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Use of diethanolammonium–tetrachloridopalladate(II) complex in bioorganic modelling as artificial metallopeptidase in the reaction with *N*-acetylated *L*-methionylglycine dipeptide. NMR and DFT study of the hydrolytic reaction

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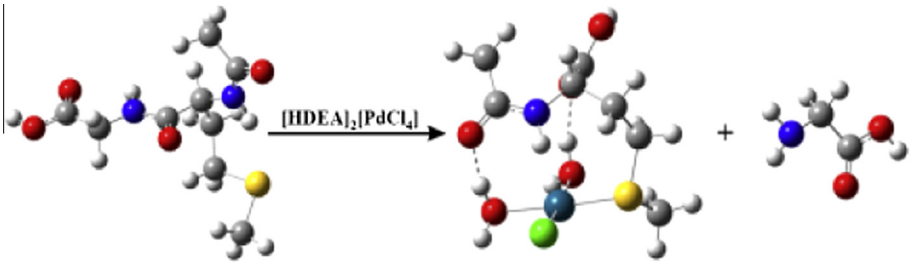
HIGHLIGHTS

- Hydrolytic activity of the palladium(II) complex with AcMet-Gly was investigated.
- The reaction was monitored by ^{1}H NMR spectroscopy.
- Regioselective cleavage of the peptide bond was achieved.
- The possible mechanism of this reaction was investigated using DFT.

GRAPHICAL ABSTRACT

Hydrolytic activity of the diethanolamine palladium(II) complex was tested in the reaction with AcMet-Gly at pH = 2.0 and 60 °C. DFT study was applied in order to explore the mechanism of this hydrolytic reaction.



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ABSTRACT

Hydrolytic activity of diethanolammonium–tetrachloridopalladate(II) complex was tested in the reaction with AcMet-Gly at pH = 2.0 and 60 °C. The reaction was monitored using ^{1}H NMR spectroscopy, during the course of 45 h. It was shown that regioselective cleavage of amide bond involving the carboxylic group of methionine is achieved under these experimental conditions. DFT study was performed, in order to explore the mechanism of this hydrolytic reaction. This study contributes to a better understanding of the mechanism of the peptide bond hydrolysis of the methionine-containing peptides, and generally interaction of Pd(II) with –SR groups of biological relevant molecules.

1. Introduction

Hydrolytic cleavage of peptides and proteins plays important functional and regulatory role in many physiological processes. Proteolysis is responsible for many fundamental biological processes, such as control of the cell cycle, transcription, antigen processing, and apoptosis. However, it is worth pointing out that the peptide bond is exceptionally unreactive, and that the half-life for its hydrolysis in solution with pH 4–8 is several hundred years at room temperature [1,2].

Selective hydrolysis of peptide bond can be achieved with enzymes and synthetic reagents, such as acids, bases, and metal complexes. Several proteolytic enzymes are used for the cleavage, but

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among them only trypsin is highly regioselective [3]. The enzymes application is limited due to their special requirements related to the temperature and pH. Also, they are sometimes inapplicable because they cleave peptide bond indiscriminately, in several sites, giving unwanted short fragments. Today, the selective hydrolysis of this bond can be achieved by several methods. Among them, transition-metal complexes have been used as artificial metalloproteases, whereby Pd(II) and Pt(II) complexes deserve special attention [4–12]. Both of the mentioned types of the complexes posses similar properties, but due to much faster ligand substitution (10^5), palladium(II) complexes are more suitable for reaction monitoring and mechanism elucidation [13]. A key requirement for the complex to act as an artificial metallopeptidase is to have at least two coordination sites available for the substitution. Namely, during hydrolytic reaction one site is used for anchoring to the side chain of the amino acid in the peptide, and second for interaction with the proximate scissile peptide bond. Therefore, these coordination places should be occupied by weak ligands which can be easily substituted in a given time [14–16]. It has been shown that peptides containing methionine [4,6,14,16,17–19] or histidine [7,20–27] in the side chain, can be good model molecules for the study of their interactions with different Pd(II) and Pt(II) complexes, due to the fact that these complexes are able to bind to the sulfur or nitrogen, and promote hydrolysis of peptide bond. However, the mechanism of these hydrolytic reactions has not been completely elucidated. Elucidation of the mechanism of the peptide bond hydrolysis is also important for better understanding of some side effects which cause Pt and Pd complexes, such as nephrotoxicity, since both of these biological processes are associated with interaction with –SR or –SH groups.

In this paper we report the study of the hydrolytic reaction of earlier prepared diethanolammonium–tetrachloridopalladate(II) complex ($[HDEA]_2[PdCl_4]$) [28] with *N*-acetylated *L*-methionylglycine dipeptide (AcMet-Gly). To examine the structures of the proposed reaction participants, this hydrolytic reaction was studied by using ^{1}H NMR spectroscopy and density functional theory.

2. Experimental

2.1. Reagents

The compounds D_2O, DNO_3 and $PdCl_2$, were obtained from Aldrich Chemical Co. All common chemicals were of reagent grade. Diethanolamine, dipeptide L-methyonylglycine (Met-Gly), were obtained from Sigma Chemical Co. The terminal amino group in Met-Gly was acetylated by a standard method to obtain AcMet-Gly [5].

2.2. Measurements

Reactions of AcMet-Gly with Pd(II) complexes were followed by ^{1}H NMR spectroscopy, using a Varian Gemini 200 MHz spectrometer, in D_2O solutions containing TSP (sodium trimethylsilylpropionate) as the internal reference. All pH measurements were made at 25 °C. The pH meter (Iskra MA 5704) was calibrated with Fischer certified buffer solutions of pH 4.00. The results were not corrected for the deuterium isotope effect.

2.3. Computational method

All calculations were conducted using Gaussian09 [29] with the M06 hybrid meta functional [30]. The 6-311+G(d,p) basis set was used for C, H, O, N, and Cl, whereas def2-TZVPD [31] was employed for the Pd center. This triple-zeta-valence basis set contains polarization and diffuse functions, as well as effective core potential. Geometrical parameters of all investigated species in water were optimized using the CPCM solvation model (Polarizable Conductor Calculation Model, $e = 78.36$). All calculated structures were verified to be local minima (all positive eigenvalues) for ground state structures by frequency calculations. The natural bond orbital analysis [32] (Gaussian NBO version) was performed for all structures. The ^{1}H NMR properties of the crucial reaction product were predicted, and the chemical shifts for all hydrogen atoms relative to TMS were calculated. For the simulation of the ^{1}HNMR spectrum, the model mentioned above, which involves different basis sets for nonmetals and the Pd center, was not suitable. For this reason, the M06/LANL2DZ method was used for the prediction of the ^{1}H NMR properties.

3. Results and discussion

In our previous study we reported that $[HDEA]_2[PdCl_4]$ complex [28] shows selective hydrolytic activity in the reaction with *N*-acetylated *L*-histidylglycine dipeptide (AcHis-Gly). Bearing this in mind, we assumed that this complex can also act as artificial metallopeptidase in the reaction with methionine-containing dipeptide. To confirm our presumption, the reaction with AcMet-Gly dipeptide was performed. The Pd(II) complex and dipeptide were mixed in equimolar amounts, 20 mM in D_2O, at pH = 2 and 60 °C. The reaction was monitored by ^{1}H NMR spectroscopy, which proved to be a very useful tool for studying complex hydrolytic reactions. The reaction products were distinguished on the basis of the chemical shifts of the S-methyl protons of methionine, methylene glycine protons (from non-hydrolyzed dipeptide substrate and free glycine), and protons of the methylene groups from the diethanolamine units (Fig. 1). Immediately after mixing the reactants, spontaneous coordination of the Pd(II) to the sulfur atom of methionine occurs, yielding the intermediate Pd(II) complex **A** (Fig. 2). This is documented with the simultaneous decline of the resonance at 2.11 ppm (S-methyl protons of the free dipeptide) and the growth of the resonance at 2.50 ppm (corresponding to the S-methyl protons of the complex **A**, Fig. 1) [13].

The appearance of a new singlet in the ^{1}H NMR spectrum at 3.62 ppm was an indication that the hydrolytic reaction occurs. Namely, during the reaction the resonance at 4.00 ppm of methylene glycine protons from the non-hydrolyzed dipeptide decreased, while the singlet at 3.62 ppm for methylene protons of the free glycine increased. After 45 h of heating of the reaction mixture at 60 °C, the intensity of the singlet at 3.62 ppm was not changed, Fig. 1. In addition, a new singlet at 3.68 ppm and a multiplet at 3.86 ppm appeared in the NMR spectrum. We assumed that these signals can be assigned to the salt $[C_6H_{17}O_4N_2]^+$ (**D** in Fig. 2) which is formed in the reaction of outgoing glycine with diethanolamine ligand (Fig. 2). Indeed, the same compound was formed in a separate reaction, where equimolar amounts of diethanolamine and glycine were mixed, confirming that the new singlet at 3.68 ppm belongs to the methylene protons of glycine, while the triplet at 3.86 ppm belongs to the protons of $-CH_2-N$ of diethanolammonium moiety. The formation of the salt **D** is not unexpected due to the fact that under our reaction conditions (pH = 2) glycine amino group ($pK_a \sim 9.6$) is protonated and the carboxyl group ($pK_a \sim 2.3$) is partially deprotonated. Therefore, carboxylate oxygen is more available for the reaction with diethanolamine [9,33]. It is worth pointing out that some of the liberated glycine reacts with the catalyst to form a small amount of the Pd-Gly complex, which was detected by ^{1}H NMR spectroscopy at 3.52 ppm [27]. Under these reaction conditions, free acetic acid was not detected by NMR spectroscopy, confirming that the reaction is regioselective.

Success of the hydrolytic process is determined on the basis of the integrated resonance of methylene protons from free glycine

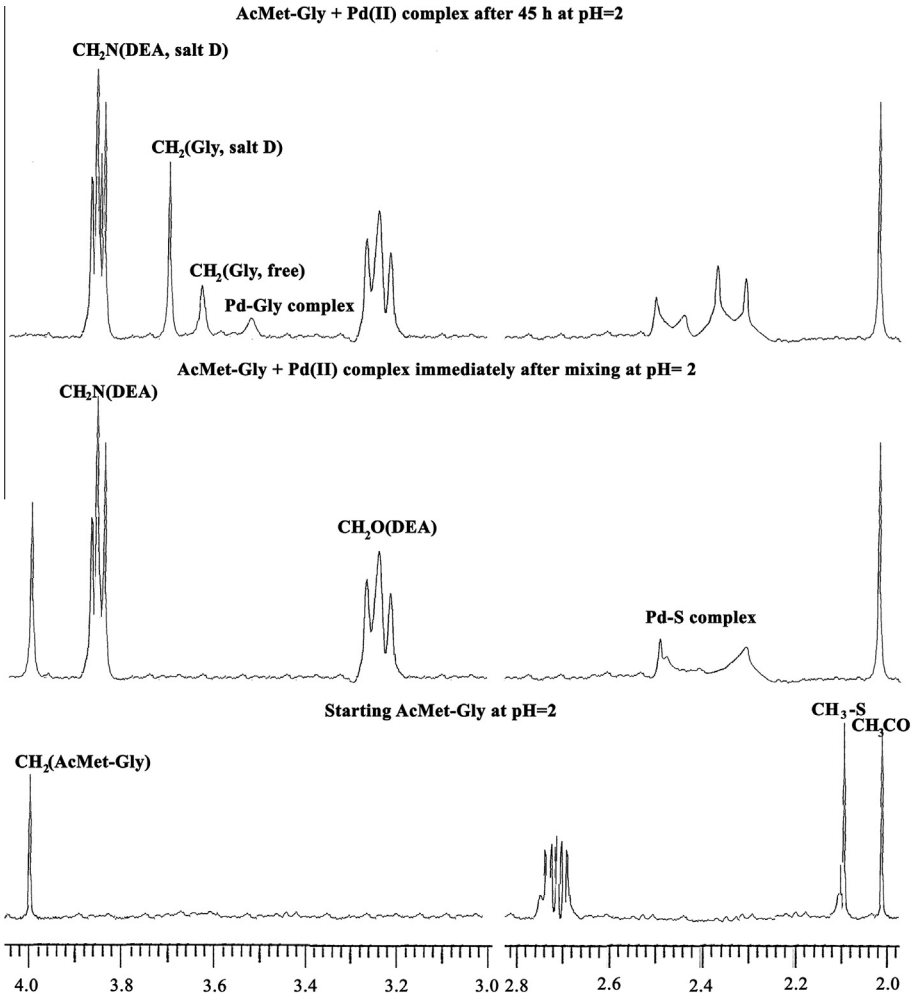

Fig. 1. Parts of ^{1}H NMR spectra for the hydrolytic reaction of AcMet-Gly with $[HDEA]_2[PdCl_4]$ complex as a function of time, in D_2O as solvent. The chemical shifts are given in ppm relative to TSP.

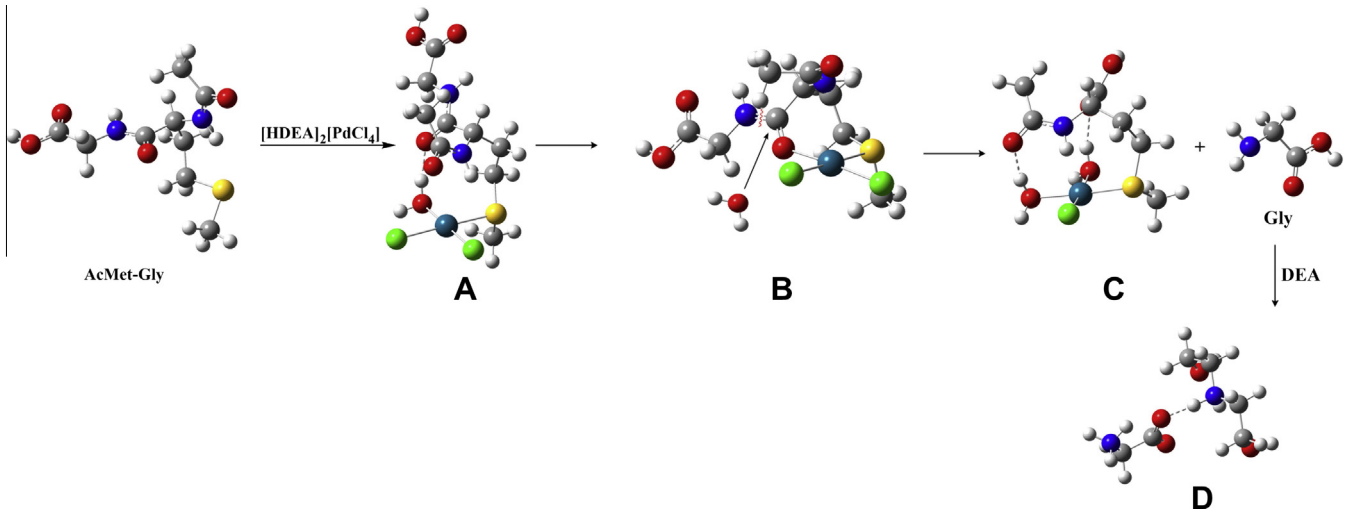

Fig. 2. Optimized structures of the starting dipeptide, intermediates and products.

and glycine from unreacted dipeptide. After 45 h, about 90% of the starting dipeptide was hydrolyzed.

Bearing in mind the results from reference [17] we applied density functional theory to examine the structures of the intermediates and reaction products, and propose possible mechanism of this reaction. Taking into account the fact that we used $[PdCl_4]^{2-}$ as promoter of the hydrolytic process, known to give mononuclear palladium(II)-sulfur complex as active form in the hydrolytic

Table 1
Selected bond distances (Å) in the crucial intermediate and products.

Dipeptide	
C–O (from peptide bond)	1.22
C–N (from peptide bond)	1.35
B	
Pd–S	2.35
Pd–Cl	2.32
Pd–Cl	2.36
Pd–O (O from peptide bond)	2.12
C–O (from peptide bond)	1.24
C–N (peptide bond)	1.33
C	
Pd–S	2.31
Pd–Cl	2.30
Pd–O(H_2O) (both)	2.14

reactions of methionine-containing peptides [17], as well as our experimental findings, we assumed possible structure of the intermediate **A** (Fig. 2). The calculated chemical shift for the S-methyl protons singlet amounts 2.93 ppm, while for the methylene glycine protons this value is 4.34 ppm. The Pd(II)-S peptide complex **A**, contains water molecule as a ligand, which is a good leaving group. Departure of this ligand enables the Pd(II) ion to come close to the unreactive amide bond. In this way the amide oxygen coordinates to the Pd(II), forming the hydrolytically active intermediate **B**. The carbonyl group of methionine moiety becomes more polarized (the NBO charges on the oxygen and carbon of this group are −0.63, and 0.74), which facilitates the external nucleophilic attack of the molecule of solvent water. Regioselective cleavage of the amide bond, involving the carboxylic group of methionine is achieved, and glycine is liberated. As a result of the hydrolytic reaction of Pd(II)-anchored complex **B** (regioselective cleavage of the amide bond and substitution of the chlorido ligand with water molecule) the complex **C** is formed. The calculated chemical shift for the S-methyl protons amounts 2.55 ppm, which is in accord with the signal at 2.49 ppm from the experimental ^{1}H NMR spectrum. Such good agreement between the experimental and calculated ^{1}H NMR spectra indicates that the optimized geometry of **C** corresponds to the structure of the reaction product. The NBO analysis of the product **C** shows that Pd forms covalent bonds with sulfur and chlorido ion, whereas the lone electron pairs from the p orbitals of ligating atoms participate with more than 70% in the bonds around palladium. The lone pairs on the oxygen atoms of the ligating water molecules delocalize into the formally empty almost pure palladium p orbital, enabling coordinative interactions. The selected bond distances in the starting dipeptide, intermediate **B**, as well as for the product **C** are given in Table 1.

4. Conclusion

Hydrolytic activity of the diethanolammonium-tetrachloridopalladate(II) complex with methionine-containing dipeptides was tested in the reaction with AcMet-Gly at pH = 2.0 and 60 °C. Based on the ^{1}H NMR spectroscopy monitoring, it is shown that regioselective cleavage of peptide bond involving the carboxylic group of methionine is achieved under these experimental conditions, during the course of 45 h. Under these reaction conditions, free acetic acid was not detected by NMR spectroscopy, confirming that the reaction is regioselective. DFT method was applied for better explanation of the mechanism of this hydrolytic reaction, and provides a better insight into the coordination chemistry of methionine-containing peptides. Good agreement between the experimental and calculated ^{1}H NMR spectra for the proposed intermediates **A** and **B** and the reaction product **C** confirms the proposed mechanism. This study contributes to the better understanding of the mechanism of the peptide bond hydrolysis of the methionine-containing peptides, and generally interaction of Pd(II) with –SH or –SR groups, as well as indicates a direction for the development of new palladium(II) complexes for their future application in bioorganic chemistry and structural biology.

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