PERGAMON

www.elsevier.nl/locate/poly

Polyhedron 19 (2000) 959–963

POLYHEDRON

Selective hydrolysis of the unactivated peptide bond in *N*-acetylated L-histidylglycine catalyzed by various palladium(II) complexes: dependence of the hydrolysis rate on the steric bulk of the catalyst

Miloš I. Djuran *, Snežana U. Milinković

Department of Chemistry, Faculty of Science, University of Kragujevac, R. Domanovića 12, P.O. Box 60, 34000 Kragujevac, Yugoslavia

Received 10 December 1999; accepted 24 February 2000

Abstract

Hydrolytic reactions between various palladium(II) complexes of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$ in which L is a chelating diamine (ethylenediamine, en; 1,2-propylenediamine, 1,2-pn; *N*-methylethylenediamine, Meen; isobutylenediamine, ibn and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Me_4en) or *S*,*N*-coordinated amino acid (*S*-methyl L-cysteine, MeS-L-HCys and L-methionine, L-HMet) and *N*-acetylated L-histidylglycine (MeCO-His-Gly), were studied by ^{1}H NMR spectroscopy. The reactions were carried out in the pH range 2.0–2.5 and at 60°C. In all these reactions, a palladium(II) complex bound to a histidine residue effects the regioselective cleavage of the amide bond involving the carboxylic group of histidine. We found that the rate of hydrolysis decreases as the steric bulk of the palladium(II) complex increases (en > 1,2-pn > Meen > MeS-L-HCys > ibn > L-HMet > Me_4en). The observed rates of hydrolytic reaction are discussed in terms of steric hindrance of the chelating diamine or sulfur-containing amino acid on the palladium(II) complexes. This study is an important step in the development of new palladium(II) complexes as artificial metallopeptidases.

Keywords: Palladium(II) complexes; Catalysts; Peptides; Hydrolysis

1. 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 a 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.

Transition-metal complexes hold promise as new reagents for protein cleavage by various mechanisms. Several cobalt(III) complexes coordinate to peptides and facilitate hydrolysis, but they cleave only the *N*-terminal amino acid [2]. However, practical applications usually require cleavage of internal amide bonds. Studies with some iron-EDTA complexes that are covalently attached to amino acid side chains revealed some mechanistic features of these hydrolytic reactions, but they did not result in practical methods for analytical biochemistry [3–12]. Although in some cases the cleavage was fast, the synthetic work involved in the attachment and the additional chemicals required for cleavage limit the applicability of this method. Recently, Kostić and coworkers have shown that platinum(II) [13] and palladium(II) [14–21] aqua complexes can be promising reagents for the hydrolytic cleavage of peptides and proteins. These complexes bind to the heteroatom in the side chain of methionine [13–17] or histidine [18–21] and promote cleavage of the amide bond involving the carboxylic group of this anchoring amino acid. Palladium(II) complexes, as cleavage agents, differ from the well known complexes of other transition metals because their attachment to substrates is achieved simply by mixing, the hydrolysis reactions have half-lives as short as 15 min, and the complexes are easily removed from the cleaved substrates.

The consistent regioselectivity in the cleavage of methionine or histidine containing peptides promoted by palladium(II) aqua complexes and the mechanism of this hydrolytic reaction are 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 peptide and protein cleavage promoted by such complexes. This paper reports a ^{1}H NMR investigation of the hydrolysis reac-

* Corresponding author. Tel.: +381-34-335-039; fax: +381-34-335-040; e-mail: djuran@uis0.uis.kg.ac.yu or djuran@knez.uis.kg.ac.yu

0277-5387/00/$ - see front matter
PII S0277-5387(00)00342-9

tion of a model histidine-containing peptide, *N*-acetylated L-histidylglycine, catalyzed by a series of palladium(II) complex cations of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$ in which L is a chelating diamine ligand, which differ in the number of methyl groups at the nitrogen or carbon atom, or *S,N*-coordinated amino acid. The observed rates of reaction are explained in terms of steric hindrance of the chelating ligand on the palladium(II) complexes.

2. Experimental

2.1. Materials

Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm. The compounds D_2O, DNO_3, NaOD, and $K_2[PdCl_4]$ were obtained from Aldrich Chemical Co. All common chemicals were of reagent grade. Dipeptide L-histidylglycine, amino acids, *S*-methyl-L-cysteine and L-methionine and all diamines were obtained from Sigma Chemical Co. The terminal amino group in His-Gly was acetylated by standard methods [14].

2.2. Synthesis of palladium(II) complexes

The palladium(II) complexes of the type *cis*-$[Pd(L)Cl_2]$ (L is ethylenediamine, en; *N*-methylethylenediamine, Meen; 1,2-propylenediamine, 1,2-pn; *S*-methyl L-cysteine, MeS-L-HCys; L-methionine, L-HMet; *N,N,N′,N′*-tetramethylethylenediamine, Me_4en; isobutylenediamine, ibn) were synthesized according to the procedures published in the literature [22–24]. K_2PdCl_4 was dissolved in water and mixed with an equimolar amount of diamine or amino acid. 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 are in accordance with theoretical values calculated for *cis*-$[Pd(L)Cl_2]$ complexes. The chloro complexes were converted into the corresponding diaqua complexes by treatment with 2 equiv. of $AgNO_3$ at pH 2.0 according to a published method [25]. In each case, the solid AgCl was removed by filtration in the dark, and fresh stock solution of the aqua complex was used in further experiments.

2.3. Reactions of peptide with palladium(II) complexes

Reactions of MeCO-His-Gly with palladium(II) complexes were followed 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 mM in each reactant. The pH was varied in the range 2.0–2.5. All reactions were carried out at 60°C.

2.4. pH 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-trimethylsilylpropane-1-sulfonate) as internal reference were recorded with Varian Gemini 200 spectrometer.

3. Results and discussion

In the present study hydrolytic reactions between various complexes of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$ (L is ethylenediamine, en; 1,2-propylenediamine, 1,2-pn; *N*-methylethylenediamine, Meen; *S*-methyl L-cysteine, MeS-L-HCys; isobutylenediamine, ibn; L-methionine, L-HMet; *N,N,N′,N′*-tetramethylethylenediamine, Me_4en) and *N*-acetylated L-histidylglycine, MeCO-His-Gly, were studied by ^{1}H NMR spectroscopy. The palladium(II) complexes are as shown in Fig. 1. The different chelate ligands (L) in these complexes are inert to substitution and are expected to remain bound to the palladium(II) atom during the reactions with the amino acids and peptides. The course of the hydrolytic reaction at pH 2.0 is shown in Scheme 1. As was shown in previous studies [18–20,26], acidic solutions are needed to suppress the formation of hydroxo-bridged oligomeric palladium(II) complexes, which are catalytically inactive.

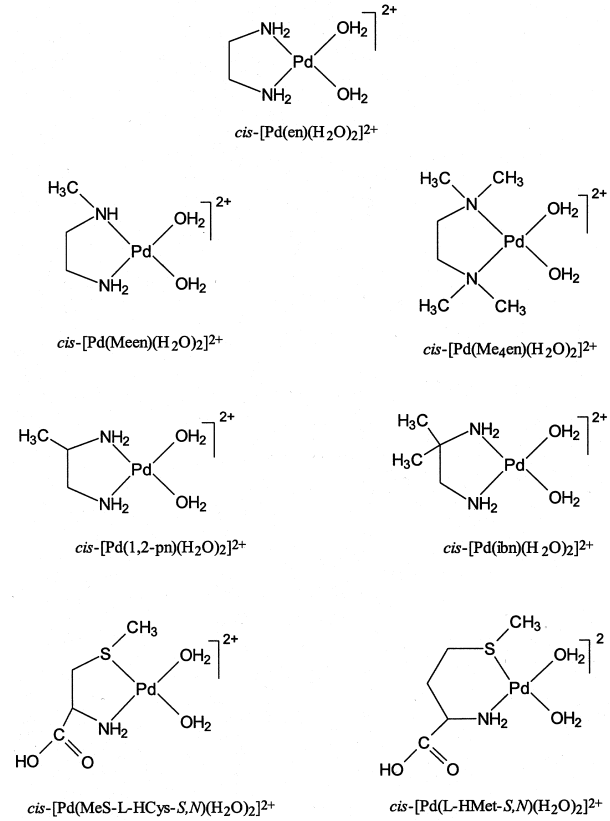
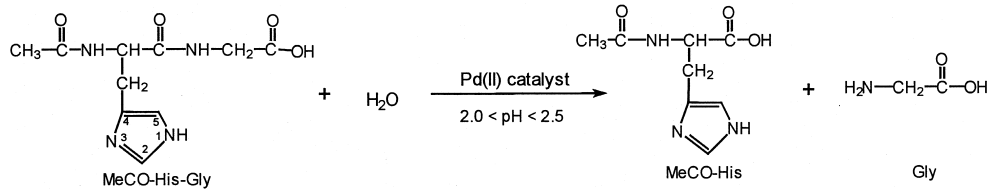


Fig. 1. Palladium(II) complexes used in the reaction with MeCO-His-Gly.



Scheme 1.

3.1. Binding of the catalysts to the substrate

The previous study with dipeptide MeCO-Gly-His and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ showed that at pH < 3 five palladium(II)-peptide products formed [18–20]. These complexes were distinguished on the basis of the chemical shifts of the two imidazole protons, H-2 and H-5. The two major complexes are linkage isomers of each other with unidentate coordination of palladium(II) via the N-3 or N-1 atom to the imidazole ring. The three minor complexes contain more than one palladium(II) atom per dipeptide or involve more than one donor atom in the dipeptide. The experiments with MeCO-His-Gly selectively methylated at the N-1 or N-3 atom of imidazole and *cis*-$[Pd(en)(H_2O)_2]^{2+}$ showed that only the palladium(II) complex with monodentate coordination to the N-3 atom of imidazole can effect the cleavage of the amide bond involving the carboxylic group of histidine (Fig. 2); none of the four other modes of coordination is effective [19]. The cleavage of the amide bond is regioselective and the reaction is completed in less than 72 h. Also, the experiments with different histidine-containing peptides and different palladium(II) complexes showed that only unidentate coordination of the peptide via the N-3 atom of the imidazole to the Pd(II) ion effects hydrolytic cleavage of the amide bond. This was explained through the fact that this coordination mode permits the necessary close approach of the palladium(II) ion and of its aqua ligand to the scissile peptide bond [18,20].

When an equimolar amount of *cis*-$[Pd(L)(H_2O)_2]^{2+}$, where L is Meen, Me_4en, or 1,2-pn, was incubated with MeCO-His-Gly at 60°C and 2.0 < pH < 2.5, five NMR-detectable complexes formed. The complexes were distinguished on the basis of the chemical shifts of imidazole protons. These complexes are identical with those for the reaction of this peptide with *cis*-$[Pd(en)(H_2O)_2]^{2+}$ [18–20]. In the reaction between *cis*-$[Pd(ibn)(H_2O)_2]^{2+}$ and MeCO-His-Gly at 2.0 < pH < 2.5, only four complexes formed. The complex with bidentate coordination of peptide via the N-3 atom of the imidazole ring and deprotonated peptide nitrogen atom was not detected in solution even after the reaction mixture stayed for 5 days at 60°C. This is in accordance with the fact that two methyl groups on the ibn ligand cause steric crowding on palladium(II) and do not allow its interaction with the peptide nitrogen atom. Mixing of *cis*-$[Pd(L)(H_2O)_2]^{2+}$, where L is MeS-L-HCys or L-HMet, with an equimolar amount of MeCO-His-Gly under the same experimental conditions resulted in a spontaneous formation of three NMR-detectable complexes. In the two complexes peptide is a unidentate ligand coordinated via the N-3 or N-1 atom of the imidazole ring to palladium(II). In the third palladium(II)-peptide complex bidentate coordination of the peptide occurs via the N-3 atom of imidazole and the deprotonated peptide nitrogen atom of the terminal amino group of histidine.

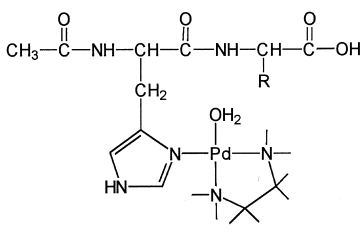


Fig. 2. Catalytically active form of the palladium(II)-peptide complex.

3.2. Regioselective cleavage of the peptide

The reactions between palladium(II) complexes of the type *cis*-$[Pd(L)(H_2O)_2]^{2+}$ and MeCO-His-Gly were carried out at 60°C and at 2.0 < pH < 2.5. The obtained results compared with those for *cis*-$[Pd(en)(H_2O)_2]^{2+}$ [18–20]. In all these reactions only the cleavage of the amide bond involving the carboxylic group of histidine was observed; see Scheme 1. In the reaction between the palladium(II) complex and MeCO-His-Gly, the ^{1}H NMR resonance at δ 3.96 ppm in the peptide decreased, while that at $\delta \sim 3.80$ ppm for free glycine increased. Upon addition of glycine to the reaction mixture its resonance is enhanced. The concentrations of the peptide and the hydrolysis products were determined from the known initial concentration of MeCO-His-Gly and from integrated resonance of the free glycine. Some of the liberated glycine reacts with the catalyst to form a small amount of the bis(bidentate) complex *cis*-$[Pd(L)(Gly\text{-}N,O)]^+$, easily detected by ^{1}H NMR spectroscopy by the resonance at δ 3.52 ppm. Indeed, the same complex is formed upon mixing of equimolar amounts of *cis*-$[Pd(L)(H_2O)_2]^{2+}$ and glycine.

3.3. Steric effects of the catalyst

The seven Pd(II) complexes in Fig. 1 differ in the chelate ligand. The palladium(II) complexes *cis*-$[Pd(Meen)(H_2O)_2]^{2+}$, *cis*-$[Pd(Me_4en)(H_2O)_2]^{2+}$, *cis*-$[Pd(1,2\text{-}pn)(H_2O)_2]^{2+}$ and *cis*-$[Pd(ibn)(H_2O)_2]^{2+}$ in relation to *cis*-$[Pd(en)(H_2O)_2]^{2+}$ have a bidentate ligand with different numbers of methyl groups at the nitrogen or carbon atom. These methyl groups contribute to the steric bulk of the palladium(II) complex. From Fig. 3 it can be concluded that the

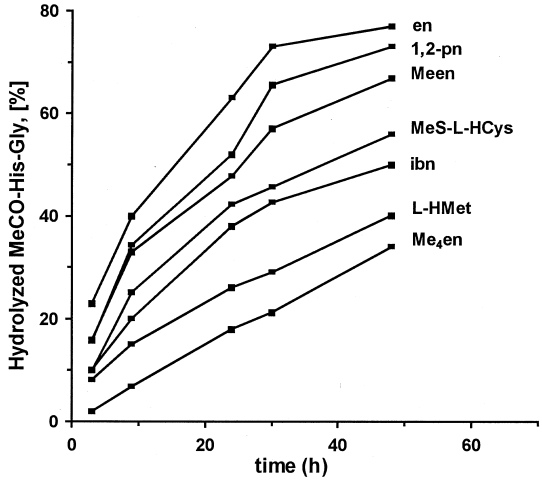


Fig. 3. Time dependence of hydrolytic reaction of MeCO-His-Gly with different palladium(II) complexes at $2.0 < pH < 2.5$ and at 60°C.

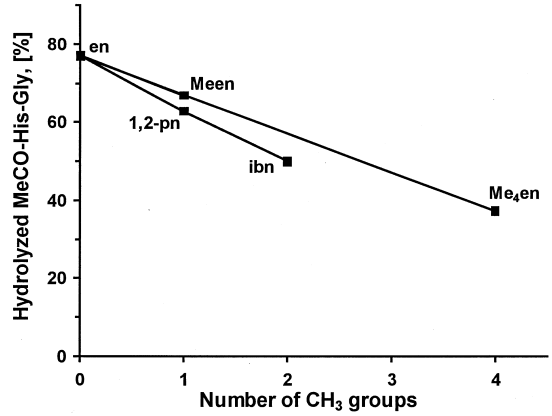
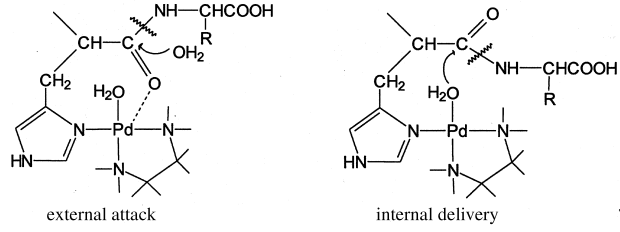


Fig. 4. Dependence of the hydrolysis rate on the steric bulk of the palladium(II). The reactions were carried out for 48 h at $2.0 < pH < 2.5$ and at 60°C.



Scheme 2.

rate of hydrolysis decreases as the steric bulk of the palladium(II) complex increases (en > 1,2-pn > Meen > MeS-L-HCys > ibn > L-HMet > Me_4en). The reaction between *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and MeCO-His-Gly after 48 h at 60°C is almost two times faster in comparison with *cis*-$[Pd(Me_4en)(H_2O)_2]^{2+}$, or one and a half times faster in relation to *cis*-$[Pd(ibn)(H_2O)_2]^{2+}$. On comparing the ligand 1,2-pn with Meen, or Me_4en with ibn, we found that the former are more sterically demanding because the methyl groups bound directly to the nitrogen atoms are closer to the metal center, hindering the approach of a nucleophile. It is very illustrative to compare the rate of reactivity of MeCO-His-Gly with en, 1,2-pn and ibn, or this peptide with en, Meen and Me_4en palladium(II) complexes, see Fig. 4. It is obvious that increasing the number of methyl groups at the nitrogen or carbon atom of the chelate ligand decreases the amount of hydrolyzed His-Gly peptide bond. For comparison, parallel work has been done with MeCO-His-Gly and *cis*-[Pd(MeS-L-HCys-*S,N*)$(H_2O)_2]^{2+}$ and *cis*-[Pd(L-HMet-*S,N*)-$(H_2O)_2]^{2+}$. These two complexes differ in the ring size of the chelate ligand. The MeS-L-HCys-palladium(II) complex with five-membered chelate ring is more reactive than L-HMet-palladium(II) with six-membered ring in the square-planar plane. This inhibition of the hydrolytic reaction can be attributed to the steric bulk of the palladium(II) complex. A similar trend to our results has emerged in a previous study of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and MeCO-His-X peptides containing different C-terminal amino acids (X is Gly, Ala, Ser, Thr, Leu, Phe, Tyr) [18]. It was found that the rate constant of the hydrolytic reaction decreases smoothly as the volume of the leaving amino acid increases.

Summing up together our results with different palladium(II) complexes and previous results with different peptides [18], we can say that inhibition of the hydrolytic reaction can be attributed to the steric bulk of the palladium(II) complex as well as to the steric bulk of the leaving amino acid. These findings can be explained in terms of two possible limiting mechanisms for hydrolytic cleavage of the peptide bond promoted by palladium(II) [18–20], see Scheme 2. In the mechanism involving external attack by water, the palladium(II) atom, as a Lewis acid, polarizes the carbonyl group in the scissile peptide bond. In the mechanism involving internal attack by water, the palladium(II) atom delivers an aqua ligand to the scissile bond. As can be seen, both mechanisms require a close approach of the pendant catalyst to the adjacent peptide bond, and in both cases the steric bulk of the leaving amino acid as well as palladium(II) complex hinder this crucial interaction.

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, IA, USA) and his coworkers for providing K_2PdCl_4 and the peptides. We thank also B. Mojsilović for assistance with recording the ^{1}H NMR spectra.

References

[1] L.R. Croft, Handbook of Protein Sequence Analysis, Wiley, Chichester, 2nd edn., 1980.
[2] J. Suh, Acc. Chem. Res. 25 (1992) 273 and Refs. therein.
[3] T.M. Rana, C.F. Meares, Proc. Natl. Acad. Sci. USA 88 (1991) 10578.
[4] T.M. Rana, C.F. Meares, J. Am. Chem. Soc. 113 (1991) 1859.

[5] T.M. Rana, C.F. Meares, J. Am. Chem. Soc. 112 (1990) 2457.
[6] M.R. Ermácora, J.M. Delfino, B. Cuenoud, A. Schepartz, R.O. Fox, Proc. Natl. Acad. Sci. USA 89 (1992) 6383.
[7] I.E. Platis, M.R. Ermácora, R.O. Fox, Biochemistry 32 (1993) 12761.
[8] A. Schepartz, B. Cuenoud, J. Am. Chem. Soc. 112 (1990) 3247.
[9] D. Hoyer, H. Cho, P.G. Schultz, J. Am. Chem. Soc. 112 (1990) 3249.
[10] N. Ettner, W. Hillen, G.A. Ellestad, J. Am. Chem. Soc. 115 (1993) 2546.
[11] J.B. Ghaim, D.P. Greiner, C.F. Meares, R.B. Gennis, Biochemistry 34 (1995) 11311.
[12] M.R. Ermácora, D.W. Ledman, R.O. Fox, Nat. Struct. Biol. 3 (1996) 59.
[13] I.E. Burgeson, N.M. Kostić, Inorg. Chem. 30 (1991) 4299.
[14] L. Zhu, N.M. Kostić, Inorg. Chem. 31 (1992) 3994.
[15] L. Zhu, N.M. Kostić, J. Am. Chem. Soc. 115 (1993) 4566.
[16] L. Zhu, N.M. Kostić, Inorg. Chim. Acta 217 (1994) 21.
[17] E.N. Korneeva, M.V. Ovchinnikov, N.M. Kostić, Inorg. Chim. Acta 243 (1996) 9.
[18] T.N. Parac, N.M. Kostić, J. Am. Chem. Soc. 118 (1996) 51.
[19] T.N. Parac, N.M. Kostić, J. Am. Chem. Soc. 118 (1996) 5946.
[20] S.U. Milinković, T.N. Parac, M.I. Djuran, N.M. Kostić, J. Chem. Soc., Dalton Trans. (1997) 2771.
[21] X. Chen, L. Zhu, H. Yan, X. You, N.M. Kostić, J. Chem. Soc., Dalton Trans. (1996) 2653.
[22] H. Hohmann, R. van Eldik, Inorg. Chim. Acta 174 (1990) 87.
[23] A. Caubet, V. Moreno, E. Molins, C. Miravitlles, J. Inorg. Biochem. 48 (1992) 135.
[24] R.C. Warren, J.F. McConnell, N.C. Stephenson, Acta Crystallogr., Sect. B 26 (1970) 1402.
[25] G. Mehal, R. van Eldik, Inorg. Chem. 24 (1985) 4165.
[26] A.F.M. Siebert, Ph.D. Dissertation, University of Bochum, Germany, 1995, p. 166.