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Crystallographic evidence of anion···π interactions in the pyrazine bridged $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex and a comparative study of the catalytic ability of mononuclear and binuclear platinum(II) complexes in the hydrolysis of *N*-acetylated L-methionylglycine

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ABSTRACT

This paper reports on the synthesis and the X-ray characteristics of the binuclear $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex (en is ethylenediamine, acting as a bidentate ligand; pz is a bridging pyrazine ligand). This complex was converted into the corresponding aqua complex, $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and ^{1}H NMR spectroscopy was applied for a comparison of its catalytic activities in the hydrolysis of *N*-acetylated L-methionylglycine (Ac-L-Met-Gly) dipeptide with those for the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex. The peptide and the corresponding platinum(II) complex were reacted in different molar ratios and all reactions were performed at $2.0 < pH < 2.5$ in D_2O solvent at 37 °C. The course of these hydrolytic reactions is discussed and the difference in the catalytic ability between the mononuclear and binuclear Pt(II) complexes was correlated with the presence of the different hydrolytically active platinum(II)–peptide complexes formed during their reactions with the Ac-L-Met-Gly dipeptide.

1. Introduction

Pyrazine, a weak base, acts as a monodentate or bridging bidentate ligand in transition metal chemistry. As a bridging bidentate ligand it commonly exhibits a simple and controllable coordination mode and possesses small steric hindrance, therefore it has been recently utilized as a building unit for molecular architectures with transition metals [1–3]. Another interesting property of this ligand is modification of its basicity as a consequence of coordination to transition-metal fragments [4]. Combined together, the two properties make the pyrazine ligand a good alternative to triazine in studies on anion···π interactions in metal complexes and their utilization for building new molecular architectures. The involvement of pyrazine and oxo anions in anion···π interactions has already been reported [5,6]. In this paper we demonstrate the presence of such interactions between chloride and pyrazine units.

The term anion···π interaction to describe an attractive and non-covalent contact geometry, derived from quantum chemistry calculations, in which an anion is placed above the center of an aromatic ring appeared in the literature ten years ago and initiated a continuous discussion concerning its presence and structure determining role [7,8]. On the basis of the results derived from theoretical calculations and searches of the Cambridge Crystallographic Database (CSD), the authors have demonstrated the existence of anion···π interactions involving mainly electron deficient aromatic systems, although the involvement of non-electron-deficient aromatic rings was also considered as possible whenever the aromatic ring was simultaneously interacting with a cation [9]. The existence of anion···π interactions in metal complexes stemming from encapsulation of a chloride anion by four pyridine rings has been noted in 2004 [10]. Notably, the authors pointed out that the observed chloride···pyridine anion···π interactions were favored because the pyridine rings are coordinated to copper ions, which enhances their electron-poor character. Following this report, other papers demonstrating the enhancing role of metal coordination in anion···π interactions have appeared [6,11,12]. To date, there are many reports on this subject due to the various possible applications of these interactions in supramolecular chemistry, including design of anion receptors and transporters across bilayer membranes [13,14]. The most recent perspective on the subject has been published by Reedijk and coworkers [15]. Notably, anion···π interactions have been reported

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as being persistent in solution and in the gas phase [16], and a review on the studies of these interactions in solution has appeared only very recently [17].

From the other side, recent studies showed that the thiolate-bridged $(Me_4N)_2[Pd_2(\mu\text{-}SPh)_2Cl_4]$ complex in the reactions with different methionine-containing peptides of the type Ac-L-Met–X (X is Gly, Val, Phe or Ala) was a very effective hydrolytic reagent in the cleavage of these dipeptides in non-aqueous solvents [18]. 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 [19–24]. The above mentioned findings suggested that polynuclear platinum(II) complexes could also be good reagents for amide bond hydrolysis in reactions with methionine-containing peptides.

In the present study we have synthesized and structurally characterized the new pz-bridged binuclear $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex. This complex was converted into the corresponding aqua complex, $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and ^{1}H NMR spectroscopy was applied for comparison of its catalytic activity in the hydrolysis of *N*-acetylated L-methionylglycine (Ac-L-Met-Gly) dipeptide with that of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex. All the reactions were investigated at 2.0 < pH < 2.5 and at 37 °C in D_2O solution.

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), pyrazine (or 1,4-diazine), pz and $K_2[PtCl_4]$ were obtained from Aldrich Chemical Co. All common chemicals were of reagent grade. The dipeptide L-methionylglycine (L-Met-Gly) was obtained from Sigma Chemical Co. The terminal amino group in this peptide was acetylated by a standard method [25]. The $[Pt(en)Cl_2]$ complex was synthesized according to a procedure published in the literature [26]. The purity of the complex was checked by elemental microanalyses and NMR (^{1}H and ^{13}C) spectroscopy.

2.2. Preparation of $[Pt(en)(dmf)Cl]NO_3$

The $[Pt(en)Cl_2]$ complex was converted into the corresponding monodimethylformamide (dmf) complex $[Pt(en)(dmf)Cl]NO_3$ by treatment with 0.98 equivalents of $AgNO_3$, according to a previously published method [27]. To a solution of 55.3 mg (0.325 mmol) of $AgNO_3$ in 5 cm^3 of dmf was added a suspension of 108.3 mg (0.332 mmol) of $[Pt(en)Cl_2]$ 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 the resulting pale yellow dmf solution of $[Pt(en)(dmf)Cl]NO_3$ was used as the starting material for the preparation of the required pyrazine-bridged binuclear platinum(II) complex, $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$.

2.3. Preparation of the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex

A dmf solution of the pyrazine ligand (pz) (13.29 mg, 0.166 mmol) was added dropwise to a solution of $[Pt(en)(dmf)Cl]NO_3$. The mixture was stirred at room temperature in the dark for 3 h. The solvent was then rotary evaporated and the residue was 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 $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ was removed by filtration, washed with methanol and then ether, and air-dried. Yield 48.61 mg (40%). *Anal.* Calc. for $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2 = C_8H_{20}N_6Cl_4Pt_2$ (FW = 732.25): C, 13.12; H, 2.75; N, 11.48. Found: C, 13.16; H, 2.98; N, 11.19%. NMR (^{1}H and ^{13}C) characterization (D_2O, 200 MHz). ^{1}H NMR (δ, ppm): 2.68–2.79 (*m*, 4CH_2, en), 9.03 (*s*, 4CH, pz); ^{13}C NMR (δ, ppm): 52.34 (4CH_2, en), 153.46 (C2, C3, C4 and C5, pz).

2.4. Preparation of $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$

The $[Pt(en)Cl_2]$ and $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complexes were converted into the corresponding aqua complexes by treatment with 1.98 and 3.98 equivalents of $AgNO_3$, respectively, according to a previously published method [28]. 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 subsequent experiments.

2.5. Analyses

Elemental microanalyses for carbon, hydrogen and nitrogen were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade.

2.6. pH 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.

2.7. ^{1}H NMR measurements

The ^{1}H NMR spectra of D_2O solutions containing TSP (sodium trimethylsilylpropane-3-sulfonate) as the internal reference were recorded with a Varian Gemini 2000 spectrometer (200 MHz). Fresh solutions of the $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes and Ac-L-Met-Gly were prepared separately (the initial concentration of each reactant was 40 mM) and then mixed in an 5 mm NMR tube, and spectra were recorded at appropriate time intervals. All reactions were performed at 2.0 < pH < 2.5 and at 37 °C. The reactions between the platinum(II) complexes and Ac-L-Met-Gly dipeptide were investigated in the following molar ratios: $[Pt(en)(H_2O)_2]^{2+}$:Ac-L-Met-Gly = 1:1, 2:1 and 1:2 and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$:Ac-L-Met-Gly = 1:1 and 1:2. The ^{1}H NMR spectra were acquired using the WATERGATE sequence for water suppression. Typical acquisition conditions were as follows: 90° pulses, 24 000 data number points, 4 s acquisition time, 1 s relaxation delay, collection of 16–128 transients and final digital resolution of 0.18 Hz per point. All proton NMR spectra were processed using the Varian V NMR software (version 6.1, revision C). The chemical shifts are reported in ppm.

2.8. X-ray analysis of the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex

Diffraction data for the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex were measured with a Xcalibur kappa-geometry diffractometer using CrysAlisPro software [29] and monochromated Mo Kα radiation (λ = 0.7173 Å). Crystal data and experimental details are summarized in Table 1. The structure was solved by direct methods using SHELXS-86 [30] and refined by full-matrix least-squares calculations on F^2 with SHELXL-97 [30]. The intensity data were corrected for absorption effects [29]. Anisotropic displacement parameters were refined for all non-hydrogen atomic positions. Hydrogen atoms attached to the carbon and nitrogen atoms were placed in calculated positions (methylene C–H = 0.97, aromatic C–H = 0.93 and amine N–H = 0.90 Å). During the refinement isotropic displacement parameters for H-atoms were assigned 20% higher than the isotropic equivalent for the atom to which the H-atom was bonded. All

Table 1
Crystal data for $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$.

Crystal data	
Chemical formula	$C_8H_{20}Cl_2N_6Pt_2{\cdot}2(Cl)$
M_r	732.28
Crystal system, space group	monoclinic, $P2_1/n$
T (K)	295
a (Å)	5.07703(9)
b (Å)	12.6594(2)
c (Å)	14.1839(2)
β (°)	97.7545(16)
V (Å^3)	903.29(2)
Z	2
Radiation type	Mo Kα
μ (mm^{-1})	16.06
Crystal size (mm)	0.20 × 0.20 × 0.10
Data collection	
Diffractometer	Xcalibur
Absorption correction	Multi-scan
T_{min}, T_{max}	0.405, 1.000
No. of measured, independent and observed $[I > 2\sigma(I)]$ reflections	20103, 1588, 1545
R_{int}	0.026
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.595
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.014, 0.035, 1.20
No. of reflections	1588
No. of parameters	91
No. of restraints	0
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.65, −0.35

Table 2
Selected geometrical parameters [Å] for $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$.

Pt1–N1	2.032(3)	Pt1–N3	2.018(3)
Pt1–N2	2.040(3)	Pt1–Cl1	2.2980(10)
N1–Pt1–N2	83.24(12)	N1–Pt1–Cl1	92.82(10)
N1–Pt1–N3	175.61(12)	N2–Pt1–Cl1	175.67(9)
N2–Pt1–N3	93.51(11)	N3–Pt1–Cl1	90.52(8)
Pt1–N1–C1–C2	−41.0(4)	C2–N2–Pt1–N1	13.2(2)
N1–C1–C2–N2	52.1(4)	N2–Pt1–N1–C1	15.7(3)
C1–C2–N2–Pt1	−38.5(4)		

H-atoms were refined as riding. SIEMENS [31] and MERCURY [32] computer graphics programs were used to prepare the drawings. Selected bond distances and angles are reported in Table 2. Hydrogen-bond parameters are listed in Table 3. Atomic coordinates, anisotropic displacement parameters and tables of all bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre (Deposition No. CCDC 895153).

3. Results and discussion

3.1. X-ray structure of the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex

The molecular structure and labeling scheme are shown in Fig. 1 and selected bond distances and angles are listed in Table 2. X-ray analysis has unequivocally confirmed that the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex is a binuclear complex of Pt(II) bridged by the pyrazine ligand. In the crystal the complex is positioned on an inversion center and thus possesses C_i symmetry. Each platinum(II) atom exhibits an approximately square planar coordination, with one Pt–Cl bond, one Pt–N bond of pyrazine (pz), and two Pt–N bonds of the same chelating diamine (en) ligand. The Pt···Pt distance is 6.7890(3) Å, comparable with the mean value of 6.815(8) Å obtained from 17 observations for crystal structures

Table 3
Hydrogen bond parameters for $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$.

D–H···	*D*–H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*–H···*A* (°)
N1–H1B···Cl2	0.90	2.44	3.291 (3)	156.9
N1–H1A···Cl2^{i}	0.90	2.41	3.230 (3)	150.9
N2–H2A···Cl2^{ii}	0.90	2.46	3.313 (3)	157.4
N2–H2B···Cl2^{iii}	0.90	2.34	3.211 (3)	161.9

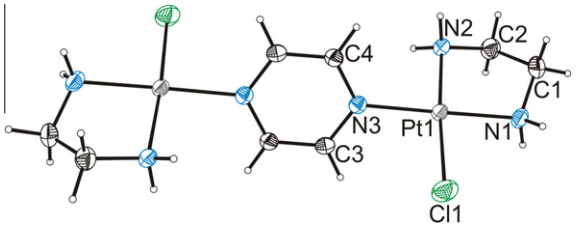

Fig. 1. Perspective view of the dinuclear complex cation $\{[Pt(en)Cl]_2(\mu\text{-pz})\}^{2+}$. Atomic displacement ellipsoids are drawn at the 40% probability level. The molecule possesses a center of symmetry, so only the asymmetric part of it has been labeled. The other half of the molecule is generated from the original coordinates by symmetry transformation $1-x, 1-y, -z$.

containing a discrete pyrazine-bridged Pt(II) dimer, deposited in the CSD [33]. The C_i molecular symmetry ensures the mutually *trans* orientation of the two Cl and (en) ligands. The atoms involved in the Pt(II) square coordination deviate less than 0.045 Å from the mean plane through the three nitrogens and Cl1. The Pt atom deviates from this plane by only 0.010(1) Å and from the pyrazine best plane by 0.084(6) Å. The Pt–N distances are in the expected range, with the Pt–N(diamine) distances only slightly longer (2.032(3) and 2.040(3) Å) than the Pt–N(pyrazine) distance of 2.018(3) Å, probably a consequence of the more negative charge on the pyrazine nitrogen as compared with the diamine nitrogens, and the Pt–Cl bond measures 2.298(3) Å. The N1–Pt–Cl(1), Cl1–Pt–N3 and N3–Pt–N2 angles are 92.8(1)°, 90.5(1)° and 93.5(1)°, respectively, while the N1–Pt–N2 angle in the five-membered chelate ring is only 83.2(1)°, a value similar to the mean N–Pt–N angle in other platinum complexes with ethylene diamine (mean value of 83.33(6)° for 207 observations in structures with $R < 5\%$ [33]). The coordinated pyrazine ring is oriented out of the Pt(II) square plane. The dihedral angle between the pyrazine-ring plane and the square plane around Pt(II) amounts to 58.4(1)°, and the Cl1–Pt–N3–C3 and Cl1–Pt–N3–C4 torsion angles are −60.6(3)° and 122.9(3)°, respectively. The diamine rings adopt the usual *twist* conformation (one λ and the other δ), with an approximate twofold axis passing through the C1–C2 bond (Cremer and Pople parameters calculated with PARST included in the WinGX package [34] are 0.439(4) Å and −92.0(4)°).

3.2. Crystal packing of the $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex

The complex cations form columns extending along the *a*-direction, with distances of 5.077(1) Å between Pt(II) ions and the pz rings (Fig. 2). The uncoordinated chloride anions (Cl2) are located above and below the pyrazine rings and are involved in anion–π interactions with these aromatic rings. Pyrazine rings are typically less electron poor than triazines, where the majority of anion–π interactions have been observed, but it has been demonstrated, both experimentally and theoretically, that coordination to the metal dramatically enhances the disposition of aromatic rings toward anion···π binding [6,11,12,35]. Accordingly, in the investigated complex the pyrazine ring is activated by coordination to

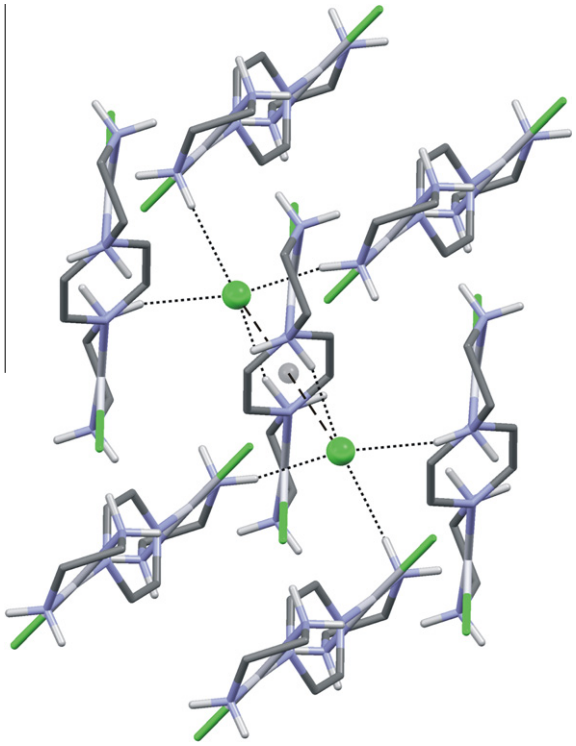

Fig. 2. Specific intermolecular interactions in the crystal structure of $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ involving complex cations and uncoordinated chloride anions. NH···Cl hydrogen bonds are marked by dotted lines while anion···π interactions are shown by broken lines joining the chloride anion (green ball) with the centre of gravity of the pyrazine ring (gray ball). For clarity, only H-atoms involved in hydrogen bonds are displayed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

two Pt(II) metal centers. The Cl2···C_g distance (C_g stands for the center of gravity of the pyrazine ring) amounts to 3.410 Å, while the angle between the normal to the pz ring and the Cl2···C_g···Cl2 line is 12.9°. For comparison, Mascal et al. have calculated an equilibrium distance of 3.2 Å between chloride and the aryl centroid in the Cl^-···1,3,5-triazine complex, with the anion positioned on the C3-axis above the triazine ring [8]. Moreover, the Cl2 anion acts as a hydrogen bond acceptor from N–H groups belonging to four different complex cations situated in its neighborhood. The hydrogen bond parameters are listed in Table 3. Notably, the coordinated chloride anion (Cl1) is not involved in hydrogen bonding or any other specific intermolecular interactions.

3.3. ^{1}H NMR investigation of the hydrolytic reactions of the N-acetylated dipeptide Ac-L-Met-Gly in the presence of $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ and $[Pt(en)(H_2O)_2]^{2+}$ complexes

The hydrolytic cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide in the presence of two platinum(II) complexes, binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ (en = bidentate coordinated ethylenediamine and pz = bridging pyrazine (or 1,4-diazine) ligand) and the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ was investigated by ^{1}H NMR spectroscopy. The corresponding peptide and platinum(II) complexes were reacted in 1:1 and 2:1 M ratios and all reactions were performed at $2.0 < pH < 2.5$ in D_2O as the solvent and at 37 °C. Additionally, the reaction between $[Pt(en)(H_2O)_2]^{2+}$ and the Ac-L-Met-Gly dipeptide was realized with an excess of the platinum(II) complex (2:1 M ratio, respectively). As was shown in previous studies [36–39], acidic solutions are needed to suppress the formation of hydroxo-bridged oligomeric Pt(II) complexes, which are catalytically inactive.

3.3.1. Reactions of platinum(II) complexes with an equimolar amount of Ac-L-Met-Gly

The mixing of $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with an equimolar amount of Ac-L-Met-Gly dipeptide under the above-mentioned conditions resulted in the spontaneous coordination of these two platinum(II) complexes to the methionine sulfur atom of the dipeptide. Different reaction pathways for the hydrolytic cleavage of this dipeptide in the reactions with the two Pt(II) complexes are presented in Fig. 3. The coordination of Ac-L-Met-Gly through the sulfur atom of methionine shifted the resonance for the methyl protons of free methionine at 2.11 ppm downfield to 2.54 ppm after its coordination to Pt(II) and this reaction was almost completed after 10 min (Table 4). These chemical shifts are in accordance with those previously reported for the reaction of platinum(II) complexes with different methionine-containing molecules [40–43]. The monodentate coordination of Ac-L-Met-Gly to the $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes resulted in the formation of two platinum(II)–peptide complexes $[Pt(en)(Ac\text{-L-Met-Gly-}S)(H_2O)]^{2+}$ (**1**) and $\{[Pt(en)(Ac\text{-L-Met-Gly-}S)](\mu\text{-pz})[Pt(en)(H_2O)]\}^{4+}$ (**2**) (Fig. 3). Complexes **1** and **2** are intermediate products and they promote the regioselective cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide. This hydrolytic reaction can be followed successfully using ^{1}H NMR spectroscopy by observing the glycine protons in complexes **1** and **2** and these protons for free glycine. The resonance at 3.99 ppm, corresponding to the glycine protons of the dipeptide attached to platinum(II) in complexes **1** and **2**, decreased while that at 3.77 ppm for free glycine increased. Upon addition of glycine to the reaction mixture, the resonance at 3.77 ppm was enhanced. Due to the fact that after 10 min all the Ac-L-Met-Gly was bound to platinum(II) (no resonance at 2.11 ppm was observed in the ^{1}H NMR spectrum), it can be assumed that the concentrations of complexes **1** and **2** were equal to the initial concentration of the dipeptide. According to this, the hydrolytic cleavage of the Met-Gly amide bond in complexes **1** and **2** was determined by integration of the resonances for the glycine protons of the dipeptide attached to the platinum(II) (3.99 ppm) and for the protons of the free glycine (3.77 ppm) (Table 4). The changes in concentrations of free glycine and non-hydrolyzed complexes **1** and **2** were followed over time and the sum of their concentrations was always equal to the initial concentration of Ac-L-Met-Gly. The hydrolysis of the Met-Gly amide bond was followed over 24 h and no change in the chemical shifts of the resonances at 2.54 and 2.64 ppm for the methyl protons of the methionine and methylene protons of ethylenediamine, respectively, were observed, indicating that the ligands were bound to platinum(II) all the time (complexes **3** and **4**; Fig. 3 and Table 4). However, when the reaction between $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ and the Ac-L-Met-Gly dipeptide was followed for more than 24 h, some changes in the aromatic region of the ^{1}H NMR spectrum occurred. These changes were manifested through the fact that the singlet at 9.03 ppm for the bridging pyrazine ligand decreased while two symmetric multiplets in the range 8.75–9.00 ppm slowly increased (Table 4). The appearance of these two new multiplets indicates that one Pt(II)–N(pyrazine) bond in complex **2** was broken and that the four pyrazine protons of this ligand coordinated in a monotopic fashion to Pt(II) (complex **4**, Fig. 3) were split into two multiplets because of vicinal and long-range coupling. Symmetric multiplets for monodentate coordinated pyrazine were also observed for *cis*- and *trans*-$[Pt(NH_3)_2(pz)_2](NO_3)_2$ and for the $[Pt(tmda)(pz)_2](NO_3)_2$ (tmda is *N*,*N*,*N*′,*N*′-tetramethylenediamine) complex [1]. The platinum(II) complex **4** (Fig. 3), with a monodentate bound pyrazine ligand, was very stable and no resonance at

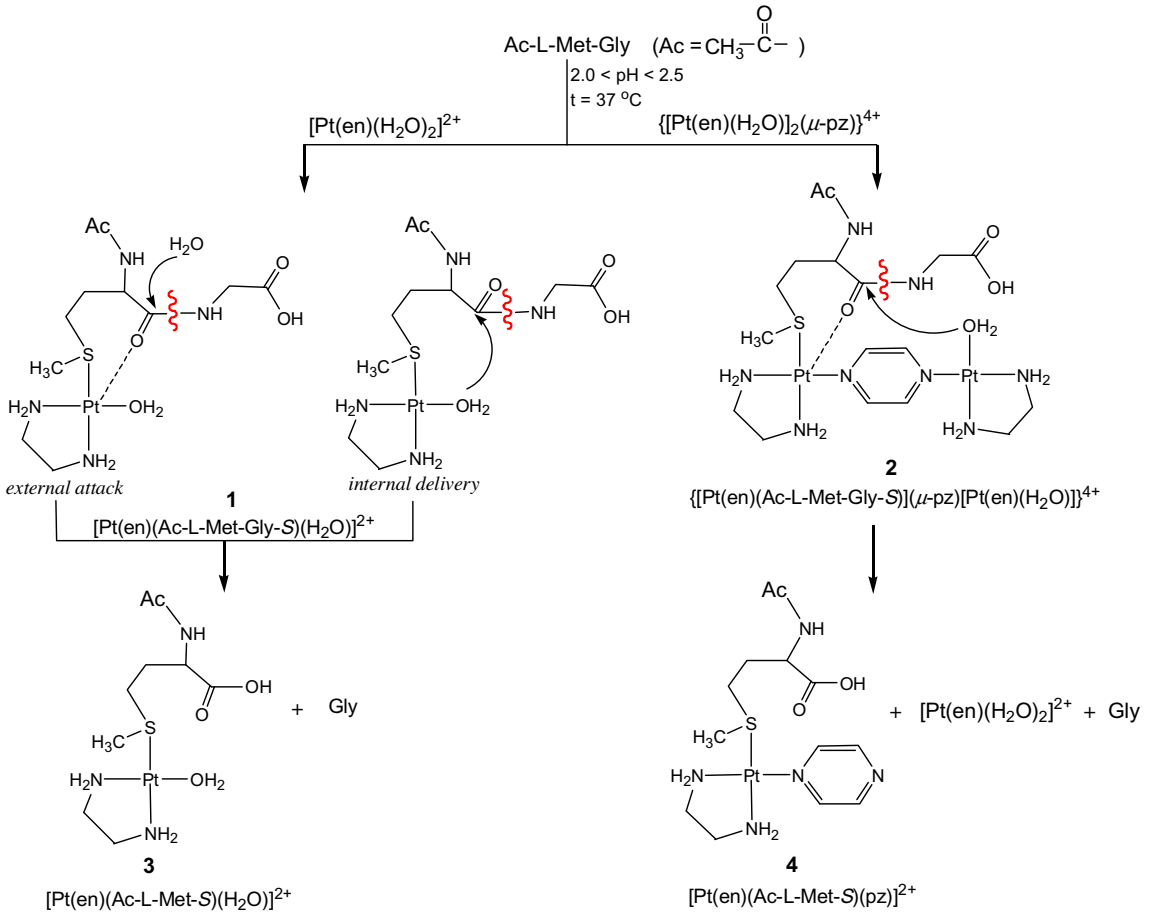

Fig. 3. Schematic presentation of the hydrolytic reactions of the Ac-L-Met-Gly dipeptide with an equimolar amount of the platinum(II) complex.

Table 4
Characteristic ^{1}H NMR chemical shifts observed in the reactions of the $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\text{-pz})\}^{4+}$ complexes with Ac-L-Met-Gly at 2.0 < pH < 2.5 in D_2O and at 37 °C. All spectra were recorded in D_2O solutions containing TSP (sodium trimethylsilylpropane-3-sulfonate) as the internal reference.

Dipeptide/Pt(II) complex/reaction product	Characteristic ^{1}H NMR resonances (δ, ppm)		
Ac-L-Met-Gly	2.11 (*s*, $MetCH_3$)	3.99 (*s*, $GlyCH_2$)	
$\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$	2.64 (*s*, $enCH_2$)	9.03 (*s*, pzCH)	
$[Pt(en)(H_2O)_2]^{2+}$	2.64 (*s*, $enCH_2$)		
$[Pt(en)(Ac\text{-L-Met-Gly-}S)(H_2O)]^{2+}$ (**1** and **7**)	2.54 (*s*, $MetCH_3$)	2.84 (*s*, $enCH_2$)	
$\{[Pt(en)(Ac\text{-L-Met-Gly-}S)](\mu\text{-pz})[Pt(en)(H_2O)]\}^{4+}$ (**2**)	2.54 (*s*, $MetCH_3$)	2.70, 2.84 (2*s*, $enCH_2$)	9.03 (*s*, pzCH)
$[Pt(en)(Ac\text{-L-Met-}S)(H_2O)]^{2+}$ (**3**)	2.54 (*s*, $MetCH_3$)	2.84 (*s*, $enCH_2$)	
$[Pt(en)(Ac\text{-L-Met-}S)(pz)]^{2+}$ (**4**)	2.53 (*s*, $MetCH_3$)	2.89 (*s*, $enCH_2$)	8.75–9.00 (*m*, pzCH)
$[Pt(en)(Ac\text{-L-Met-Gly-}S)_2]^{2+}$ (**5**)	2.54 (*s*, $MetCH_3$)	2.89 (*s*, $enCH_2$)	
$\{[Pt(en)(Ac\text{-L-Met-Gly-S})]_2(\mu\text{-pz})\}^{4+}$ (**6**)	2.53 (*s*, $MetCH_3$)	2.89 (*s*, $enCH_2$)	9.03 (*s*, pzCH)
Free glycine (Gly)	3.77 (*s*, $GlyCH_2$)		
Free pyrazine (pz)	8.66 (*s*, pzCH)		

8.66 ppm for free pyrazine [1] was detected in the ^{1}H NMR spectrum, even after this reaction was prolonged to 10 days. The time dependences of the hydrolytic cleavage of the Met-Gly amide bond in the reactions between different molar ratios of the platinum(II) complex and Ac-L-Met-Gly dipeptide are given in Fig. 4. When an equimolar amount of $[Pt(en)(H_2O)_2]^{2+}$ was mixed with this dipeptide, only 35% of the Met-Gly amide bond was cleaved after 2 h. However, in the reaction with the binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex, this cleavage was two times faster and about 70% of the Met-Gly amide bonds were cleaved in the same time. Finally, after 24 h in the presence of the binuclear Pt(II) complex, more than 83% of the Met-Gly amide bond hydrolyzed, while with the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex, about 56% of this bond had cleaved. However, when the reaction of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex with Ac-L-Met-Gly dipeptide was performed under the above-mentioned experimental conditions in an excess of the Pt(II) complex (2:1 M ratio), the cleavage of the peptide was faster than with equimolar amounts of these reactants. The hydrolysis of the dipeptide in the presence of two equivalents of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex was still slower than that with an equimolar amount of the binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex (see Fig. 4). The better catalytic ability of the binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{ -pz})\}^{4+}$ complex in comparison with the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex can be attributed to the structural differences of their hydrolytically active platinum(II)–dipeptide complexes (complexes **1** and **2**; Fig. 3). Hydrolysis of the Met-Gly amide bond in the presence of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex can occur by two possible limiting mechanisms, both represented in complex **1** (Fig. 3) [44–46]. The first possibility is that the platinum(II) ion interacts

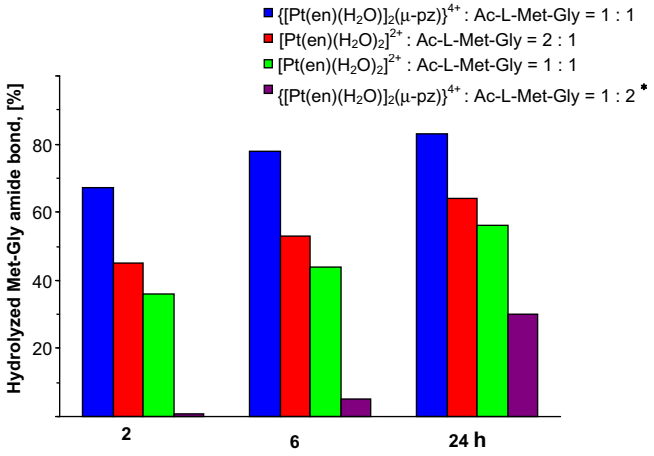

Fig. 4. Time dependence of the hydrolytic cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide with platinum(II) complexes at 2.0 < pH < 2.5 and at 37 °C. *No hydrolysis of the amide bond was observed in the reaction of $[Pt(en)(H_2O)_2]^{2+}$ with an excess of dipeptide.

with the oxygen atom of the scissile amide bond. This interaction polarizes the carbonyl group and activates its carbon atom toward attack by a water molecule from the solvent (*external attack*). For the reaction to occur by this mechanism, the platinum(II) and carbonyl oxygen atoms should be proximate. The other possibility is that an aqua ligand on platinum(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, the aqua ligand on platinum(II) should be proximate to the carbonyl carbon of the scissile amide bond. Additionally, it was found that a coordinated aqua ligand in Pd(II) and Pt(II) complexes plays an important role in promoting the regioselective cleavage of the amide bond in histidine- and methionine-containing peptides [36,44–50]. Two platinum(II) centers bridged with one aromatic pyrazine ligand in the hydrolytically active complex **2** are more efficient in the hydrolysis of the scissile amide bond than even two equivalents of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex (Fig. 4). The two platinum(II) centers in complex **2** can simultaneously participate in the cleavage of the scissile amide bond, one by polarizing the carbonyl oxygen atom and another by delivering a water molecule. Meanwhile the single Pt(II) ion in the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ complex is incapable in promoting these two interactions simultaneously. Our latest results for the good catalytic ability of the binuclear platinum(II) complex in the cleavage of the Ac-L-Met-Gly dipeptide are also in accordance with those previously reported for the reaction of a binuclear palladium(II) complex having thiolate bridging ligands with different methionine-containing peptides [19] and for different polynuclear complexes with nucleic acids and phosphate esters [20,51].

3.3.2. Reactions of platinum(II) complexes with an excess of Ac-L-Met-Gly

The hydrolytic cleavage of the Met-Gly amide bond in the presence of the $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes was studied with an excess of the Ac-L-Met-Gly dipeptide.

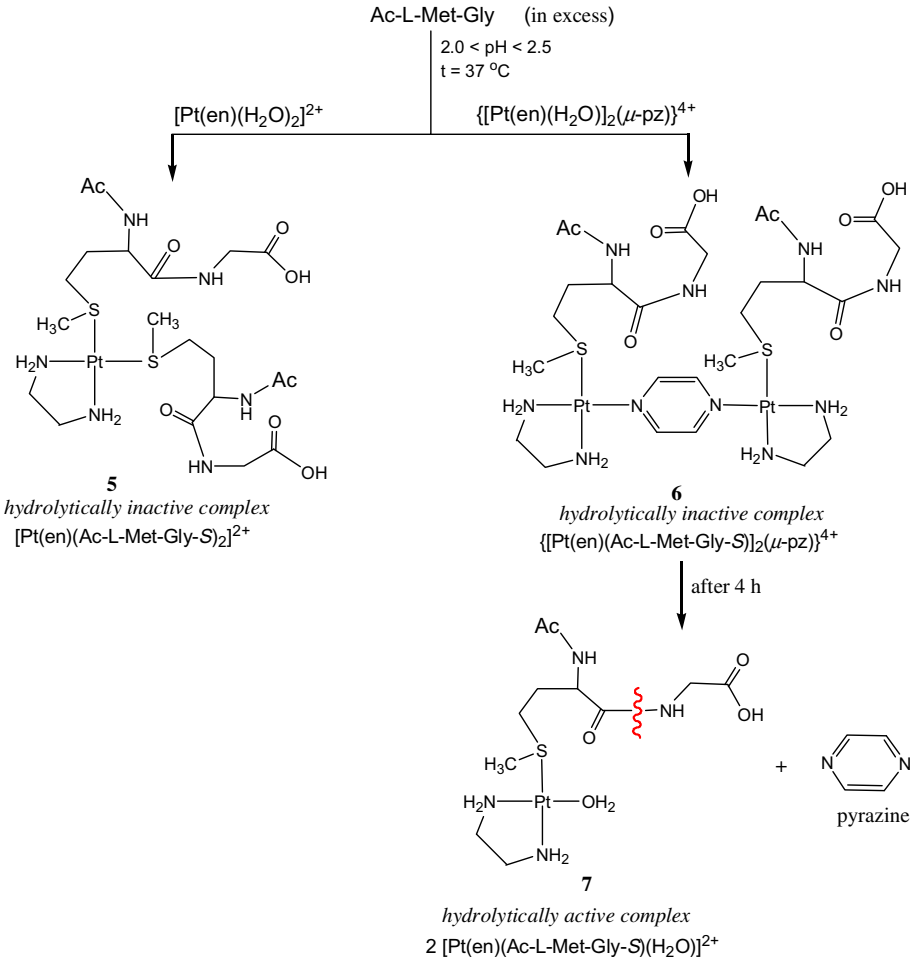

Fig. 5. Reaction pathways of the $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complexes with an excess of the Ac-L-Met-Gly dipeptide (1:2 M ratio, respectively) at 2.0 < pH < 2.5 and at 37 °C.

The two platinum(II) complexes, $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and the corresponding dipeptide were mixed in a 1:2 M ratio, respectively, and all reactions were performed at $2.0 < pH < 2.5$ and at 37 °C. The formation of the $[Pt(en)(Ac\text{-L-Met-Gly-}S)_2]^{2+}$ (**5**) and $\{[Pt(en)(Ac\text{-L-Met-Gly-}S)]_2(\mu\text{-pz})\}^{4+}$ (**6**) complexes was evidenced by the simultaneous decline of the resonance at 2.11 ppm, arising from the *S*-methyl protons of free Ac-L-Met-Gly, and the growth of the resonance at 2.54 ppm, corresponding to the *S*-methyl protons of the peptide coordinated to Pt(II) through the sulfur atom (complexes **5** and **6**; Fig. 5 and Table 4). These reactions were followed over time and no resonance at 2.11 ppm for the free peptide was observed in the ^{1}H NMR spectra after 2 h. In addition, during this time, no cleavage of any amide bond in Ac-L-Met-Gly was observed, indicating that the platinum(II)–peptide complexes **5** and **6** are hydrolytically inactive. Moreover, in the reaction between $[Pt(en)(H_2O)_2]^{2+}$ and Ac-v-Met-Gly, the hydrolytic reaction was not observed, even when the reaction was prolonged to 4 days. Additionally, no changes in the ^{1}H NMR spectrum for this reaction were observed during this time, confirming that complex **5** is very stable under the employed experimental conditions. However, the hydrolytically inactive complex **6**, formed in the reaction between the $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex and the Ac-L-Met-Gly dipeptide, was unstable under these experimental conditions and after 4 h of reaction, it was converted into the hydrolytically active $[Pt(en)(Ac\text{-L-Met-Gly-}S)(H_2O)]^{2+}$ complex **7**. This complex is structurally identical with complex **1**, obtained in the reaction between equimolar amounts of the mononuclear $[Pt(en)(H_2O)_2]^{2+}$ and Ac-L-Met-Gly dipeptide (see Fig. 3). The conversion of **6** into **7** was evident in the ^{1}H NMR spectrum by the simultaneous decline of the singlet at 9.03 ppm, due to protons of the bridging pyrazine ligand, and the growth at 8.66 ppm, due to the free pyrazine ligand (Table 4). This conversion was very slow and only 30% of free pyrazine was detected in the solution after 48 h. Along with the resonance for the free pyrazine ligand, a new resonance at 3.77 ppm was also detected in the ^{1}H NMR spectrum. This resonance was assigned to free glycine protons and its intensity increased upon addition of glycine to the reaction mixture. This undoubtedly confirmed that inactive complex **6** was converted into the hydrolytically active species **7**, which further promotes the cleavage of the Met-Gly amide bond. The amounts of the hydrolysis products in this reaction were determined from the known initial concentration of Ac-L-Met-Gly and from the integrated resonance of the free glycine. It was found that in the reaction of the $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex with a two times bigger amount of the peptide, only 30% of the Met-Gly bond had hydrolyzed after 24 h (see Fig. 4). However, when this reaction was prolonged for a further 24 h, the amount of the hydrolyzed amide bond was about 50%.

4. Conclusions

The X-ray elucidation of the pyrazine-bridged $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ binuclear complex confirmed the *trans* configuration of its complex cation and the existence of anion$\cdots\pi$ interactions between the uncoordinated chloride and electron-deficent pyrazine ring. The reactions of $[Pt(en)(H_2O)_2]^{2+}$ and $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ with the Ac-L-Met-Gly dipeptide at $2.0 < pH < 2.5$ and at 37 °C are remarkably selective in the cleavage of the amide bond involving the carboxylic group of methionine. In comparison with the single Pt(II) ion in the $[Pt(en)(H_2O)_2]^{2+}$ complex, the 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. Moreover, when the reaction of the platinum(II) complexes was investigated with an excess of the dipeptide, only the binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex was shown to be a capable catalytic agent in the hydrolysis of the dipeptide. The better catalytic ability of the binuclear $\{[Pt(en)(H_2O)]_2(\mu\text{-pz})\}^{4+}$ complex than the corresponding mononuclear Pt(II) complex can be attributed to the presence of different hydrolytically active Pt(II)–peptide complexes formed during the reaction with the Ac-L-Met-Gly dipeptide. These results together with those previously reported for the reaction of a binuclear palladium(II) complex having thiolate bridging ligands [18] should be taken into consideration when designing new polynuclear platinum(II) and palladium(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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Appendix A. Supplementary material

CCDC 895153 contains the supplementary crystallographic data for $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

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