[Pd(en)(*N*,*S*-oxytocin)]$^{2+}$: 585.2 (^{104}Pd).

associated with cleavage of the His−Gly bond. This was proposed previously,[12] but now we have experimental evidence. Although the carbonyl oxygen atom is a weak ligand, its coordination is facilitated by the chelate effect. In acidic solution (pH ~ 1.0) used in the hydrolysis studies of peptides and horse heart cytochrome *c*,[12] the imidazole nitrogen in **6** and **7** was detached from Pd(II) and protonated (pK_a ~ 6). The activated His−Gly bond in **6** was attacked by external water causing hydrolysis.

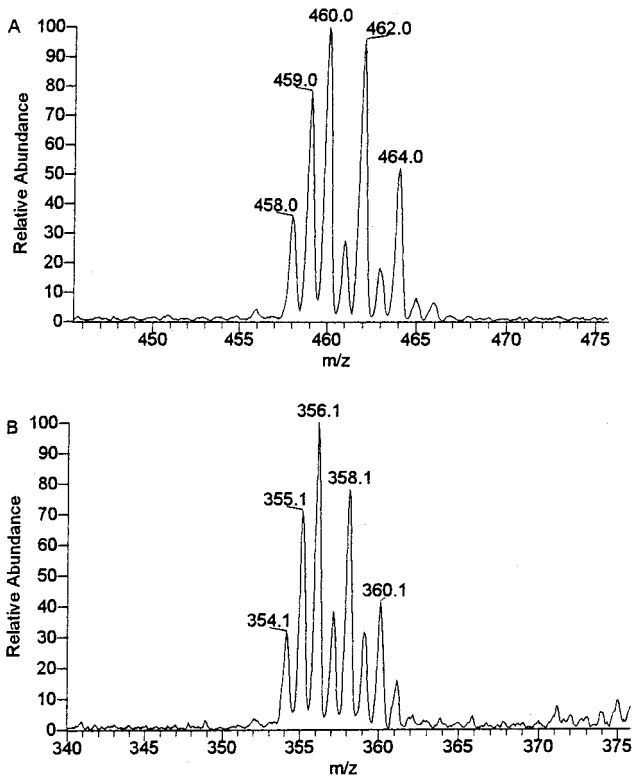
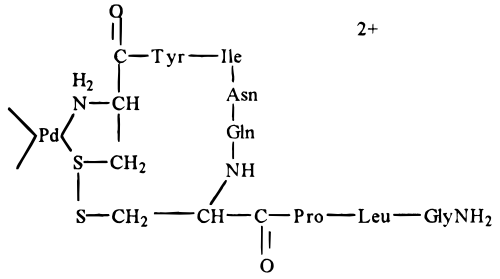


Figure 6. ESMS spectra measured 10 min after mixing of AcMetH with *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ (A) or with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (B), with different isotopic masses of Pd(II). (A) Measured *m*/*z* values: 458.0, 459.0, 460.0, 462.0, and 464.0. Calculated *m*/*z* values of $C_{13}H_{24}NO_4S_3Pd$ for *cis*-[Pd(dtco-3-OH)(*S*,*N*-AcMetH]$^+$: 458.0 (^{104}Pd). (B) Measured *m*/*z* values: 354.1, 355.2, 356.1, 358.1, and 360.1. Calculated *m*/*z* values of $C_9H_{20}N_3O_3SPd$ for *cis*-[Pd(en)(*S*,*N*-AcMetH)]$^+$: 354.0 (^{104}Pd).

Formation of Complexes between *cis*-[Pd(dtco-3-OH)(H$_2$O)$_2$]$^{2+}$ or *cis*-[Pd(en)(H$_2$O)$_2$]$^{2+}$ and Oxytocin. Ten minutes after mixing oxytocin with *cis*-$[Pd(dtco\text{-}3\text{-}OH)(H_2O)_2]^{2+}$ or *cis*-$[Pd(en)(H_2O)_2]^{2+}$, in a 1:1 mole ratio of oxytocin to the Pd(II) complex, we measured the ESMS for the mixed solution. As seen from Figure 5, the isotopic peaks separated by 0.5 *m*/*z* unit are attributed to doubly charged cationic complexes. The molecular masses calculated for *cis*-[Pd(dtco-3-OH)(*S*,*N*-oxytocin)]$^{2+}$ and *cis*-[Pd(en)(*S*,*N*-oxytocin)]$^{2+}$with different isotopic masses of Pd(II) are exactly equal to the values observed by ESMS. We also measured the binding energy (eV) of sulfur 2p of disulfide group in oxytocin and its solid complex of Pd(II), *cis*-[Pd(en)(*S*,*N*-oxytocin)]$^{2+}$. There are two binding energies of sulfur 2p observed in the complex. One is 163.8 eV equal to that of oxytocin itself (163.6 eV). The other is 166.4 eV, which is attributed to sulfur 2p bound to Pd(II). Although disulfide is a weak nucleophile and a poor ligand, the coordination of disulfide to transition metals such as Ni(II), Pd(II), Cu(II), and Cd(II) has been reported.[34−36] It has also been reported that the nickel(II) ion is coordinated by one sulfur atom of the disulfide group.[36] ESMS and XPS determinations cannot tell which sulfur atom of the disulfide group in the oxytocin is preferable for bonding to Pd(II), however, it is generally believed that a five-membered chelate ring is more stable thermodynamically. Therefore, a structure for the Pd(II) complexes of oxytocin is proposed in which Pd(II) coordinates to the oxytocin through a *N*-terminal

(34) Waiton, R. A. *Coord. Chem. Rev.* **1980**, *31*, 183.
(35) Thich, J. A.; Mastropalo, D.; Potenza, J.; Schugar, H. J. *J. Am. Chem. Soc.* **1974**, *96*, 728.
(36) Riley, P. E.; Seff, K. *Inorg. Chem.* **1972**, *11*, 2993.

amino group and one sulfur atom of a cysteinyl residue located at *N*-terminal, forming a five-membered chelate (**8**). Because



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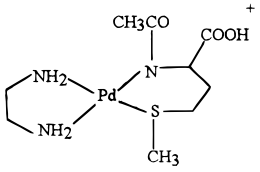
the disulfide has a weaker trans-effect compared with thiolato anion and thioether group, the en ligand in *cis*-[Pd(en)(*S*,*N*-oxytocin)]$^{2+}$ is still coordinated to Pd(II) even in acidic solution. This kind of coordination keeps the Cys−Pro bond intact. We monitored the reaction during incubation of the mixed solution at 40 °C and pH ∼ 1.0 by means of HPLC and ESMS measurements after removing Pd(II) as PdI_2. No fragments associated with Cys−Pro bond cleavage were detected by either method.

Interaction of Pd(II) Complexes with AcMetH or Acet-GlyH. ESMS was measured 10 min after mixing of Pd(II) complexes with AcMetH or AcMet-GlyH in a 1:1 mole ratio at pH ∼ 1.0 and at room temperature. As Figure 6 shows, the reaction of Pd(II) complexes with AcMetH or AcMet-GlyH proceeds as follows:

$$cis\text{-}[PdL(H_2O)_2]^{2+} + \text{AcMetH} \rightarrow cis\text{-}[PdL(S,N\text{-AcMetH})]^{+} + 2H_2O \quad (1)$$

$$cis\text{-}[PdL(H_2O)_2]^{2+} + \text{AcMet-GlyH} \rightarrow cis\text{-}[PdL(S,N\text{-AcMet-GlyH})]^{+} + 2H_2O \quad (2)$$

Here, L is en or dtco-3-OH. The diaqua palladium(II) complexes react with AcMetH or AcMet-GlyH to form a six-membered chelate through the thioether and through the deprotonated amide nitrogen of methionine. The latter occurs rather than using a deprotonated amide nitrogen of glycine in AcMet-GlyH because this would involve seven-membered ring formation. This type of coordination was also confirmed by ^{1}H NMR measurements. When AcMetH and AcMet-GlyH reacted with Pd(II) the chemical shifts of SCH_3 and CH_3CO groups (δ, ppm) moved from 2.11 and 2.04 downfield to 2.50 and 2.06, respectivly. The molecular mass was determined when the mixed solution of *cis*-[Pd(en)$(H_2O)_2]^{2+}$ with AcMet-GlyH in mole ratio 1:1 was incubated at 40 °C and pH ∼ 1.0 for 30 min. As shown in Figure 7A, the *cis*-[Pd(en)(*S*,*N*-AcMetH)]$^+$ (**9**) was observed in addition to the *cis*-[Pd(en)(*S*,*N*-AcMet-GlyH)]$^+$ (**10**) present in solution. ^{1}H NMR studies (monitoring the signal at 3.37 ppm)



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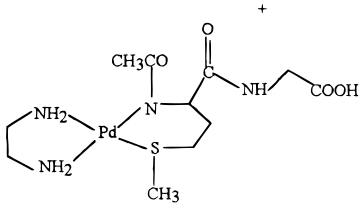

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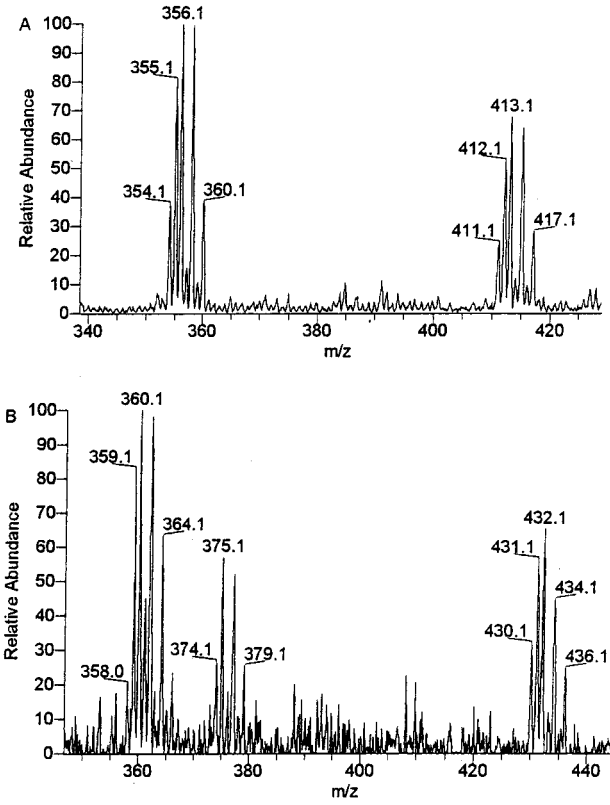
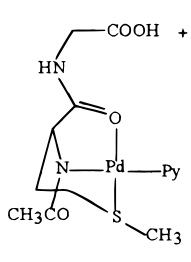


Figure 7. ESMS spectra measured after a half-hour or 10 min incubation of mixed solution of AcMet-GlyH with *cis*-[Pd(en)$(H_2O)_2]^{2+}$ (A) or with *trans*-[Pd$(py)_2(H_2O)_2]^{2+}$ (B) at 40 °C and at pH ∼ 1.0. (A) Measured *m*/*z* values with different isotopic masses of Pd(II): 411.1, 412.1, 413.1, 415.1, and 417.1. Calculated *m*/*z* values of $C_{11}H_{23}N_4O_4$-SPd for *cis*-[Pd(en)(*S*,*N*-AcMet-GlyH]$^+$: 411.0 (^{104}Pd). Measured *m*/*z* values: 354.1, 355.1, 356.1, 358.1, and 360.1. Calculated *m*/*z* values of $C_9H_{20}N_3O_3$SPd for *cis*-[Pd(en)(*S*,*N*-AcMetH]$^+$: 354.0 (^{104}Pd). (B) Measured *m*/*z* values: 430.1, 431.1, 432.1, 434.1, and 436.1. Calculated *m*/*z* values of $C_{14}H_{20}N_3O_4$SPd for [Pd(py)(*S*,*N*,*O*-AcMet-GlyH)]$^+$: 430.0 (^{104}Pd). Measured *m*/*z* values: 373.1, 374.1, 375.1, 377.1, and 379.1. Calculated *m*/*z* values of $C_{12}H_{17}N_2O_3$SPd for [Pd(py)(*S*,*N*,*O*-AcMetH)]$^+$: 373.0 (^{104}Pd). Measured *m*/*z* values: 358.0, 359.1, 360.1, 362.1, and 364.1. Calculated *m*/*z* values of $C_9H_{20}NO_5$SPd for cis-[Pd(*S*,*N*-AcMetH)-$(CH_3OH)_2]^+$: 358.0 (^{104}Pd).

showed that the en ligand was detached from Pd(II) at pH ∼ 1.0 and attached to Pd(II) again at pH ∼ 5. This result is consistent with that obtained by ESMS measurements. When we used *trans*-[Pd$(py)_2(H_2O)_2]^{2+}$, instead of *cis*-[Pd(en)$(H_2O)_2]^{2+}$, to incubate with AcMet-GlyH at 40 °C and pH ∼ 1.0 for 10 min, the reaction products were different. From ESMS measurements the molecular masses (Figure 7B) and simulation of the isotope distribution patterns showed that the complexes [Pd(py)(*S*,*N*,*O*-AcMet-GlyH)]$^+$ (**11**), [Pd(py)(*S*,*N*,*O*-AcMetH)]$^+$ (**12**) and [Pd(*S*,*N*-AcMetH)$(CH_3OH)_2]^+$ (**13**) were formed. In



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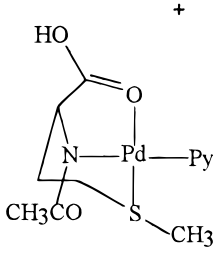

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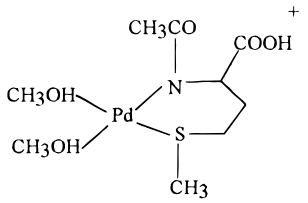

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our previous studies, it was found that *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ is one of the most effective promoters for the cleavage of Met−aa peptide bonds.[11] Two pyridine ligands were detached and protonated soon after mixing of *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ with dipeptides of type AcMet-aaH (aaH = amino acid) in acidic solution. As pointed out, it is to be expected that from ESMS measurements at pH ∼ 5, one pyridine will be attached to Pd(II) again in the species **11** and **12**. The discovery of the $[Pd(py)(S,N,O\text{-AcMet-GlyH})]^+$ complex, in which for the first time, Pd(II) coordinates to the carbonyl oxygen of methionine, is probably a clue to the mechanism associated with cleavage of Met−aa bonds. A fused six-membered and five-membered ring in **6** and **11** seems to favor coordination of the carbonyl oxygen of histidine in **6** and methionine in **11,** resulting in the activation of His−Gly and Met−Gly bonds toward hydrolysis.

Conclusion

A feature of these studies is that the intact ions formed by interaction of Pd(II) complexes with sulfur-containing peptides were always observed, emphasizing the "soft" nature of the ESMS process. Both precise molecular masses and structural information can be obtained. The sulfhydryl group, thioether and disulfide in sulfur-containing peptides coordinate to Pd(II) complexes in a different fashion: the thiolato anion in GSH bridges to two Pd(II); the thioether in *s*-methyl-cysteine-containing peptides (GSMe and AcCysMe-His-GlyH) anchors to Pd(II), followed by coordination of Pd(II) to deprotonated amide nitrogen located at the *C*-terminal to form a six-membered chelate. However, the thioether in methionine-containing peptides (AcMetH and AcMet-GlyH) anchors to Pd(II), followed by coordination of Pd(II) to deprotonated amide nitrogen located at the *N*-terminal to form a six-membered chelate ring too. One sulfur atom in oxytocin coordinates to Pd(II), forming a five-membered chelate ring via a *N*-terminal amino group and one sulfur atom of a cysteinyl residue at *N*-terminal. The discovery of the complexes $[Pd(S,N,O\text{-AcMet-GlyH})]^+$ and $[Pd(S,N,N,O\text{-AcCysMe-His-GlyH})]^+$ in which Pd(II) coordinates to a carbonyl oxygen of methionine or histidine is of interest and is probably related to the cleavage of Met−aa bond in the former and His-aa bond in the latter.

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