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Synthesis of different pyrazine-bridged platinum(II) complexes and ^{1}H NMR study of their catalytic abilities in the hydrolysis of the *N*-acetylated L-methionylglycine

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ABSTRACT

Four binuclear $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$-type complexes have been synthesized and characterized by elemental microanalyses and NMR (^{1}H and ^{13}C) spectroscopy (L is ethylenediamine, en; (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn; *trans*-(±)-1,2-diaminocyclohexane, dach and pz is bridging pyrazine ligand). The chlorido complexes were converted into the corresponding aqua species, $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and ^{1}H NMR spectroscopy was applied to study their reactions with the *N*-acetylated L-methionylglycine, Ac-L-Met-Gly. The $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex and dipeptide were reacted in 1:1 and 1:2 M ratios, respectively, and all reactions were performed in the pH range 2.0–2.5 and at 37 °C. In the reactions with equimolar amounts of the reactants all Pt(II) aqua complexes bind to the methionine side chain of Ac-L-Met-Gly dipeptide and promote the cleavage of the amide bond involving the carboxylic group of methionine. It was found that the amount of hydrolyzed dipeptide strongly depends from the steric bulk of bidentate coordinated diamine ligand L in $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex (en > 1,2-pn > ibn > dach). However, in the reaction with an excess of dipeptide the influence of the nature of diamine ligand L on this hydrolytic process could not be observed due to the fact that slow decomposition of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex was occured.

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1. Introduction

Hydrolytic reactions of uncatalyzed peptide bonds are extremely slow. Enzymes are commonly used as cleavage agents for this reactions but investigation of the hydrolytic reactions promoted by metal complexes suggested that they can be applied for cleavage of unactivated amide bonds very efficiently. Recent years have witnessed an increasing interest in the study of the interactions of platinum(II) [1–3] and palladium(II) [2–16] complexes with sulfur- and histidine-containing peptides and proteins [17–20] as effective catalyst for the hydrolytic cleavage of the above mentioned peptides.

In general, it was shown that aqua complexes of these metal ions spontaneously bind to the heteroatom in the side chain of methionine [1–8] or histidine [2,3,9–16,21] and promote cleavage of the amide bond involving the carboxylic group of the anchoring amino acid. The hydrolytic reactions of methionine-containing peptides with different palladium(II) complexes were investigated [3,8,9,21–23] and it was shown that different promoters produce different hydrolytically active palladium(II)–peptide complexes. Thus, in the reactions of methionine-containing peptides with $[PdCl_4]^{2-}$, the active form was a mononuclear palladium(II)–peptide complex, while with $[Pd(H_2O)_4]^{2+}$ and $[Pd(en)(H_2O)_2]^{2+}$ complexes, binuclear hydrolytically active palladium(II)–peptide complexes bridged with two methionine side chains were formed. Moreover, it was shown that these binuclear palladium(II)–peptide complexes are more efficient than the corresponding mononuclear complex in promoting the hydrolysis of the scissile amide bond in methionine-containing peptides. In accordance to this, the reactions of the thiolate-bridged $(Me_4N)_2[Pd_2(\mu\text{-SPh})_2Cl_4]$ complex with different methionine-containing peptides of the type Ac-L-Met-X (X is Gly, Val, Phe or Ala) showed that this complex was an effective catalyst for the rapid cleavage of methionine-containing dipeptides in non-aqueous solvents [24]. An important advantage of dimerization is the possibility of cooperation between the metals, as was shown for hydrolysis of DNA, RNA, and their models catalyzed by polynuclear metal complexes and metalloenzymes [25–30]. Very recently in one of our laboratories we compared hydrolytic abilities of two Pt(II) complexes, mononuclear $[Pt(en)(H_2O)_2]^{2+}$ and binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, in the reaction with Ac-L-Met-Gly dipeptide. It was shown that two Pt(II) ions bridged with one aromatic pyrazine ligand in the $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex are more efficient in the hydrolysis of the Ac-L-Met-Gly dipeptide, even when the hydrolytic reaction was performed with an excess of the mononuclear complex. Our

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latest findings for $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex [31] together with those for different binuclear Pd(II) complexes [24] showed that polynuclear Pt(II) and Pd(II) complexes can be perspective catalytic reagents for amide bond hydrolysis in the reactions with methionine-containing peptides.

In this work, an attempt was made to gain further insight into selective hydrolysis of the methionine-containing peptides in the presence of different binuclear platinum(II) complexes. For these purposes, the ^{1}H NMR spectroscopy was applied to study the influence of the chelating diamine ligand L in $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$-type complexes (L is ethylenediamine, en; (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn; *trans*-(±)-1,2-diaminocyclohexane, dach and pz is bridging pyrazine ligand) on the hydrolytic cleavage of the Ac-L-Met-Gly dipeptide.

2. Experimental

2.1. Materials

Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm^{-1}. The compounds D_2O, DNO_3, NaOD, ethylenediamine (en), (±)-1,2-propylenediamine (1,2-pn), isobutylenediamine (ibn), *trans*-(±)-1,2-diaminocyclohexane (dach), pyrazine (or 1,4-diazine), pz and $K_2[PtCl_4]$ were obtained from the Aldrich Chemical Co. All common chemicals were of reagent grade. The dipeptide L-methionylglycine (L-Met-Gly) was obtained from the Sigma Chemical Co. The terminal amino group in this dipeptide was acetylated by a standard method [4].

2.2. Preparation of $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$-type complexes (L is en, 1,2-pn, ibn or dach)

The binuclear platinum(II) complexes of the type $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ were synthesized from the corresponding mononuclear $[Pt(L)Cl_2]$ complexes by modification of the procedure published in the literature [31,32].

Preparation of $[Pt(L)Cl_2]$: All mononuclear Pt(II) complexes were prepared with minor modification of a method previously used in our laboratory for the preparation of a series of $[M(L)Cl_2]$ complexes (M is Pt(II) or Pd(II); L is bidentate coordinated diamine or amino acid) [15,23,33]. K_2PtCl_4 was dissolved in water and mixed with an equimolar amount of diamine ligand (L). The pH of the solution was adjusted to ca. 3 by addition of 1 M HCl and 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 warm water and than cooling at room temperature. The experimental results of the elemental analyses for C, H and N parameters for all investigated Pt(II) complexes are in accordance with theoretical values calculated for $[Pt(L)Cl_2]$ complexes.

Preparation of $[Pt(L)(dmf)Cl]NO_3$: The solid $[Pt(L)Cl_2]$ complex was converted into the corresponding monodimethylformamide (dmf) $[Pt(L)(dmf)Cl]NO_3$ complex by treatment with 0.98 equivalents of $AgNO_3$ on the following manner. To a solution of 55.3 mg (0.325 mmol) of $AgNO_3$ in 5 cm^3 of dmf was added a suspension of 0.332 mmol of $[Pt(L)Cl_2]$ in 10 cm^3 of dmf. The mixture was stirred overnight at room temperature in the dark. The precipitated AgCl was removed by filtration and resulting pale yellow dmf solution of $[Pt(L)(dmf)Cl]NO_3$ was used as the starting material for the preparation of the required pyrazine-bridged platinum(II) complexes, $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$.

Preparation of $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$: The dmf solution of the pyrazine ligand (pz) (13.29 mg, 0.166 mmol) was added dropwise to the solution of $[Pt(L)(dmf)Cl]NO_3$. The mixture was stirred at room temperature in the dark for 12 h. The solvent was then rotary evaporated and the residue washed with ether. The crude product was dissolved in a minimal amount of 0.5 mol/dm^3 LiCl aqueous solution. The obtained solution was left overnight in the dark. The pale-yellow precipitate of $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ was removed by filtration, washed with methanol and then ether, and air-dried. Depending of the type diamine ligand L the yield of $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ complex was between 30–40%. *Anal.* Calc. for $\{[Pt(1,2\text{-pn})Cl]_2(\mu\text{-pz})\}Cl_2 = C_{10}H_{24}N_6Cl_4Pt_2$ (FW = 760.31): C, 15.80; H, 3.18; N, 11.05. Found: C, 15.45; H, 3.19; N, 10.82%. *Anal.* Calc. for $\{[Pt(ibn)Cl]_2(\mu\text{-pz})\}Cl_2 = C_{12}H_{28}N_6Cl_4Pt_2$ (FW = 788.36): C, 18.28; H, 3.58; N, 10.66. Found: C, 17.84; H, 3.63; N, 10.47%. *Anal.* Calc. for $\{[Pt(dach)Cl]_2(\mu\text{-pz})\}Cl_2 = C_{16}H_{32}N_6Cl_4Pt_2$ (FW = 840.43): C, 22.87; H, 3.84; N, 10.00. Found: C, 22.56; H, 3.94; N, 9.56%.

2.3. Preparation of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$

The $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ complexes were converted into the corresponding aqua complexes by treatment with 3.98 equivalents of $AgNO_3$, according to a previously published method [34]. In each case, the formed solid AgCl was removed by filtration in the dark, and the fresh solutions of the aqua complexes were kept in a refrigerator and used in the further experiments.

2.4. Measurements

All pH measurements were realized at ambient temperature using an Iskra MA 5704 pH meter calibrated with Fischer certified buffer solutions of pH 4.00 and 7.00. The results were not corrected for the deuterium isotope effect.

The NMR spectra of D_2O solution containing TSP (sodium trimethylsilylpropane-3-sulfonate) as the internal reference were recorded with a Varian Gemini 2000 spectrometer (200 MHz). Fresh solutions of aqua complexes and dipeptide were prepared separately and then mixed in 1:1 and 1:2 M ratios, respectively. The initial concentration of dipeptide and aqua complexes solutions were 40 mM. All reactions were performed in the pH range 2.0–2.5 and at 37 °C. Elemental microanalyses for carbon, hydrogen and nitrogen were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade.

3. Results and discussion

Four binuclear $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$-type complexes have been synthesized and characterized by elemental microanalyses and NMR (^{1}H and ^{13}C) spectroscopy (L is (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn and *trans*-(±)-1,2-diaminocyclohexane, dach; see Fig. 1). The schematic presentation of the reaction for the syntheses of these complexes is given in Fig. 2. The spectroscopic results of these complexes are in accordance with those for similar complexes in literature [15,23,32] and with proposed formula $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ (Table 1). As it was shown in Fig. 1, all investigated Pt(II) complexes have the same bridging pyrazine ligand but different chelating diamine ligand L. In the present study we investigated the influence of this chelating ligand L on the hydrolysis of the amide bond in the *N*-acetylated L-methionylglycine dipeptide (Ac-L-Met-Gly). The terminal amino group in this peptide was acetylated to protect its binding to the Pt(II) atom. In order to investigate the influence of structural changes in the chelating diamine ligand L on the hydrolysis of Ac-L-Met-Gly dipeptide, all chlorido Pt(II) complexes were converted into the corresponding aqua species, $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$. The platinum(II) aqua complex and dipeptide were reacted in 1:1 and 1:2 M ratios, respectively, and all reactions were performed in the pH range 2.0–2.5 and at 37 °C. As was shown in our previous

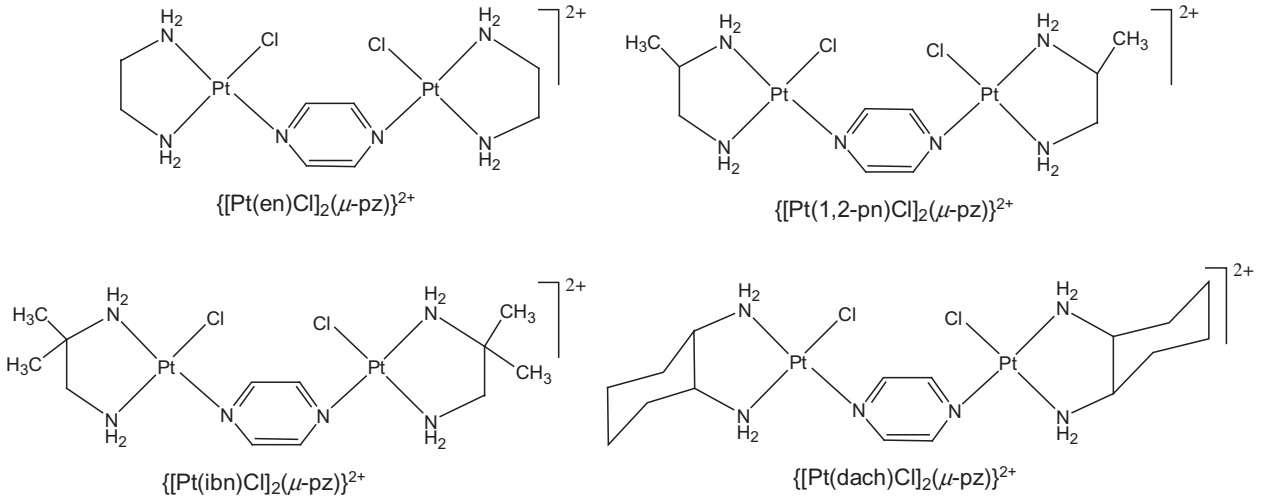

Fig. 1. Schematic drawing of the binuclear $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$ complexes. These complexes were converted into the corresponding aqua species, $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and used in the reactions with the *N*-acetylated L-methionylglycine.

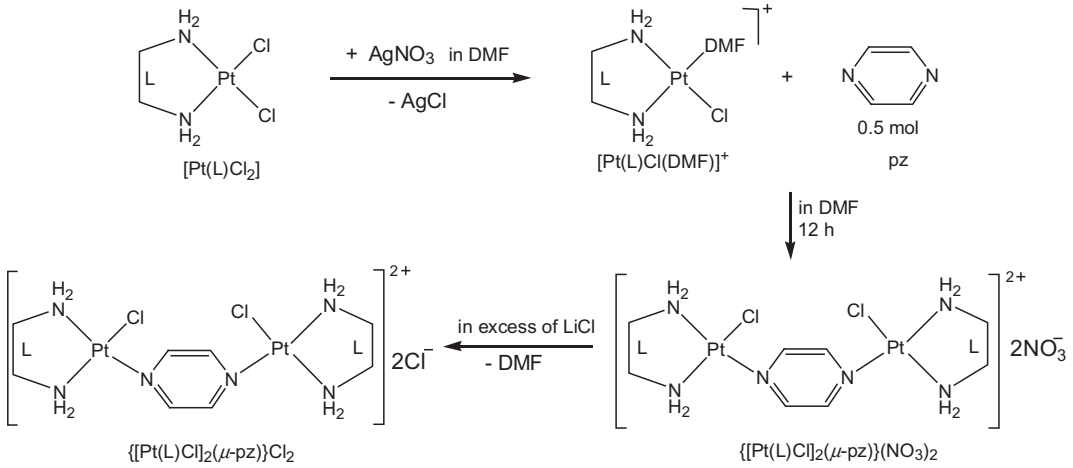

Fig. 2. The schematic presentation of the reaction for the synthesis of binuclear $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$-type complexes (L is ethylenediamine, en; (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn; *trans*-(±)-1,2-diaminocyclohexane, dach and pz is bridging pyrazine ligand).

Table 1
Characteristic NMR (^{1}H and ^{13}C) chemical shifts for the $\{[Pt(L)Cl]_2(\mu\text{-pz})\}Cl_2$-type complexes. These chemical shifts are in accordance with those previously reported for similar complexes [15,23,32].

Platinum(II) complex	Characteristic ^{1}H NMR resonances (δ, ppm)		Characteristic ^{13}C NMR resonances (δ, ppm)	
$\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$	2.64 (*s*, enCH$_2$)	9.03 (*s*, pzCH)	52.34 (enCH$_2$)	153.46 (pz)
$\{[Pt(1,2\text{-pn})Cl]_2(\mu\text{-pz})\}Cl_2$	1.34 (*d*, 1,2-pnCH$_3$), 2.45–2.98 (*m*, 1,2-pnCH$_2$), 3.11–3.32 (*m*, 1,2-pnCH),	9.01 (*s*, pzCH)	17.83 (1,2-pnCH$_3$), 54.52 (1,2-pnCH$_2$), 59.86 (1,2-pnCH),	153.44 (pz)
$\{[Pt(ibn)Cl]_2(\mu\text{-pz})\}Cl_2$	1.42–1.48 (*m*, ibnCH$_3$), 2.66 (*s*, ibnCH$_2$),	9.13 (*s*, pzCH)	25.96 (ibnCH$_3$), 60.09 (ibnCH$_2$), 63.61 (ibnCH),	153.36 (pz)
$\{[Pt(dach)Cl]_2(\mu\text{-pz})\}Cl_2$	1.27–1.62 (*m*, dachCH$_2$, C4,C5), 1.76–2.08 (*m*, dachCH$_2$,C3,C6), 2.45–2.61 (*m*, dachCH,C1,C2),	9.00 (*s*, pzCH)	26.55 (dach, C4,C5), 34.56 (dach, C3,C6), 65.15 and 65.30 (dach, C1,C2),	153.31(pz)

studies [11,21,22], acidic solutions are needed to suppress the formation of hydroxo-bridged oligomeric Pt(II) complexes, which are catalytically inactive. All chelate diamine ligands L in $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes are inert to substitution and expected to remain bound to the platinum(II) atom during the reactions with Ac-L-Met-Gly dipeptide. The reactions of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with Ac-L-Met-Gly were followed by applying ^{1}H NMR spectroscopy.

3.1. The reactions of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with an equimolar amount of Ac-L-Met-Gly dipeptide

Recently we investigated the reactions of binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex with methionine-containing peptides [31]. This pyrazine-bridged Pt(II) complex was shown as very effective hydrolytic reagent in the cleavage of these peptides. As continuation of this study, here we compared the rate of hydrolysis of the Ac-L-Met-Gly dipeptide in the presence of $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex with those for three new binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes having different bidentate diamine ligand L. When an equimolar amount of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex was incubated with Ac-L-Met-Gly, under the above mentioned experimental conditions, all reactions resulted in formation of platinum(II)–dipeptide complex $\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ in a yield of 95% for less than 30 min (Fig. 3). The monodentate binding of the platinum(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 dipeptide and the growth of a resonance at 2.54 ppm corresponding to these protons for the dipeptide coordinated to platinum(II) through the sulfur atom. These chemical shifts are in accordance with those previously reported for the reactions of platinum(II) complexes with different methionine-containing molecules [1–3,35]. In all investigated reactions the intermediate $\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ complex is hydrolytically active species and promotes the regioselective cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide. These hydrolytic reactions can be followed successfully using ^1H NMR spectroscopy by observing the methylene glycine protons in $\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ complex and these protons for the free glycine, see Fig. 4. As can be seen from this figure, the resonance at 3.99 ppm corresponding to the methylene glycine protons of the dipeptide attached to platinum(II) in

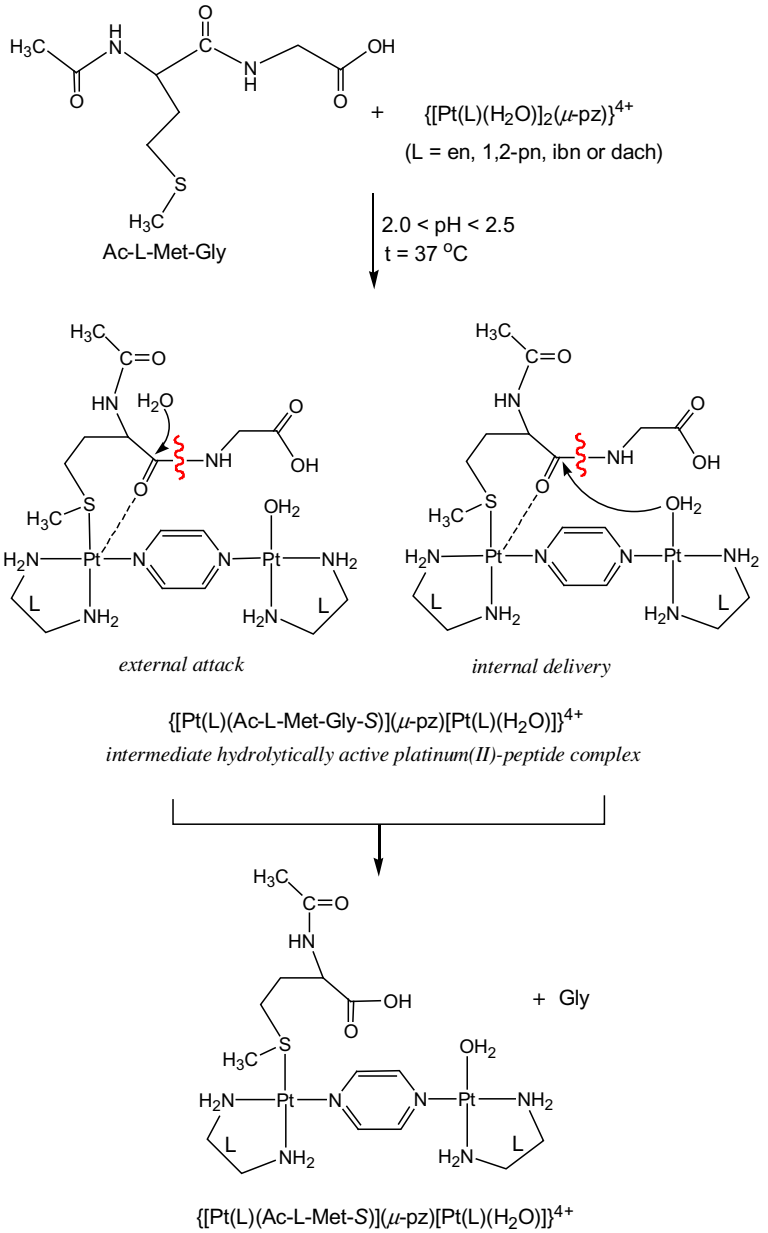

Fig. 3. The reaction scheme of the hydrolytic reaction of the Ac-L-Met-Gly dipeptide with binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$-type complexes. The corresponding Pt(II)-aqua complex and dipeptide were mixed in a 1:1 M ratio and all reactions were performed at 2.0 < pH < 2.5 and at 37 °C.

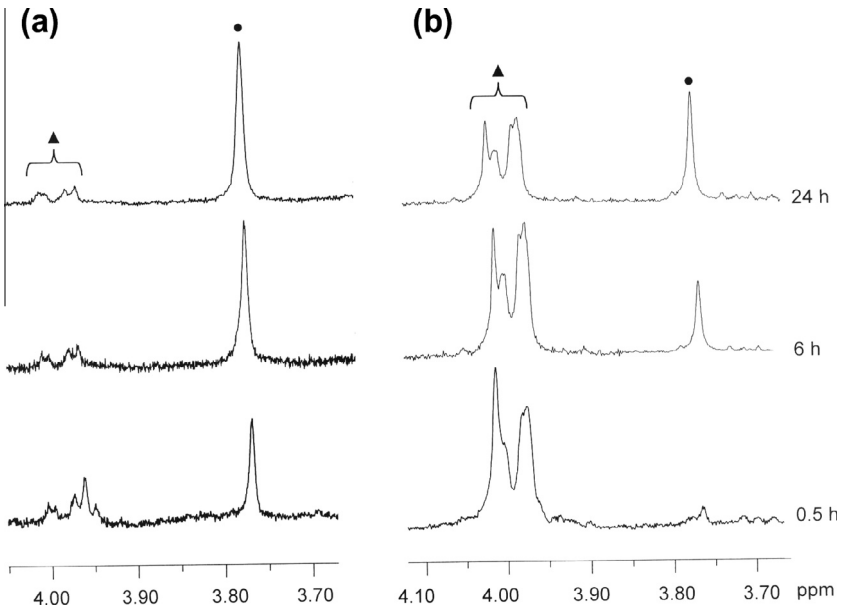

Fig. 4. Parts of the ^{1}H NMR spectra recorded during the reaction of $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ (a) and $\{[Pt(dach)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ (b) complexes with an equimolar amount of Ac-L-Met-Gly dipeptide as a function of time in the pH range 2.0–2.5 and at 37 °C. The resonances assigned as (▲) and (●) correspond to the methylene glycine protons of Ac-L-Met-Gly dipeptide monodentate bound to the binuclear platinum(II) complex and these protons for the free glycine, respectively.

$\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ complex decreased, while that at 3.77 ppm for these protons in the free glycine increased. Upon addition of amino acid glycine to the reaction mixture, the resonance at 3.77 ppm was enhanced. The amounts of the non-hydrolyzed dipeptide coordinated to platinum(II) complex and hydrolysis products were determined from the known initial concentration of Ac-L-Met-Gly and from the integrated resonance for the methylene protons of the free glycine. The changes in concentrations of the free glycine and non-hydrolyzed dipeptide in $\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ complex were determined every 30 min during 24 h. During this time the total amounts of $\{[Pt(L)(Ac\text{-}L\text{-}Met\text{-}Gly\text{-}S)](\mu\text{-pz})[Pt(L)(H_2O)]\}^{4+}$ complex and free glycine was always equal to the initial concentration of Ac-L-Met-Gly dipeptide. The time dependence of the hydrolytic cleavage of the Met-Gly amide bond in the reactions between different binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes and Ac-L-Met-Gly dipeptide is given in Fig. 5. From this figure it can be concluded that the amount of the hydrolyzed Ac-L-Met-Gly dipeptide decreased in the following order: en > 1,2-pn > ibn > dach. Therefore, the amount of hydrolyzed Ac-L-Met-Gly dipeptide in the reaction with $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex after 30 min is almost two or one and a half times larger than with $\{[Pt(ibn)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ and $\{[Pt(1,2\text{-pn})(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex, respectively. However, during the same time the amount of hydrolyzed Ac-L-Met-Gly dipeptide in the presence of $\{[Pt(dach)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ was eight times smaller than with $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex. Finally, after 24 h in the presence of the $\{[Pt(dach)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex only 30% of the Met-Gly amide bond hydrolyzed, while with the $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex more than 80% of this bond had cleaved. Difference in the hydrolytic abilities of the investigated binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes can be attributed to the steric bulk of their bidentate coordinated diamine ligand L. In Fig. 6 we compared the catalytic abilities of en, 1,2-pn,

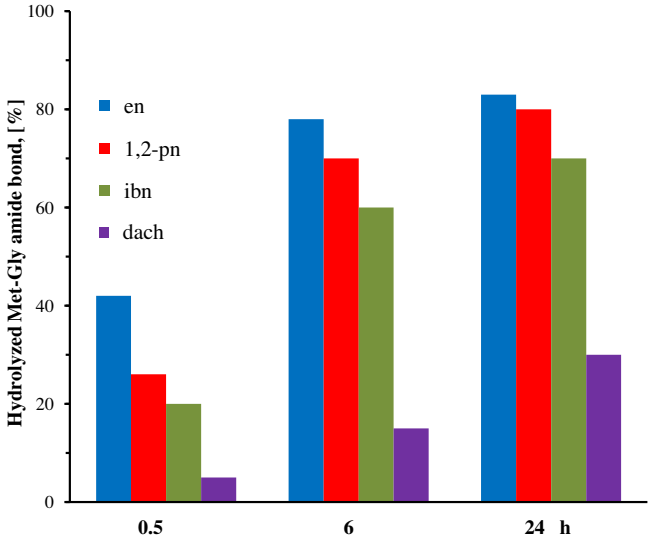

Fig. 5. Time dependence of the hydrolytic cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide with an equimolar amount of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$-type complex.

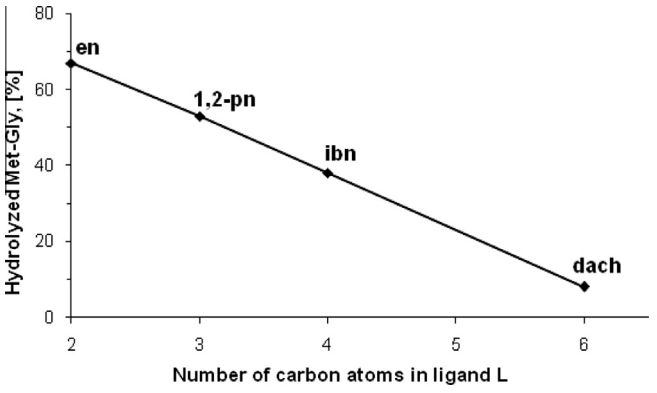

Fig. 6. Dependence of the amount of hydrolyzed Ac-L-Met-Gly dipeptide on the number of carbon atom in the structural skeleton of ligand L of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex. The amount of the hydrolyzed dipeptide was determined after 2 h of reaction.

ibn and dach Pt(II) binuclear complexes in the cleavage of the Met-Gly amide bond of Ac-L-Met-Gly dipeptide after 2 h of reaction. In spite of the fact that all diamine ligands L in the investigated $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes form five-membered chelate ring, it is obvious that the amount of hydrolyzed peptide was decreased by increasing the number of carbon atom in the structural skeleton of ligand L. Our latest findings that inhibition of the hydrolytic reaction can be achieved by structural modification of the catalyst are in accordance with those for different mononuclear Pd(II) complexes and histidine-containing peptides [15]. Summing up together our results related to the hydrolytic cleavage of methionine-containing peptides in the presence of different binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with previous results with histidine-containing peptides and different mononuclear $[Pd(L)(H_2O)_2]^{2+}$ complexes (L is bidentate coordinated diamine ligand) [15], we can conclude that inhibition of the hydrolytic reaction can be attributed to the steric bulk of the catalyst. These findings can be explained in terms of two possible limiting mechanisms for hydrolytic cleavage of the peptide bond promoted by Pd(II) and Pt(II) complexes [10–12], see Fig 3. First possibility is that the platinum(II) atom coordinated to the methionine side chain polarizes the carbonyl group in the scissile peptide bond and activates its carbon atom toward attack by water molecule from the solvent (*external attack*). For the reaction to occur by this mechanism the Pt(II) and carbonyl oxygen atoms should be proximate. Another possibility is that an aqua ligand from the second Pt(II) atom in the binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex is delivered to the carbon atom in the amide bond (*internal delivery*). For the cleavage of the amide bond to occur by this mechanism an aqua ligand at Pt(II) should be proximate to the carbonyl carbon of the scissile amide bond. As can been seen, any of these proposed mechanism requires a close approach of the pendant catalyst to the adjacent peptide bond and this interaction can be hindered by the steric bulk of diamine ligand L in $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex.

3.2. The reactions of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with an excess of Ac-L-Met-Gly dipeptide

The reaction between $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$-type complexes and the Ac-L-Met-Gly was followed in an excess dipeptide. The corresponding Pt(II) complex and dipeptide were mixed in 1:2 M ratio, respectively, and all reactions were performed under the above mentioned experimental conditions. The first ^{1}H NMR spectrum ran after 30 min of reaction indicated that the decomposition of the corresponding binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex occurred. The singlet appeared in the region at 9.00–9.13 ppm for the bridging pyrazine ligand of $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex (chemical shifts of this singlet is dependent from the type of Pt(II) complex) decreased while two symmetric multiplets in the range 8.75–9.00 ppm increased. The appearance of these two new multiplets indicates that one Pt(II)–N(pyrazine) bond of Pt(II) complex was broken and that the four pyrazine protons of this ligand coordinated in a monotopic fashion to Pt(II) ion were split into two multiplets because of vicinal and long-range coupling [36]. The decomposition of the binuclear complex was finished after 4 h and final product in this reaction was mononuclear platinum(II) complex with a monodentate bound pyrazine ligand. This mononuclear complex was stable during time and no resonance at 8.66 ppm for free pyrazine ligand [31] was detected in the ^{1}H NMR spectrum after 2 days. However, during this time slow hydrolysis of the Met-Gly amide bond in Ac-L-Met-Gly dipeptide was occurred. This hydrolytic reaction was promoted by the presence mononuclear Pt(II) complex resulted from decomposition of the corresponding binuclear complex. Considering this, we were not able to correlate the rate of hydrolysis of Ac-L-Met-Gly dipeptide with the nature of the chelating diamine ligand L in $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex.

4. Conclusions

In this paper, it was demonstrated that by modification of the binuclear $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex by the introduction of a sterically hindered diamine ligand L, inhibition of the hydrolytic cleavage of the amide bond involving the carboxylic group of methionine can be achieved. These results should be taken into consideration when designing new polynuclear platinum(II) complexes as effective agents in the hydrolysis of methionine-containing peptides. Studies aimed at investigating these new possible synthetic metallopeptidases are in progress.

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