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^{1}H NMR study of the reactions between carboplatin analogues [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] and various methionine- and histidine-containing peptides under physiologically relevant conditions

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ABSTRACT

^{1}H NMR spectroscopy was applied to the study the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes (en is ethylenediamine, Me-mal and Me$_2$-mal are bidentate coordinated anions of 2-methylmalonic and 2,2-dimethylmalonic acids, respectively) with *N*-acetylated Ac-L-Met-Gly and Ac-L-Met-L-His-type peptides (Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH$_2$ and Ac-L-Met-Gly-Gly-L-His-Gly). The use of Me-mal and Me$_2$-mal Pt(II) complexes in the above reactions allows convenient monitoring of their biscarboxylate group via methyl peaks by ^{1}H NMR measurements. All reactions were realized at 37 °C with equimolar amounts of the Pt(II) complex and the dipeptide at pH 7.40 in 50 mM phosphate buffer in D$_2$O. In all these reactions the ring-opened Me-mal and Me$_2$-mal Pt(II) adducts as an intermediate products were detected in solution for more than 48 h. We found that during this time in the reaction with Ac-L-Met-Gly these monodentate bound malonate ligands have been replaced by water molecule leading to the formation of the corresponding aqua Pt(II)–peptide complex which further promotes the regioselective cleavage of the peptide. However, in the reaction with Ac-L-Met-L-His-type peptides a selective intramolecular replacement of these malonate anions by the N3 imidazole nitrogen atom from histidine residue was occurred. This replacement reaction leads to the formation of the *S*,*N*3-macrochelate Pt(II)–peptide complex which was shown as very stable and hydrolytically inactive for more than two weeks.

1. Introduction

Recent years have witnessed an increasing interest in the study of the interactions of platinum(II) and palladium(II) complexes with sulfur- and histidine-containing peptides and proteins [1–4]. This interest was induced through the facts that interactions of platinum(II) complexes with methionine or cysteine residues in peptides and proteins are thought to be responsible for a variety of biological effects, such as deactivation of platinum(II) antitumor complexes, development of cellular resistance to platinum drugs and to toxic side effects, such as nephrotoxicity [5]. The presence of histidine has also been established in a large number of enzyme active centers [6] and the histidyl residue is probably the most important metal-binding site in biological systems [7,8]. Moreover, interest in the study of the interactions of platinum(II) [9–11] and palladium(II) [10–24] complexes with methionine- and histidine-containing peptides and proteins also become of cardinal importance after the discovery that their aqua complexes can be promising reagents for the hydrolytic cleavage of the above-mentioned peptides. In general, it was shown that these complexes bind to the heteroatom in the side chain of methionine [9–16] or histidine [10,11,17–25] and promote cleavage of the amide bond involving the carboxylic group of the anchoring amino acid. The influence of different factors, such as pH, temperature, solvent and steric effects of the substrate or catalyst, on this hydrolytic reaction has been extensively investigated in the past two decades. Up to now, most of these investigations were performed in strong acidic media, whereas only a few reports concerning this hydrolytic reaction between methionine- and histidine-containing peptides and platinum(II) antitumor complexes under physiologically relevant conditions have been reported [26–30]. The findings that Pt(II) complexes can cleave peptides and proteins under physiologically relevant conditions of the pH and temperature can have importance for a better understanding of the toxic side effects of Pt(II) antitumor drugs. Our recent studies of the reactions between various Pt(II) complexes of the type [Pt(L)Cl$_2$] and [Pt(L)(cbdca-*O*,*O*′] (L is ethylenediamine, en; (±)-*trans*-1,2-diaminocyclohexane, dach; (±)-1,2-propylenediamine, 1,2-pn and cbdca is cyclobutane-1,1-dicarboxylate) and Ac-L-Met-Gly dipeptide showed that hydrolysis of the Met-Gly amide bond in this peptide occurred under physiological conditions of pH and temperature (pH 7.40 and 37 °C) [31].

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The cleavage of this peptide was much slower in the reaction with [Pt(L)(cbdca-*O*,*O*′] than with [Pt(L)Cl_2] complexes. Difference in the catalytic abilities between these two type of Pt(II) complexes was attributed to the different stability of their intermediate [Pt(L)(cbdca-*O*)(Ac-L-Met-Gly-*S*)]$^-$ and [Pt(L)Cl(Ac-L-Met-Gly-*S*)] complexes, respectively. The higher stability of the initially formed [Pt(L)(cbdca-*O*)(Ac-L-Met-Gly-*S*)]$^-$ complex with respect to the analogue [Pt(L)Cl(Ac-L-Met-Gly-*S*)] intermediate product is in accordance with the slow replacement of its cbdca ligand by a water molecule, finally resulting in the formation of the hydrolytically active [Pt(L)(H_2O)(Ac-L-Met-Gly-*S*)]$^+$ complex. These findings are in accordance with those previously reported that very stable ring-opened [Pt(NH_3)$_2$(cbdca-*O*)(L-HMet-*S*)] complex was detected during the reaction of carboplatin with L-HMet and that similar ring-opened species was also found in the urine of animals treated with carboplatin (carboplatin, [Pt(NH_3)$_2$(cbdca-*O*,*O*′)], is a widely used second generation anticancer drug) [32]. Malonate complexes of Pt(II)-am(m)ine exhibited antitumor activity without the nephrotoxic effects of cisplatin, *cis*-[Pt(NH_3)$_2$$Cl_2$] [33,34].

We report here reactions of peptides with methionine (Ac-L-Met-Gly) and both methionine and histidine in the side chains (Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH_2 and Ac-L-Met-Gly-Gly-L-His-Gly) with carboplatin analogues, [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)]. Our use of 2-methylmalonate (Me-mal) and 2,2-dimethylmalonate (Me$_2$-mal) in the place of cyclobutane-1,1-dicarboxylate (cbdca) allows convenient monitoring the biscarboxylate group via methyl peaks by ^{1}H NMR measurements. Also, [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes showed better solubility in comparison with analogue [Pt(L)(cbdca-*O*,*O*′)] (L is en, dach and 1,2-pn).

2. Experimental

2.1. Materials

Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm^{-1}. The compounds D_2O, ethylenediamine (en), malonic acid (H_2mal), 2-methylmalonic acid (Me-H_2mal), 2,2-dimethylmalonic acid (Me$_2$-H_2mal) 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 dipeptide L-methionyl-L-histidine (L-Met-L-His) and pentapeptide L-methionylglycylglycyl-L-histidylglycine (L-Met-Gly-Gly-L-His-Gly) were obtained from the Bachem A.G. The tetrapeptide *N*-acetylated-L-methionylglycyl-L-histidylglycineamide (Ac-L-Met-Gly-L-His-GlyNH_2) was synthesized by manual solid phase peptide synthesis using Fmoc-chemistry [35,36]. The peptide was purified using semi-preparative RP-HPLC, and analyzed by analytical HPLC and electrospray ionization mass spectrometry. The terminal amino group in this peptide was acetylated by a standard method [12]. The [Pt(en)Cl_2] complex was synthesized according to a procedure published in the literature [37–39]. The purity of the complex was checked by elemental microanalyses. *Anal.* Calc. for [Pt(en)Cl_2] = $C_2H_8N_2Cl_2Pt$ (FW = 326.08): C, 7.37; H, 2.47; N, 8.59. Found: C, 7.32; H, 2.50; N, 8.52%.

2.2. Syntheses of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal)] complexes

The [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes were synthesized by modification of the procedure published in the literature [40].

The chlorido complex [Pt(en)Cl_2] was converted into the corresponding diaqua complex by treatment with 1.95 equivalents of $AgNO_3$ at pH 2.00, according to a published method [41]. The solid AgCl was removed by filtration in the dark. To the clear solution of the [Pt(en)(H_2O)$_2$]$^{2+}$ complex, equimolar amounts of the solid Me-H_2mal (or Me$_2$-H_2mal) acid and two equivalents of $NaHCO_3$ were added. The mixture was stirred at 60 °C for 3 h. All the complexes were crystallized from water by cooling in a refrigerator. The pure complexes were obtained by recrystallization from small amount of water. The yield was between 50% and 60%. *Anal.* Calc. for [Pt(en)(Me-mal-*O*,*O*′)] = $C_6H_{12}N_2O_4Pt$ (FW = 371.25): C, 19.41; H, 3.26; N, 7.55. Found: C, 19.50; H, 3.22; N, 7.48%. *Anal.* Calc. for [Pt(en)(Me$_2$-mal-*O*,*O*′)] = $C_7H_{14}N_2O_4Pt$ (FW = 385.28): C, 21.82; H, 3.66; N, 7.27. Found: C, 21.54; H, 3.42; N, 7.43%.

2.3. NMR (^{1}H and ^{13}C) characterization (D_2O, 200 MHz)

[Pt(en)(Me-mal-*O*,*O*′)]: ^{1}H NMR, δ (ppm) 2.57 (*s*, 4H, 2CH_2 from en), 4.16 (*q*, H, α-CH from Me-mal), 1.37 (*d*, 3H, CH_3 from Me-mal); ^{13}C NMR, δ (ppm) 16.54 (CH_3 from Me-mal), 50.79 (CH_2 from en), 53.68 (CH from Me-mal), 183.30 (C=O from Me-mal).

[Pt(en)(Me$_2$-mal-*O*,*O*′)]: ^{1}H NMR, δ (ppm) 2.59 (*s*, 4H, 2CH_2 from en), 1.77 (*s*, 6H, 2CH_3 from Me$_2$-mal); ^{13}C NMR, δ (ppm) 27.87 (CH_3 from Me$_2$-mal), 50.68 (CH_2 from en), 55.32 (C from Me$_2$-mal), 185.63 (C=O from Me$_2$-mal).

These data are in accordance with those reported previously for the corresponding Pt(II) complexes [39,42].

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 reactions of Ac-L-Met-Gly, Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH_2 and Ac-L-Met-Gly-Gly-L-His-Gly with the platinum(II) complexes in 50 mM phosphate buffer at pH 7.40 in D_2O were followed by ^{1}H NMR spectroscopy using a Varian Gemini 2000 spectrometer (200 MHz). Sodium trimethylsilylpropane-3-sulfonate (TSP) was used as an internal reference. The final solution was 10 mM in each reactant. All reactions were performed at 37 °C.

Equimolar amounts of the platinum(II) complex and the peptide were mixed in an 5 mm NMR tube and the rate constants were determined from the proton NMR data recorded at appropriate time intervals. The formation of the ring-opened complexes **1a** and **1b** (Fig. 1) in the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with Ac-L-Met-Gly and Ac-L-Met-L-His dipeptides, respectively, was fitted to a second-order process [43] by plotting $x/a_o(a_o - x)$ against t (a_o is the initial concentration of the Pt(II) complex and x is the concentration of the corresponding ring-opened complex at time t). The concentrations of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes were determined by integration of the resonances for the CH_3 protons of bidentatly coordinated Me-mal at 1.37 and Me$_2$-mal at 1.77 ppm and those for these protons at 1.24 for Me-mal and 1.27 ppm for Me$_2$-mal both coordinated in monotopic fashion of the corresponding ring-opened Pt(II)–peptide complex.

The deatachment of Me-mal and Me$_2$-mal ligands from the ring-opened Pt(II)–peptide complexes **1a** and **1b** leading to the formation of the aqua Pt(II)–peptide complex **2** and macrochelate complex **4** (Fig. 1), respectively, was fitted to a first-order process [43] by plotting $\ln(a_o/a_t)$ against t (a_o is the concentration of the corresponding ring-opened Pt(II)–peptide complex and a_t is the concentration of this complex at time t). The concentrations of the ring-opened complexes **1a** and **1b** were determined by integration of the resonances for the CH_3 protons of the Me-mal at 1.24 and Me$_2$-mal at 1.27 ppm coordinated in monotopic fashion to

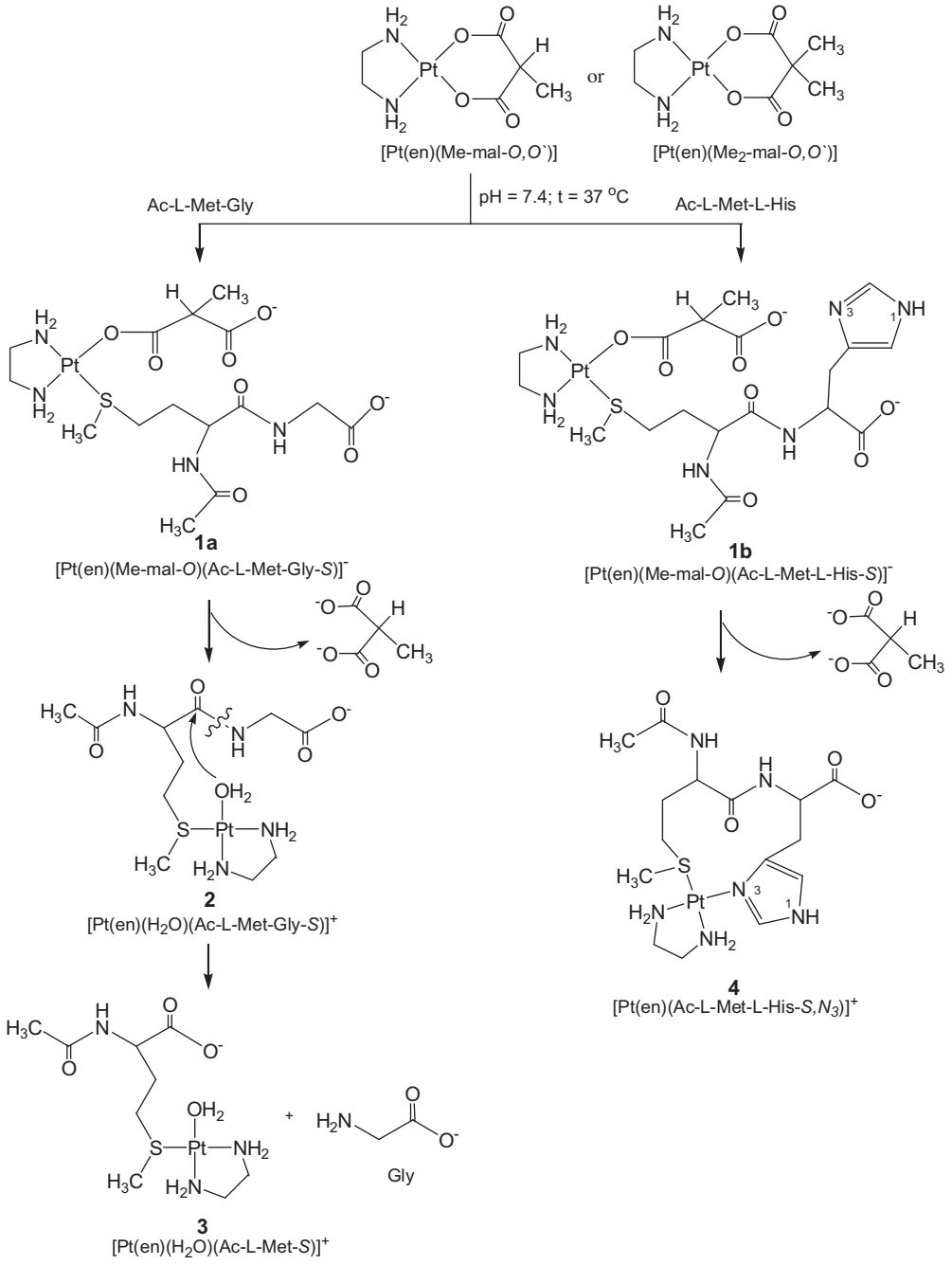

Fig. 1. Different pathways of the reaction of the Ac-L-Met-Gly and Ac-L-Met-His-type peptides with [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] complexes. The corresponding Pt(II) complex and peptide were mixed in a 1:1 M ratio and all reactions performed at pH 7.40 and at 37 °C in 50 mM phosphate buffer in D_2O.

the platinum(II) and those for these protons of the free Me-mal and Me_2-mal ligands at 1.31 and 1.34 ppm, respectively.

3. Results and discussion

The reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] complexes (en is ethylenediamine, Me-mal and Me_2-mal are bidentate coordinated anions of 2-methylmalonic and 2,2-dimethylmalonic acids, respectively, with *N*-acetylated Ac-L-Met-Gly and Ac-L-Met-L-His-type peptides (Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH_2 and Ac-L-Met-Gly-Gly-L-His-Gly) were studied by ^{1}H NMR spectroscopy. All reactions were performed with equimolar amounts of the platinum(II) complex and peptide at the pH 7.40 in 50 mM phosphate buffer in D_2O and at 37 °C. Under these experimental conditions ethylenediamine ligand remains bound to the Pt(II) [33], while bidentate coordinated Me-mal and Me_2-mal ligands in the platinum(II) complexes undergo substitution during their reactions with peptides. In the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] complexes with Ac-L-Met-Gly very slow hydrolytic cleavage of the Met-Gly amide bond was observed, while in the reactions between these Pt(II) complexes and peptides containing both L-methionine and L-histidine amino acids in the side chains no hydrolysis of these peptides was observed and only macrochelate platinum(II)–peptide complexes formed as a final products. In all these reactions an intermediate Pt(II)–peptide

Table 1
Characteristic proton NMR chemical shifts for the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] with Ac-L-Met-Gly dipeptide at pH 7.40 in 50 mM phosphate buffer in D_2O and at 37 °C.

Reactants/products	Characteristic ^{1}H NMR chemical shifts (δ, ppm; *J*, Hz)[a]				
	α-CH-mal	CH_3-mal	$(CH_3)_2$-mal	CH_3-Met	Gly-CH_2
Ac-L-Met-Gly				2.11(*s*)	3.78 (*s*)
[Pt(en)(Me-mal-*O*,*O*′)]	4.16 (*q*, *J* = 6.92)	1.37 (*d*, *J* = 6.96)			
[Pt(en)(Me_2-mal-*O*,*O*′)]			1.77 (*s*)		
[Pt(en)(Me-mal-*O*)(Ac-L-Met-Gly-*S*)]$^-$ **(1a)**	3.29 (*q*, *J* = 7.02)	1.24 (*d*, *J* = 7.14)		2.38 (*s*)	3.78 (*s*)
[Pt(en)(Me_2-mal-*O*)(Ac-L-Met-Gly-*S*)]$^-$ **(1a)**			1.27 (*s*)	2.38 (*s*)	3.78 (*s*)
[Pt(en)(H_2O)(Ac-L-Met-Gly-*S*)]$^+$ **(2)**				2.38 (s)	3.78 (*s*)
Free Me-mal		1.31(*d*)			
Free Me_2-mal			1.34 (*s*)		
Free Gly					3.56 (*s*)

[a] In all complexes the multiplet for methylene protons of en is centered at 2.70 ppm.

Table 2
Observed rate constants for the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] complexes with Ac-L-Met-Gly and Ac-L-Met-L-His dipeptides. All rate constants were obtained from ^{1}H NMR measurements at pH 7.40 in 50 mM phospahate buffer in D_2O and at 37 °C.

Reactions	Second-order rate constants for formation of the ring-opened complexes **1a** (Ac-L-Met-Gly) and **1b** (Ac-L-Met-L-His) $\mathbf{10^3 k_2/M^{-1} s^{-1}}$	First-order rate constants for conversion of **1a** into **2** (Ac-L-Met-Gly) and **1b** into **4** (Ac-L-Met-L-His) $\mathbf{10^6 k/s^{-1}}$
[Pt(en)(Me-mal-*O*,*O*′)] + Ac-L-Met-Gly	(26.20 ± 0.62)	(3.02 ± 0.02)
[Pt(en)(Me_2-mal-*O*,*O*′)] + Ac-L-Met-Gly	(4.05 ± 0.04)	(1.45 ± 0.02)
[Pt(en)(Me-mal-*O*,*O*′)] + Ac-L-Met-L-His	(11.95 ± 0.31)	(5.22 ± 0.03)
[Pt(en)(Me_2-mal-*O*,*O*′)] + Ac-L-Met-L-His	(6.19 ± 0.02)	(5.98 ± 0.03)

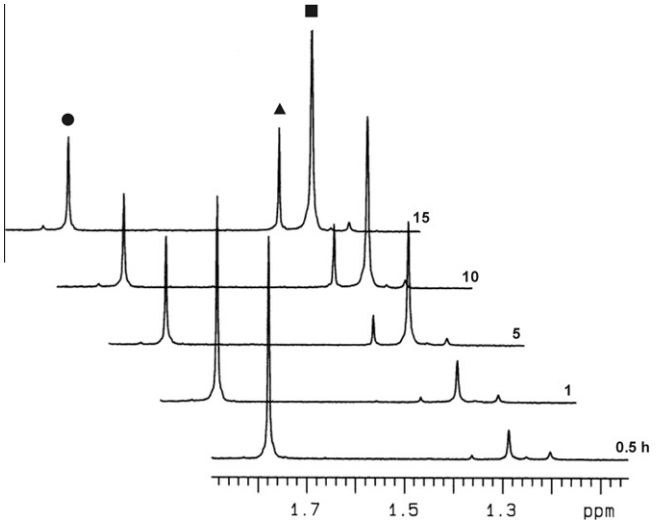

Fig. 2. Parts of the ^{1}H NMR spectra recorded during the reaction of [Pt(en)(Me_2-mal-*O*,*O*′)] with an equimolar amount of Ac-L-Met-Gly dipeptide as a function of time at pH 7.40 and at 37 °C in 50 mM phosphate buffer in D_2O with TSP as the internal standard. The resonances assigned as (■), (▲) and (●) correspond to the methyl malonate protons of the [Pt(en)(Me_2-mal-*O*)(Ac-L-Met-Gly-*S*)]$^-$, free Me_2-mal and [Pt(en)(Me_2-mal-*O*,*O*′)], respectively.

products containing ring-opened Me-mal and Me_2-mal rings were detected in solution.

3.1. Reactions of Pt(II) complexes with Ac-L-Met-Gly

The schematic presentation of the reactions of Ac-L-Met-Gly dipeptide with two platinum(II) complexes, [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)], is given in Fig. 1. When an equimolar amount of the Pt(II) complex was incubated with Ac-L-Met-Gly dipeptide under the above-mentioned experimental conditions, the first product observed in solution after 15 min of the reaction was an intermediate complex **1a** (Fig. 1). The formation of this intermediate product containing monodentate coordinated Me-mal (or Me_2-mal) ligand was evidenced in the ^{1}H NMR spectrum by the simultaneous decline of the resonances at 2.11 ppm, arising from the *S*-methyl protons of free Ac-L-Met-Gly, and growth of the resonance at 2.38 ppm, corresponding to these protons for the dipeptide coordinated to Pt(II) through the sulfur atom (Table 1). Additionally, formation of the intermediate complex **1a** for the reaction of Ac-L-Met-Gly dipeptide with [Pt(en)(Me-mal-*O*,*O*′)] was followed from difference in the chemical shifts of the resonances for α-CH at 4.16 (*q*, *J* = 6.92) and CH_3 malonate protons at 1.37 ppm (*d*, *J* = 6.96 Hz) for [Pt(en)(Me-mal-*O*,*O*′)] and those for these protons of **1a** at 3.29 (*q*, *J* = 7.02) and 1.24 ppm (*d*, *J* = 7.14 Hz), respectively (Fig. 1 and Table 1). These chemical shifts are in accordance with those previously reported for the ring-opened Me-mal-*O* adducts containing monodentate coordinated thioethers in the reactions of the [Pt(en)(Me-mal-*O*,*O*′)] complex with Ac-L-Met and Met-Gly [33]. Formation of **1a** in the reaction of Ac-L-Met-Gly with [Pt(en)(Me_2-mal-*O*,*O*′)] was observed in ^{1}H NMR spectrum from difference in the chemical shifts of the singlet for the methyl Me_2-mal protons at 1.77 ppm for [Pt(en)(Me_2-mal-*O*,*O*′)] and that for these protons at 1.27 ppm due to the complex **1a** (Fig. 1 and Table 1). However, in the present study the formation of the ring-opened complex in the reaction of [Pt(en)(mal-*O*,*O*′)] (mal is bidentate coordinated anion of malonic acid) with Ac-L-Met-Gly could not be followed by ^{1}H NMR spectroscopy based on the α-CH_2 protons of malonate anion because of the rapid exchange of its methylene protons with deuterium from D_2O solvent. Also, this exchange occurred when pure malonic acid was dissolved in D_2O. The coordination of malonic acid to Pt(II) complex additionally activates H/D exchange [44,45].

The formation of the ring-opened adducts **1a** in the above investigated reactions was followed during time and second-order rate constant for the reaction of [Pt(en)(Me-mal-*O*,*O*′)] with Ac-L-Met-Gly, $k_2 = (26.20 \pm 0.62) \times 10^{-3}\ M^{-1}\ s^{-1}$, was six times lager from that for the reaction of this peptide with analogue [Pt(en)(Me_2-mal-*O*,*O*′)] complex, $k_2 = (4.05 \pm 0.04) \times 10^{-3}\ M^{-1}\ s^{-1}$ (Table 2).

It was found that complex **1a** under the above mentioned experimental conditions was converted into the hydrolytically active complex **2** (Fig. 1). This conversion proceeds through the

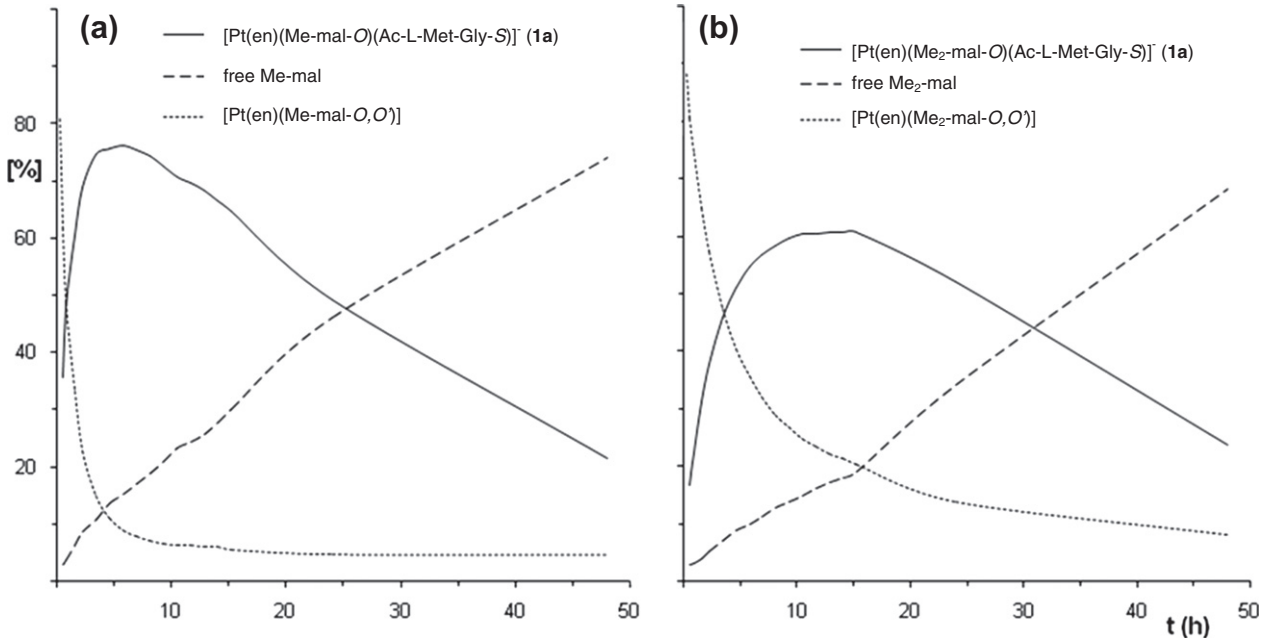

Fig. 3. Time dependence of relative percentages of Me-mal and Me_2-mal species (based on the CH_3 malonate protons) for the reactions of [Pt(en)(Me-mal-*O*,*O*′)] **(a)** and [Pt(en)(Me_2-mal-*O*,*O*′)] **(b)** complexes with an equimolar amount of Ac-L-Met-Gly dipeptide at pH 7.40 in 50 mM phosphate buffer in D_2O and at 37 °C.

detachment of the monodentate coordinated Me-mal and Me_2-mal ligands from Pt(II) and their replacement by water molecules. The conversion of **1a** into **2** is evident in the ^{1}H NMR spectrum by the simultaneous decline of the doublet at 1.24 and singlet at 1.27 ppm due to the methyl protons of monodentate coordinated Me-mal and Me_2-mal ligands, respectively, and the growth of these resonances at 1.31 and 1.34 ppm for the free Me-mal and Me_2-mal ligands, respectively (Table 1). The parts of ^{1}H NMR spectra measured during time for the reaction between [Pt(en)(Me_2-mal-*O*,*O*′)] and Ac-L-Met-Gly are presented in Fig. 2. The plots illustrating the changes in concentration [%] of **1a** and free Me-mal and Me_2-mal ligands during 48 h are presented in Fig. 3. From this figure it can be seen that concentration of **1a** was increased in the first 5 h for the Me-mal and 15 h for the Me_2-mal ring opened complex. After this time the concentrations of these ring-opened adducts were decreased finally being about 25% after 48 h. The first-order rate constants for the conversion of **1a** into **2** were determined by ^{1}H NMR measurements, $k = (3.02 \pm 0.02) \times 10^{-6}$ s^{-1} for Me-mal and $k = (1.45 \pm 0.02) \times 10^{-6}$ s^{-1} for Me_2-mal (see Section 2 and Table 2).

We found that complex **2**, obtained after displacement of monodentate coordinated Me-mal and Me_2-mal ligands from complex **1a** by water molecule, promote the regioselective cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide (Fig. 1). This hydrolytic reaction is very slow and it could be followed successfully by ^{1}H NMR spectroscopy. The resonance at 3.78 ppm corresponding to the glycine protons of the non-hydrolyzed peptide decreased while that at 3.56 ppm for free glycine increased (Table 1). Upon addition of glycine to the reaction mixture, its resonance was enhanced. The amount of hydrolyzed Met-Gly amide bond in the complex **2** was determined by integration of the resonance for the glycine protons in the non-hydrolyzed peptide and that for the free glycine. In this study we compared the catalytic abilities in the cleavage of the Ac-L-Met-Gly dipeptide for Pt(II) complexes with malonate-type ligands, [Pt(en)(mal-*O*,*O*′)], [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)], with those for previously investigated [Pt(en)Cl_2] and [Pt(en)(cbdca-*O*,*O*′)] complexes [31] (Fig. 4). From this figure in can be concluded that the catalytic abilities of these Pt(II) complexes increase in the following order: [Pt(en)(cbdca-*O*,*O*′)] < [Pt(en)(Me_2-mal-*O*,*O*′)] < [Pt(en)(Me-mal-*O*,*O*′)] < [Pt(en)(mal-*O*,*O*′)] < [Pt(en)Cl_2]. This finding can be attributed to the steric influence of the leaving ligands on the catalytic properties of the Pt(II) complexes indicating that cbdca ligand is more sterically demanding in comparison with bidentate malonate-type or monodentate chlorido ligands. This is clearly demonstrated in the reaction of Ac-L-Met-Gly dipeptide with three malonate Pt(II) complexes which differ in the number of the methyl groups attached on the α-malonate carbon atom. The rate of the cleavage of this dipeptide was decreased in the following order: mal > Me-mal > Me_2-mal (see inserted chart in Fig. 4). This undoubtedly confirms that inhibition of the hydrolytic

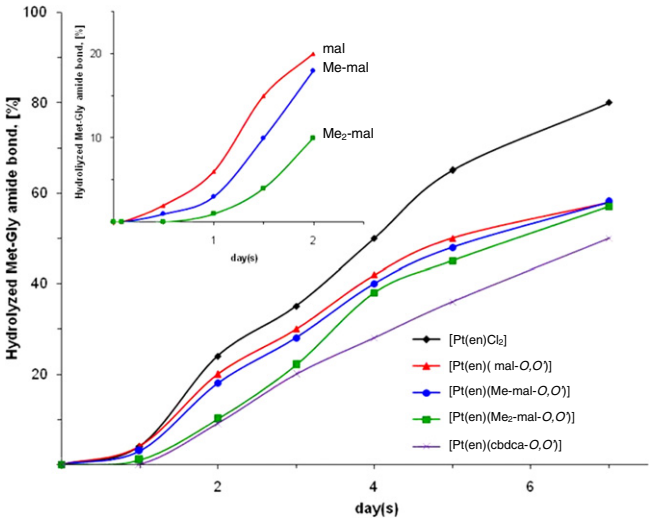

Fig. 4. The time dependence of the hydrolytic cleavage of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptide with different Pt(II) complexes. All reactions were realized at 37 °C with equimolar amounts of the Pt(II) complex and the dipeptide at pH 7.40 in 50 mM phosphate buffer. The inserted figure shows the hydrolysis of the Met-Gly bond in the presence of three malonato–Pt(II)-type complexes during 2 days.

Table 3
Characteristic ^{1}H NMR chemical shifts (δ, ppm) for the Ac-L-Met-L-His-type peptides and corresponding macrochelate Pt(II)-peptide complexes **4** formed in the reaction with [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes at pH 7.40 and at 37 °C in D_2O. The chemical shifts of monodentate coordinated Me-mal and Me$_2$-mal ligands of the ring-opened complexes **1b** are identical with those for complex **1a** shown in Table 1.

Peptide/macrochelate Pt(II)-peptide complex **4**	Imidazole protons		*S*-CH$_3$	Gly1CH$_2$	Gly2CH$_2$	Gly3CH$_2$
	C2H	C5H				
Ac-L-Met-L-His	8.44	7.20	2.10			
[Pt(en)(Ac-L-Met-L-His-*S*,*N*3)]$^+$	8.13	7.08	2.58,2.60			
Ac-L-Met-Gly1-L-His-Gly2-NH$_2$	8.31	7.19	2.11	3.93	3.93	
[Pt(en)(Ac-L-Met-Gly-L-His-Gly-NH$_2$-*S*,*N*3)]$^+$	8.09	7.02, 6.97	2.57	3.98	3.93	
Ac-L-Met-Gly1-Gly2-L-His-Gly3	8.61	7.34	2.11	4.04	3.96	3.80
[Pt(en)(Ac-L-Met-Gly-Gly-L-His-Gly-*S*,*N*3)]$^+$	8.07 7.09	7.09	2.59	4.00	3.94	3.81

reaction was effected by the methyl group in the leaving malonate ligand. The same conclusion can be drawn from our kinetic data presented in Table 2. From this table we can see that replacement of monodentate coordinated Me$_2$-mal ligand by water molecule in the ring-opened complex **1a** (Fig. 1) was two times slower in respect to the corresponding Me-mal complex, both resulting in the formation of the hydrolytically active complex **2**. Difference in the replacement of these two ligands can be correlated with different stability of their ring-opened complexes.

3.2. Reactions of Pt(II) complexes with Ac-L-Met-L-His-type peptides

In the second part of this study we used ^{1}H NMR spectroscopy to investigate the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with peptides containing both L-methionine and L-histidine amino acids in the side chains, namely Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH$_2$ and Ac-L-Met-Gly-Gly-L-His-Gly (Ac-L-Met-L-His-type peptides). When an equimolar amount of Pt(II) complex was incubated with the corresponding Ac-L-Met-L-His-type peptide under the above mentioned experimental conditions only one Pt(II)–peptide complex in these reactions was observed in solution as a final product after 48 h (see Fig. 1, complex **4**). As it was shown in Fig. 1 complex **4** has bidentate coordinated peptide via the N3 atom of the imidazole ring and methionine sulfur atom. The complex **4** was showed as very stable product during time and no hydrolysis of amide bonds in the above investigated peptides had been observed during two weeks. The formation of this macrochelate complex proceeds in two steps. The first step of this reaction is monodentate coordination of Ac-L-Met-L-His-type peptide to the Pt(II) through the methionine sulfur atom. This reaction yields to the formation of the ring-opened complex **1b** which was evidenced in the ^{1}H NMR spectrum from difference in the chemical shifts of the methyl protons for the free at 2.10–2.11 and these protons for the Pt(II)–sulfur bound peptide at 2.57–2.60 ppm after 15 min (Table 3). These chemical shifts are in accordance with those previously reported for the reactions of Pt(II) complexes with different methionine-containing peptides [10,30]. The monodentate coordination of Ac-L-Met-L-His-type peptides to the [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] caused the opening of the malonate rings in these complexes. This ring-opening process can be followed in the ^{1}H NMR spectrum from difference in the chemical shifts of the methyl malonate protons for bidentate and those for these protons of monodentate bound Me-mal and Me$_2$-mal ligands. These chemical shifts are almost identical with those for the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with Ac-L-Met-Gly dipeptide (see previous section and Table 1). The second-order rate constants for the formation of **1b** were determined from ^{1}H NMR measurements (Table 2). The doublet at 1.37 and singlet at 1.77 ppm due to the methyl malonate protons of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes, respectively, were decreased during time and new resonances at 1.24 and at 1.27 ppm for these protons in the ring-opened [Pt(en)(Me-mal-*O*)(Ac-L-Met-L-His-*S*)] and [Pt(en)(Me$_2$-mal-*O*)(Ac-L-Met-L-His-*S*)] complexes, respectively, had been appeared. The concentrations of the starting Pt(II) complex and corresponding ring-opened species were determined during time by integration of the above-mentioned resonances. The second-order rate constants for the reactions of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with Ac-L-Met-L-His are similar with those for the reactions between these Pt(II) complexes and Ac-L-Met-Gly dipetide, $k_2 = (11.95 \pm 0.31) \times 10^{-3}$ M^{-1}s^{-1} for Me-mal and $k_2 = (6.19 \pm 0.02) \times 10^{-3}$ M^{-1} s^{-1} for Me$_2$-mal (Table 2).

The second step of the reaction between Pt(II) complexes and Ac-L-Met-His-type peptides is conversion of **1b** into **4** (Fig. 1). This conversion proceeds through the intramolecular replacement of monodentate coordinated Me-mal and Me$_2$-mal ligands from **1b** with the N3 imidazole nitrogen atom of the histidine residue. The conversion of **1b** into **4** is evident in the ^{1}H NMR spectrum by the simultaneous decline of the resonances due to the methyl protons of the Me-mal and Me$_2$-mal coordinated in monotopic fashion to the Pt(II) and growth of those for these protons of free Me-mal and Me$_2$-mal ligands. The chemical shifts for these resonances are almost identical with those observed for the conversion of **1a** into **2** in the reaction of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with Ac-L-Met-Gly dipeptide (Table 1). Additionally, the conversion of **1b** into **4** can be followed in the ^{1}H NMR spectrum from difference in the chemical shifts of the C2H and C5H imidazole protons for the free and N3-bound histidine side chain to Pt(II) (Table 3). From Table 3 it can be seen that the C2H and C5H resonances are upfield shifted after peptide coordination through the N3 nitrogen atom of the imidazole to Pt(II). The higher field chemical shifts for the C2H ($\Delta\delta$ = 0.22–0.31 ppm) with respect to the C5H proton ($\Delta\delta$ = 0.12–0.17 ppm) can be attributed to the fact that this proton is closer to the N3 binding center [46–48]. The first-order rate constants for conversion of **1b** into **4**, $k = (5.22 \pm 0.03) \times 10^{-6}$ s^{-1} for Me-mal and $k = (5.98 \pm 0.03) \times 10^{-6}$ s^{-1} for Me$_2$-mal, are lager from those for the conversion of **1a** into **2**, $k = (3.02 \pm 0.02) \times 10^{-6}$ s^{-1} for Me-mal and $k = (1.45 \pm 0.02) \times 10^{-6}$ s^{-1} for Me$_2$-mal (Table 2). This can be attributed to the fact that N3 nitrogen atom of imidazole ring from Ac-L-Met-L-His dipeptide is better nuclephile than water molecule in the reaction with Ac-L-Met-Gly dipeptide. We found that the same *S*,*N*3-coordination mode of the Pt(II) occurred in the reactions with Ac-L-Met-L-His, Ac-L-Met-Gly-L-His-GlyNH$_2$ and Ac-L-Met-Gly-Gly-L-His-Gly peptides. Also, no influence of Gly residue (one or two) inserted between Met and His anchoring amino acids on the formation rate of complex **4** was observed.

4. Conclusions

From the present investigation, it can be stated that the reaction of [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me$_2$-mal-*O*,*O*′)] complexes with *N*-acetylated Ac-L-Met-Gly and Ac-L-Met-L-His-type peptides

under physiological conditions (pH 7.40 and 37 °C) primarily proceeds with the formation of the ring-opened malonate adducts containing monodentate methionine bound peptide to the Pt(II). These ring-opened species are present in solution for more than 48 h and during that time the monodentate coordinated malonate ligand (Me-mal or Me_2-mal) has been slowly replaced intermolecularly by water for Ac-L-Met-Gly or intramolecularly by the N3 nitrogen atom from the histidine residue for Ac-L-Met-L-His-type peptides. Replacement of the Me-mal and Me_2-mal ligands by water molecules leads to the formation of the hydrolytically active aqua Pt(II)–peptide complex which further promotes slow hydrolysis of the Met-Gly amide bond in the Ac-L-Met-Gly dipeptde. However, intramolecular replacement of these malonate anions by the histidine residue of Ac-L-Met-L-His-type peptides leads to the formation of very stable and hydrolytically inactive macrochelate Pt(II)–peptide complex. The previous results confirming that the ring-opened carboplatin adducts containing monodentate thioethers were also observed in the reactions of the anticancer drug carboplatin with a variety of sulfur-containing amino acids [49,50] together with those for the presently investigated reactions of carboplatin analogues [Pt(en)(Me-mal-*O*,*O*′)] and [Pt(en)(Me_2-mal-*O*,*O*′)] with methionine-containing peptides contribute to the current hypothesis that the ring-opened adducts of chelated dicarboxylate platinum anticancer complexes with methionine derivatives could play important role in their mechanism of action.

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