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Hydrolysis of the amide bond in methionine-containing peptides catalyzed by various palladium(II) complexes: Dependence of the hydrolysis rate on the steric bulk of the catalyst

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ABSTRACT

^{1}H NMR spectroscopy was applied to study the reactions of *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complexes (L is en, pic and dpa) with the *N*-acetylated tripeptides L-methionylglycylglycine, MeCOMet–Gly–Gly, and glycyl–L-methionyl–glycine, MeCOGly–Met–Gly. All reactions were performed in the pH range 2.0–2.5 with equimolar amounts of the *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complex and the tripeptide at 60 °C. The hydrolytic reactions of the *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complexes with MeCOMet–Gly–Gly were regioselective and only the amide bond involving the carboxylic group of methionine was cleaved. However, in the reactions of these three Pd(II) complexes with MeCOGly–Met–Gly, two amide bonds, Met–Gly and MeCO–Gly, were cleaved. From UV–Vis spectrophotometry studies, it was found that the rate-determining step of these hydrolytic reactions is the monodentate coordination of the corresponding Pd(II) complex to the sulfur atom of the methionine side chain. The rate of the cleavage of these amide bonds is dependent on the nature of the bidentate coordinated diamine ligand L (en > pic > dpa). The hydrolytic reaction of *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes with MeCOMet–Gly–Gly, containing the methionine side chain in the terminal position of the peptide, is regioselective while in the reaction of these Pd(II) complexes with MeCOGly–Met–Gly, none selective cleavage of the peptide occurs. This study contributes to a better understanding of the selective cleavage of methionine-containing peptides employing palladium(II) complexes as catalysts.

1. Introduction

Recent years have witnessed an increasing interest in the study of the interactions of platinum(II) and palladium(II) complexes with sulfur- and histidine-containing peptides and proteins [1–4]. Interest in the study of these interactions also became of capital importance after the discovery that platinum(II) [5] and palladium(II) [6–13] aqua complexes can be promising reagents for the hydrolytic cleavage of the above-mentioned peptides. These complexes bind to the heteroatom in the side chain of methionine [5–9] or histidine [10–13] and promote cleavage of the amide bond involving the carboxylic group of this anchoring amino acid. The mechanism of this regioselective hydrolytic reaction catalyzed by platinum(II) and palladium(II) aqua complexes is not yet completely understood. For clarification of this mechanism, it was necessary to investigate the influence of different factors, such as pH, temperature and steric effects of the substrate and catalyst, on this hydrolytic reaction.

The hydrolytic reactions of methionine-containing peptides with palladium(II) complexes were studied by Kostić and coworkers in great detail [14]. From NMR spectra, they concluded that different promoters produce different hydrolytically active palladium(II)–peptide complexes. When the promoter was $[PdCl_4]^{2-}$, the active form is a mononuclear palladium(II)–peptide complex (a), while when the promoter was $[Pd(H_2O)_4]^{2+}$, a dinuclear complex with the two sulfur atoms of the two methionine side chains as bridges and water molecules in unspecified terminal positions was produced (b). It was shown that this dinuclear complex is more efficient than the corresponding mononuclear complex in promoting hydrolysis of the scissile amide bond. Additionally, it was also found that with methionine-containing peptides and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ (en is ethylenediamine), *cis*-$[Pd(Me_4en)(H_2O)_2]^{2+}$ (Me_4en is *N,N,N′,N′*-tetramethylethylenediamine) and *trans*-$[Pd(py)_2(H_2O)_2]^{2+}$ (py is pyridine) complexes, the nitrogen coordinated ligands were replaced with water molecules and, presumably, the same active complex (b) as from $[Pd(H_2O)_4]^{2+}$ was formed [15].

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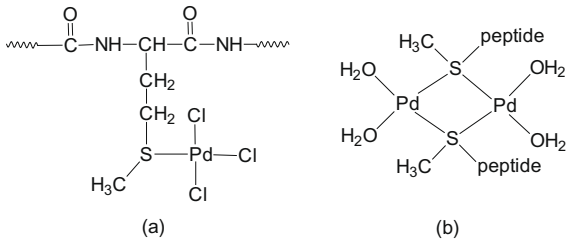


The latter results are in accordance with those recently obtained in one of our laboratories [16] for the reaction of MeCOMet–Gly with different *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes (L is bidentate coordinated en; 1,2-propylenediamine, 1,2-pn; *N*-methylethylenediamine, Meen; *iso*-butylenediamine, ibn; or Me_4en). With all these complexes, in the pH range 2.0–2.5 and at 60 °C, the rate of the regioselective cleavage of the amide bond involving the carboxylic group of methionine was approximately the same and the amount of the hydrolyzed MeCOMet–Gly peptide was between 80% and 88% after 4 h. This was explained by the existence of the same catalytically active form in the reaction of *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complexes with MeCOMet–Gly peptide complex obtained by the replacement of the chelated diamine ligand from the initially formed dinuclear palladium(II)–peptide complex. The above results for MeCOMet–Gly peptide are not in accordance with those obtained for *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes and the MeCOHis–Gly peptide under the same experimental conditions. The observed rates of hydrolytic reaction for the MeCOHis–Gly peptide were investigated in terms of steric hindrance of the chelating diamine on the palladium(II) complexes and the results showed that the rate of the hydrolysis decreased as the steric bulk of the palladium(II) complex increased (en > 1,2-pn > ibn > 1,2-dach > Meen > Me_4en) [17]. Different effects of the palladium(II) catalyst on the hydrolysis rate in reactions with histidine- and methionine-containing peptides can be explained by the different reaction mechanisms of the hydrolytic cleavage of the amide bonds in these peptides.

A better knowledge of the coordination chemistry of methionine-containing peptides with palladium(II) complexes is necessary for an understanding of the effects of palladium(II) complexes on the rate of hydrolysis and the mechanism of the hydrolytic reaction of methionine-containing peptides.

The present study deals with the hydrolysis of the peptide bond in the *N*-acetylated tripeptides: L-methionylglycylglycine, MeCOMet–Gly–Gly, and glycyl–L-methionyl–glycine, MeCOGly–Met–Gly, catalyzed by various palladium(II) complexes of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$, in which L is the bidentate coordinated ethylenediamine (en), 2-picolylamine (pic) and 2,2-dipyridylamine (dpa) ligand.

2. Experimental

2.1. Reagents

Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm^{-1}. The compounds D_2O, DNO_3, NaOD, ethylenediamine (en), 2-picolylamine (pic), 2,2′-dipyridylamine (dpa) and K_2PdCl_4 were obtained from the Aldrich Chemical Co. All common chemicals were of reagent grade. The tripeptides L-methionylglycylglicine (Met–Gly–Gly) and glycyl–L-methionylglycine (Gly–Met–Gly) were obtained from the Sigma Chemical Co. The terminal amino group in the peptides was acetylated by standard methods [6]. As all the solutions were made in D_2O; hence, all the aqua ligands were actually D_2O. However, for simplicity and consistency with our previous publications, the D_2O ligand is shown as H_2O.

2.2. Synthesis of the Pd(II) complexes

The palladium(II) complexes of the type *cis*-$[Pd(L)Cl_2]$ (L is en, pic or dpa ligand) were synthesized according to the procedures published in the literature [18–20]. K_2PdCl_4 was dissolved in water and mixed with an equimolar amount of ligand (L). The pH of the solution was adjusted to *ca.* 3 by addition of 1 M HCl and the mixture was stirred at 80 °C for 2 h. All complexes were crystallized from water at room temperature. The pure complexes were obtained by recrystallization from a small amount of water and cooling. The experimental results of the elemental analysis for C, H and N parameters for all palladium(II) complexes were in accordance with the theoretical values calculated for *cis*-$[Pd(L)Cl_2]$. The chlorido complexes were converted into the corresponding diaqua complexes by treatment with 1.95 equiv. of $AgNO_3$ at pH 2.0 according to a previously published method [21]. In each case, the formed solid AgCl was removed by filtration in the dark and fresh stock solutions of the aqua complexes were stored in a refrigerator until used for reaction with the peptides.

2.3. ^1H NMR measurements

Proton NMR spectra of D_2O solutions containing TSP (3-trimethylsilylpropane-1-sulfonate) as the internal reference were recorded with a Varian Gemini 200 spectrometer. Equimolar amounts of the palladium(II) complex and the peptide were mixed in an NMR tube. The final solution was 10 mM in each reactant. The pH was varied in the range 2.0–2.5. All reactions were performed at 60 °C.

2.4. UV–Vis measurements

The reactions between *N*-acetylated L-methionine, MeCOMet and the *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes (L is en, pic or dpa) were followed on a Perkin Elmer Lambda 35 double-beam spectrophotometer equipped with thermostated 1.00-cm quartz Suprasil cells by measuring the change in absorbance at suitable wavelengths as a function of time. The concentration of the palladium(II) complex was held constant at 1.0×10^{-4} M and the concentration of MeCOMet was varied between 2.2×10^{-3} and 6.5×10^{-3} M. The pH of the reaction mixture was 2.5. After preliminary repetitive scan experiments in the 260–400 nm range to search for isobestic points and spectral changes, the kinetics were studied by measuring the change in absorbance at 280, 292 and 282 nm for *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$, respectively, as a function of time. All reactions were performed at 298 K. The pseudo-first-order rate constants (k_{obs}, s^{-1}) were obtained graphically from a plot of $\ln(A_\alpha - A_t)$ *versus* time [22] (A_t and A_α are the absorbance of the reaction mixture at time t and at the end of the reaction, respectively usually after 10 half-lives).

2.5. pH measurements

All pH measurements were realized at 25 °C using an Iskra MA 5704 pH meter, which had been calibrated with Fischer certified buffer solutions of pH 4.00. The results were not corrected for the deuterium isotope effect.

3. Results and discussion

In the present study, the hydrolytic reactions between various palladium(II) complexes of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$ (L is bidentate coordinated ethylenediamine, en; 2-picolylamine, pic; 2,2-dipyridylamine, dpa) and the *N*-acetylated tripeptides

L-methionylglycylglycine, MeCOMet–Gly–Gly, and glycyl–L-methionyl–glycine, MeCOGly–Met–Gly were studied by ^{1}H NMR spectroscopy. The structures of the palladium(II) complexes and peptides are shown in Fig. 1. The three Pd(II) complexes in Fig. 1 differ in the chelate ligand. The palladium(II) complexes *cis*-[Pd(pic)$(H_2O)_2]^{2+}$ and *cis*-[Pd(dpa)$(H_2O)_2]^{2+}$ in relation to the *cis*-[Pd(en)$(H_2O)_2]^{2+}$ complex are more sterically demanding because the aromatic pyridine rings present in the first two complexes are bound directly to the metal center, hindering the approach of a nucleophile. The tripeptides MeCOMet–Gly–Gly and MeCOGly–Met–Gly differ in the position of the methionine side chain which could also have some effects on their reactivity with palladium(II) complexes, as well as on the selectivity of this hydrolytic reaction. The reactions between these palladium(II) complexes and methionine-containing tripeptides were performed at 60 °C and at 2.0 < pH < 2.5. As was shown in previous studies [10–12,23], acidic solutions are required to suppress the formation of hydroxo-bridged oligomeric palladium(II) complexes, which are catalytically inactive. In the reactions of MeCOMet–Gly–Gly with the investigated *cis*-[Pd(L)$(H_2O)_2]^{2+}$-type complexes, regioselective cleavage of the amide bond involving the carboxylic group of methionine was observed, while in the reactions between MeCO-Gly–Met–Gly and the investigated Pd(II) complexes, two amide bonds, Met–Gly and MeCO–Gly, were cleaved; see Fig. 2. The mixing of the palladium(II) complex with an equimolar amount of the peptide under the above-described experimental conditions resulted in the spontaneous coordination of the palladium(II) complex to the sulfur atom of the methionine residue. The binding of the palladium(II) to the methionine side chain was registered from the simultaneous decline of the resonance at 2.11 ppm due to the *S*-methyl protons of the free peptide and the growth of a resonance at 2.54 ppm, corresponding to the *S*-methyl protons of the peptide coordinated to palladium(II) through the sulfur atom.

3.1. Reactions of palladium(II) complexes with MeCOMet–Gly–Gly

The schematic presentation of the reactions of *cis*-[Pd(L)$(H_2O)_2]^{2+}$-type complexes (L is en, pic or dpa) with the MeCO-Met–Gly–Gly tripeptide is given in Fig. 2. The hydrolytically active palladium(II)–peptide complexes in these reactions is the dinuclear complex **2**, with the two sulfur atoms of the two methionine side chains as bridges and water molecules in unspecified terminal positions. Previous studies with *cis*-[Pd(L)$(H_2O)_2]^{2+}$-type complexes and methionine-containing peptides showed that a similar *S*-bridged dinuclear complex was also formed at pH < 3 [14–16]. The complexes **2** were formed from the intermediate dinuclear palladium(II)–peptide complex **1** through the detachment of the chelate ligand L from Pd(II) and its replacement by two water molecules. The replacement of the ligand L by water molecules in the investigated reactions was very fast and it was additionally supported by the *trans*-effect of the bridged sulfur atom of the methionine side chain and the acidic medium (2.0 < pH < 2.5) [24]. In the reaction between the MeCOMet–Gly–Gly tripeptide and *cis*-[Pd(en)$(H_2O)_2]^{2+}$ complex, this replacement reaction was observed in the ^{1}H NMR spectrum by the simultaneous decline of the singlet at 2.86 ppm, due to protons of the bidentate

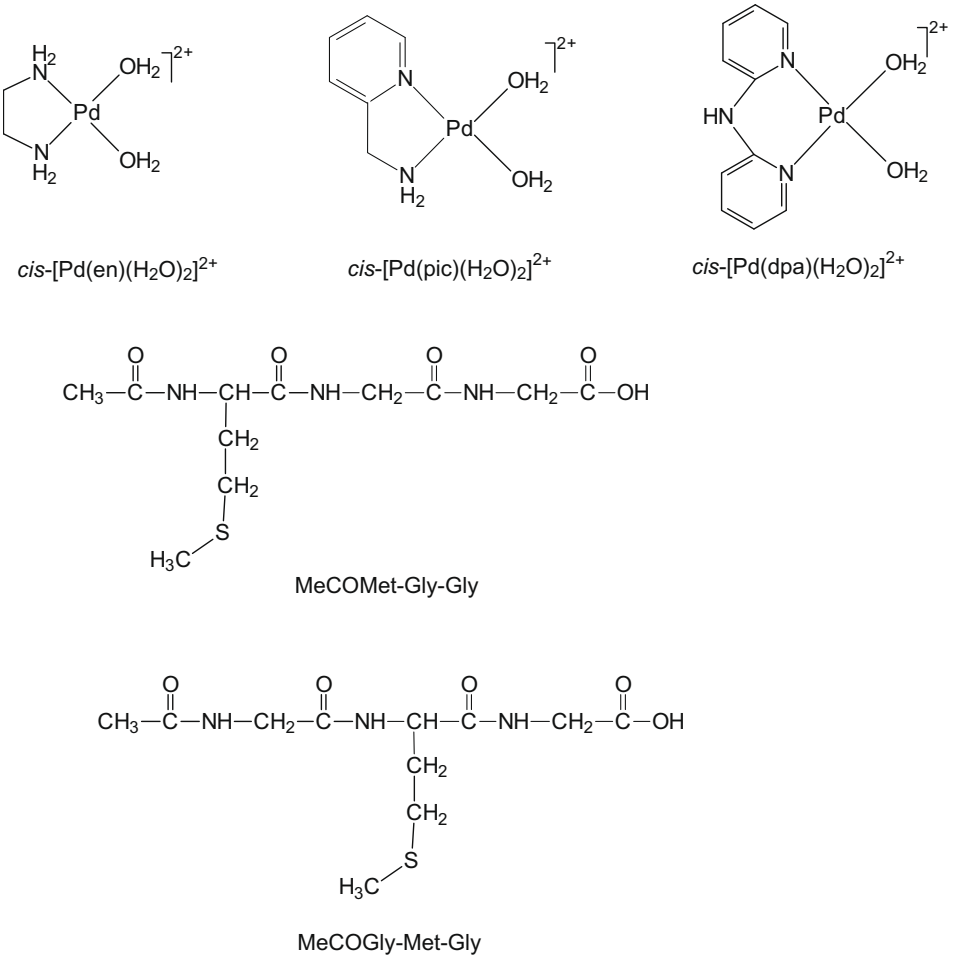


Fig. 1. The palladium(II) complexes and methionine-containing peptides employed in this study.

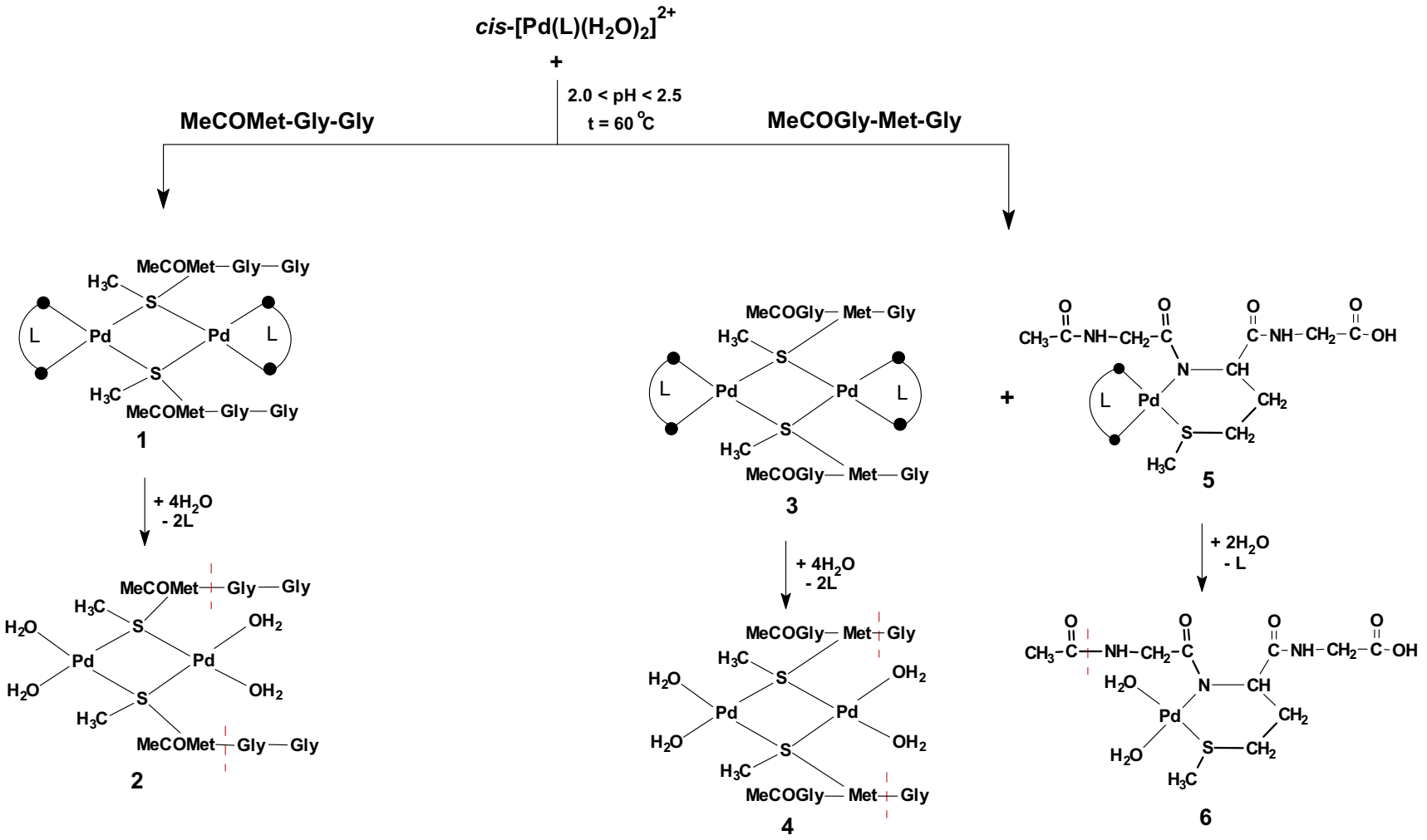


Fig. 2. The reaction scheme of *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes (L = en, pic and dpa) with MeCOMet–Gly–Gly and MeCOGly–Met–Gly peptides in the pH range 2.0 < pH < 2.5 and at 60 °C.

coordinated en ligand, and the growth of the signal at 3.36 ppm, due to free H_2en^{2+} ligand. In the reactions of this tripeptide with the other two complexes, *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$, owing to the overlap of the signals corresponding to the protons of the free pic and dpa ligands with those of the palladium(II)–peptide complex **1**, giving a multiplet in the region 7.00–8.50 ppm, the displacement of these two ligands by water molecules could not be followed by ^{1}H NMR spectroscopy.

It was found that in the reactions of MeCOMet–Gly–Gly with the investigated *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes, the ^{1}H NMR resonance at δ 3.98 ppm, corresponding to the glycine protons (glycine next to the methionine) of the none hydrolyzed peptide decreased, while that at δ 3.89 ppm for free Gly–Gly increased. Upon addition of Gly–Gly to the reaction mixture, the resonance at 3.89 ppm was enhanced. The amounts of unreacted tripeptide and the hydrolysis products were determined from the known initial concentration of MeCOMet–Gly–Gly and from the integrated resonance of the free Gly–Gly. The catalytic ability of each *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complex in the investigated reactions was determined by measuring the amount of the hydrolyzed peptide with time under the same experimental conditions; see Fig. 3a. From this figure, it can be concluded that the rate of hydrolysis decreased as the steric bulk of the palladium(II) complex increased (en > pic > dpa). In the reaction between *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and MeCOMet–Gly–Gly more than 95% of the Met–Gly amide bond had been hydrolyzed after 4.5 h. The reaction of this tripeptide with the other two complexes was much slower with 55% of the Met–Gly amide bond having been cleaved during this time with the *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ complex and only 15% with the *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complex (Fig. 3a). The reaction of MeCOMet–Gly–Gly with these three Pd(II) complexes was followed for 30 h. It was found that with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(pic)(H_2O)_2]^{2+}$, the hydrolytic cleavage of MeCOMet–Gly–Gly was almost completed while with the *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complex only 35% of this tripeptide had been cleaved during this time. No cleavage of the other amide bonds in MeCOMet–Gly–Gly was observed during this time.

3.2. Reactions of the palladium(II) complexes with MeCOGly–Met–Gly

When an equimolar amount of *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complex was incubated with MeCOGly–Met–Gly at 2.0 < pH < 2.5 at 60 °C, two palladium(II)–peptide complexes, **3** and **5**, were formed in a molar ratio 3:1, respectively; see Fig. 2. The complexes **3** and **5** are intermediate products and after detachment of the chelate ligand L from Pd(II) and its replacement by water molecules, these complexes were converted into the hydrolytically active palladium(II)–peptide complexes **4** and **6**, respectively. In the present study, this replacement reaction was followed in the same manner as for the reaction of the *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes with the MeCOMet–Gly–Gly tripeptide (see previous section). The hydrolytically active complex **4** was responsible for the cleavage of the Met–Gly amide bond in the MeCOGly–Met–Gly tripeptide. The new signal in the ^{1}H NMR spectrum at 3.71 ppm was assigned to the methylene protons of the free glycine obtained by the cleavage of the Met–Gly amide bond. This signal increased with time and the concentrations of the peptide and the hydrolysis products were determined from the known initial concentration of the MeCOGly–Met–Gly tripeptide and from the integrated resonance of the free glycine. The catalytic ability of each of the *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes was determined by measuring the amount of the hydrolyzed peptide with time under the same experimental conditions. In the reaction of the *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes with MeCOGly–Met–Gly, the rate of hydrolysis decreased as the steric bulk of the palladium(II) complex increased (en > pic > dpa); see Fig. 3b. For the reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ with MeCOGly–Met–Gly, 40% of the Met–Gly amide bond had been hydrolyzed after 4.5 h. The reaction with *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ was two times slower and during this time only 20% of the peptide had been cleaved. Moreover, in the reaction with the *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complex, no hydrolysis of the Met–Gly amide bond in the same peptide was observed (Fig. 3b).

The mononuclear complex **5**, which is responsible for the cleavage of the MeCO–Gly amide bond in MeCOGly–Met–Gly, was obtained by coordination of the deprotonated amide nitrogen atom as the second step after monodentate coordination of the palladium(II) catalyst to the methionine sulfur atom. Palladium(II) is one of the most effective transition-metal ions in displacing an amide proton [25]. The estimated pK_a for this reaction affected by palladium(II) is ≈2, and displacement was observed even in a solution with pH < 2.0 [25–31]. The absence of cleavage of the Gly–Met bond in this peptide is in accordance with the fact that binding of the methionine sulfur–anchored palladium(II) atom to the deprotonated nitrogen in the amide bond stabilizes the C–N bond and inhibits its cleavage [8,25,32]. However, it was found in the present study that this complex plays very important role in the cleavage of the MeCO–Gly amide bond in MeCOGly–Met–Gly. The cleavage of this amide bond occurred after conversion of

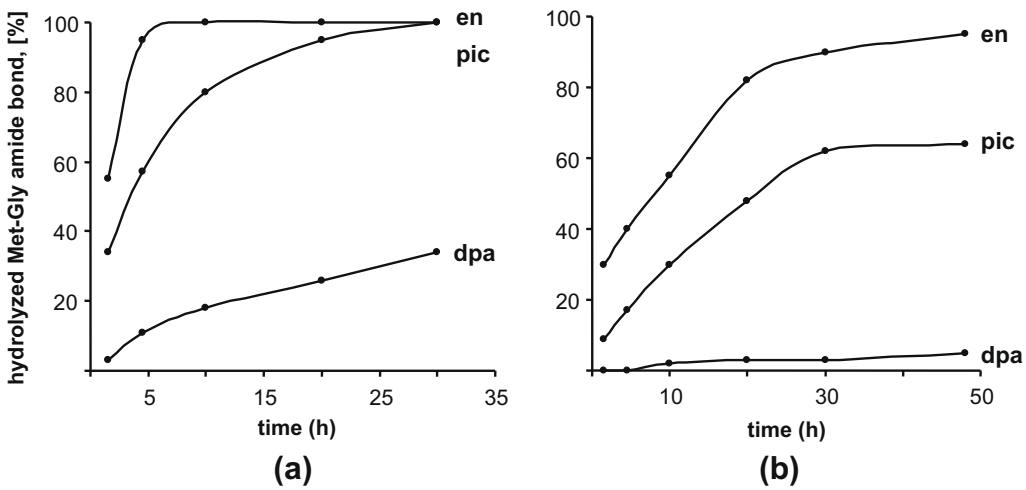
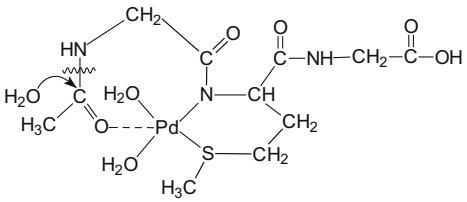
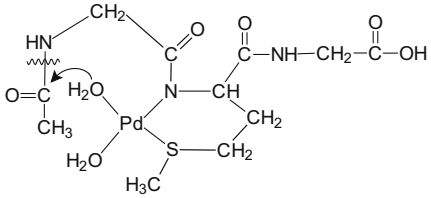


Fig. 3. The time dependence of the hydrolytic cleavage of the Met–Gly amide bond in methionine-containing peptides with different *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes (L is en, pic or dpa) at 2.0 < pH < 2.5 and at 60 °C: (a) MeCOMet–Gly–Gly and (b) MeCOGly–Met–Gly.

complex **5** into complex **6** by replacement of the ligand L with two water molecules. This replacement reaction is additionally supported by the *trans* influence of the negative charge on the deprotonated amide nitrogen and the acidic medium. Due to the fact that complexes **3** and **5** are simultaneously present in the reaction mixture, the replacement of L can be followed as an overall process. Hydrolysis of the MeCO–Gly amide bond can occur by two limiting mechanisms as represented in the scheme below. The first possibility is that palladium(II) acts as a Lewis acid and forms a chelate involving a deprotonated amide nitrogen of the amino group of methionine and the oxygen atom of the scissile amide bond. The interaction with the oxygen atom polarizes the carbonyl group and activates the carbon atom toward attack by a water molecule from the solvent (*external attack*). For the reaction to occur by this mechanism, the palladium(II) and carbonyl oxygen atoms should be proximate.



external attack



internal delivery

Another possibility is that an aqua ligand on the palladium(II) is delivered to the carbon atom in the amide bond (*internal attack*). For the cleavage of the amide bond to occur by this mechanism, an aqua ligand at palladium(II) should be proximate to the carbonyl carbon of the scissile amide bond. In the reaction between *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes and the MeCOGly–Met–Gly tripeptide, the ^1H NMR resonance at 2.05 ppm due to the CH_3 protons of the acetyl group in the substrate decreased, while that at 2.08 ppm for the CH_3 protons of free acetic acid increased. Upon addition of acetic acid to the reaction mixture, this resonance was enhanced. The concentrations of the peptide and the hydrolysis products were determined from the known initial concentration of MeCOGly–Met–Gly and from the integrated resonance of free acetic acid. When this reaction was performed with *cis*-$[Pd(en)(H_2O)_2]^{2+}$, approximately 15% of the MeCO–Gly amide bond had been hydrolyzed after 30 h. This reaction is dependent on the nature of the ligand L and the number of hydrolyzed MeCO–Gly amide bonds decreased in the order en > pic > dpa.

4. Conclusions

From the present investigation of the reactions of *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes with two methionine-containing tripeptides, MeCOMet–Gly–Gly and MeCOGly–Met–Gly, at 2.0 < pH < 2.5 and at 60 °C, the following conclusions can be drawn. The rate of the hydrolytic cleavage of the amide bond in the reactions of *cis*-$[Pd(L)(H_2O)_2]^{2+}$-type complexes with the two methionine-containing peptides is strongly dependent on the nature of the bidentate coordinated diamine ligand L. It was found that the rate of hydrolysis decreased as the steric bulk of the palladium(II) complex increased (en > pic > dpa); see Figs. 3 and 4. The first step of these hydrolytic reactions is the monodentate coordination of the corresponding Pd(II) complex to the sulfur atom of the methionine side chain. The rate reactivity of the Pd(II) complexes with the methionine side chain is also the rate-determining step for the hydrolytic cleavage of the amide bonds in the investigated peptides. For confirmation of this statement, in the present study, separate experiments between the corresponding *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complex and *N*-acetylated L-methionine, MeCO-Met, were investigated by UV–Vis spectrophotometry. The reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complexes with MeCOMet was followed at pH 2.5 and at room temperature by measuring the change in absorbance at suitable wavelengths as a function of time. The use of a large excess of ligand ([MeCOMet]:[Pd(II) complex] > 20:1) provided for pseudo-first-order rate conditions, which allowed k_{obs} to be calculated at four different MeCOMet concentrations. The second-order rate constants were determined from a plot of k_{obs} *versus* [MeCO-Met]. Dinuclear Pd(II)–amino acid complexes with the two sulfur atoms of the two methionine residues as bridges in the reactions between these three Pd(II) complexes and MeCOMet were expected [14–16]. The reaction of MeCOMet with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ was two times faster ($k_2 = 1.75 \times 10^{-1}\ M^{-1}\ s^{-1}$) than with *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ ($k_2 = 8.29 \times 10^{-2}\ M^{-1}\ s^{-1}$) and approximately 36 times faster than with *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ ($k_2 = 4.87 \times 10^{-3}\ M^{-1}\ s^{-1}$). These results are in accordance with the fact that the latter two complexes are more sterically demanding than the *cis*-$[Pd(en)(H_2O)_2]^{2+}$ complex. The obtained results from the UV–Vis measurements can be taken as additional arguments for the fact that inhibition of the hydrolytic reaction of the methionine-containing peptides is strongly dependent on the steric bulk of the *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complex.

Additionally, from the present study, it can be concluded that position of the methionine side chain in the peptide plays a role in the cleavage selectivity as well as in the rate of this hydrolytic reaction. Hydrolytic reaction of *cis*-$[Pd(en)(H_2O)_2]^{2+}$, *cis*-$[Pd(pic)(H_2O)_2]^{2+}$ and *cis*-$[Pd(dpa)(H_2O)_2]^{2+}$ complexes with MeCOMet–Gly–Gly tripeptide under the above-mentioned experimental conditions was regioselective and only the amide bond involving the carboxylic group of the methionine was cleaved (Fig. 2). However, the hydrolytic reaction of these three Pd(II) complexes with MeCOGly–Met–Gly tripeptide was none selective and under the investigated conditions, two amide bonds, Met–Gly and MeCO–Gly, were cleaved

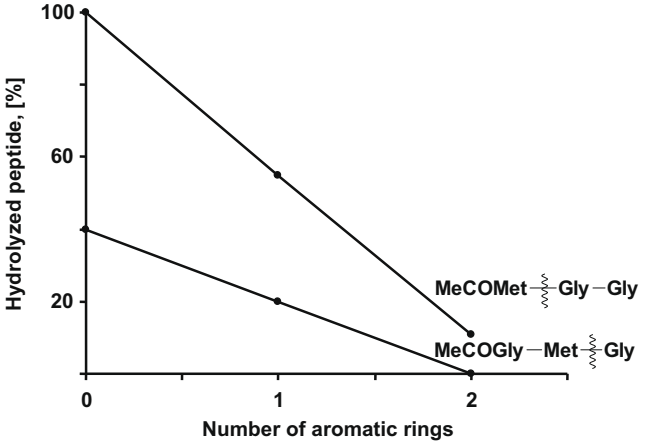


Fig. 4. The dependence of the rate of hydrolysis of the Met–Gly amide bond in methionine-containing peptides on the steric bulk of the palladium(II). The reactions were performed for 4.5 h at 2.0 < pH < 2.5 and at 60 °C.

(Fig. 2). The cleavage of these two amide bonds is a parallel process and also strongly dependent on the steric bulk of the Pd(II) complex.

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