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ARTICLE

Versatility and trends in the interaction between Pd(II) and peptide hydroxamic acids

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Primary and secondary di- and tripeptide hydroxamic acids, Ala-Ala-NHOH, Ala-Ala-N(Me)OH, Ala-Gly-Gly-NHOH and Ala-Gly-Gly-N(Me)OH were synthesized and their interaction with Pd(II) (as a Pt(II) model but with faster ligand exchange reactions) was studied in aqueous solution in presence of Cl^- competitor ion by pH-potentiometric and ^1H NMR methods. To the best of our knowledge, this is the first detailed solution study on Pd(II)-peptide hydroxamate systems revealing that, except Ala-Gly-Gly-NHOH, the other three ligands act not only as coordination compounds, but also the hydrolysis of the coordinated ligands and formation of protonated hydroxylamine and Pd(II) complexes of the corresponding peptides under acidic conditions occured. The hydrolysis was rather slow with Ala-Gly-Gly-N(Me)OH (more than one week), just a bit faster with Ala-Ala-NHOH, so speciation studies could also be performed successully on the systems containing one of the latter two ligands. This was, however, hindered for the Pd(II)-Ala-Ala-N(Me)OH system, where, in addition to the quite fast hydrolysis of the ligand, the reduction of Pd(II) to elementary metal by the N(Me)-hyroxylamine formed was also observed. Speciation studies with Ala-Gly-Gly-NHOH revealed the predominance of a very stable 4N-donor complex, (NH_2,$2N_{amide}$, $N_{hydr.}$) over a wide pH-range. This is also capable of binding metal ion excess with the hydroxymate (O,O) set in dinuclear species. The formation of this latter type complex is hindered with the secondary analogue, Ala-Gly-Gly-N(Me)OH, where, in addition to the 3N donor atoms, the hydroxamate-O is also involved in the coordination of the most stable complex. However, the formation of mixed hydroxo species at high pH and a bis-complex in a rather slow process with (NH_2,N_{amide})$_2$ bonding mode in the presence of ligand excess was proven. Although the 3N coordination (NH_2,N_{amide}, N_{hydx}) results in a highly stable complex with the dipeptide derivative, Ala-Ala-NHOH, the fourth coordination site remains free for accepting an NH_2 moiety from excess ligand, or hydroxide ion at high pH. Likewise, the hydroxymate (O,O) set remains free to bind metal ion excess in a trinuclear species. The results of this study may also contribute to the design and synthesis of novel Pt(II) complexes with anticancer potential.

Introduction

Hydroxamic acids bear the characteristic weak acidic function -C(O)N(R)OH (R = H in primary, alkyl or aryl moiety in secondary derivatives).[1,2] The most well-known natural representatives of these very important bioligands belong to the siderophore's family and play a crucial role in the microbial iron uptake.[3] A wide range of potential applications of microbial siderophores have been considered and some of these compounds, e.g. the desferrioxamine B, are used in medicine for many years.[4] Not only the natural hydroxamic acid based molecules, but also numerous synthesized ones are known to show biological activities, such as hypotensive, anti-cancer, anti-malarial, or anti-fungal properties, as well as effective and selective inhibitory effect on numerous metalloenzymes like ureases, matrix metalloproteinases and histone deacetylases.[1,5,6] Some may also behave as NO donors under certain conditions[7]. The versatile biological activity is due in some part to the significant H-bonding ability,[6,8] but first of all to the metal-binding ability of these biomolecules. This fact has initiated a large number of investigations on metal complexes of hydroxamic acids. Mostly, 3*d* transition metals have been involved into the studies and much less literature results can be found on complexes with other metals, like platinum group metals. Although by far the most typical coordination mode of a hydroxamate function has been found to involve chelation via deprotonated hydroxyl and carbonyl oxygen atoms (O,O), providing five-membered singly deprotonated hydroxamate and doubly deprotonated hydroximato chelates. Other modes are also possible.[6,9] According to the HSAB theory, the (O,O) chelating set involves hard donor sites, therefore the most stable hydroxamato chelates are formed with hard metal ions, like Fe(III), Co(III), Al(III),[10-13] but high stability hydroxamato (under certain conditions also hydroximato) complexes are also known with borderline metal ions, such as Cu(II).[10]

In fact, there is a linear correlation between the first hydrolytic constant of the metal ions and the stability of the

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hydroxamato chelate with them.[11,13] Consequently, the stability of a hydroxamato chelate is not too high with soft metal ions. However, the hydroximato-N, in primary derivatives and in specially designed coordination environments, was found to become a very effective donor towards soft metals. This is well-demonstrated by the few results published on relevant complexes with Pd(II)[14] or with the half-sandwich type cation of Ru(II).[15,16] In the latter case, importance of the well-known H-bonding ability of the hydroxamic acid moiety in the formation of a trinuclear cluster ion, was also supported.[15]

The metal ion binding behaviour of a hydroxamate group can dramatically be altered by the incorporation of further groups, such as amino group,[17] or pyridyl-based moiety[18] in the molecule. Aminohydroxamic acids, in addition to the 5-membered hydroxamate-type (O,O)-chelate, are also capable of forming an (N,N)-chelate via the amino-N and deprotonated hydroxamate/hydroximate-N resulting in 5-membered (N,N)-type chelate with α-aminohydroxamic acids, 6-membered with β ones and 7-membered with γ derivatives. In the interaction with borderline metal ions (*e.g.* Cu(II)), both chelation modes are likely, leading to the formation of interesting clusters, the most well-known of them belong to the metallacrowns family, in which $–[M–N–O]_n–$ units are repeated in a cyclic arrangement.[19-21] However, only the (O,O)-type chelation is preferred by hard metal ions, while the N-donors are favoured by soft ones. Taking advantage of this different preference of different metal ions towards the (O,O) and (N,N) chelating sites large number of supramolecular coordination complexes have been developed during the past several years via the selective coordination to different metal centres.[22-24] Often Pd(II) as typical soft metal ion was chosen to coordinate to the (N,N) donor set and $[Pd(en)Cl_2]$ (en = ethane-1,2-diamine), in which the soft nitrogen donors of the en group occupy two coordination sites of the metal centre, was selected as palladium source.[22-24] It is well-known from the literature that even simple dipeptides (without any side-chain donor) are very effective ligands of Pd(II). Since this metal ion was reported to be one of the most effective one to promote deprotonation and coordination of peptide-amide groups,[25,26] the question arises, whether, alternatively, $[PdCl_4]^{2-}$ ion can be used as soft metal ion source to develop supramolecular entities with hydroxamic acid derivatives of simple oligopeptides. Previous results with various metal ions also supported that with these hydroxamic based derivatives the hard metal ions are coordinated exclusively to the (O,O)-chelate, while e.g. the rather soft Ni(II) prefers the nitrogens of the peptide backbone.[27-29] It is also a question, whether only complexation processes or also Pd(II)-assisted hydrolysis (as it was previously observed in the case of Pt(II)-glycinehydroxamic acid system)[30,31] occur. The above open questions initiated the aim of our study, the investigation of the interaction of Pd(II)-ions with primary and secondary di- and tripeptide hydroxamic acids in solution, namely with Ala-Gly-Gly-NHOH, Ala-Gly-Gly-N(Me)OH, Ala-Ala-NHOH and Ala-Ala-N(Me)OH (their structures are shown in Chart 1).

Experimental

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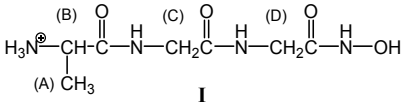
Materials and methods

Methanol and THF were purified and dried according to literature methods.[32] Ala-Ala-NHOH·TFA and Ala-Ala-N(Me)OH·TFA were prepared as previously described.[28-29] Z-Ala-Gly-Gly-OH was purchased from Bachem, hydroxylamine- and N-methylhydroxylamine-hydrochloride from Fluka, Pd/C (10 % (m/m)) from Merck. All chemicals have the highest purity commercially available and were used without further purification. The Pd(II) stock solution was prepared from $K_2[PdCl_4]$ (Merck) using deionised and ultra-filtered water obtained from a Milli-Q RG (Millipore) water purification system. The metal ion solution also contained two equivalents of hydrochloric acid to prevent hydrolysis. The exact concentration of the stock solution was determined by pH-potentiometric titration using Gran's method.[33]

Synthesis of the ligands

Z-Ala-Gly-Gly-NHOH (1). Hydroxylamine hydrochloride (1.92 g, 27.62 mmol) was dissolved in dry methanol (20 mL) and chilled in an ice-bath. KOH (1.55 g, 27.62 mmol) was added as pellets and chilled to 0 °C under nitrogen. The reaction mixture was stirred for 15 min, meanwhile a white precipitate (KCl) was formed. In another flask Z-Ala-Gly-Gly-OH (3.11 g, 9.22 mmol) was dissolved in dry THF (150 mL) under nitrogen, and chilled in an ice-bath to 0 °C. Ethyl chloroformiate (2.11 mL, 22.17 mmol) followed by N-methylmorpholine (2.65 mL, 24.10 mmol) was added and stirred for 30 min under nitrogen, meanwhile a fine white precipitate (N-methylmorpholinium chloride) was formed. The free hydroxylamine solution was filtered into a three-neck flask kept in an ice-bath under nitrogen, while the mixed anhydride solution was filtered into a dropping funnel under nitrogen. The latter solution was added dropwise to the former one within 5 min; the obtained opalescent reaction mixture was stirred under nitrogen overnight and kept in an ice-bath. After removal the solvent, yellowish oil was obtained, which was dissolved in water and extracted at pH ~ 3–4 with EtOAc (5 × 100 mL). The collected organic phases were dried on $MgSO_4$, after that the solution



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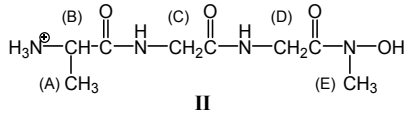

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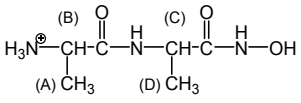
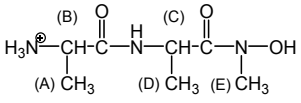


III



IV

Chart 1 Structures of the fully protonated ligands

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was evaporated to one third and cooled to –20 °C, while fluffy precipitate was obtained. The crude product was filtered off and recrystallized from MeCN. Yield: 0.72 g (22 %). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 10.46 (1H, s, CONHOH), 8.84 (1H, s, CONHOH), 8.04 (1H, t, NH), 8.02 (1H, t, NH), 7.55 (1H, d, NH), 7.36 (5H, m, C_6H_5), 5.02 (2H, q, C_6H_5-CH_2), 4.06 (1H, q, CH), 3.73 (2H, d, CH_2), 3.63 (2H, d, CH_2), 1.21 (2H, d, CH_3).

Z-Ala-Gly-Gly-N(Me)OH (2). Z-Ala-Gly-Gly-N(Me)OH was prepared as described for **1** by using N-methylhydroxylamine hydrochloride (2.31 g, 27.66 mmol) and obtained after recrystallization of the crude product from EtOAc. Yield: 1.16 g (34 %). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 9.95 (1H, s, OH), 8.15 (1H, t, NH), 7.81 (1H, t, NH), 7.50 (1H, d, NH), 7.35 (5H, m, C_6H_5), 5.02 (2H, q, C_6H_5-CH_2), 4.06 (1H, q, CH), 3.98 (2H, s, CH_2), 3.75 (2H, d, CH_2), 3.10 (3H, s, N-CH_3), 1.22 (2H, d, CH_3).

Ala-Gly-Gly-NHOH·TFA (3). Z-Ala-Gly-Gly-NHOH (380 mg, 1.08 mmol) was dissolved in dry methanol (20 ml) then TFA (100 µL, 1.3 mmol) and Pd/C (10 % (w/w), 110 mg) were added. The mixture was stirred under H_2 (2 bar) for 4 h. The catalyst was filtered off and the solvent was removed. *Abs.* diethyl ether was added to the residue, then the solvent was evaporated under vacuum. The product is a white hygroscopic solid. Yield: 330 mg (92 %). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 4.11 (1H, q, CH), 3.99 (2H, q, CH_2), 3.87 (2H, s, CH_2), 1.51 (3H, d, CH_3).

Ala-Gly-Gly-N(Me)OH·TFA (4). Ala-Gly-Gly-N(Me)OH·TFA was prepared as described for **3** using 500 mg Z-Ala-Gly-Gly-N(Me)OH. The product is a white hygroscopic solid. Yield: 444 mg (94 %). Analysis: ^{1}H NMR (400 MHz, D_2O) δ (ppm): 4.23 (2H, s, CH_2), 4.17 (1H, q, CH), 4.07 (2H q, CH_2), 3.25 (3H, s, N-CH_3), 1.57 (3H, d, CH_3).

pH-potentiometric and spectroscopic studies

The pH-potentiometric titrations were carried out at t = 25.0 °C using 0.10 M KCl ionic strength, which was adjusted to 0.20 M by addition of KNO_3. The application of that mixture of salts to keep the ionic strength constant was taken from previous work on Pd(II)-peptides.[25] In fact, in the absence of any chloride ions the complexation was found to occur already under very acidic conditions (below the measurable pH-range), but in the presence of the competitor chloride ions, the complexation processes with the peptides were shifted into the measurable pH range. On the other hand, more than 0.10 M chloride concentration was found to prevent the solution equilibrium studies due to slow complexation processes.[25] Carbonate free, ca. 0.2 M KOH solution of known concentration was used as titrant. The exact concentration of the HCl and KOH was determined by potentiometric titrations. A Mettler Toledo DL 50 titrator equipped with a Mettler Toledo combined electrode (DGI114SC) was used for the pH-metric measurements. The electrode system was calibrated according to Irving et al.[34] The water ionization constant was pK_w = 13.76 ± 0.01 under the conditions used. The initial volume of the samples was 15.0 mL, the ligand concentrations were varied in the range 2.0-3.0 mM, the metal ion to ligand ratios were 1:2, 1:1, 1.5:1 and 2:1, where the equimolar solutions contained 5-10 % excess of ligand. The samples were stirred and completely deoxygenated by bubbling purified argon. The titrations were performed in the pH-range 2.0-11.0 in equilibrium controlled mode (where equilibrium was assumed to be reached when dpH/dt was less than 0.015 mV/11 s). The waiting time between two titration points was found to fall into the range of 150-1800 s because of the quite slow equilibrium processes. For this reason, relatively high fitting parameters were obtained during the calculations. The stability constants (β_{pqr} = $[Pd_pH_qL_r]/[Pd]^p[H]^q[L]^r$) were determined via fitting the titration curves by the PSEQUAD and SUPERQUAD programs.[35-36] The stability constants for the complexes of Pd(II) with chloride ions ($\log\beta_1$= 4.47, $\log\beta_2$ = 7.76, $\log\beta_3$ = 10.17, $\log\beta_4$ = 11.54 for the species $[PdCl]^+$, $[PdCl_2]$, $[PdCl_3]^-$, $[PdCl_4]^{2-}$, respectively) were taken from the literature.[37] These fixed values were involved into the equilibrium models during the calculations, but stability constants for various ternary complexes, involving chloride ions, were not involved. Consequently, the stability constants determined for the various complexes formed between Pd(II) and the peptide hydroxamic acids are conditional values, valid under the experimental conditions used, and the free coordination site(s) of the metal ion is(are) occupied by chloride ion(s) or water molecule(s). A negative stoichiometric number for H in the formulae of the Pd(II) complexes indicates a proton, which dissociates only from the complex but neither from the ligand nor from the metal aqua ion separately. This proton can be liberated from a water molecule in the coordination sphere of the metal ion bound by the ligand, from a hydroxamate group or from the very weakly acidic amide group(s) of the bound ligands. For the Pd(II)-Ala-Ala-NHOH 1:1 system, a repeated titration was also performed by acidifying the sample to pH ~ 2.0 after titration then the measurement was repeated after four days.

^{1}H NMR titrations were carried out on a Bruker Avance 400 instrument at t = 25.0 °C in the presence of 0.10 M KCl and 0.10 M KNO_3 (I_{tot} = 0.20 M). The chemical shifts are reported in ppm (δ_H) from TSP as internal standard. Titrations were performed using 5-10 individual samples in D_2O (99.8%) at c_{lig} = 5.0 mM, the metal ion to ligand ratios were 1:2, 1:1, 1.5:1 and 2:1, the pH was varied within the range 1.7 – 11.5. The pH of the samples was set up with NaOD or DNO_3 solutions. The pH* values (direct pH-meter readings in a D_2O solution of a pH-meter calibrated in H_2O according to Irving et al.[34]) were converted to pH values using the following equation: pH = 0.936·pH* + 0.412.[38] NMR spectroscopy was also used to determine the fraction of each species allowing further additional equilibrium information about those systems, where the signals of each complexes were well-separated. Regarding the slow exchange in the NMR time scale, each species has an individual resonance. At this condition the integrated peak areas assigned to the different species were converted to molar fraction and are also displayed on the speciation curves. In case of Pd(II)-Ala-Ala-NHOH system, fast exchange in the NMR time scale was observed between $[PdL]^+$ and $[PdH_{-1}L]$, where a single resonance can be observed at the molar fraction weighted average of the chemical shifts of the two species.

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Time-dependent investigations were also carried out on all of the systems. The first spectra were registered within 1 hr from preparation (freshly prepared samples), then the measurements were repeated several times on the individual samples over a period of 7-9 days. The pH of the samples was checked before the NMR measurements, but because the samples were kept in NMR tube on air the pH was shifted slightly in many cases which were taken into account during the evaluation

Results and discussion

H^+ complexes of the ligands

The fully protonated ligands can release two protons (from the ammonium and hydroxamic functions) in the pH range ca. 7-10. Although the dissociation constants of the studied peptide hydroxamic acids were determined in previous works in our laboratory,[28-29,39] the slightly different conditions used in the present work and to obtain the exact concentration of the stock solutions made the determination of the constants necessary. The dissociation constants (p*K* values) calculated via fitting the pH-potentiometric titration curves are shown in Table 1. The values relating to the two functions of a ligand are quite close to each other, indicating that the deprotonation processes of $-NH_3^+$ and -CON(R)OH groups overlap considerably. Since the present macroconstants are in good agreement with the earlier ones,[28-29] and in these cited works the microconstants (showing the basicity of the individual groups) were also determined, the same basicity-trend under our conditions is assumed, namely, the $-NH_3^+$ group shows increased acidity compared to the -CON(R)OH moiety.[28] A comparison of the corresponding values does not show any measurable difference between the values relating to (i) the primary versus secondary, (ii) dipeptides *versus* tripeptides.

Table 1 Protonation constants (log K_i) of the ligands at 25.0 °C, I_{tot} = 0.20 M (0.10 M KCl + 0.10 M KNO_3)[a]

Ligand	log K_1	log K_2
Ala-Gly-Gly-NHOH	8.80(1)	7.70(1)
	8.82[1]	7.70[1]
Ala-Gly-Gly-N(Me)OH	8.55(1)	7.73(1)
	8.63[1]	7.66[1]
Ala-Ala-NHOH	8.80(1)	7.70(1)
	8.88[2]	7.66[2]
Ala-Ala-N(Me)OH	8.74(1)	7.78(1)
	8.74[3]	7.74[3]

[a]3σ standard deviations are in parentheses
[1] Ref. 39 (t = 25.0 °C, I = 0.20 M KCl)
[2] Ref. 28 (t = 25.0 °C, I = 0.20 M KCl)
[3] Ref. 29 (t = 25.0 °C, I = 0.20 M KCl)

Pd(II) complexes of peptide hydroxamic acids

Equilibrium studies on the Pd(II)-peptide hydroxamate systems were performed by pH-potentiometric titrations and ^{1}H NMR method to obtain the speciation. It was clearly indicated by the experimental findings that, except the Ala-Gly-Gly-NHOH containing system, not only complexation, but also other processes, e.g. Pd(II) promoted irreversible hydrolysis of the coordinated ligands were observed in time-dependent experiments under acidic conditions. As a consequence, the equilibrium results reported below are valid for freshly prepared samples. Ligand dependent differences were observed in the rate of the irreversible process(es), which were slower with tripeptide derivatives than with dipeptide ones and slower with primary derivatives compared to secondary ones (*vide infra*). The only system, in which complete reversibility was found, is the Pd(II)-Ala-Gly-Gly-NHOH one. Representative examples of pH-potentiometric titration curves registered for this system are shown in Fig. 1.

Pd(II)-Ala-Gly-Gly-NHOH system

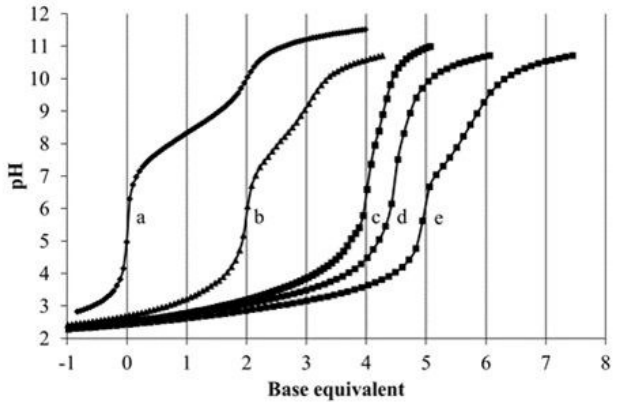

Fig. 1 Representative titration curves of H^+–Ala-Gly-Gly-NHOH (a) and Pd(II)–Ala-Gly-Gly-NHOH systems at 1:2 ratio (b), 1:1 ratio (c), 1.5:1 ratio (d) and 2:1 ratio (e); negative base equivalent refers to an excess of acid in the sample

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Table 2 Stability constants (logβ) of Pd(II) complexes of peptide hydroxamic acids at I_{tot} = 0.20 M (0.10 M KCl + 0.10 M KNO_3) at 25.0 °C [a]

Species	Ala-Gly-Gly-NHOH	Ala-Gly-Gly-N(Me)OH	Ala-Ala-NHOH
$[PdL]^+$	22.70(1)	22.60(2)	22.84(3)
$[PdH_{-1}L]$	18.98(3)	18.69(2)	20.25(2)
$[PdH_{-2}L]^-$	13.98(6)	12.2(1)	11.5(1)
$[Pd_2H_{-3}L]$	22.96(5)	–	–
$[Pd_2H_{-4}L]^-$	13.9(2)	–	–
$[Pd_3H_{-4}L_2]$	–	–	44.4(3)
$[PdL_2]$	–	–	33.4(3)
$[PdH_{-1}L_2]^-$	–	–	24.8(3)
Fitting parameter	0.0165	0.0250	0.0284
Number of fitted data	597	214	283
$pK_{[PdL]}$	3.72	3.92	2.59
$pK_{[PdH-1L]}$	5.00	6.50	8.8

[a] 3σ standard deviations in parentheses

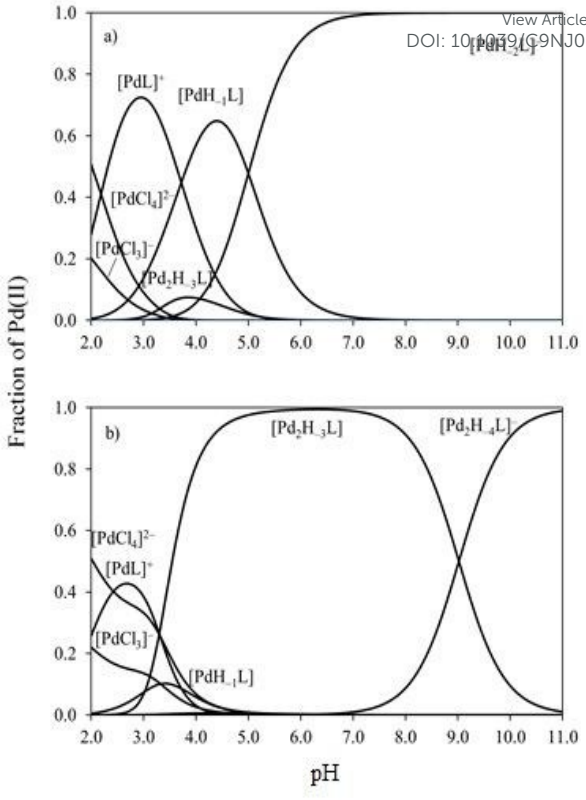

Fig. 2 Concentration distribution curves of the Pd(II)–Ala-Gly-Gly-NHOH system at (a) 1:1 and (b) 2:1 metal ion to ligand ratio, at c_{lig} = 3.0 mM

As it can be seen in Fig. 1, the titration curves indicate the beginning of the complexation at pH ~ 2, and already under acidic conditions metal ion induced proton release of the ligand takes place in overlapping steps. Fitting all of the titration curves together led to the speciation model and overall stability constants summarized in Table 2, 1st column. These pH-metric results were used to calculate the concentration distribution curves seen in Fig. 2. These curves were also calculated under biologically more relevant concentrations ($c_{Pd(II)}$ = 20 μM, in presence of c_{Cl^-} = 100 mM and c_{Cl^-} = 4 mM), which can be seen in Figures S1-S4. At lower metal ion concentration and high chloride concentration the competition with chloride ions is more significant than it was assumed under the conditions of pH-metric studies. On the other hand, at low chloride concentration the complexation processes are shifted into the lower pH-range. These observations are generally true for all of the investigated systems. As it is seen in Fig. 1c, in approximately equimolar solution, in addition to the release of the two dissociable protons of the ligand, two extra deprotonation processes take place. Fig. 2a clearly shows that the complexation starts with the formation of the $[PdL]^+$ complex, then an increase in pH results in the formation of $[PdH_{-1}L]$ and $[PdH_{-2}L]^-$ in overlapping processes; the latter complex predominates above pH ~ 6 at this ratio. The four equivalents base consumption by the end of the titration indicates the metal ion induced deprotonation of the terminal ammonium, the two amides and the hydroxamic groups and, consequently, a 4N-

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Table 3 Chemical shifts (δ) of non-dissociable protons of the ligand in ppm measured in the Pd(II)-Ala-Gly-Gly-NHOH system at different metal ion to ligand ratios and pH values

Ratio ($c_{Pd(II)}$:c_L)	pH	(A)	(B)	(C)	(D)	Species
0:1	3.11	1.559	4.168	4.036	3.914	H_2L^+
	6.63	1.548	4.142	4.033	3.914	H_2L^+/HL
	8.19	1.405	3.835	3.999	3.897	HL
	9.44	1.290	3.585	3.963	3.828	HL/L^-
	10.22	1.278	3.557	3.956	3.800	L^-
1:1	2.36	1.559	4.168	4.036	3.914	H_2L^+
		1.398	3.582	3.977	3.914	$[PdL]^+$
	3.18	1.559	4.168	4.036	3.914	H_2L^+
		1.398	3.582	3.977	3.914	$[PdL]^+$
		1.360	3.600	3.832	3.814	$[PdH_{-1}L]$
	4.98	1.360	3.600	3.832	3.814	$[PdH_{-1}L]$
		1.374	3.651	3.876	3.779	$[PdH_{-2}L]^-$
	7.14	1.374	3.651	3.876	3.779	$[PdH_{-2}L]^-$
1.5:1	2.47	1.559	4.168	4.036	3.914	H_2L^+
		1.398	3.582	3.977	3.914	$[PdL]^+$
	4.00	1.398	3.582	3.977	3.914	$[PdL]^+$
		1.360	3.600	3.832	3.814	$[PdH_{-1}L]$
		1.365	3.602	3.841	3.723	$[Pd_2H_{-3}L]$
2:1	2.47	1.559	4.168	4.036	3.914	H_2L^+
		1.398	3.582	3.977	3.914	$[PdL]^+$
	6.12	1.360	3.600	3.832	3.814	$[PdH_{-1}L]$
		1.365	3.602	3.841	3.723	$[Pd_2H_{-3}L]$
	8.53	1.365	3.602	3.841	3.723	$[Pd_2H_{-3}L]$
		1.363	3.651	3.886	3.752	$[Pd_2H_{-4}L]^-$
	10.84	1.363	3.651	3.886	3.752	$[Pd_2H_{-4}L]^-$

coordination (NH_2, N_{amide}, N_{amide}, $N_{hydr.}$) in the $[PdH_{-2}L]^-$. (More precisely this complex has a $[Pd(H_{-3}L)H]^-$ composition, where the dissociable hydroxamate-OH is still protonated.) The assumed binding mode is shown in Chart 2/II. Fig. 1 also shows that this ligand is capable of binding metal ion excess. This observation is in agreement with the results obtained previously for the Cu(II)-complexes of this tripeptide hydroxamic acid.[39] One-equivalent of Pd(II) excess is necessary to substitute the dissociable proton of the hydroxamate moiety from the total amount of the ligand up to pH ~ 6 (see curves d and e in Fig. 1). However, at 2:1 metal ion to ligand ratio all together two equivalents of base were titrated, in addition to the one up to pH ~ 6, another one up to pH ~ 10, which indicates additional metal ion-induced deprotonation process. The best fit of the titration curves was obtained with the involvement of $[Pd_2H_{-3}L]$ and $[Pd_2H_{-4}L]^-$ species in the model. Out of the processes the first one corresponds to the formation of $[Pd_2H_{-3}L]$ dinuclear complex (Fig. 2/b and Chart 2/III), while the second one to the formation of $[Pd_2H_{-4}L]^-$ mixed hydroxido species (Chart 2/IV). Similarly to the appropriate Cu(II) system[39] there was no indication for the formation of trinuclear species with similar binding mode as XI in Chart 2. At 1:2 metal ion to ligand ratio the titration curve (Fig. 1/b) does not show any extra metal ion induced deprotonation processes, therefore formation of bis-complexes was not indicated by the pH-metric experimental results.

^{1}H NMR study was also performed for the determination of the binding mode of each complex. The reported ^{1}H NMR spectra were registered within one-hour equilibration; therefore the obtained data are comparable with the pH-potentiometric results. The chemical shifts of the species are reported in Table 3. In the NMR spectra at 1:1 Pd(II) to ligand ratio there are three new sets of signals beside the signals of the uncomplexed ligand. The obtained spectra demonstrate that each complex has an individual signal in the NMR spectra, indicating that the species show slow exchange in the NMR time scale. This is an additional support to the fact that the Pd(II) is coordinated by N-atoms in the 1:1 complex. A comparison between the speciation curves in Fig. 2a and the chemical shifts as well as integrated peak areas of the NMR signals recorded at various pH-s clearly show that the above-mentioned three signal-sets correlate well with the formation of the $[PdL]^+$, $[PdH_{-1}L]$ and $[PdH_{-2}L]^-$ complexes. At pH = 2.36 the quartet of B in Table 3 showed a significant upfield shift compared to the one of the free ligand supporting the (NH_2, N_{amide}) binding mode for the $[PdL]^+$ complex. The exact stoichiometry of this complex is $[Pd(H_{-1}L)H]^+$, where one of the peptide moiety is already deprotonated, while the dissociable proton is still on the hydroxamic function. This finding is also in good agreement with the earlier results on palladium(II) complexes of simple di- and tripeptides, where the interaction also starts with a (NH_2, N_{amide}) coordination at pH ~ 2.[25] As the pH increases new signals appear in two sets, which are assigned to the formation of $[PdH_{-1}L]$ (with the exact stoichiometry of $[Pd(H_{-2}L)H]$) and $[PdH_{-2}L]^-$ complexes. Their parallel existence is seen until pH = 7.14, where the $[PdH_{-2}L]^-$ becomes the dominant one. At 2:1 metal ion to ligand ratio above pH ~ 4 a new set of signals can also be detected indicating the formation of a dinuclear $[Pd_2H_{-3}L]$ complex (Chart 2/III), while above pH 8.5 another signal can be assigned to the formation of the $[Pd_2H_{-4}L]^-$ species (Chart 2/IV). The NMR measurements were repeated in 7 days. During this period of time the samples were kept in the tubes. Consequently, the pH values of the samples changed due to dissolution of carbon-dioxide from the air, but the signals detected in each sample were found to be changed according to the slight change in the pH only. As a consequence, it can be stated, that no other processes apart from the reversible complexation take place in measurable extent in this system.

Pd(II)-Ala-Gly-Gly-N(Me)OH system

Substitution of the hydrogen at the hydroxamic-N by a methyl moiety results in the elimination of this nitrogen as a potential coordinating donor atom. Consequently, one ligand can provide only three N-donors as a maximum, which are, unlike

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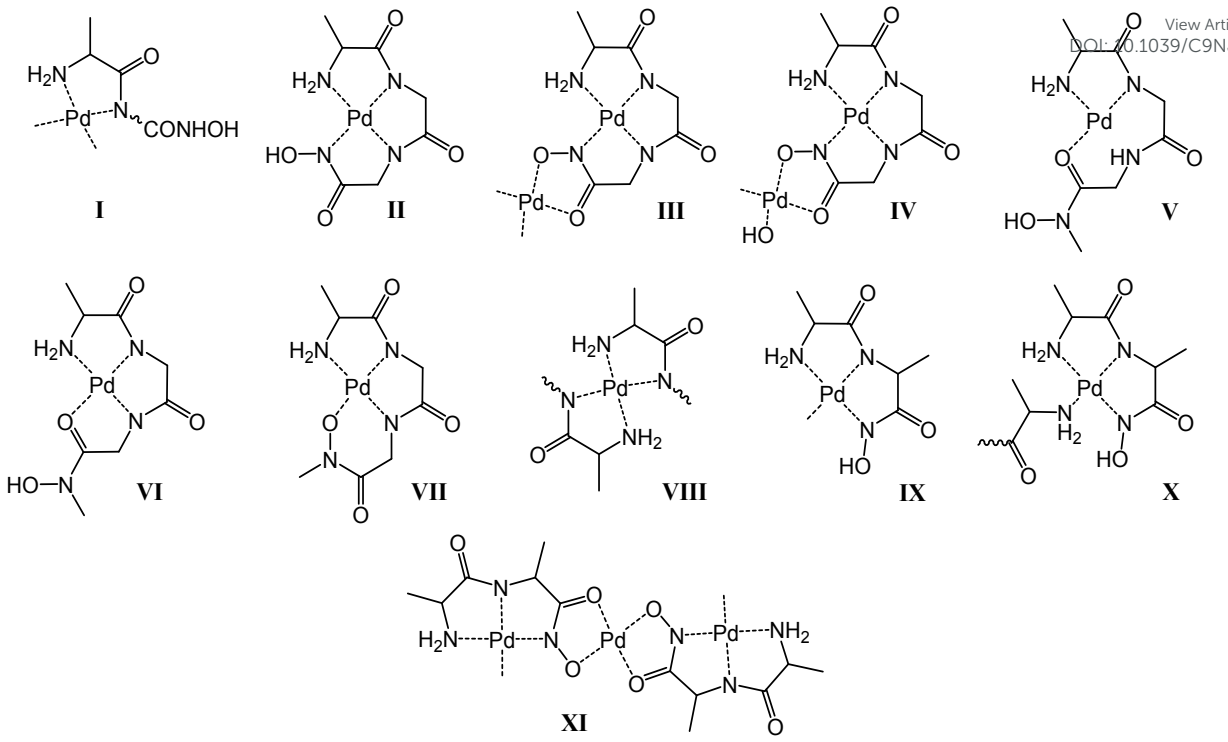

Chart 2 Suggested binding modes of the complexes

Table 4 Chemical shifts (δ) of non-dissociable protons of the ligand in ppm measured in the Pd(II)-Ala-Gly-Gly-N(Me)OH system at different metal ion to ligand ratios and pH values

Ratio ($c_{Pd(II)}$:c_L)	pH	(A)	(B)	(C)	(D)	(E)	Species
0:1	3.02	1.557	4.164	4.050	4.215	3.238	H_2L^+
	7.11	1.536	4.111	4.047	4.211	3.234	H_2L^+/HL
	8.36	1.379	3.772	4.012	4.178	3.215	HL
	9.48	1.293	3.580	3.991	4.134	3.188	HL/L^-
	11.60	1.281	3.550	3.989	4.123	3.178	L^-
1:1	2.53	1.557	4.164	4.050	4.215	3.238	H_2L^+
		1.428	3.564	4.232	4.029	3.260	$[PdL]^+$
	4.05	1.428	3.564	4.232	4.029	3.260	$[PdL]^+$
		1.359	3.592	4.049	3.839	3.208	$[PdH_{-1}L]$
	6.51	1.359	3.592	4.049	3.839	3.208	$[PdH_{-1}L]$
		1.348	3.576	3.884	3.863	3.178	$[PdH_{-2}L]^-$
	8.10	1.348	3.576	3.884	3.863	3.178	$[PdH_{-2}L]^-$
1:2	2.74	1.557	4.164	4.050	4.215	3.238	H_2L^+
		1.428	3.564	4.232	4.029	3.260	$[PdL]^+$
	6.00	1.557	4.164	4.050	4.215	3.238	H_2L^+
		1.359	3.592	4.049	3.839	3.208	$[PdH_{-1}L]$
		1.407	3.600	3.742	4.239	3.258	$[PdL_2]$
	7.91	1.550	4.143	4.038	4.212	3.238	H_2L^+/HL
		1.407	3.600	3.742	4.239	3.258	$[PdL_2]$
		1.348	3.576	3.884	3.863	3.178	$[PdH_{-2}L]^-$

with the above discussed primary derivative Ala-Gly-Gly-NHOH, not able to take all the four equatorial coordination sites of one Pd(II). The experimental findings obtained for the Pd(II)-Ala-Gly-Gly-N(Me)OH system by pH-potentiometric method are as follows: (i) This ligand is not able to bind metal ion excess at all suggesting some involvement of the hydroxamic function in the coordination already at equimolar conditions. (ii) As it is illustrated in Fig. S5, the titration curve registered at 1:1 metal ion to ligand ratio shows three equivalents base-consumption in the pH range ~ 2.5-4.5, which indicates three overlapping processes in this region. In a separated step, in the range of ca. pH 5-9 an additional equivalent base is consumed. This latter process is shifted to higher pH compared to that with the analogous primary ligand (*cf.* Figs. 1 and S5). (iii) The titration curve, registered at 1:2 metal ion to ligand ratio, does not show any "extra" metal-induced process compared to those observed at 1:1 ratio.

All of the pH-potentiometric experimental data were fitted together and the results obtained are shown in the second column of Table 2. The speciation model indicates that only mononuclear complexes can be detected in this system and the formulae of them are $[PdL]^+$, $[PdH_{-1}L]$ and $[PdH_{-2}L]^-$. Due to the very similar basicity of the functions in the two investigated tripeptide derivatives (see Table 1), simple comparison of the corresponding stability constants is possible. The similarity of the stability constants suggests analogy in the coordination modes in the complexes $[PdL]^+$, $[PdH_{-1}L]$ formed with Ala-Gly-Gly-NHOH and Ala-Gly-Gly-N(Me)OH. Namely, in both systems, a 5-membered chelate via (NH_2, N_{amide}) donor set can be assumed as main coordinating mode in the former species, while (NH_2, N_{amide}, N_{amide}) joined chelates in the latter one. As it could be expected, the stability

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constants of $[PdH_{-2}L]^-$ are significantly different in the two systems. In fact, the value for the complex formed with the secondary derivative is ca. two-orders of magnitude lower than the one with the primary counterpart. 4N coordination was suggested in the complex of the primary derivative (see above), but, because the hydroxamate nitrogen of the secondary ligand, Ala-Gly-Gly-N(Me)OH, is unavailable for coordination, most likely, the deprotonated hydroxamate-O occupies the fourth coordination site in $[PdH_{-2}L]^-$ (see structure VII in Chart 2). This binding mode is the same as it was determined in the corresponding Cu(II) complex of Ala-Gly-Gly-N(Me)OH.[39] This also explains why binding of any Pd(II) excess is not possible by this ligand.

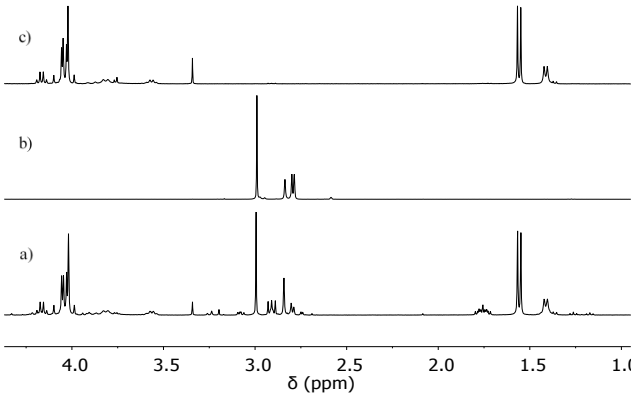

Fig. 3 ^{1}H NMR spectra of Pd(II)–Ala-Gly-Gly-N(Me)OH = 1:1 in 9 days (a), freshly prepared samples of Pd(II)-HN(Me)-OH = 1:1 (b) and Pd(II)–Ala-Gly-Gly-OH = 1:1 (c) at pH = 2.4

^{1}H NMR measurements were carried out at various metal ion to ligand ratios, pH values and time-dependence of the interaction was also monitored on samples summarized in Fig. S6. Although the registered NMR spectra supported the formation of all the three species suggested by pH-metry, some different and unexpected results were found, too. If the spectra were registered in 1 hour after the preparation of the samples, the following main results were obtained: (i) Compared to those of the free ligand, NMR spectra registered at equimolar conditions, showed a new set of signals in the range pH = 1.93-2.53, which was assigned to $[PdL]^+$ (the values of the chemical shifts are summarized in Table 4). In this pH range the B quartet of the ligand showed a significant upfield shift, the E singlet a downfield shift. Based on this observation, it was assumed, that the carbonyl oxygen of the –CON(Me)OH moiety is also coordinated to the metal ion beside the main coordinating donors, NH_2- and N_{amide}, while the hydroxamic function is still protonated. Therefore the exact composition of this complex is $[Pd(H_{-1}L)H]^+$ (structure V in Chart 2), although the coordination of the carbonyl oxygen of the neighbouring amide function cannot be completely ruled out either.

(ii) The new signals appeared in the pH range 6.46-7.20 were assigned to $[PdH_{-1}L]$ with (NH_2, N_{amide}, N_{amide}, $O_{carb.}$) binding mode, as a consequence the precisely assigned formula of this species is $[Pd(H_{-2}L)H]$ (Chart 2/VI). A further increase of pH above ca. 8 resulted in the deprotonation and coordination of hydroxamic OH, what is confirmed by the upfield shift of the E peak ($\Delta\delta$ = –0.029 ppm) in the mentioned pH-range. Consequently, both the NMR and pH-metric results show that under equimolar conditions, above pH ca. 8, $[PdH_{-2}L]^-$ is the major complex in this system. (iii) At twofold excess of ligand, in the pH-range ca. 6.0 - 9.5, in addition to the signals belonging to the free ligand and 1:1 complexes, one additional low intensity "extra" set could be observed. Although, pH-potentiometry did not indicate the formation of bis-complex at ligand excess, still its appearance after 1 hour under the mentioned conditions seems a reasonable interpretation of these NMR results. For further information in this question time dependence of the spectra was monitored (as detailed in Fig. S6). In fact, the intensity of the "extra" signals was increased for five days, when the maximum intensity was reached. This result shows that the formation of a bis-complex is rather slow, five days being necessary for its complete formation. The bis-complex has a single set of signals, which indicating its symmetrical structure, therefore, this complex should contain the two ligands in the same binding mode. Most probably, each of the two ligands is coordinating via a five-membered (NH_2, N_{amide})-chelate (see structure VIII in Chart 2). (iv) Below pH ~ 5, no additional signals were observed in the spectra registered after 1 hour at 1:2 metal ion to ligand ratio, compared to 1:1. However, in one day, at both ratios new signals were detected in the acidic region and their intensity increased continuously during the period of the investigation, 7-9 days (see Fig. S6). Taking into account the previous result on the hydrolysis of the ligand in the Pt(II)-glycinehydroxamate complex to glycine and hydroxylamine,[31] the possibility of a similar process in our system was assumed. Spectra registered on "nine-days samples" were compared with those of Pd(II)-HN(Me)OH and Pd(II)-Ala-Gly-Gly-OH and for illustration are shown in Fig. 3.

As Fig. 3 shows, the new signals observed under the above conditions in the Pd(II)-Ala-Gly-Gly-N(Me)OH system correspond to HN(Me)OH and that of Pd(II)-Ala-Gly-Gly-OH. Although detailed investigation on the mechanism was not performed, the facts that the hydrolysis was observed only below pH 5 and the pH-dependence of the relative intensity of E signals (see Table 4) fits the speciation curve of [PdL], support the kinetic activity of this complex in the hydrolysis. (see Fig. 4). Because in [PdL], in addition to the (NH_2, N_{amide}) chelate also the coordination of the hydroxamic $O_{carb.}$ was supported by the NMR results, a similar hydrolysis of our coordinated ligand might be possible that it was published in the above already mentioned literature for glycinehydroxamic acid in its Pt(II) complex.[31‡]

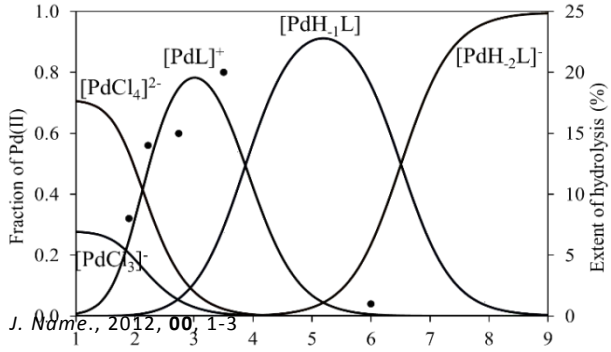

Fig. 4 The extent of ligand hydrolysis (expressed in the ratio of the N-CH_3 signals of the ligand and the free HN(Me)OH) after 24 hours (dots) on concentration distribution curve of Pd(II)-Ala-Gly-Gly-N(Me)OH = 1:1 system

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Pd(II)-dipeptide hydroxamic acid systems

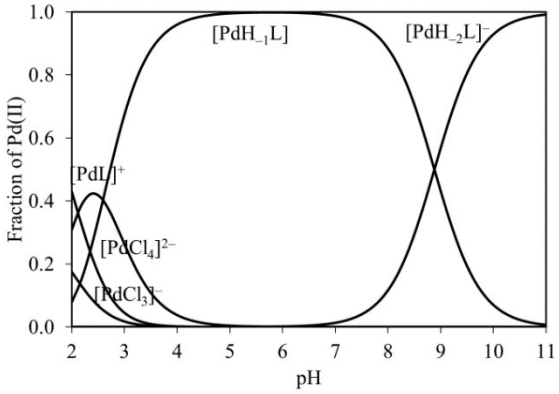

Fig. 5 Concentration distribution curves of Pd(II)–Ala-Ala-NHOH system at $c_{Pd(II)}$ = c_{lig} = 3.0 mM

Although Ala-Ala-NHOH has three N-donor atoms (NH_2, N_{amide}, $N_{hydr.}$) and two hydroxamate O-donors to interact with Pd(II) all the four coordination sites of Pd(II) cannot be occupied by them in a mononuclear species. The pH-metric measurements were performed as it is detailed in the Experimental part and provided the following experimental findings: (i) The pH-potentiometric titration curve registered in an equimolar sample showed the consumption of three equivalents of base until pH ~ 5, and an additional one above pH 7 (Fig. S7, curve d).

(ii) Interestingly, this latter process was not observed in the titration curve registered on an equimolar sample, which was once already titrated than acidified for a 4 days period prior to this "repeated titration" (see curve c in Fig. S7). This finding was a clear indication for the hydrolysis of the hydroxamic function from the coordinated ligand during the mentioned period of time. (iii) Like the primary tripeptide hydroxamic acid, Ala-Gly-Gly-NHOH, this ligand was also found to be able to bind Pd(II) excess, but significant differences were observed between the two systems in this question. Namely, while one equivalent Pd(II) excess was bound by the former molecule, it was only half equivalent for the dipeptide hydroxamic acid (*cf.* Fig. 1 and S7). (iv) Unlike with the two tripeptide hydroxamic acids, additional pH-effect compared to the equimolar ratio was observed in the titration curve registered at 1:2 metal ion to ligand ratio with Ala-Ala-NHOH, what is a direct indication for the measurable formation of bis-complex(es) being detectable by pH-metry in this system.

Despite the clear indication for the hydrolysis of the coordinated ligand under acidic conditions after a period of few days (see point (ii) above), yet the pH-metric curves registered in freshly prepared samples were adequate to carry out equilibrium calculations. The best fit of all titration curves provided the speciation model and overall stability constants presented in Table 2, 3rd column. The obtained results showed the measurable existence of $[PdL]^+$, $[PdH_{-1}L]$, $[PdH_{-2}L]^-$, $[PdL_2]$ and $[PdH_{-1}L_2]$ mononuclear, as well as $[Pd_3H_{-4}L_2]$ trinuclear complexes. By using these results, concentration distribution curves were calculated and, for illustration, those obtained at 1:1 and 1.5:1 metal ion to ligand ratio are shown in Fig. 5 and Fig. 6b., while the concentration distribution curves at 1:2 metal to ligand ratio can be seen in fig. S8. The stability constant of $[PdL]^+$ is very similar to those determined for the corresponding complexes formed with the two tripeptide hydroxamic acids (see Table 2), suggesting the most likely existence of a 5-membered (NH_2, N_{amide}) chelate (and the exact stoichiometry of $[Pd(H_{-1}L)H]^+$) (Chart 2/I).

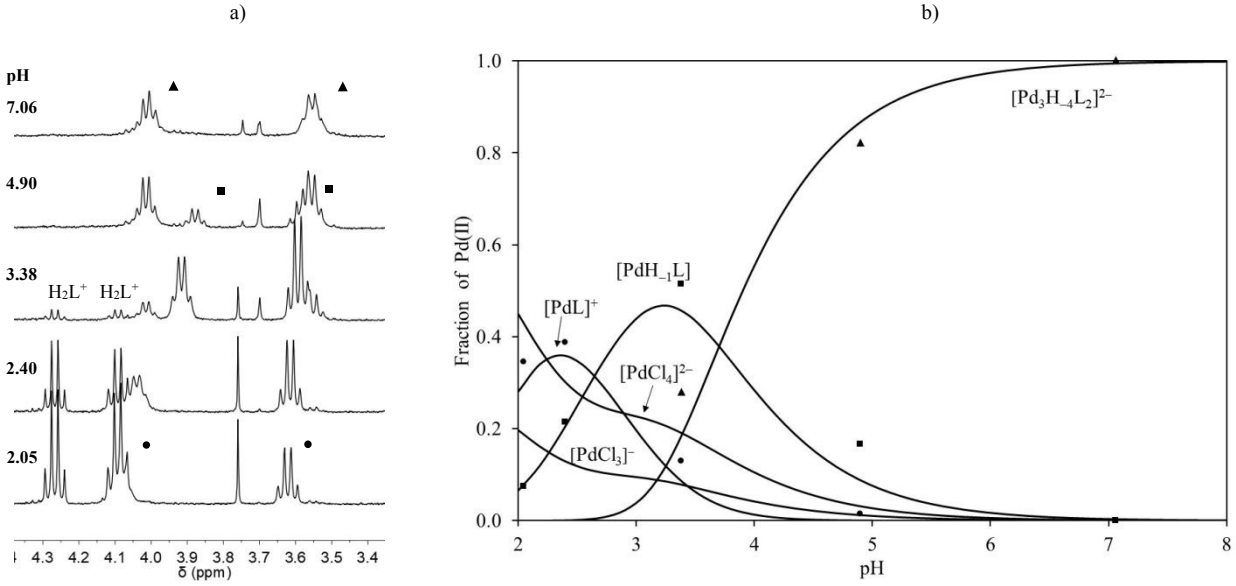

Fig. 6 pH-dependence of the ^{1}H NMR spectra of the Pd(II)-Ala-Ala-NHOH = 1.5:1 system (a); concentration distribution curves calculated using the stability constants in Table 2 (solid lines, c_L = 5 mM.) and from ^{1}H NMR spectra at 1.5:1 metal ion to ligand ratio (b); $[PdL]^+$ (●), $[PdH_{-1}L]$ (■), $[Pd_3H_{-4}L_2]^{2-}$ (▲)

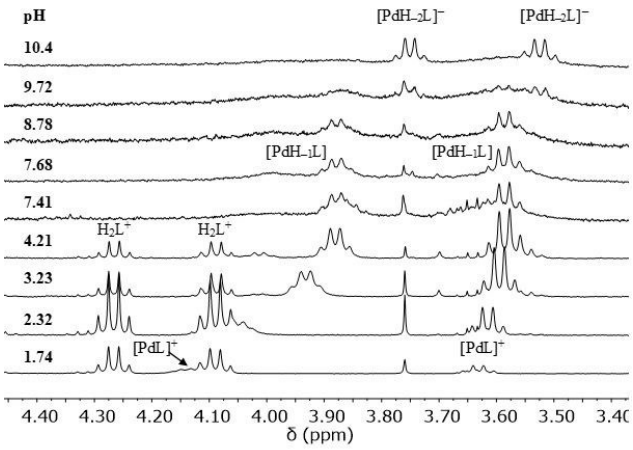

Fig. 7 pH-dependence of the ^{1}H NMR spectra of Pd(II)-Ala-Ala-NHOH system at 1:1 metal ion to ligand ratio

Deprotonation of [PdL] (most probably at the hydroxamic-NH function) occurs already in the pH-range, pH < 5, resulting in the formation of [PdH$_{-1}$L] (Chart 2/IX). In the last deprotonation process [PdH$_{-2}$L]$^-$ is formed and predominates in equimolar sample above pH 8. Simply the pH-potentiometric effect does not provide information for the question, whether, [PdH$_{-2}$L]$^-$ is either a mixed hydroxo species, or a (NH_2, N_{amide}, N_{amide}) coordinated one with doubly deprotonated hydroxymate function. However, the latter binding mode is supported by the lack of this fourth deprotonation process in the "repeated titration" on the "4-day sample", where the hydrolysis of the hydroxamic function from the coordinated ligand and formation of the simple dipeptide was assumed (see above in point (ii)). In the presence of Pd(II) excess the deprotonation and coordination of the hydroxamate group is assumed above pH ~ 3-4 and a trinuclear complex, [Pd$_3$H$_{-4}$L$_2$] is formed. The coordination mode is suggested to be analogous with the one found previously in the corresponding Cu(II) complex[28] and shown in Chart 2/ XI). In the case of twofold ligand excess, formation of bis complexes was shown by the pH-metric results. The stoichiometry of these are [PdL$_2$] and [PdH$_{-1}$L$_2$], and each of them most probably contains a [PdH$_{-1}$L] unit, to which the second ligand coordinates at the fourth equatorial site via its terminal amino-N donor, and contains the hydroxamic function still in protonated or already in the deprotonated form. Consequently, the exact stoichiometries are [Pd(H$_{-1}$L)(HL)] and [Pd(H$_{-1}$L)(L)], respectively (see Chart 2/X). The formation of all the complexes discussed above was supported by ^{1}H NMR measurements, monitoring the dependence of the chemical shifts on the metal ion to ligand ratio and pH. The obtained chemical shifts are shown in Fig. S9. Several new information obtained from the NMR results are as follows: (i) At 1:1 metal ion to ligand ratio one pair of quartets was detected beside the quartets of the free ligand at pH ca. 1.7 which was assigned to [PdL]$^+$. On increasing the pH up to ca. 4.2, where the [PdH$_{-1}$L] was found to become the major species, not only the intensity of these signals was increased, but surprisingly, they showed a continuous up-field shift. This indicates fast exchange between these two species in the NMR time scale, which is a significant difference compared to the tripeptide hydroxamic acid containing systems, where each of the complexes showed individual signals. (see Fig 7.) Based on this difference, it is assumed that the third coordination site of the metal ion in [PdH$_{-1}$L] might be occupied (at least in a transient species) by a rather labile bond, e.g. by O_{hydr}. Getting additional information in this question (e.g. from time-dependent measurements), were hindered by the above hydrolytic process, which were also found to occur in this acidic pH-range. (ii) At 1.5:1 Pd(II) to ligand ratio above pH ca. 3.4, a new pair of quartets appeared at δ = 4.015 ppm and δ = 3.556 ppm, which were assigned to [Pd$_3$H$_{-4}$L$_2$] complex. To demonstrate the good fit between the pH-metric and NMR speciation profiles Fig. 6 is shown. (iii) In the case of ligand excess above pH = 5.8 two pairs of new signals appeared beside the signals of the free ligand, which were assigned to the formation of bis-complex(es) (see Fig. S10). Existence of two sets of signals suggested that the coordinated ligands have different chemical environments in the bis-complexes. This finding supports the coordination mode discussed above based on the pH-metric results. Similarly, different binding modes of the two coordinated ligands in the Pd(II)-tripeptide bis-complexes were previously published, where, one of the ligands was assumed to bind via tridentate, while the other via a monodentate manner.[25]

The hydrolysis of the coordinating ligand) detected after 4 days with Ala-Gly-Gly-N(Me)OH) was already measurable after 2 days with Ala-Ala-NHOH. Significant changes in the NMR spectra were monitored in the pH-range 2-7 although some minor changes in the spectra could also be seen at neutral pH.

Pd(II)-Ala-Ala-N(Me)OH system

During the titration of the Pd(II)-Ala-Ala-N(Me)OH system, above pH 4.5 elementary palladium was formed as fine black powder. This also occurred during the NMR measurements, as a Pd-mirror was formed on the inner surface of the NMR tube. For this reason, equilibrium study on this system was not

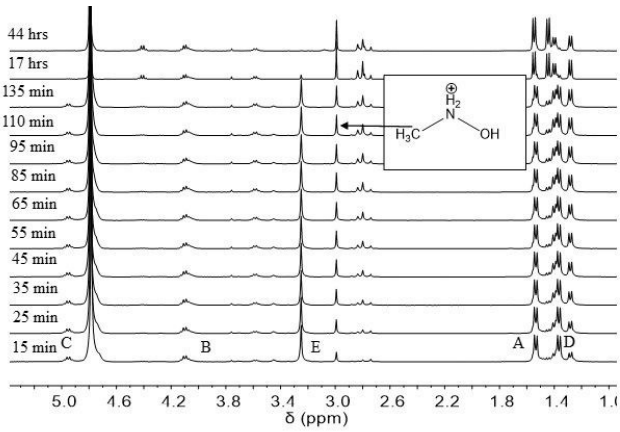

Fig. 8 Time dependence of the ^{1}H NMR spectra of Pd(II)-Ala-Ala-N(Me)OH 1:1 system at pH = 2.0 showing the formation of $H_2N(Me)OH^+$ and the corresponding Pd(II)-dipeptide complex

possible. ^{1}H NMR spectra showed that beside the signals of the free ligand, the registered new signals correspond to the protonated HN(Me)OH and the Pd(II)-Ala-Ala-OH complex (Fig. 8). This finding indicates the very fast hydrolysis of the coordinated Ala-Ala-N(Me)OH. The protonated hydroxylamine formed in the hydrolytic process can reduce Pd(II) to metallic palladium.

Conclusions

The present study provides important new information relating to the Pd(II)-peptide hydroxamic acids interactions. This is the first case, when $[PdCl_4]^{2-}$ as metal ion source was used. The previous results refer to systems, in which the metal ion source was $[Pd(en)Cl_2]$, so the formation of ternary complexes with a peptide hydroxamic acid was studied.[18] This study indicates that peptide hydroxamic acids are very effective chelators of Pd(II) where the nitrogen atoms are the most preferred donors by the metal ion. For primary ligands, in addition to the amino and amide-nitrogen(s), the deprotonated hydroxamate-N was also found to be an effective donor. Out of the investigated molecules only the primary tripeptide derivative, Ala-Gly-Gly-NHOH, is capable of saturating the coordination sphere of the Pd(II) by N-donors in a mononuclear complex. In fact, this 4N-coordinated species with very high stability predominates in equimolar solution, pH > 6 (Chart 2, II). Because in this complex the hydroxamic oxygens are not involved in the coordination, in the presence of metal ion excess a second Pd(II) will also be bound via the 5-membered hydroximate-O,O chelate resulting in the formation of a dinuclear species (Chart 2, III and IV).

Both Ala-Gly-Gly-N(Me)OH and Ala-Ala-NHOH are capable of providing up to three N-donors, (NH_2,N_{amide},N_{amide}) by the former and (NH_2,N_{amide},$N_{hydr.}$) by the latter ligand. In these systems, in mononuclear complexes, saturation of the fourth coordination site by a strongly coordinating donor can be achieved by the $O_{hydr.}$ (Chart 2, VII) in the former case, but, due to steric reasons this could happen with the latter ligand only in intermolecular interaction. As a result, unlike with Ala-Ala-NHOH (Chart 2, XI), binding of metal ion excess is not possible by Ala-Gly-Gly-N(Me)OH. Steric reason might be responsible for the difference between the stoichiometry of the complexes formed in presence of metal ion excess with Ala-Gly-Gly-NHOH and Ala-Ala-NHOH. Namely, dinuclear species was found to form with the tripeptide derivate but a trinuclear one with the dipeptide derivate.

With the two 3N-donor ligands, formation of bis-complexes was also found in presence of ligand excess. Interestingly, the formation of the bis-complex was very slow with the secondary tripeptide derivative, Ala-Gly-Gly-N(Me)OH, and monitoring the necessary structural changes identical binding mode of the two coordinating ligands (Chart 2, VIII) was supported by the NMR results. For the primary dipeptide derivative, however, the bis complex formation was relatively fast and the results supported the different binding modes of the two coordinating ligands. In this latter complex, simple monodentate coordination of the second ligand via its terminal amino-N to $[PdH_{-1}L]$ is assumed (Chart 2, X).

Unlike in the Pd(II)-Ala-Gly-Gly-NHOH system, parallel to complexation, Pd(II)-assisted hydrolysis of the coordinated ligands was also found under acidic conditions in the other three systems. The results supported the formation of protonated hydroxylamine and the corresponding peptides in these processes. Hydrolysis hindered the equilibrium measurements for the Pd(II)-Ala-Ala-N(Me)OH system, where the ligand can donate maximum two N-donors during the complexation and reduction of Pd(II) to Pd(0) by the hydroxylamine formed was also observed. Fortunately, the significantly slower hydrolysis with the two 3N-donor ligands, Ala-Ala-NHOH and Ala-Gly-Gly-N(Me)OH, allowed us to carry out equilibrium measurements (speciation studies) on these systems (as it is discussed above). Although detailed study of the hydrolysis was beyond the scope of this work, an order of the rate of hydrolysis could be determined. No indication for the hydrolysis was found with Ala-Gly-Gly-NHOH while some days were necessary to observe the measurable extent of it in the Pd(II)-Ala-Gly-Gly-N(Me)OH system. The process was faster in the Pd(II)-Ala-Ala-NHOH, while very fast for Pd(II)-Ala-Ala-N(Me)OH system. Plausible suggestion was also made for the kinetically active species in the case of Pd(II)-Ala-Gly-Gly-N(Me)OH. Since Pd(II) and Pt(II) share very similar coordination behaviour (square planar complexes and soft donor atom preference) but different ligand exchange kinetics the results obtained in this study may provide useful information for the design of hydroxamate based Pt(II) complexes with antitumor potential.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

Ala – alanyl
DMSO – dimethyl-sulfoxide
EtOAc – ethyl-acetate
Gly – glycyl
L – completely deprotonated forms of the ligands studied
MeCN –acetonitrile
N_{amide} – deprotonated amide group
$N_{hydr.}$ – deprotonated hydroxamic-N
NH_2 – terminal amino moiety of the ligand
$O_{carb.}$ – carbonyl-O
$O_{hydr.}$ – deprotonated hydroxamic-O
Pd – $Pd(II)_{aq.}$/Cl
TFA – trifluoroacetic acid
THF – tetrahydrofuran
Z – benzyloxycarbonyl

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Notes and references

‡ In the cited work protonation of the (NH_2, $N_{hydr.}$)-bound $[PtL]^+$ to $[PtHL]^{2+}$ with (NH_2, $O_{carb.}$) binding mode was found to be the key step of the mechanism. Hydrolysis of the ligand was assumed to occur in this transient species, by nucleophilic attack of an uncoordinated water on the coordinated and activated carbonyl group.[31]

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