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Potentiometric and NMR studies on palladium(II) complexes of oligoglycines and related ligands with non-co-ordinating side chains

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Palladium(II) complexes of peptides including Gly-Gly, Gly-Ala, Ala-Gly, Gly-Phe, Phe-Gly, triglycine, Gly-Gly-Ala and tetraglycine were studied by potentiometric and NMR spectroscopic methods. It was found that the reaction of free palladium(II) ion with dipeptides is almost complete below pH 2, therefore pH-metry can not be directly used for stability constant determinations. High excess of chloride ions (0.1 mol dm^{-3}) was used to shift complex formation between $[PdCl_4]^{2-}$ and peptides into the measurable pH range. The formation of the species [PdL(Cl)], $[PdLH_{-1}Cl]^-$ and $[PdLH_{-2}]^-$ with (NH_2,N^-,CO_2^-) co-ordination was detected with all dipeptides in equimolar solutions. Bis complexes were formed in the presence of an excess of ligand, but their stoichiometry varied as a function of the amino acid sequences of the peptides. In the case of dipeptides with C-terminal Gly residues (X-Gly) the species $[PdL_2H_{-2}]^{2-}$ with 4N co-ordination of two bidentate peptide molecules was formed by the physiological pH, while the stoichiometry of the bis complexes of dipeptides with non-co-ordinating side chains at the C termini (Gly-X) corresponds to $[PdL_2H_{-1}]^-$ containing both tridentate and monodentate peptide ligands. Equimolar solutions of palladium(II) and tripeptides are characterized by the formation of the species $[PdLH_{-2}]^-$ with (NH_2,N^-,N^-,CO_2^-) co-ordination, while $[PdL_2H_{-2}]^{2-}$ containing two bidentate ligands is the main species in the presence of an excess of tripeptides.

It is well known that peptides are versatile and effective ligands to bind metal ions under physiological conditions.[1–3] The formation of stable metal complexes of peptides, however, is connected to the involvement of amide nitrogen in metal binding, especially in the absence of side-chain donor groups. As a consequence, the co-ordination of peptide molecules to metal ions requires deprotonation and co-ordination of the peptide amide groups. Palladium(II) ion was reported to be one of the most effective metal ions to promote this type of interaction. Substitution of the hydrogen of the CONH groups of dipeptides by palladium(II) ion occurs in such acidic solution that the pK value is difficult to measure accurately and a value of around 2 was suggested for simple dipeptides.[4–6] It corresponds to an increase of more than twelve orders of magnitude in the acidity of the amide group in the presence of palladium(II) and results in the remarkable thermodynamic stability of palladium(II)–peptide complexes. One of the consequences of the high stability is that equilibrium data are scarcely available on the interaction of palladium(II) with peptide molecules, but ^{1}H NMR and CD spectroscopies are very convenient and widely used techniques to study the palladium(II) complexes of various di- and tri-peptides.[7–17]

The most important findings in this field have been discussed in several reviews,[18–20] and it has been widely accepted that palladium(II) forms stable square planar, diamagnetic complexes with dipeptides, in which the terminal amino, deprotonated amide nitrogen and terminal carboxylate oxygen donor atoms are the metal binding sites. Of course, in the presence of strongly co-ordinating side chains like thioether of methionyl[21,22] or imidazole of histidyl[23–25] residues the co-ordination of the carboxylate oxygen is replaced by the sulfur or nitrogen donor atoms, respectively, and it generally results in further increase of the thermodynamic stability of the corresponding peptide complexes. On the other hand, the co-ordination chemistry of simple dipeptides with non-co-ordinating side chains seems to be rather simple and unique. The results mainly come from NMR spectroscopic studies, which is an especially well suited technique to study palladium(II)–peptide interaction because the relatively slow exchange reactions of the co-ordinated tridentate peptide ligands makes it easy to distinguish the NMR parameters of the co-ordinated and "free" ligands. The tridentate binding of peptide molecules in acidic solutions of palladium(II) is widely accepted and well resolved NMR spectra provide a good base for the calculation of various rotamer populations in solution.[8–10,12,16,17] NMR Studies have also demonstrated that the fourth co-ordination site of palladium(II) complexes of dipeptides can easily be occupied by a water molecule or chloride ion or any other monodentate ligands including hydroxide ions in slightly basic solution. Further increase of pH generally results in breakage of Pd–O (carboxylate) bonds and dihydroxo species can be formed.[11] In the presence of an excess of peptide ligands the formation of 4N-co-ordinated bis complexes was also suggested, but this interaction was reported to occur only in strongly alkaline media.[8,9,12] None of these previous studies provides, however, stability constants on the palladium(II) complexes and a complete metal ion speciation of the corresponding systems is not available. The first potentiometric equilibrium study on the palladium(II) complexes of a peptide molecule was published only recently from our laboratory, describing the Pd^{II}–Pro-Gly-Ala-His system[26] in acidic media. It is evident from this and from some other previous studies that the difficulties of stability constant measurements come from the extra high stability of palladium(II) peptide complexes, which results in the very low pH range (< 2) of complex formation. In the case of tripeptides or higher oligomers the difficulties are enhanced by kinetic factors, namely by the slow formation kinetics of the complexes. On the other hand, it is also evident that the reliable metal ion speciation of the multicomponent systems of polydentate ligands is a very useful tool for the interpretation of spectral studies in solution. A similar study on the copper complexes of oligoglycines and related ligands using potentiometric, EPR and UV-VIS techniques was published recently.[27] This study revealed that the combined

application of potentiometric and spectroscopic techniques made it possible to detect new species in solution, which were declared as well known, *e.g.* the formation of bis complexes was demonstrated in the interaction of copper(II) with tri- or tetrapeptides.

Now in this paper we report a potentiometric method developed for the determination of stability constants of palladium(II) complexes. It was used to determine the metal ion speciation of several palladium(II) dipeptide systems (Gly-Gly, Gly-Ala, Ala-Gly, Gly-Phe, Phe-Gly) and also the tripeptides Gly-Gly-Gly and Gly-Gly-Ala and, in part, tetraglycine. The reliability of the speciation was verified by ^{1}H NMR studies. In addition to the first general characterization of the solution equilibria of palladium(II) peptide complexes, the results obtained in this study revealed that in the case of palladium(II) even the presence of non-co-ordinating side chains results in significant differences in the metal binding capabilities of ligands, *e.g.* differences between complexes of type X-Gly and Gly-X dipeptides.

Experimental

Materials

The peptides Gly-Gly, Gly-L-Ala, L-Ala-Gly, Gly-L-Phe, L-Phe-Gly, Gly-Gly-Gly, Gly-Gly-L-Ala and tetraglycine were purchased from Bachem and their purity checked *via* potentiometric titrations. Stock solutions of palladium(II) ions were prepared from $K_2[PdCl_4]$ (Fluka) and two equivalents of acid (HNO_3) were added to suppress hydrolytic processes.[28]

Determination of stability constants

Theoretical background. The difficulties of the stability constant measurements on palladium(II)–peptide complexes come from the very high thermodynamic stability of the corresponding species. The NMR spectra of solutions containing palladium(II) and dipeptide in equimolar concentration indicate almost complete complex formation around pH 2. As a consequence, the potentiometric titration curves of samples containing $[PdCl_4]^{2-}$ ion and dipeptide in the form $[H_2L]^+$ correspond to strong acid–base titration curves, which are not suitable for calculation of stability constants. Increasing the acidity of the samples suppresses complex formation, but the error of measurement of pH and the uncertainties in the constancy of ionic strength are increased very much below pH 2. Therefore, we decided to apply an indirect (or competitive) method using a relatively high concentration of chloride ion, which also suppresses the interaction between palladium(II) ions and peptide molecules. Under these experimental conditions the equilibrium reactions can be described by eqn (1)

$$p\mathrm{Pd} + q\mathrm{L} + r\mathrm{H} + s\mathrm{Cl} \rightleftharpoons \mathrm{Pd}_p\mathrm{L}_q\mathrm{H}_r\mathrm{Cl}_s \qquad (1)$$
$$\beta_{pqrs} = [\mathrm{Pd}_p\mathrm{L}_q\mathrm{H}_r\mathrm{Cl}_s]/[\mathrm{Pd}]^p[\mathrm{L}]^q[\mathrm{H}]^r[\mathrm{Cl}]^s$$

(charges are omitted for clarity). The application of this formulae requires the exact knowledge of the stability constants for the interaction of Pd^{2+} with Cl^- ion, which was taken from the literature[29] (log $\beta_1 = 4.47$, log $\beta_2 = 7.76$, log $\beta_3 = 10.17$ and log $\beta_4 = 11.54$ for the species $[PdCl]^+$, $PdCl_2$, $[PdCl_3]^-$ and $[PdCl_4]^{2-}$, respectively). On the other hand, the application of eqn. (1) provides the overall stability constants for the ternary systems, *e.g.* in the case of dipeptides the main species is $[PdLH_{-1}Cl]^-$ (log $\beta_{11\text{-}11}$). To characterize the real thermodynamic equilibrium of the interaction of palladium(II) with the dipeptide the stability constant of the species $[PdLH_{-1}]$ (log $\beta_{11\text{-}10}$) should be obtained, for which equilibrium (2) should be

$$[\mathrm{PdLH}_{-1}] + \mathrm{Cl}^- \rightleftharpoons [\mathrm{PdLH}_{-1}\mathrm{Cl}]^- \qquad (2)$$
$$K_{\mathrm{Cl}} = [\mathrm{PdLH}_{-1}\mathrm{Cl}]/[\mathrm{PdLH}_{-1}][\mathrm{Cl}]$$

considered. Indirect potentiometry can be used again to determine the values of K_{Cl}. The measurement of K_{Cl} is based upon the fact that $[PdLH_{-1}]$ can be prepared in chloride-free solutions, then titrated with a monodentate nitrogen base in the presence and absence of chloride ion. Namely, in the case of Gly-Gly equimolar amounts of $K_2[PdCl_4]$ and dipeptide were mixed and pH of the solution was raised to 5 (3 equivalents of base required). Part of this stock solution was treated with $AgNO_3$ to precipitate all chloride ions and silver chloride was filtered off. Two different stock solutions were obtained containing the species $[PdLH_{-1}]$ or $[PdLH_{-1}Cl]^-$ in known concentrations. Samples of these stock solutions were mixed with an equimolar amount of uridine (pK = 9.15) and titrated with base at a constant ionic strength of 0.2 mol dm^{-3} KNO_3. The presence of chloride ion shifts the complex formation with uridine to higher pH values and two different equilibrium constants K_U and K_U^{Cl} can be calculated, eqns. (3) and (4). The difference between

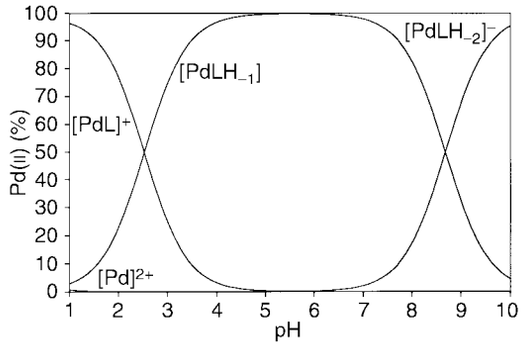

Fig. 1 Concentration distribution in the palladium(II)–Gly-Gly system calculated by the assumption of $c_{Cl} = 0$.

$$[\mathrm{PdLH}_{-1}] + \mathrm{U}^- \rightleftharpoons [\mathrm{PdLH}_{-1}\mathrm{U}]^- \ (K_U) \qquad (3)$$

$$[\mathrm{PdLH}_{-1}\mathrm{Cl}]^- + \mathrm{U}^- \rightleftharpoons [\mathrm{PdLH}_{-1}\mathrm{U}]^- + \mathrm{Cl}^- \ (K_U^{Cl}) \qquad (4)$$

these equilibrium constants depends on the binding constant (K_{Cl}) and the concentration of chloride ion. Using a constant chloride concentration ($c_{Cl} = 4c_{Pd}$) the value of K_{Cl} can be obtained as in eqn. (5). In the case of the Pd^{II}–Gly-Gly-uridine-

$$K_{\mathrm{Cl}} = (K_U - K_U^{Cl})/K_U^{Cl}[\mathrm{Cl}^-] \qquad (5)$$

Cl^- system the values of log $K_U = 7.09$ and log $K_U^{Cl} = 6.75$ were obtained ($c_{Cl} = 0.012$ mol dm^{-3}), from which log $K_{Cl} = 1.99$ can be calculated. It is quite close to the value of log $K_{Cl} = 1.90$ published by Kim and Martin[17] on the basis of spectrophotometric measurements. It is also obvious from their studies that the equilibrium constants for the binding of chloride ions are the same for other dipeptides having the same charge and donor atoms around palladium, as is the case for Ala-Gly and Phe-Gly. Taking into account the value of log K_{Cl}, the stability constants (log $\beta_{11\text{-}10}$) of the species $[PdLH_{-1}]$ can be calculated by eqn. (2) and involved in Table 1. These values can then be used to calculate the metal ion speciation of palladium–dipeptide systems in the absence of chloride ion, which is demonstrated by Fig. 1 for Pd^{II}–Gly-Gly.

It is unambiguously represented by Fig. 1 that there is no chance of direct pH-potentiometric measurements of stability constants of palladium(II) complexes of Gly-Gly because the complex formation is almost complete around pH 1. It is also obvious that deprotonation of the amide group of dipeptides takes place below pH 1 if no other ligand is present which can form stable adducts with palladium(II). The presence of halide ions, however, shifts the deprotonation of amide groups to less acidic pH ranges, which makes potentiometric detemination of stability constants possible.

Potentiometric studies on Pd^{II}–peptide systems. Potentiometric measurements were carried out in 10 cm^3 samples at

three different metal ion to ligand ratios, 1:1, 1:2 and 1:3, at constant palladium(II) (c_{Pd} = 0.0025 mol dm^{-3}) and chloride ion (c_{Cl} = 0.1 mol dm^{-3}) concentrations. The ionic strength was adjusted to 0.2 mol dm^{-3} with the addition of KNO_3. The reason for the application of a mixed background electrolyte (Cl^- + NO_3^-) is the fact that the increase of chloride concentration above 0.1 mol dm^{-3} resulted in significant increase in the periods to reach equilibrium during potentiometric titrations, while the necessity of a relatively high chloride concentration was discussed in the previous paragraph. The measurements were made with an automatically controlled Radiometer ABU 91 titration system containing the pH-meter and automatic burette and equipped with a Russel CWR/320/757 combined electrode. The titrations were performed with carbonate free potassium hydroxide solution of known concentration and 40 to 80 experimental points were collected at all ratios. The equilibration of the titration points generally required 2 to 10 min. All pH-metric measurements were made at 298 ± 0.1 K and argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide and for stirring the solutions. The pH readings were converted to hydrogen ion concentration as described previously[30,31] and the stability constants calculated by the application of a general computational program (PSEQUAD).[32]

NMR studies. Proton NMR spectra of the solutions of the "free" ligands ($c_L \approx 0.01$ mol dm^{-3}) and those containing $K_2[PdCl_4]$ and peptides at 1:1 and 1:2 ratios in D_2O were measured as a function of pD, which was determined by the use of Radiometer pH-meters and Russel CWR/320/757 combined electrodes and addition of 0.4 to the pH-meter readings. The spectra were recorded on BRUKER MA 360 MHz and AMX 300 MHz instruments using TSP (sodium 3-trimethylsilylpropionate) as internal standard.

Results and discussion

Palladium(II) complexes of dipeptides

Stability constants of palladium(II) complexes of various peptides were determined by potentiometric titrations in the presence of a high excess (about fortyfold) of chloride ions. Even in this case the complex formation processes take place in rather acidic media, but its range is at least partly shifted to pH > 2, which makes pH-metry an adequate method for stability constant determination. Representative examples of pH-metric titration curves are shown in Fig. 2 for the Pd^{II}–Gly-Ala and –Ala-Gly systems.

It can be seen from Fig. 2 that in equimolar solutions of palladium(II) and dipeptides one extra base is titrated, which corresponds to the formation of the well characterized species $[PdLH_{-1}]$ (or $[PdLH_{-1}Cl]^-$) with (NH_2,N^-,CO_2^-) coordination. This species is formed with all dipeptides studied in this work and transformed into $[PdLH_{-2}]^-$ in the pH range of 8 to 10. However, the titration curves of Gly-Ala and Ala-Gly are different in the presence of an excess of dipeptides. It is clear from Fig. 2(a) that in the Pd^{II}–Ala-Gly system one more extra base per metal ion is consumed around pH 6, which corresponds to the stoichiometry of $[PdL_2H_{-2}]^{2-}$. On the other hand, the base consumption of the solutions containing palladium(II) ion and Gly-Ala in the same concentrations (Fig. 2b) corresponds only to formation of the species $[PdL_2H_{-1}]^-$ below pH 10. As a consequence, the species [PdL(Cl)], $[PdLH_{-1}Cl]^-$, $[PdLH_{-2}]^-$, $[PdL_2H_{-1}]^-$ and $[PdL_2H_{-2}]^{2-}$ (L stand for the monoanionic forms of the dipeptides) were involved in the computational model in all cases and the corresponding stability constants are collected in Table 1.

Table 1 reveals that the 1:1 complexes ([PdL(Cl)], $[PdLH_{-1}Cl]^-$ and $[PdLH_{-2}]^-$) are formed with all of the five dipeptides and even their stability constants are rather close to each other.

Table 1 Stability constants of the palladium(II) complexes of dipeptides (I = 0.1 mol dm^{-3} Cl^- + 0.1 mol dm^{-3} NO_3^-, 298 K, standard deviations in parentheses)

Species	Gly-Gly	Gly-Ala	Ala-Gly	Gly-Phe	Phe-Gly
[HL]	8.11(1)	8.17(1)	8.12(1)	8.08(1)	7.53(1)
$[H_2L]^+$	11.21(2)	11.30(2)	11.31(2)	11.07(3)	10.62(3)
[PdL(Cl)]	18.08(2)	18.00(8)	17.96(3)	17.94(8)	17.58(7)
$[PdLH_{-1}Cl]^-$	15.56(1)	16.01(2)	15.09(2)	16.09(2)	15.20(3)
$[PdLH_{-2}]^-$	4.89(5)	4.80(10)	4.38(9)	5.30(10)	4.50(10)
$[PdL_2H_{-1}]^-$	19.30(10)	19.80(5)	18.70(10)	20.10(9)	19.20(10)
$[PdL_2H_{-2}]^{2-}$	13.90(5)	—	13.37(5)	—	13.70(5)
$[PdLH_{-1}]^a$	13.57	14.02	13.10	14.10	13.21

^{a} Corrected for c_{Cl} = 0 with log K_{Cl} = 1.99.

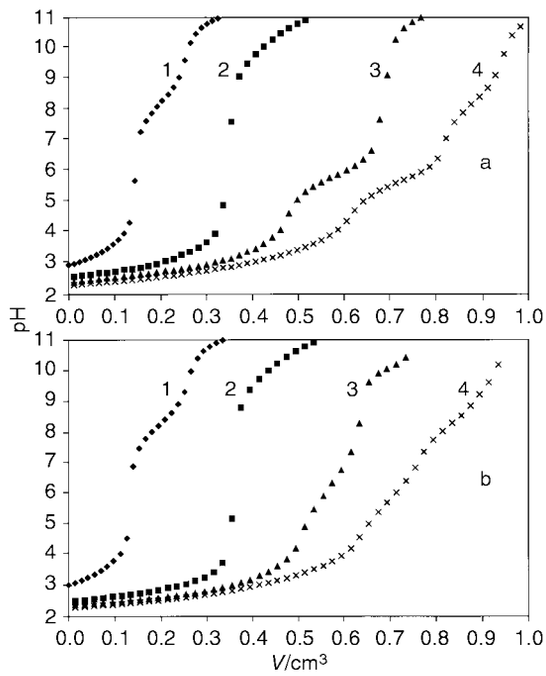

Fig. 2 pH-Metric titration curves of (a) Pd^{II}–Ala-Gly and (b) Pd^{II}–Gly-Ala systems. (1) "free" ligand, (2) 1:1 ratio ($c_{Pd} = c_L$ = 2.5 mmol dm^{-3}), (3) 1:2 ratio (c_{Pd} = 2.5, c_L = 5 mmol dm^{-3}), (4) 1:3 ratio (c_{Pd} = 2.5, c_L = 7.5 mmol dm^{-3}). The volume of 1 equivalent base per metal ion corresponds to (a) 0.101, (b) 0.121 cm^3.

The pK values for the deprotonation process $[PdL(Cl)] \rightleftharpoons [PdLH_{-1}Cl]^- + H^+$ correspond well to the deprotonation of the non-bonded carboxylic groups, supporting that the binding sites are (NH_2,N^-,Cl^-), (NH_2,N^-,CO_2^-,Cl^-) and (NH_2,N^-, CO_2^-, OH^-) for the species [PdL(Cl)], $[PdLH_{-1}Cl]^-$ and $[PdLH_{-2}]^-$, respectively.

In contrast with the uniformity of the complex formation processes of equimolar solutions the dipeptides can be classified into two categories as regards the formation and stoichiometry of bis complexes. Namely, in the case of Gly-Gly, Ala-Gly and Phe-Gly (having a Gly residue at the C terminus) the formation of the 1:1 complexes is followed by some $[PdL_2H_{-1}]^-$ on increasing pH and then $[PdL_2H_{-2}]^{2-}$ will predominate in a wide pH range, while in the case of Gly-Ala and Gly-Phe (having side chains at the α-carbon atom of the C terminus) only the species $[PdL_2H_{-1}]^-$ is formed below pH 10. These differences are especially well represented by the species distribution curves of Pd^{II}–Ala-Gly and –Gly-Ala systems and are shown in Fig. 3.

It is clear from Fig. 3(a) that $[PdL_2H_{-2}]^{2-}$ is almost completely formed by pH 7 in Pd^{II}–Ala-Gly system and the same holds for the other dipeptides containing a glycyl residue at the C terminus. However, the interaction of Gly-Ala and Gly-Phe is characterized by the formation of only $[PdL_2H_{-1}]^-$ in the same

Table 2 The NMR chemical shifts (ppm) measured in the Pd^{II}–Gly-Gly system at different metal ion to ligand ratios and pD values

Ratio $c_{Pd}:c_L$	pD	CH_2 (N termini)	CH_2 (C termini)	Main species
0 ("free" ligand)	2.20	3.901	4.069	$[H_2L]^+$
	4.55	3.860	3.835	$[HL]/[H_2L]^+$
	5.32	3.856	3.812	[HL]
	5.96	3.856	3.809	[HL]
	8.03	3.755	3.803	$[HL]/[L]^-$
	8.92	3.555	3.794	$[HL]/[L]^-$
	9.81	3.401	3.786	$[L]^-$
1:1	2.20	3.901	4.069	$]H_2L]^+$
		3.455	3.854	$[PdLH_{-1}]$
		3.455	4.044	$[PdL]^+$
	2.80	3.898	4.060	$[H_2L]^+$
		3.452	3.853	$[PdLH_{-1}]$
		3.452	4.040	$[PdL]^+$
	5.40	3.463	3.875	$[PdLH_{-1}]$
1:2	2.20	3.901	4.069	$[H_2L]^+$
		3.455	3.854	$[PdLH_{-1}]$
		3.455	4.043	$[PdL]^+$
	4.40	3.864	3.851	$[HL]/[H_2L]^+$
		3.463	3.876	$[PdLH_{-1}]$
	6.00	3.854	3.807	[HL]
		3.461	3.875	$[PdLH_{-1}]$
		3.461	3.875	$[PdL_2H_{-1}]^-$
		3.499	3.790	
		3.527	3.438	$[PdL_2H_{-2}]^{2-}$
	7.90	3.523	3.438	$[PdL_2H_{-2}]^{2-}$
	9.00	3.523	3.438	$[PdL_2H_{-2}]^{2-}$

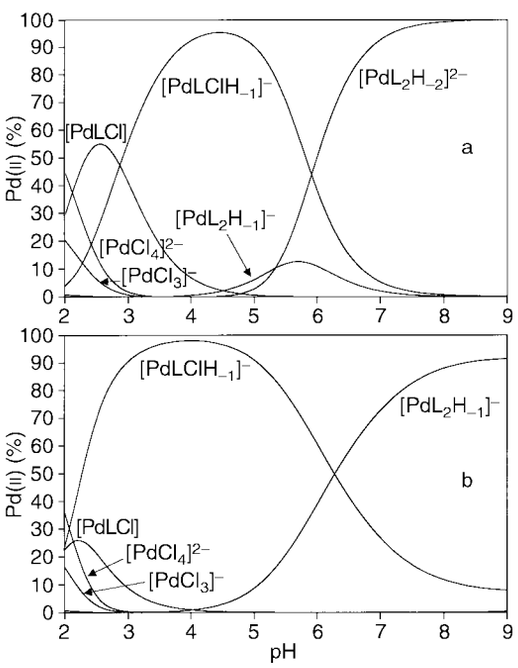

Fig. 3 Concentration distribution curves of (a) Pd^{II}–Ala-Gly and (b) Pd^{II}–Gly-Ala systems at 1:2 ratio in the presence of chloride ion (c_{Pd} = 2.5 and c_{Cl} = 100 mmol dm^{-3}).

pH range. Previous studies on the palladium(II) complexes of peptides have clarified the existence of 4N-co-ordinated bis complexes, but strongly alkaline media were suggested for their formation.[8,9,12] This observation is, however, in a good agreement with the earlier findings obtained for the dipeptide complexes of copper(II). The formation of the species $[CuL_2H_{-2}]^{2-}$ was reported to occur selectively with the dipeptides X–Gly, while the same species of Gly–X were not considered even in strongly alkaline media, and it was explained by steric effects.[33] Comparison of the species distribution curves in the acidic pH range gives further insight into the weaker binding of carboxylate in the palladium(II) complex of Ala-Gly. Namely, the species

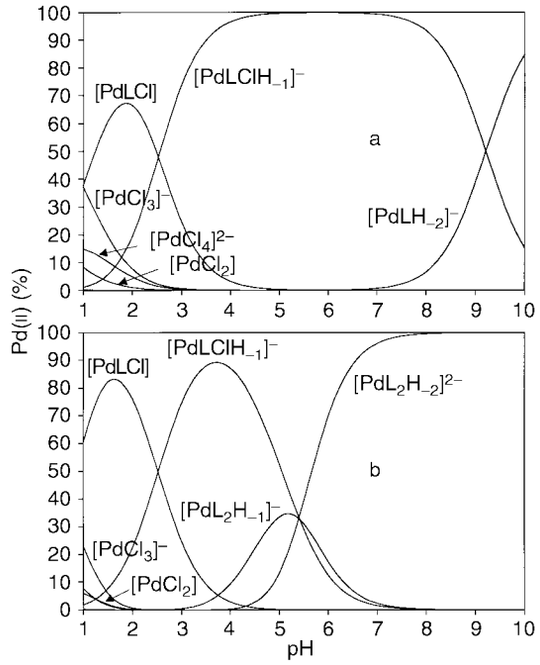

Fig. 4 Concentration distribution in the palladium(II)–Gly-Gly system under the experimental conditions of the NMR studies. (a) 1:1 ratio, $c_{Pd} = c_L = 10$, $c_{Cl} = 40$ mmol dm^{-3}; (b) 1:2 ratio, $c_{Pd} = 5$, $c_{Cl} = 20$ mmol dm^{-3}.

$[PdLH_{-1}Cl]^-$ containing the tridentate peptide molecule is formed in the pH ranges 2 to 4 and 1 to 3 for Ala-Gly and Gly-Ala, respectively. As a consequence, the concentration of the species with bidentate peptide molecule and protonated carboxylate [PdL(Cl)] is relatively high in the case of Ala-Gly and the pK calculated for non-bonded carboxylate (pK = 2.87) corresponds well to that of the "free" ligand. On the other hand, the concentration of [PdL(Cl)] is almost negligible with Gly-Ala supporting that co-ordination of carboxylate takes place in very acidic media. The same conclusion can be drawn for the complexes of Gly-Phe and Phe-Gly.

The ^1H NMR studies on the Pd^{II}–Gly-Gly, –Gly-Ala and –Ala-Gly systems definitely support these differences in the binding modes in the physiological pH range. Chemical shifts of the palladium(II) complexes of dipeptides are collected in Tables 2 and 3. It is clear from Table 2 and from the corresponding speciation curves in Fig. 4 that equimolar solutions of palladium(II) and Gly-Gly show the existence of three different species in the acidic pH range and they can be assigned to [PdL(Cl)], $[PdLH_{-1}Cl]^-$ and the fully protonated form of the ligand $[H_2L]^+$.

The relative intensities of these peaks indicate significant concentration of the "free" ligand around pH 2, which is in agreement with the assumption that the presence of chloride ion shifts complex formation to less acidic pH values and potentiometry is an adequate method for stability constant determination. The intensity of the signals of the "free" ligand and [PdL] decreases with increasing pH and $[PdLH_{-1}]$ will be the only species above pH 4. (The change in the concentration of chloride ion does not affect the NMR parameters of the co-ordinated ligand, therefore $[PdLH_{-1}]$ and $[PdLH_{-1}Cl]^-$ can not be distinguished by NMR, and they will be referred to as $[PdLH_{-1}]$). At 1:2 metal ion to ligand ratio and in acidic solutions the NMR signals can be assigned to the same 1:1 species discussed above with a high ratio of unco-ordinated ligand. Around pH 5 the new sets of signals can be assigned to the presence of $[PdL_2H_{-1}]^-$ containing tridentate and monodentate Gly-Gly residues in the complex. The NMR peaks of this species exist only in a narrow pH range, between 4 and 7, but the intensity of the doublet, which corresponds to the species $[PdL_2H_{-2}]^{2-}$, continuously increases above pH 6 and becomes

Table 3 The NMR chemical shifts (ppm) measured in the Pd^{II}–Gly-Ala system at different metal ion to ligand ratios and pD values

Ratio $c_{Pd}:c_L$	pD	CH_2 (Gly)	CH (Ala)[a]	CH_3 (Ala) (doublet)	Main species
0 ("free" ligand)	2.21	3.847	4.420	1.425, 1.445	$[H_2L]^+$
	6.30	3.826	4.178	1.350, 1.370	[HL]
	10.60	3.340	4.177	1.344, 1.365	$[L]^-$
1:1	2.20	3.848	4.426	1.427, 1.448	$[H_2L]^+$
		3.469, 3.484[b]	4.002	1.400, 1.420	$[PdLH_{-1}]$
	5.90	3.461, 3.477[b]	4.008	1.403, 1.423	$[PdLH_{-1}]$
1:2	2.20	3.847	4.423	1.426, 1.447	$[H_2L]^+$
		3.464, 3.480[b]	4.002	1.398, 1.419	$[PdLH_{-1}]$
	4.64	3.821	4.188	1.344, 1.365	$[HL]/[H_2L]^+$
		3.458, 3.474[b]	4.012	1.401, 1.420	$[PdLH_{-1}]$
	8.91	3.459, 3.474[b]	4.017	1.404, 1.420	$[PdL_2H_{-1}]^-$
		3.383	4.163	1.335, 1.356	

[a] Average for the quartet. [b] CH_2 Gly protons give the AB spectrum when indicated.

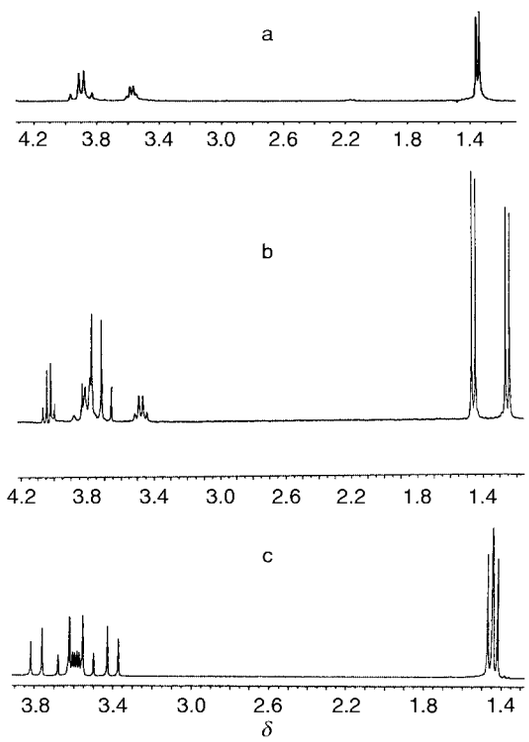

Fig. 5 The NMR spectra of the Pd^{II}–Ala-Gly system at different ratios and pD values. (a) 1:1 ratio, $c_{Pd} = c_L = 10$, $c_{Cl} = 40$ mmol dm^{-3}, pD 2.80; (b) 1:2 ratio, $c_{Pd} = 5$, $c_{Cl} = 20$ mmol dm^{-3}, pD 4.90; (c) 1:2 ratio, $c_{Pd} = 5$, $c_{Cl} = 20$ mmol dm^{-3}, pD 9.40.

the only set of NMR signals above pH 7 as is demonstrated by the predominance of the species in the concentration distribution curve (Fig. 4b).

The analysis of the NMR spectra of the Pd^{II}–Ala-Gly system led to the same conclusions. It is demonstrated by Fig. 5, where the NMR spectra are collected at three different ratios and pH values. Spectrum (5a) corresponds to species $[PdLH_{-1}]$ containing the tridentately bonded (NH_2,N^-,CO_2^-) dipeptide molecules, (b) was obtained at 1:2 metal ion to ligand ratio at pD 4.90, where the formation of several species overlapped ($[PdLH_{-1}]$, $[PdL_2H_{-1}]^-$, and "free" ligand) and (c) was obtained in the same solution at pD 9.40 and in this case the "free" ligand, $[PdLH_{-1}]$ and $[PdL_2H_{-1}]^-$ can not be detected, but the symmetry of the spectrum corresponds to the exclusive formation of the 4N-co-ordinated $[PdL_2H_{-2}]^{2-}$ species.

Spectral parameters of the NMR signals obtained in Pd^{II}–Gly-Ala system are collected in Table 3 and can be assigned to the appropriate species by comparison of the corresponding speciation curves (Fig. 3). The formation of $[PdLH_{-1}]$ is exclusive again in equimolar solutions. However, in case of an excess of ligand only three sets of signals can be assigned, which corresponds to the formation of $[PdLH_{-1}]$ and $[PdL_2H_{-1}]^-$ together with the presence of "free" ligand, and those occur at any pH value in agreement with the concentration distribution curve, which shows that the concentration of $[PdL_2H_{-1}]^-$ never reaches 100%. Of course, at very high pH values (pH > 12) further changes can be observed, which correspond to the formation of the species $[PdL_2H_{-2}]^{2-}$ in agreement with earlier findings.

The NMR chemical shifts and speciation curves clearly indicate a significant difference in the complex formation processes of Gly-Ala and Ala-Gly, which is reflected in the presence of $[PdL_2H_{-2}]^{2-}$ around pH 7. The same observation holds for the interaction of palladium(II) ions with Gly-Phe and Phe-Gly. The stability constants of these complexes are also collected in Table 1 and show similar tendencies as discussed for the peptides of alanine. Another important feature of this interaction is the good resolution of NMR spectra of palladium(II) complexes, which makes it possible to calculate rotamer populations of the tridentate co-ordinated ligands and was published many years ago.[9]

Palladium(II) complexes of tri- and tetra-peptides

The complex formation processes between transition metal ions and oligopeptides are generally characterized by the formation of 1:1 complexes *via* the co-ordination of a terminal amino group and subsequent amide nitrogens with the stoichiometries of [ML], $[MLH_{-1}]$, $[MLH_{-2}]$ and $[MLH_{-3}]$. However, on the basis of combined application of potentiometric and EPR studies we found recently that copper(II) can form bis complexes with tri- and tetra-glycine.[27] Of course, these species ($[CuL_2H_{-1}]^-$ and $[CuL_2H_{-2}]^{2-}$) were present only in intermediate pH ranges and in relatively low concentrations. The binding modes of these species were interpreted by the involvement of tridentate and monodentate peptide ligands in metal binding. Such species, at least in principle, can be formed with all metal ions, when the successive deprotonation and co-ordination of amide nitrogens take place in well separated processes. In this case the co-ordination spheres of $[MLH_{-1}]$ of tripeptides and $[MLH_{-1}]$ and $[MLH_{-2}]^-$ of tetrapeptides are unsaturated and monodentate binding of a second ligand is possible. Steric requirement is another factor, which influences the formation of the bis complexes, therefore in addition to simple oligoglycines (triglycine and tetraglycine) we decided to study the palladium(II) complexes of Gly-Gly-Ala for comparison.

Potentiometric studies were performed under the same experimental conditions as described for dipeptides. However, it is important to note that complex formation processes of tripeptides (and especially those of tetra- or higher oligomers) are hampered by the much slower formation kinetics than those of dipeptides. In the case of tripeptides the equilibration

Table 4 Stability constants of the palladium(II) complexes of the tripeptides Gly-Gly-Gly and Gly-Gly-Ala and tetraglycine ($I = 0.1$ mol dm^{-3} Cl^- + 0.1 mol dm^{-3} NO_3^-, 298 K, standard deviations in parentheses)

Species	Gly-Gly-Gly	Gly-Gly-Ala	Tetraglycine
[HL]	7.93(1)	7.94(1)	7.98(2)
$[H_2L]^+$	11.25(2)	11.22(2)	11.12(2)
[PdL(Cl)]	17.91(4)	17.91(2)	18.25(3)
$[PdLH_{-1}Cl]^-$	14.64(3)	14.45(3)	14.81(5)
$[PdLH_{-2}]^-$	9.07(5)	8.99(4)	10.13(4)
$[PdLH_{-3}]^{2-}$	−1.15(7)	−2.40(10)	2.45(6)
$[PdL_2]$	23.0(2)	23.7(1)	[a]
$[PdL_2H_{-1}]^-$	19.81(8)	19.60(10)	[a]
$[PdL_2H_{-2}]^{2-}$	13.40(7)	15.74(5)	[a]

[a] Detected by NMR.

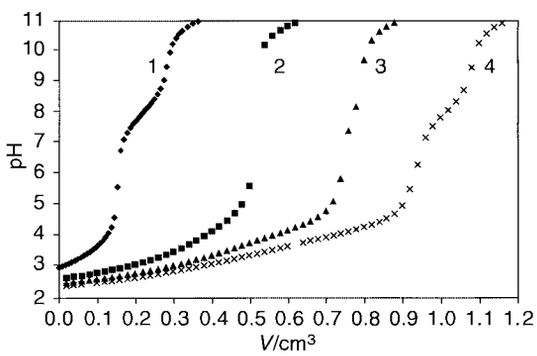

Fig. 6 Potentiometric titration curves of the Pd^{II}–Gly-Gly-Ala system. (1) "free" ligand, (2) 1:1 ratio ($c_{Pd} = c_L = 2.5$ mmol dm^{-3}), (3) 1:2 ratio ($c_{Pd} = 2.5$, $c_L = 5$ mmol dm^{-3}), (4) 1:3 ratio ($c_{Pd} = 2.5$, c_L 7.5 mmol dm^{-3}). The volume of 1 equivalent base per metal ion corresponds to 0.128 cm^3.

generally required less then 15 min for each titration point, thus reliable equilibrium parameters can be obtained and they are collected in Table 4. However, in the case of tetraglycine, especially in the systems containing an excess of ligand, equilibrium was not reached on a reasonable timescale. This probably comes from the fact that bis complexes are formed in this case too, but it requires a rearrangement of co-ordination sites around the metal ion and it takes place in a relatively slow reaction. Therefore in this paper we report the potentiometric analysis of equimolar solutions of Pd^{II} and tetraglycine, while other aspects of palladium(II)–tetrapeptide interactions will be covered by further studies. The potentiometric titration curves are demonstrated by the Pd^{II}–Gly-Gly-Ala system in Fig. 6 and almost the same curves were obtained for triglycine. The resulting metal ion speciation curves are shown in Fig. 7.

It can be seen from Fig. 6 that titration of the equimolar solution of Pd^{II} and Gly-Gly-Ala results in two equivalents of an extra base consuming process in the acidic pH range. In agreement with Fig. 7(a) it suggests that the species $[PdLH_{-2}]^-$ should be formed completely below pH 6. Chemical evidence and previous studies on palladium(II) complexes of tripeptides[11] suggest the (NH_2,N^-,N^-,CO_2^-) binding mode in this species and this is also supported by our NMR studies. The species $[PdLH_{-2}]^-$ remains intact in a very wide pH range and further base consuming processes take place only at high pH values (>10). The species $[PdLH_{-3}]^{2-}$ was reported to be a hydroxo complex, in which binding of the carboxylate residue is replaced by a hydroxo group in highly alkaline media. Another important feature of the equimolar solutions is that we can easily estimate the deprotonation constants for the amide groups of tripeptides. The exact determination of this pK value requires the knowledge of binding constants of chloride ions in the various species, but it is not possible in this case because $[PdLH_{-1}]$ is not a single species at any pH value. However, we

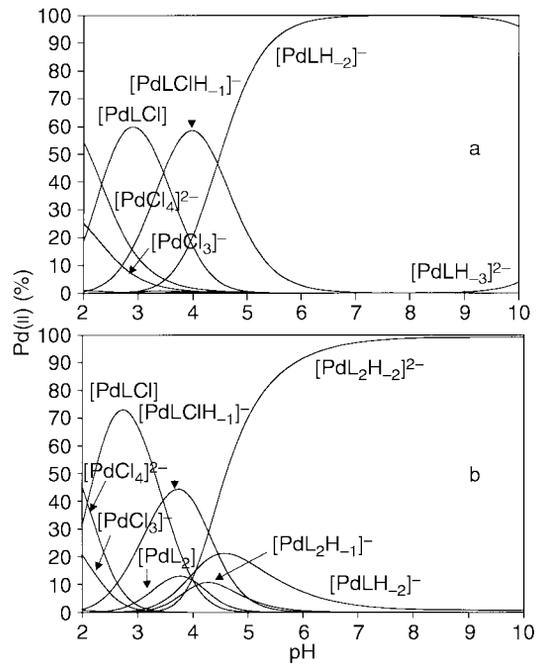

Fig. 7 Concentration distribution curves of the Pd^{II}–Gly-Gly-Ala system at (a) 1:1 and (b) 1:2 ratio in the presence of chloride ion ($c_{Pd} = 2.5$, $c_{Cl} = 100$ mmol dm^{-3}).

can assume that it should be similar to that of Gly-Gly (log $K_{Cl} = 1.99$). Taking into account this value p$K_1 = 3.27$ and 3.46 can be obtained for the process $[PdL]^+ \rightleftharpoons [PdLH_{-1}] + H^+$ in the case of Gly-Gly-Gly and Gly-Gly-Ala, respectively. It can correspond to either the deprotonation of the first amide or of the non-co-ordinated carboxylic groups. The NMR studies definitely confirm that deprotonation predominantly originates from the latter process suggesting that the first amide is already bonded to the metal ion at very low pH values. Deprotonation and co-ordination of the second amide nitrogen takes place in the pH range of 4 to 5 (see Fig. 7) in the presence of chloride ion. If one takes into account again the binding constants of chloride ions the values of p$K_2 = 3.58$ and 3.47 can be calculated for Gly-Gly-Gly and Gly-Gly-Ala, respectively, but they can only be used in a chloride-free environment. The actual pK values of the amide groups always depend on the concentration of halide ions or any other ligands present in the solution.

Both the pH-metric titration curves and NMR measurements suggest the formation of bis complexes in the presence of an excess of ligand. The formation of the hydroxo complexes in equimolar solutions was interpreted by the substitution of carboxylate with hydroxide ion suggesting that terminal carboxylate is only a loosely bonded donor atom of the fully deprotonated quadridentate tripeptide molecule. As a consequence, the carboxylate residue can be substituted by a second ligand and this is demonstrated by the titration curves, too. Namely, the pH-metric titration curves of 1:2 solutions do not indicate the presence of "free" ligand above pH 5, while it is present at 1:3 ratio. Therefore, potentiometric data could only be fitted if the species $[PdL_2]$, $[PdL_2H_{-1}]^-$ and $[PdL_2H_{-2}]^{2-}$ were also involved in the computational model (see Table 4). The corresponding metal ion speciation curves (see Fig. 7) clearly indicate that the species $[PdL_2]$ and $[PdL_2H_{-1}]^-$ are formed only in low concentrations. As a consequence, it is difficult to get an unambiguous proof of the binding modes of these species, but chemical evidence and the results obtained on copper(II) complexes with the same stoichiometry strongly support that one of the ligands is tridentate (or bidentate), while the other is monodentate (NH_2,N^-,CO_2^-(H^+) + (–NH_2). The species $[PdL_2]$ and $[PdL_2H_{-1}]^-$ probably differ only in the degree of protonation of one of the carboxylate residues. On the other hand, it is obvious that $[PdL_2H_{-2}]^{2-}$ is the main species in the Pd^{II}–dipeptide

Table 5 The NMR chemical shifts (ppm) measured in the Pd^{II}–Gly-Gly-Ala system at different metal ion to ligand ratios and pD values

Ratio $c_{Pd}:c_L$	pD	CH_2 (N termini)	CH_2 (internal)	CH (Ala)[a]	CH_3 (Ala)	Main species
0 ("free" ligand)	2.30	3.895	4.027	4.393	1.417, 1.438	$[H_2L]^+$
	2.80	3.889	4.022	4.376	1.407, 1.429	$[H_2L]^+$
	3.80	3.891	4.015	4.276	1.370, 1.389	$[HL]/[H_2L]^+$
	6.80	3.900	4.018	4.172	1.337, 1.357	[HL]
	9.40	3.474	3.986	4.177	1.335, 1.356	$[HL]/[L]^-$
	10.40	3.410	3.981	4.177	1.335, 1.356	$[L]^-$
1:1	2.76	3.894	4.022	4.380	1.404, 1.425	$[H_2L]^+$
		3.453	3.759, 3.783	4.415	1.452, 1.471	[PdL]
	3.92	3.892	4.013			[HL][b]
		3.465	3.74, 3.78	4.340	1.427, 1.446	$[PdLH_{-1}]$
	7.70	3.479, 3.496	3.853, 3.866	4.055	1.408, 1.427	$[PdLH_{-2}]^-$
	9.55	3.479, 3.496	3.857, 3.870	4.053	1.414, 1.432	$[PdLH_{-2}]^-$
1:2	2.96	3.886	4.022	4.368	1.406, 1.426	$[H_2L]^+$
		3.455	3.77, 3.79	4.415	1.448, 1.465	[PdL]
	3.80	3.897	4.015	4.262	1.365, 1.380	$[HL]/[H_2L]^+$
		3.457	3.74, 3.77	4.237	1.425, 1.445	$[PdLH_{-1}]$
	7.10	3.478, 3.497	3.887, 3.936	4.200	1.398, 1.417	$[PdL_2H_{-2}]^{2-}$
		3.519, 3.568	3.912, 4.019			

[a] Average for the quartet. [b] Present in low concentration.

systems in the presence of an excess of ligand. Its binding mode, however, can not be determined from potentiometric results, because the stoichiometries $[Pd(LH_{-2})L]^{2-}$ (one tridentate and one monodentate ligand) and $[Pd(LH_{-1})_2]^{2-}$ (two bidentate ligands) equally fit the experimental titration curves. The NMR studies on the Pd^{II}–Gly-Gly-Ala system definitely confirm the existence of the latter binding mode and the corresponding data are collected in Table 5.

The chemical shifts in Table 5 strongly support that deprotonation and co-ordination of the amide groups of tripeptides is shifted to higher pH values than those reported for dipeptides. Namely, the NMR spectra of the equimolar solutions of palladium(II) and Gly-Gly-Ala at pD 2.76 show the presence of "free" and bonded ligands in comparable concentration supporting that pK of the first amide group is above 2. The "free" ligand is still present at pD 3.90, but its concentration is very low. The most important changes of the NMR spectra are connected to the CH protons of the Ala residue in the pH range of 3 to 4, suggesting that the $[PdL]^+$ to $[PdLH_{-1}]$ transformation comes from the deprotonation of the unbonded carboxylic group. The NMR peaks of the "free" ligand disappear by pH 5 and only one set of signals can be assigned above pH 6, which corresponds to (NH_2,N^-,N^-,CO_2^-) co-ordination in the species $[PdLH_{-2}]^-$.

The NMR spectra of the samples containing the excess of tripeptides are much more complicated especially in the pH range of 3 to 5. (See the corresponding speciation curves in Fig. 7, for comparison.) It is clear, however, that the most intense peaks can be assigned to those of the "free" ligand and the species $[PdL]^+$ and $[PdLH_{-1}]$. On the other hand, it is a bit surprising that the "free" ligand can not be detected above pH 6 at 1:2 ratio and only one set of signals can be assigned for the bonded tripeptide molecule. The NMR spectra of the solution containing Pd^{II} and triglycine in the same concentration can be interpreted in the same way, supporting that the species $[PdL_2H_{-2}]^{2-}$ corresponds to $[Pd(LH_{-1})_2]^{2-}$ containing two (NH_2,N^-)-co-ordinated bidentate ligands.

The stability constants obtained for the interaction of tetraglycine with palladium(II) are also included in Table 4. In this case the real thermodynamic equilibrium can be reached only at 1:1 ratio, therefore stability constants were calculated only for the species [PdL(Cl)], $[PdLH_{-1}Cl]^-$, $[PdLH_{-2}]^-$ and $[PdLH_{-3}]^{2-}$. The NMR studies, however, support again that bis complexes are present in intermediate pH ranges (pH 3 to 7), and these species are indicated by *a* in Table 4. The most important conclusion which can be drawn from the data for equimolar solutions is that the deprotonation and co-ordination of the subsequent amide nitrogens of the tetrapeptide take place in well separated processes. It is best represented by Fig. 8, where the metal ion speciation of the Pd^{II}–tetraglycine system is plotted as a function of pH in equimolar solution.

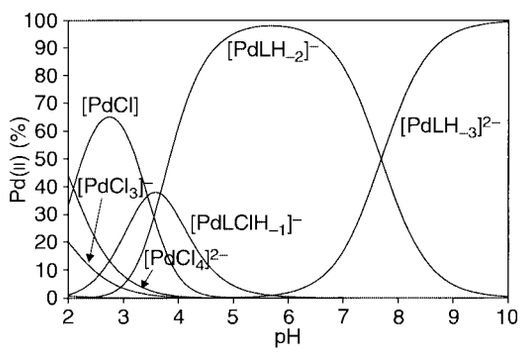

Fig. 8 Concentration distribution in equimolar solutions of Pd^{II} and tetraglycine in the presence of chloride ion ($c_{Pd} = c_L = 2.5$, $c_{Cl} = 100$ mmol dm^{-3}).

It is clear from Fig. 8 that deprotonation of the first and second amide nitrogens still takes place in acidic solution, but co-ordination of the third amide nitrogen, *i.e.* the formation of the species $[PdLH_{-3}]^{2-}$ occurs only in the neutral pH range and p$K = 7.70$ can be calculated for deprotonation of the third amide group. The resulting species $[PdLH_{-3}]^{2-}$ is a 4N complex, therefore hydrolytic processes can not be observed even in strongly alkaline media. There is a significant difference in the hydrolytic processes of the co-ordinatively saturated species $[PdLH_{-2}]^-$ and $[PdLH_{-3}]^{2-}$ of triglycine and tetraglycine, respectively. Namely, in case of triglycine or other tripeptides the fourth co-ordination site is occupied by a carboxylate residue, which can easily be substituted by hydroxide ion, while the Pd–N bonds of tetraglycine suppress hydrolytic reactions. Preliminary NMR studies, however, strongly support that bis complexes can be formed if an excess of tetraglycine is present. The study of the formation of these species is, however, significantly hampered by the slow formation kinetics and therefore we can not give a reliable concentration distribution curve. The inertness of the tetrapeptide complexes probably comes from the fact that the formation of bis complexes requires the rearrangement of co-ordination sites of the stable monomer adducts $[PdLH_{-2}]^-$ and $[PdLH_{-3}]^{2-}$.

Conclusion

Stability constants of palladium(II) complexes of oligopeptides

were determined by an indirect potentiometric method using chloride ion as a competitive ligand. The equilibrium parameters of dipeptide complexes show that deprotonation and co-ordination of amide nitrogens of dipeptides takes place around pH 1 in a chloride-free environment, but the application of a high excess of chloride ion (40-fold was applied in our experiments) shifts the complex formation processes of peptides to a well measurable pH range (2 to 4). The species $[PdLH_{-1}Cl]^-$ was the major product with all dipeptides studied (Gly-Gly, Gly-Ala, Ala-Gly, Gly-Phe and Phe-Gly), in which the metal ion is co-ordinated *via* the terminal amino, deprotonated amide nitrogen and the carboxylate oxygen donor atoms and chloride ion occupies the fourth co-ordination site. The carboxylate residue can be protonated in strongly acidic media to give [PdL(Cl)], while the chloride ion is substituted by hydroxide ion in slightly basic solutions to give $[PdLH_{-2}]^-$. However, on the basis of bis complex formation, the dipeptides can be classified into two categories. Those containing a C-terminal Gly residue (Gly-Gly, Ala-Gly and Phe-Gly) are characterized by the formation of a 4N complex, $[PdL_2H_{-2}]^{2-}$, in the physiological pH range. The two dipeptides are co-ordinated symmetrically in a bidentate mode in this complex, *via* the amino and deprotonated amide nitrogens. In the case of Gly-Ala and Gly-Phe, however, the stoichiometry of the bis complex is $[PdL_2H_{-1}]^-$ and this species contains both tridentate and monodentate dipeptide molecules. The tripeptides Gly-Gly-Gly and Gly-Gly-Ala form a 3N complex $[PdLH_{-2}]^-$ in equimolar solutions, but the bidentate co-ordination of tripeptides is characteristic in the species $[PdL_2H_{-2}]^{2-}$, which predominates in the presence of an excess of ligand. Deprotonation and co-ordination of the amide nitrogens of the tetrapeptide (Gly-Gly-Gly-Gly) is much less favoured than those of di- or tri-peptides. This is especially true for binding of the third amide nitrogen, which takes place only in the physiological pH range.

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