Monatshefte für Chemie 130, 613–622 (1999)

Monatshefte für Chemie
Chemical Monthly
© Springer-Verlag 1999
Printed in Austria

NMR Study of the Interaction of Palladium(II) Complexes with Some Histidine-Containing Peptides: Effects of the Mode of Coordination on Hydrolytic Reactions

Miloš I. Djuran* and **Snežana U. Milinković**

Department of Chemistry, Faculty of Science, University of Kragujevac, YU-34000 Kragujevac, Yugoslavia

Summary. Reactions of palladium(II) complexes (*cis*-[$PdCl_2$(*en*)], *cis*-[$PdCl_2$(*L*-HMet-S,N)] and their aqua derivatives *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$, in which *en* is ethane-1,2-diamine and *L*-HMet is *L*-methionine coordinated through nitrogen and sulfur atoms) with *L*-alanyl-*L*-histidine (Ala-His), *L*-seryl-*L*-histidine (Ser-His), and the N-acetylated dipeptides MeCO-Ala-His and MeCO-Ser-His have been studied by ^{1}H NMR spectroscopy. In the reactions between palladium(II) complexes and Ala-His or Ser-His, the tridentate coordination of peptide occurs at $1.5 < pH < 3.5$ and causes release of the *en* ligand and ring opening of the *L*-HMet chelate. The ^{1}H NMR data show that the peptides are bound to palladium(II) through imidazole N-3, deprotonated amide, and terminal amino nitrogen atoms. This tridentate coordination of the peptides is unfavourable for their hydrolysis.

The reaction between palladium(II) complexes and the N-acetylated peptides MeCO-Ala-His and MeCO-Ser-His at $1.5 < pH < 3.5$ proceeds without release of the *en* ligand or ring opening of the *L*-H Met chelate. In the reaction between the peptides and *cis*-[$PdCl_2$(*en*)] or *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$, five NMR-detectable complexes are formed, whereas with *cis*-[$PdCl_2$(*L*-HMet-S,N)] and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ only two palladium(II)-peptide complexes are observed in solution. No cleavage of the amide bonds in the reactions between the peptides and the palladium(II) aqua complexes occurs.

Keywords. Palladium(II) complexes; Peptide hydrolysis; ^{1}H NMR spectroscopy.

NMR-spektroskopische Untersuchung von Palladium(II)-Komplexen mit einigen histidinhaltigen Peptiden: Einfluß der Koordinationsverhältnisse auf Hydrolysereaktionen

Zusammenfassung. Die Reaktionen von Palladium(II)-Komplexen (*cis*-[$PdCl_2$(*en*)], *cis*-[$PdCl_2$-(*L*-HMet-S,N)], *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$, *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$; *en* = Ethan-1,2-diamin, *L*-HMet = über N und S koordiniertes *L*-Methionin) mit *L*-Alanyl-*L*-histidin (Ala-His), *L*-Seryl-*L*-histidin (Ser-His) und den N-acetylierten Dipeptiden MeCO-Ala-His und MeCO-Ser-His wurden ^{1}H-NMR-spektroskopisch untersucht. Bei der Reaktion zwischen den Pd(II)-Komplexen und Ala-His oder Ser-His erfolgt bei *pH*-Werten zwischen 1.5 und 3.5 eine tridentate Koordination des Peptids

* Corresponding author

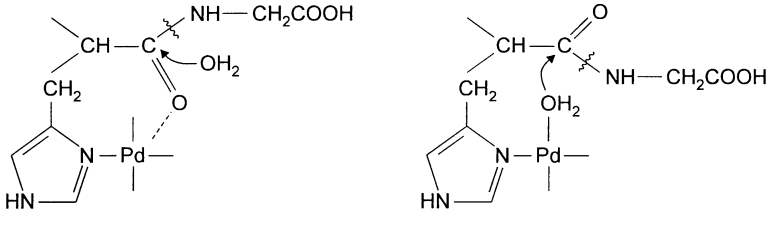
und verursacht eine Abspaltung des *en*-Liganden bzw. eine Öffnung des *L*-HMet-Chelats. Die ^{1}H-NMR-Daten zeigen, daß die Peptide über N-3 des Imidazols, das deprotonierte Amid und den terminalen Aminostickstoff an das Palladium gebunden sind. Die koordinative Bindung der Peptide erschwert deren Hydrolyse.

Die Reaktion von Palladium(II)-Komplexen mit den N-acetylierten Peptiden MeCO-Ala-His und MeCO-Ser-His bei *pH*-Werten zwischen 1.5 und 3.5 verläuft ohne Freisetzung von *en* oder Ringöffnung des *L*-HMet-Chelats. Bei der Reaktion dieser Peptide mit *cis*-[$PdCl_2$(*en*)] oder *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ werden 5 mittels NMR-Spektroskopie detektierbare Komplexe gebildet, während im Fall von *cis*-[$PdCl_2$(*L*-HMet-S,N)] und *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ nur zwei Pd(II)-Peptid-Komplexe beobachtet werden. Bei den Reaktionen der Peptide mit den Aquokomplexen tritt keine Spaltung von Amidbindungen auf.

Introduction

Many biological processes involve hydrolysis of peptides and proteins, but relatively little is known about the mechanism of this reaction. The extreme inertness of the amide bond makes this reaction interesting from the chemical point of view. Several proteolytic enzymes are used for cleavage [1], but application of enzymes is limited by their rather narrow requirements for temperature and *pH*. However, recent studies by *Kostić* and coworkers have shown that palladium(II) aqua complexes can be promising reagents for hydrolytic cleavage of peptides and proteins [2–9]. These complexes bind to the heteroatom in the side chain of methionine [2–6] or histidine [7–9] and promote cleavage of the amide bond involving the carboxylic group of this anchoring amino acid. This hydrolytic reaction can occur by two limiting mechanisms (Scheme 1), assuming a histidine anchor; analogous interactions are possible also with the methionine anchor. In the first mechanism, palladium(II) acts as a *Lewis* acid and forms a chelate involving a heteroatom in the side chain of the anchoring amino acid and the oxygen atom in the amide group. The interaction with the oxygen atom polarizes the carbonyl group and activates the carbon atom toward external attack by water. Since water for the hydrolysis of the amide bond comes from the solvent, this mechanism is called external attack.

In the second mechanism, an aqua ligand on palladium(II) is delivered to the carbon atom in the amide bond. Since the water for the hydrolysis comes from the palladium(II) complex attached to the side chain of the anchoring amino acid, this mechanism is called internal attack. Also possible is a combined mechanism, in



external attack internal delivery

Scheme 1

which the palladium(II) atom activates the amide bond by binding to the carbonyl oxygen atom and also delivers an aqua ligand to that same bond.

Recent results by *Kostić* and *coworkers* [7, 8] have shown that in the reactions of *cis*-$[Pd(H_2O)_2(en)]^{2+}$ with peptides of the type MeCO-His-*X*, in which *X* is Gly, Ala, Leu, Ser, Thr, Tyr, or Phe, complete hydrolysis of the amide bond involving the carboxylic group of histidine occured after 24 hours at 60°C and $1.46 \leq pD \leq 2.61$. In the reaction with peptides containing C-terminal histidine, as in MeCO-Gly-His, *cis*-$[Pd(H_2O)_2(en)]^{2+}$ does not effect hydrolysis. This regioselective cleavage of the amide bond involving the carboxylic group of histidine was also observed in the reactions of *cis*-$[Pd(H_2O)_2(L\text{-HMet-S,N})]^{2+}$ complexes with MeCO-His-Gly [9].

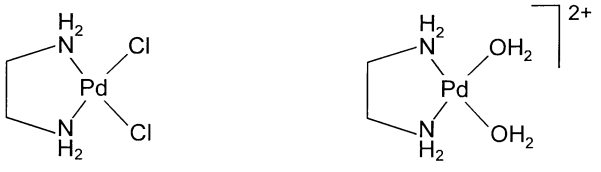
The consistent regioselectivity in the cleavage of histidine-containing peptides by palladium(II) aqua complexes is not completely understood yet. A better knowledge of the coordination chemistry of histidine-containing peptides with palladium(II) complexes is necessary for understanding the regioselectivity of protein clevage promoted by such complexes. In this study, which involves unprotected as well as protected dipeptides of the type *X*-His (*X* = Gly, Ser, and Ala), we have examined the effect of the various modes of coordination of different palladium(II) complexes on the non-occurence of the hydrolytic reaction.

Results and Discussion

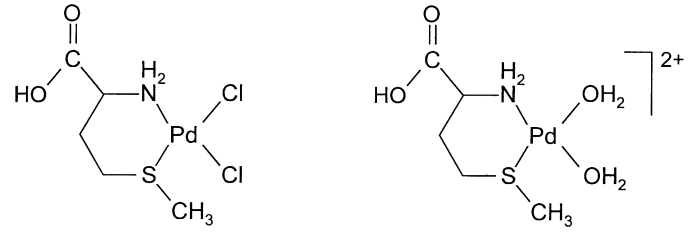
The palladium(II) complexes and peptides are shown in Scheme 2. The chelate ligands in *cis*-$[PdCl_2(en)]$ and *cis*-$[PdCl_2(L\text{-HMet-S,N})]$ as well as in their aqua derivatives are inert to substitution and expected to remain bound to the palladium(II) atom during the reactions with the amino acids and peptides. As in previous studies from this laboratory [9], acidic solutions are needed to suppress formation of hydroxo-bridged oligomeric palladium(II) complexes which are catalytically inactive. The absence of the cleavage of the amide bond in the reactions between the palladium(II) complexes and the dipeptides Ala-His, Ser-His, MeCO-Ala-His, and MeCO-Ser-His was discussed in terms of the structure of the palladium(II)-peptide complex formed.

Reactions of palladium(II) complexes with Ala-His and Ser-His

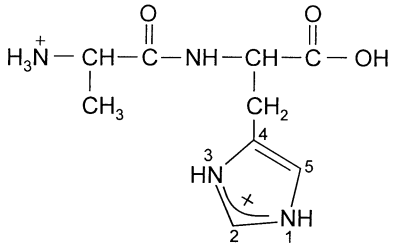
The reactions of *cis*-$[PdCl_2(en)]$, *cis*-$[PdCl_2(L\text{-HMet-S,N})]$ and their corresponding aqua complexes *cis*-$[Pd(H_2O)_2(en)]^{2+}$ and *cis*-$[Pd(H_2O)_2(L\text{-HMet-S,N})]^{2+}$ with Ala-His and Ser-His were carried out at $1.5 < pH < 3.5$ and room temperature. The tridentate coordination of the peptide and the formation of the complex **1** (Fig. 1) was observed by ^{1}H NMR spectroscopy. Previous studies with Gly-His and *cis*-$[PdCl_2(en)]$ or *cis*-$[PdCl_2(L\text{-HMet-S,N})]$ have shown that **1** was also a major product in these reactions [9–11]. The complex crystallized from the reaction between K_2PdCl_4 or *cis*-$[PdCl_2(en)]$ and Gly-His at $pH < 3$ [9, 11]. The crystal structure of [PdCl(Gly-His-N,N,N)] showed that complex is square-planar with palladium(II) bound to the terminal amino group, the deprotonated peptide nitrogen, and the N-3 atom of the imidazole ring of histidine. The fourth ligand is a chloride anion. In the reactions of *cis*-$[PdCl_2(L\text{-HMet-S,N})]$ and *cis*-$[PdCl_2(en)]$



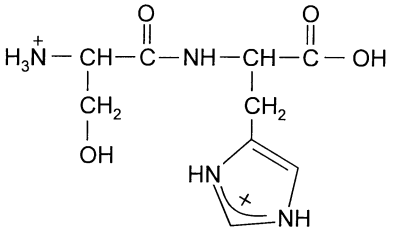
cis-[$PdCl_2$(*en*)] *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$



cis-[$PdCl_2$(*L*-HMet-S,N)] *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$



Ala-His

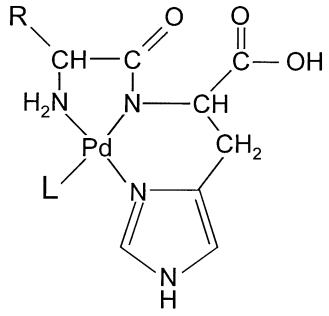


Ser-His

Scheme 2

with Gly-His, the formation of [Pd(Gly-His-N,N,N)(*L*-HMet-S)]$^+$ and [PdCl(Gly-His-N,N,N)] occurred with tridentate coordination of Gly-His and release of the *en* ligand from *cis*-[$PdCl_2$(*en*)] and ring opening of the *L*-HMet chelate in *cis*-[$PdCl_2$(*L*-HMet-S,N)] [9].

In this study, the palladium(II) chloro complexes *cis*-[$PdCl_2$(*en*)] and *cis*-[$PdCl_2$(*L*-HMet-S,N)] were treated with Ala-His and Ser-His to explore the coordination modes of peptides with a free terminal amino group. The characterization of complex **1** formed in the reaction of palladium(II) complexes with dipeptides was performed *via* determination of the chemical shifts of H-2 and H-5 of the imidazole ring and the protons of the N-terminal amino acid (Table 1) and comparison of the data with those of the corresponding complexes with Gly-His [9].



1

R = H, CH_3, CH_2OH

L = Cl^-, H_2O, HMet-*S*

Fig. 1. Schematic representation of the square planar complex obtained from reactions of palladium(II) complexes with *X*-His dipeptides (X = Gly, Ala, Ser)

Table 1. Proton NMR chemical shifts (δ, ppm) of the palladium(II)-peptide complexes obtained from reactions of *cis*-[$PdCl_2$(*en*)] and *cis*-[$PdCl_2$(*L*-HMet-S,N)] with histidine-containing peptides

Peptide/Complex	Imidazole protons		N-terminal amino acid	
	H-2	H-5		
Gly-His[a]	8.62	7.31	3.83 (Gly-CH_2)	
[Pd(Gly-His-N,N,N)Cl][a]	7.92	7.04	3.52 (Gly-CH_2)	
Ala-His	8.64	7.34	4.08 (Ala-CH)	1.53 (Ala-CH_3)
[Pd(Ala-His-N,N,N)Cl]	7.92	7.04	3.65 (Ala-CH)	1.42 (Ala-CH_3)
Ser-His	8.63	7.32	4.14 (Ser-CH)	3.97 (Ser-CH_2)
[Pd(Ser-His-N,N,N)Cl]	7.93	7.04	3.67 (Ser-CH)	3.87 (Ser-CH_2)
[Pd(Gly-His-N,N,N)(*L*-HMet-S)]$^{+}$[a]	7.94	7.04	3.52 (Gly-CH_2)	
[Pd(Ala-His-N,N,N)(*L*-HMet-S)]$^{+}$	7.95	7.03	3.63 (Ala-CH)	1.53 (Ala-CH_3)
[Pd(Ser-His-N,N,N)(*L*-HMet-S)]$^{+}$	7.93	7.04	3.66 (Ser-CH)	3.84 (Ser-CH_2)

[a] Ref. [9]

Upon mixing Ala-His or Ser-His with *cis*-[$PdCl_2$(*en*)] at $1.5 < pH < 3.5$, tridentate coordination of the dipeptide was observed. A sharp ^{1}H NMR singlet for free H_2en^{2+} at 3.37 ppm indicates complete detachment of the ethylenediamine ligand from palladium(II). The release of the *en* ligand proceeds slow enough to be monitored by ^{1}H NMR, and the total amount of the free ligand can be calculated from the integral values of the signals at 2.73 (*en* ligand chelated to palladium(II)) and 3.37 ppm (free H_2en^{2+}). In the reaction between *cis*-[$PdCl_2$(*en*)] and Ala-His, the ^{1}H NMR resonances at 4.08 ppm for Ala-CH and 1.53 ppm for Ala-CH_3 of the free peptide decreased, whereas the resonances at 3.65 and 1.42 ppm (coordinated peptide) increased. Addition of [PdCl(Ala-His-N,N,N)], (obtained from the reaction of K_2PdCl_4 with Ala-His) to the reaction mixture causes an increase of these resonances. The formation of [PdCl(Ser-His-N,N,N)] in the reaction between *cis*-[$PdCl_2$(*en*)] and Ser-His was also monitored by ^{1}H NMR. In this case, we

followed the disappearance of the resonances at 4.14 ppm for Ser-CH and 3.97 ppm for Ser-CH_2 (free peptide) and the enhancement of the resonances at 3.67 and 3.87 ppm (tridentate coordinated peptide). From the integral values of the H-5 resonance (7.32 ppm for free and 7.04 ppm for bound peptide; see Table 1) we calculated the total amount of complex **1** obtained from reaction of *cis*-[$PdCl_2$(*en*)] with Gly-His, Ala-His, and Ser-His. After 24 h at *pH* 1.7 and room temperature, the amount of **1** was *ca*. 85%, 83%, and 76% for Ser-His, Ala-His, and Gly-His, respectively. If these reactions were carried out at $pH < 1.5$, complete release of *en* and formation of **1** in 95% yield after 30 min were observed for all peptides. However, when the reactions were carried out at $3.5 < pH < 5$, release of *en* was negligible, and different palladium(II)-peptide complexes were detected in solution. The major product formed in a yield of 85% is the complex with a coordination of the peptide to palladium(II) *via* N-3 of the imidazole ring and the deprotonated amide nitrogen atom, whereas the other products are characterized by a monodentate bound peptide. These products were not investigated further.

In the reaction between *cis*-[$PdCl_2$(*L*-HMet-S,N)] and Ala-His or Ser-His, the complex of type **1** spontaneously formed. This complex contains coordinated monodentate *L*-HMet and can be formulated as [Pd(Ala-His-N,N,N)(*L*-HMet-S)]$^+$ or [Pd(Ser-His-N,N,N)(*L*-HMet-S)]$^+$. The presence of *L*-HMet in this complex was confirmed by a singlet at 2.55 ppm corresponding to the S-methyl protons of *L*-HMet coordinated to palladium(II). The signal at 2.14 ppm for the uncoordinated CH_3S group of *L*-Met could not be observed even after leaving the reaction mixture for 6 days. The formation of [Pd(Ala-His-N,N,N)(*L*-HMet-S)]$^+$ is evident from the simultaneous decline of the resonances at 4.08 and 1.53 ppm (Ala-CH and Ala-CH_3 of the free peptide) and growth of those at 3.63 and 1.53 ppm (bound peptide). In the reaction with Ser-His, the resonances of Ser-CH at 4.14 ppm and Ser-CH_2 at 3.97 ppm due to free peptide decrease, whereas these resonances of the coordinated peptide (3.66 ppm for the methine proton and 3.84 ppm for the methylene protons) increase. The reaction between the palladium(II) complex and the peptide is reversible, and after 24 h at *pH* 1.7 and room temperature, *ca*. 43% of [Pd(Gly-His-N,N,N)(*L*-HMet-S)]$^+$, 52% of [Pd(Ala-His-N,N,N)(*L*-HMet-S)]$^+$, and 72% of [Pd(Ser-His-N,N,N)](L-HMet-S)$^+$ are formed. Apparently, Ser-His containing a hydroxyl group in the side chain is much more reactive towards *cis*-[$PdCl_2$-(*L*-HMet-S,N)] than the other two dipeptides. This can be explained by the existence of a hydrogen bond between hydroxyl group of serine and the carboxylic or amino group of *L*-HMet in [Pd(Ser-His-N,N,N)(*L*-HMet-S)]$^+$ which contributes to stabilization of the complex.

In the reactions between the palladium(II) aqua complexes *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ and the dipeptides Ala-His and Ser-His, no cleavage of the amide bond was observed. The resonances at 4.08 ppm for the methine and 1.56 ppm for the methyl group of free alanine and 4.14 ppm for the methine or 3.97 ppm for the methylene protons of free serine did not appear even after one week of reaction time. The tridentate coordination of the peptides in the above reactions is unfavorable for the hydrolysis of the peptide because coordination of the amide nitrogen atom to palladium(II) strengthens the amide bond [12, 13]. External attack is unlikely because the amide oxygen atom does not interact with the palladium(II) ion. Internal attack is also unlikely because in the

complex of type **1** formed in the reaction with *cis*-[$PdCl_2$(*L*-HMet-S,N)] or *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ water from the solvent can not displace the thioether ligand. In the complex formed in the reaction with *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ the aqua ligands at palladium(II) and at the amide bond are coplanar and too distant for the reaction.

Reactions of palladium(II) complexes with MeCO-Ala-His and MeCO-Ser-His

The reactions between the palladium(II) chloro complexes *cis*-[$PdCl_2$(*en*)] and *cis*-[$PdCl_2$(*L*-HMet-S,N)] and their diaqua derivatives *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and *cis*-[$Pd(H_2O)_2$(L-HMet-S,N)]$^{2+}$ with dipeptides with N-acetylated terminal amino group (MeCO-Ala-His, MeCO-Ser-His) were carried out at $1.5 < pH < 3.5$ and room temperature. Since the amino group in these peptides was acetylated, binding of palladium(II) to this nitrogen atom was suppressed. A previous study with these two palladium(II) complexes and MeCO-Gly-His showed that at $pH < 3$ different palladium(II)-peptide products formed [7–9]. In the reaction between *cis*-[$PdCl_2$(*en*)] or *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and MeCO-Gly-His, five NMR-detectable complexes formed, whereas with *cis*-[$PdCl_2$(*L*-HMet-S,N)] and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$, only three complexes are detectable in solution [9]. These complexes were distinguished on the basis of the chemical shifts of the two imidazole protons H-2 and H-5.

When *cis*-[$PdCl_2$(*en*)] or *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ were mixed with an equimolar amount of MeCO-Ala-His or MeCO-Ser-His at $1.5 < pH < 3.5$, five complexes formed (Fig. 2); they are designated from **A** through **E**. The chemical shifts of the imidazole protons H-2 and H-5 are given in Table 2. These data are compared with those obtained for the reaction of these palladium(II) complexes with MeCO-Gly-His under the same experimental conditions [7–9]. From the chemical shift values in Table 2 it may be concluded that the palladium(II)-peptide complexes formed in the reaction with MeCO-Ala-His and MeCO-Ser-His are indentical to those for the reaction with MeCO-Gly-His.

Mixing of *cis*-[$PdCl_2$(*L*-HMet-S,N)] or *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ with an equimolar amount of MeCO-Ala-His or MeCO-Ser-His at $1.5 < pH < 3.5$ resulted in a spontaneous formation of two NMR-detectable complexes designated **B** and **D** (Fig. 2). The complexes **A**, **C**, and **E** were not detected in solution even after prolonging the reaction time for a week. The complexes **B** and **D** were present in solution even when the reaction was carried out at $0.5 < pH < 1.5$. In the complex designated **D**, the peptide coordinates to palladium(II) as a bidentate ligand *via* the N-3 atom of the imidazole moiety and the deprotonated amide nitrogen. The formation of the complex **D** in such strongly acidic solution is in accordance with the fact that palladium(II) anchored to a side chain is particularly effective in displacing the amide proton [14]. The estimated pK_a for this reaction is *ca*. 2.0, but displacement was also observed in solutions with $pH < 2.0$ [14–18].

In the reactions between the palladium(II) aqua complexes *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ and the dipeptides MeCO-Ala-His and MeCO-Ser-His, no hydrolysis of the amide bond was observed as confirmed by ^{1}H NMR spectroscopy. The resonance at 2.08 ppm (free acetic acid) and resonances for methine proton at 4.08 and 4.14 ppm (free alanine and serine) did not occur after 1

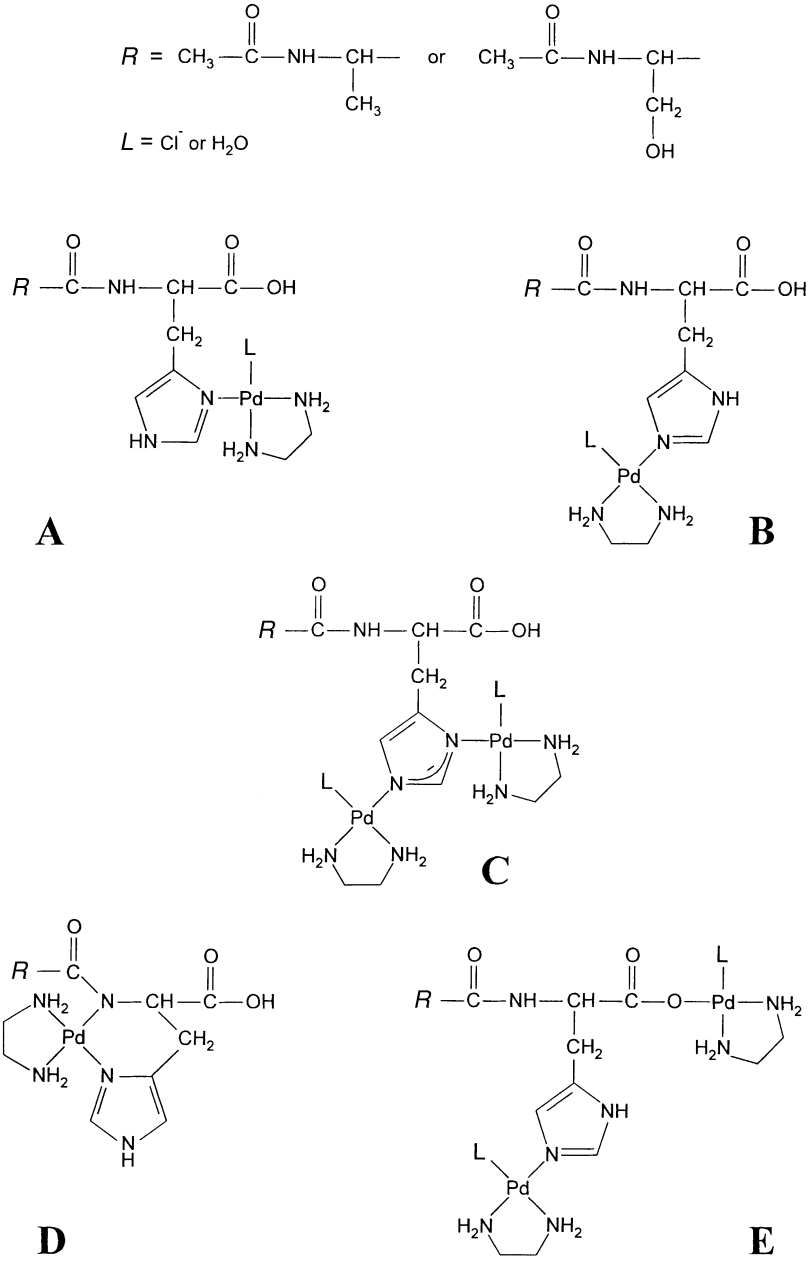


Fig. 2. Palladium(II)-peptide complexes formed in the reaction between *cis*-[$PdCl_2$(*en*)] or *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and the N-acetylated dipeptides MeCO-Ala-His and MeCO-Ser-His

week at 60°C and at $pH = 2.0$. No cleavage was also found in the reaction between *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ or *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ and MeCO-Gly-His [9]. However, in reactions with a series of MeCO-His-*X* peptides *X* = Gly, Ala, Leu, Ser, Thr, Tyr, Phe, these complexes selectively cleave the amide bond involving the carboxylic group of histidine [7, 8]. The experiments with methylated MeCO-His-*X* peptides showed that only the palladium(II) complex bound to the N-3 atom of imidazole (complex of type **A**; Fig. 2) can effect hydrolytic cleavage [8]. Indeed,

Table 2. Identification of reaction products in the reactions of palladium(II) complexes with N-acetylated histidine-containing peptides according to the chemical shifts of imidazole protons (δ, ppm)

Reactants		Type of complex/Chemical shifts of H_2 and H_5										Ref.
		A		**B**		**C**		**D**		**E**		
cis-[$PdCl_2$(*en*)]	MeCO-Gly-His	8.11	7.08	7.80	6.80	7.59	6.60	7.91	7.04	7.67	6.74	[7–9]
or +	MeCO-Ala-His	8.12	7.09	7.82	6.84	7.60	6.65	7.92	7.06	7.67	6.77	
cis-[$Pd(H_2O)_2$(*en*)]$^{2+}$	MeCO-Ser-His	8.11	7.08	7.82	6.83	7.59	6.64	7.92	7.06	7.66	6.75	
cis-[$PdCl_2$(*L*-HMet-S,N)]	MeCO-Gly-His	8.06	7.14	7.97	6.98			8.05	7.12			[9]
or +	MeCO-Ala-His			7.96	6.98			8.03	7.12			
cis-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$	MeCO-Ser-His			7.95	6.98			8.02	7.11			

only this coordination mode of peptides permits the close approach of the palladium(II) ion and of its aqua ligand to the scissile peptide bond. The non-occurrence of hydrolytic cleavage of the amide bond in the reactions between palladium(II) complexes and histidine-containing peptides in which histidine is the C-terminal amino acid is caused by the coordination modes of these peptides. The lack of cleavage of the amide bonds involving the amino group of histidine and amino group of glycine, alanine, or serine in MeCO-Gly-His, MeCO-Ala-His, and MeCO-Ser-His implies that these bonds do not interact with the palladium(II) moiety in any of the complexes **A**–**E** (Fig. 2).

Conclusions

This study contributes to the better understanding of the selective cleavage of peptides and proteins by palladium(II) aqua complexes. It has been made definitely clear that hydrolysis of the amide bond in histidine-containing peptides will not occur if histidine is a C-terminal amino acid in the sequence *X*-*Y*-His. With these results we could also prove that regioselective hydrolysis of the amide bond in peptides of the type MeCO-His-*X* is catalyzed by the proximate palladium(II) aqua complex and not by the acid in solution. This study points the way for further applications of palladium(II) complexes in biochemistry and structural biology.

Experimental

Chemicals

Distilled water was demineralized and purified to a resistance greater than 10 M$\Omega \cdot$ cm. D_2O, DNO_3, NaOD, and $K_2[PdCl_4]$ were obtained from Aldrich Chemical Co; all chemicals were of reagent grade. *L*-Methionine and the dipeptides *L*-alanyl-*L*-histidine and *L*-seryl-*L*-histidine were obtained from Sigma Chemical Co. The terminal amino group in the dipeptides was acetylated by standard methods [3].

The complexes *cis*-[$PdCl_2$(*en*)] and *cis*-[$PdCl_2$(*L*-HMet-S,N)] were synthesized by published procedures [19–21] and converted to the corresponding diaqua complexes *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$ and *cis*-[$Pd(H_2O)_2$(*L*-HMet-S,N)]$^{2+}$ by treatment with 2 equiv. of $AgNO_3$ at $pH = 2.0$ according to a published method [22]. In each case, the solid AgCl was removed by filtration in the dark, and a fresh stock solution of the aqua complex was used in further experiments.

Reactions of peptides with palladium(II) complexes

Reactions of *cis*-[$PdCl_2$(*en*)], *cis*-[$PdCl_2$(*L*-HMet-S,N)], *cis*-[$Pd(H_2O)_2$(*en*)]$^{2+}$, and *cis*-[$Pd(H_2O)_2$-(*L*-HMet-S,N)]$^{2+}$ (*en*: ethane-1,2-diamine; *L*-HMet: *L*-methionine coordinated through sulfur and nitrogen) with Ala-His, Ser-His, and the N-acetylated dipeptides MeCO-Ala-His and MeCO-Ser-His were monitored by ^{1}H NMR spectroscopy. Equimolar amounts of the palladium(II) complex and the peptide were mixed in an NMR tube. The final solution was 10 m*M* in each reactant. The *pH* was varied in the range of 1.5–3.5. All reactions were carried out at room temperature.

Measurements

All *pH* measurements were made at 298 K. The *pH* meter (Iskra MA 5704) was calibrated with *Fischer* certified buffer solutions of *pH* 4.00 and 7.00 The results were not corrected for the deuterium isotope effect. Proton NMR spectra of D_2O solutions containing *TSP* (3-trimethylsilyl-propane-1-sulfonate) as internal reference were recorded with a Varian Gemini 200 spectrometer.

Acknowledgements

This work was funded in part by the Ministry of Science and Technology of the Republic of Serbia (grant 02E35) and by the Ministry of Science and Progress in Technology of the FR Yugoslavia (grant OSI-048/1-93). We are grateful to Professor *Nenad M. Kostić* (Department of Chemistry, Iowa State University, Ames, USA) and his coworkers for providing K_2PdCl_4 and the peptides. We thank also Mrs. *B. Mojsilović* for assistance with recording the ^{1}H NMR spectra.

References

[1] Croft LR (1980) Handbook of Protein Sequence Analysis, 2nd edn. Wiley, Chichester
[2] Burgeson IE, Kostić NM (1991) Inorg Chem **30**: 4299
[3] Zhu L, Kostić NM (1992) Inorg Chem **31**: 3994
[4] Zhu L, Kostić NM (1993) J Am Chem Soc **115**: 4566
[5] Zhu L, Kostić NM (1994) Inorg Chim Acta **217**: 21
[6] Korneeva EN, Ovchinnikov MV, Kostić NM (1996) Inorg Chim Acta **243**: 9
[7] Parac TN, Kostić NM (1996) J Am Chem Soc **118**: 51
[8] Parac TN, Kostić NM (1996) J Am Chem Soc **118**: 5946
[9] Milinković SU, Parac TN, Djuran MI, Kostić NM (1997) J Chem Soc Dalton Trans 2771
[10] Kozlowski H, Matczak-Jon E (1979) Inorg Chim Acta **32**: 143
[11] Wienken M, Zangrando E, Randaccio L, Menzer S, Lippert B (1993) J Chem Soc Dalton Trans 3349
[12] Sayre LM, Reddy KV, Jacobson AR, Tang W (1992) Inorg Chem **31**: 935
[13] Sigel H, Martin RB (1982) Chem Rev **82**: 385
[14] Sigel H, Martin RB (1974) Chem Rev **74**: 471
[15] Sovago I, Martin RB (1981) J Inorg Nucl Chem **43**: 425
[16] Laussac JP, Haran R, Hadjiliadis N (1985) C R Acad Sci Ser 2 **300**: 137
[17] Menabue L, Saladini M, Sola M (1990) Inorg Chem **29**: 1293
[18] Kasselauri S, Garoufis A, Hadjiliadis M, Hadjiliadis N (1990) Coord Chem Rev **104**: 1
[19] Hohmann H, van Eldik R (1990) Inorg Chem Acta **174**: 87
[20] Caubet A, Moreno V, Molins E, Miravitlles C (1992) J Inorg Biochem **48**: 135
[21] Warren RC, McConnell JF, Stephenson NC (1970) Acta Cryst **B26**: 1402
[22] Mehal G, van Eldik R (1985) Inorg Chem **24**: 4165

Received August 31, 1998. Accepted (revised) October 28, 1998