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Polyhedron 22 (2003) 2607–2615
POLYHEDRON
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The effect of ring size of fused chelates on the stability constants and spectroscopic properties of nickel(II) and palladium(II) complexes of peptides

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Received 14 March 2003; accepted 25 April 2003

Abstract

Nickel(II) and palladium(II) complexes of di- and tri-peptides containing β-alanyl residues have been studied by potentiometric, UV–Vis and NMR spectroscopic methods. Coordination geometries of the metal ions were not affected by the chelate ring size, but the thermodynamic stability of the complexes was significantly influenced by the number and location of β-alanyl residues. The destabilizing effect of the N-terminal β-alanyl moieties was observed in all cases. The stability order for the (NH_2, N^-, N^-, COO^-) coordination mode of $[NiH_{-2}L]^-$ complexes is described as follows: GlyGly–β-Ala (5,5,6) ≥ Gly–β-AlaGly (5,6,5) ≥ GlyGlyGly (5,5,5) > β-AlaGlyGly (6,5,5) > Gly–β-Ala–β-Ala (5,6,6) > β-AlaGly–β-Ala (6,5,6), while for the corresponding palladium(II) complexes: Gly–β-AlaGly (5,6,5) > GlyGly–β-Ala (5,5,6) > Gly–β-Ala–β-Ala (5,6,6) > GlyGlyGly (5,5,5) > β-AlaGly–β-Ala (6,5,6) ≥ β-AlaGlyGly (6,5,5). The data suggest that the inclusion of β-alanine into the internal or C-terminal positions of tripeptide molecules significantly enhances the metal binding ability of the ligands.

Keywords: β-Alanine; Dipeptides; Tripeptides; Nickel(II); Palladium(II); Stability constants

1. Introduction

The equilibrium and spectroscopic parameters of the copper(II) complexes of tri- and tetra-peptides containing β-alanyl residues in different locations have been reported in our previous publication [1]. The results revealed that the presence of the six-membered chelator β-alanine significantly affects both thermodynamic stability and spectroscopic properties of copper(II) complexes. In most cases the formation of six-membered chelate rings resulted in a decrease of stability and distortion of coordination geometry, especially if β-alanyl residues were present in N-terminal or in adjacent positions. On the contrary, the formation of mixed (5,6,5) or (5,5,6) linked chelate systems of tripeptides were found to be more favoured over the pure five-membered rings. Earlier studies on the copper(II) complexes of peptides and triamines came to the same conclusions [2–8]. It was also suggested that the thermodynamic stability of the various chelate rings is influenced by the hybridisation of the coordinating donor atoms, too. It was found that the linked five-membered rings are favoured over the six-membered ones when there are two or less trigonal atoms in the rings. As a consequence, for the complexes of tripeptides with three trigonal atoms in the joined chelate rings the six-membered chelates become favoured over the five-membered ones [4].

It is also obvious that the thermodynamic stability of the various chelate systems depends on the ionic radii and coordination geometry of the metal ions, too. Up until now, the tetragonally distorted octahedral copper(II) complexes have been thoroughly studied and only a few data are available on the nickel(II) complexes [6]. The ionic radius of nickel(II) ($r_{Ni(II)} = 69$ pm) is slightly smaller than that of copper(II) ($r_{Cu(II)} = 73$ pm), while the palladium(II) ion, in the second row of the transition elements, has the highest ionic radius

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doi:10.1016/S0277-5387(03)00367-X

($r_{Pd(II)} = 86$ pm) among the three elements [9]. All three metal ions are well known to form stable complexes with peptide ligands, in which both terminal donor functions, amino and carboxylate, and the deprotonated amide groups take part in metal binding [10,11]. The coordination geometry of the peptide complexes of these metal ions are, however, significantly different. Copper(II) complexes of peptides are always characterized by the common tetragonally distorted octahedral geometry, in which the equatorial coordination sites are occupied by the peptide backbone. Nickel(II) ions form paramagnetic, octahedral complexes with dipeptides and the major species $[NiH_{-2}L_2]^{2-}$ contain two tridentate ligands [12–15]. On the contrary, the nickel(II) complexes of tripeptides are diamagnetic, square planar species and deprotonation of the amide functions takes place in a cooperative manner [10–12]. Similarly to the complexes of other ligands palladium(II) ions form four-coordinated square planar complexes with both di- and tri-peptides and this metal ion has the highest affinity for amide binding [16–18].

Now, in this paper we report the results of potentiometric equilibrium studies on the nickel(II) and palladium(II) complexes of di- and tri-peptides containing β-alanyl residues in all possible locations. The most important conclusions on the roles of chelating ring sizes were supported by UV–Vis and ^{1}H NMR measurements.

2. Experimental

2.1. Materials

The dipeptides GlyGly, Gly–β-Ala, β-AlaGly and β-Ala–β-Ala and the tripeptides GlyGly–β-Ala, Gly–β-AlaGly, β-AlaGlyGly, Glyβ-Ala–β-Ala, β-AlaGly–β-Ala, β-Ala–β-Ala–β-Ala were purchased from Bachem and used without further purification. Concentrations of the peptide stock solutions were checked by potentiometric titrations. Stock solution of nickel(II) chloride was prepared from analytical grade reagent and the concentration was checked gravimetrically via the precipitation of oximate. Stock solutions of palladium(II) ion were prepared from $K_2[PdCl_4]$ (Fluka) and 2 equiv. of nitric acid was added to avoid hydrolytic reactions.

2.2. Potentiometric studies

In the case of nickel(II) complexes the pH-potentiometric titrations were performed in 5 cm^3 samples in the metal ion concentration range 2×10^{-3}–4×10^{-3} mol dm^{-3} at the metal ion to ligand ratios between 1:1 and 1:5. In the absence of chloride ions the palladium(II) complexes of peptides are almost completely formed by pH 2 and a competitive ligand should be used to shift the complex formation into the measurable pH range. The experimental details of this procedure have already been reported in our previous publication on palladium(II) peptide complexes [18]. Chloride ions in high concentration (0.1 mol dm^{-3}) were used as the competitive ligand. The application of chloride ion suppresses hydrolytic reactions of the free metal ion and the excess chloride ions occupy all free coordination sites of the palladium(II) complexes. Thus, the species $[PdH_{-1}L]$ having the tridentately coordinated ligands corresponds to the species $[PdH_{-1}LCl]^-$. The stability constants for the interaction of palladium(II) with chloride ion were taken from the literature and the values $\log \beta_1 = 4.47$, $\log \beta_2 = 7.76$, $\log \beta_3 = 10.17$ and $\log \beta_4 = 11.54$ were used for the species $[PdCl]^+$, $[PdCl_2]$, $[PdCl_3]^-$ and $[PdCl_4]^{2-}$, respectively [19]. The binding constant of chloride ion in peptide complexes were determined in our previous studies and the value $\log K_{Cl} = 1.99$ (for the reaction: $[PdH_{-1}L] + Cl^- \rightleftharpoons [PdH_{-1}LCl]^-$) were used in all calculations [18].

The pH-metric measurements were made with an automatically controlled Radiometer ABU 91 titration system containing a 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 20–40 experimental points were collected at all ratios. Tripeptide complexes of both nickel(II) and palladium(II) ions are formed in relatively slow reactions and generally 2–10 min were required to reach equilibrium of the individual titration points. During the titration argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide and for stirring of the solutions. All pH-potentiometric measurements were carried out at a constant ionic strength of 0.2 mol dm^{-3} (pure KCl for nickel(II) and 0.1 mol dm^{-3} KCl + 0.1 mol dm^{-3} KNO_3 for palladium(II)) and at a constant temperature (298 K). The pH-readings were converted to hydrogen ion concentration [20] and the overall stability constants ($\log \beta_{pqr}$ defined by Eqs. (1) and (2)) were calculated by means of a general computational program (PSEQUAD) [21].

$$pM + qH + rL \rightleftharpoons M_pH_qL_r \quad (1)$$

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p[H]^q[L]^r} \quad (2)$$

2.3. Spectroscopic studies

UV–Vis absorption spectra of square planar nickel(II) complexes of tripeptides were recorded on a HP 8453 diode array spectrophotometer in the same concentration range as used for potentiometry. ^{1}H NMR

studies were used for the clarification of the metal binding sites of the ligands in the palladium(II)–Gly–β-Ala, palladium(II)–β-Ala Gly- and palladium(II)–Gly–β-AlaGly systems. The spectra were recorded in D_2O at 1:1 and 1:2 metal ion to ligand ratios as a function of p*D* on a Bruker AM360 FT-NMR spectrometer using tetramethylammonium tetrafluoroborate (3.18 ppm) as an internal reference. The p*D* values were determined by the use of a Radiometer pH-meter equipped with a Metrohm 6.0222.100 combined glass–calomel electrode and by addition of 0.4 to the pM-meter readings.

3. Results and discussion

3.1. Nickel(II) complexes of dipeptides

Nickel(II) complexes of the most common dipeptides have already been studied by several authors and the metal binding modes of the species $[NiL]^+$ and $[NiL_2]$ were described by (NH_2,CO)-coordination of the N-termini [12–15]. Taking into account the octahedral coordination geometry of the metal ion the formation of $[NiL_3]^-$ was also suggested in some cases [14]. The formation of tris(ligand) complexes, however, overlaps with amide deprotonation and its presence in well measured concentration requires the application of a high excess of ligand. Thus, the species $[NiL_3]^-$ can exist only in negligible concentration under our experimental conditions. Deprotonation and coordination of the amide functions generally take place above pH 8 and it results in the existence of the species $[NiH_{-1}L]$, $[NiH_{-1}L_2]^-$ and $[NiH_{-2}L_2]^{2-}$. It was also clear from the previous studies that in the absence of strongly coordinating side chain residues the nickel(II) complexes of various peptide ligands are very similar to each other.

Protonation constants of the ligands and stability constants of the nickel(II) complexes of dipeptides (GlyGly, Gly–β-Ala, β-AlaGly and β-Ala–β-Ala) were determined by potentiometric titrations and the data are collected in Table 1.

The comparison of the data in Table 1 reveals that the same conclusions are held for the nickel(II) complexes of dipeptides containing β-alanyl residues. The metal ion speciation of the four systems are similar, although small differences in the concentrations of the various species can be observed depending on the location of β-alanyl residues as it is demonstrated by Fig. 1. The overall stability constants in Table 1 cannot be directly used to compare the relative stability of each peptide complex, because the basicity of α- and β-alanine is significantly different. As it was reported for the copper(II) complexes, the metal binding abilities of the ligands may be estimated by considering the equilibrium constant of the reaction [1,4]:

Table 1
Stability constants (log β_{pqr}) of the nickel(II) complexes of dipeptides

Species	GlyGly	Gly–β-Ala	β-AlaGly	β-Ala–β-Ala
[HL]	8.13(1)	8.10(1)	9.44(2)	9.37(2)
$[H_2L]$	11.30(1)	12.15(1)	12.73(2)	13.43(1)
$[NiL]^+$	3.96(2)	4.06(2)	3.82(5)	3.70(6)
$[NiL_2]$	7.16(4)	7.43(3)	6.53(20)	6.15(20)
$[NiH_{-1}L]$	−4.91(6)	−4.94(5)	−4.59(7)	−6.02(20)
$[NiH_{-1}L_2]^-$	−2.51(7)	−2.41(6)	−2.21(8)	−3.76(20)
$[NiH_{-2}L_2]^{2-}$	−11.99(6)	−12.67(6)	−12.44(10)	−13.38(12)
log K_0	−4.17	−4.04	−5.62	−5.67
log K_{-1}	−13.04	−13.04	−14.03	−15.39
$\log(K_1/K_2)$ [a]	0.76	0.69	1.11	1.25

$T = 298$ K, $I = 0.2$ mol dm^{-3} KCl, standard deviations are in parenthesis.

[a] Ratio of stepwise stability constants $\log(K_1/K_2) = \log\beta_2 - (\log\beta_2 - \log\beta_1)$.

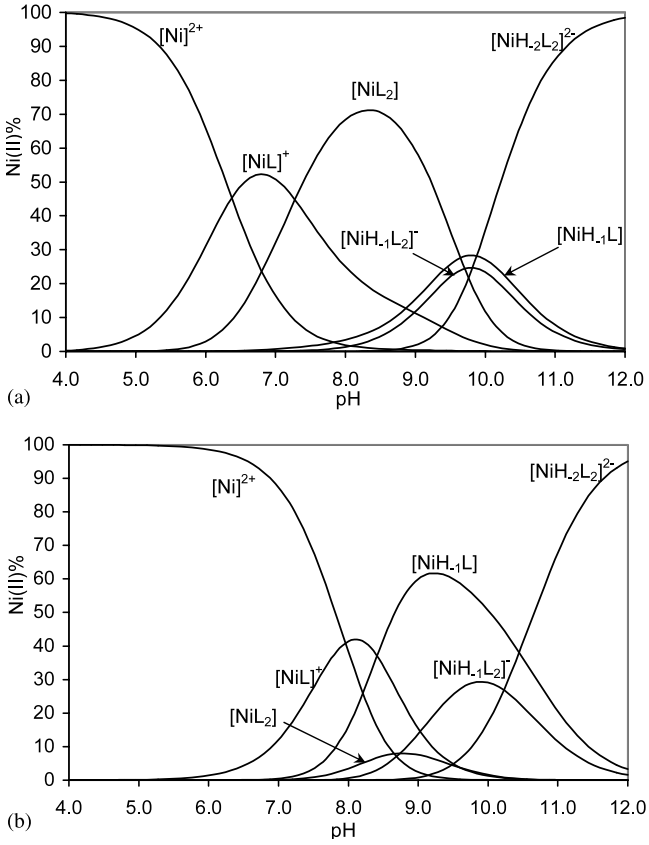


Fig. 1. Concentration distribution of the species formed in the nickel(II)–Gly–β-Ala (a) and nickel(II)–β-AlaGly (b) systems as a function of pH. ($c_{Ni(II)} = 2 \times 10^{-3}$ mol dm^{-3}, $c_L = 6 \times 10^{-3}$ mol dm^{-3}).

$$M^{2+} + HL \rightleftharpoons [MH_{-n}L]^{(1-n)+} + (n+1)H^+ \quad (3)$$

The equilibrium constant of reaction (Eq. (3)) is K_{-n} (where $n = 0, 1, 2$) and it can be calculated by the equation:

$$\log K_{-n} = \log \beta_{1-n1} - pK(HL) \quad (4)$$

Table 2
Stability constants (log β_{pqr}) of the nickel(II) complexes of tripeptides

Species	GlyGlyGly	GlyGly–β-Ala	Gly–β-AlaGly	β-AlaGlyGly	Gly–β-Ala–β-Ala	β-AlaGly–β-Ala
[HL]	7.93	7.93	8.06	9.29	8.12	9.29
$[H_2L]^+$	11.25	12.02	11.42	12.55	12.21	13.40
$[NiL]^+$	3.75(2)	3.82(2)	4.18(1)	3.53(6)	4.05(3)	3.54(5)
$[NiL_2]$	6.77(4)	6.90(3)	7.34(3)		7.44(6)	
$[NiH_{-1}L]$	−5.45(5)	−5.03(5)	−5.26(8)	−5.25(8)	−4.98(9)	−4.81(5)
$[NiH_{-2}L]^-$	−12.85(3)	−12.34(2)	−12.45(2)	−12.47(2)	−14.93(5)	−13.99(6)
log K_0	−4.18	−4.11	−3.88	−5.76	−4.07	−5.75
log K_{-1}	−13.38	−12.96	−13.32	−14.54	−13.10	−14.10
log K_{-2}	−20.78	−20.27	−20.51	−21.76	−23.05	−23.28

$T = 298$ K, $I = 0.2$ mol dm^{-3} KCl, standard deviations are in parenthesis.

The log K_0, log K_{-1} and log K_{-2} parameters are also included in Tables 1–5 and reflect the relative stabilities of the $[ML]^+$, $[MH_{-1}L]$ and $[MH_{-2}L]^-$ complexes, respectively.

There are two sets of log K_0 values in Table 1 and this suggests that the thermodynamic stability of the five-membered (NH_2,CO) chelates is much higher than the six-membered ones. The reduced stability of the six-membered chelates is even more evident in the equilibrium parameters of the bis(ligand) complexes. The increase of the ratio of stepwise stability constants corresponds to the unfavoured bis(ligand) complex formation suggesting that the presence of two six-membered chelates in the coordination sphere of nickel(II) is much less favoured than the presence of five-membered ones. As a consequence, the complex formation processes of β-AlaGly and β-Ala–β-Ala are shifted to higher pH values and the concentration of $[NiL_2]$ complexes is very low at any metal ion to ligand ratio.

Precipitation of nickel(II) hydroxide was observed at low ligand to metal ion ratios and especially in the case of dipeptides containing N-terminal β-alanyl residues. This precipitation suggests that hydroxo complex formation and amide deprotonation take place in overlapping processes and the presence of excess ligand is required for reliable equilibrium studies. Another consequence of the overlap of hydrolytic and deprotonation reactions is that the $[NiH_{-1}L]$, $[NiH_{-1}L_2]^-$ and $[NiH_{-2}L_2]^{2-}$ complexes may have isomeric forms and the log K_{-1} values cannot be unambiguously assigned

Table 3
Absorption maxima and molar absorptivity of nickel(II) complexes of tripeptides

Ligand	Species	λ (nm)	ε (dm^3 mol^{-1} cm^{-1})
GlyGlyGly	$[NiH_{-2}L]^-$	427	197
GlyGly–β-Ala	$[NiH_{-2}L]^-$	440	219
Gly–β-AlaGly	$[NiH_{-2}L]^-$	448	78
β-AlaGlyGly	$[NiH_{-2}L]^-$	443	126
Gly–β-Ala–β-Ala	$[NiH_{-2}L]^-$	459	64
β-AlaGly–β-Ala	$[NiH_{-2}L]^-$	456	93

Table 4
Stability constants (log β_{pqr}) of the palladium(II) complexes of dipeptides

Species	GlyGly [a]	Gly–β-Ala	β-AlaGly	β-Ala–β-Ala
[HL]	8.13	8.10(1)	9.44(2)	9.37(2)
$[H_2L]$	11.30	12.15(1)	12.73(2)	13.43(1)
$[PdL]^+$	16.09	17.11(4)	14.12(10)	
$[PdH_{-1}L]$	13.57	14.93(2)	11.09(2)	11.19(2)
$[PdH_{-2}L]$	4.89	6.00(5)	2.38(7)	2.52(7)
$[PdH_{-1}L_2]^-$	19.30	20.60(5)	17.43(5)	17.76(5)
$[PdH_{-2}L_2]^{2-}$	13.90			
log K_0	7.96	9.01	4.68	
log K_{-1}	5.44	6.83	1.65	1.82
pK(PdL/PdH$_{-1}$L)	2.52	2.18	3.03	
pK(OH)	8.68	8.93	8.71	8.67
log K ($PdH_{-1}L_2$/$PdH_{-1}L$)	5.73	5.67	6.34	6.57

$T = 298$ K, $I = 0.2$ mol dm^{-3} (KCl+KNO_3 = 1:1), standard deviations are in parenthesis.
[a] From Ref. [18].

to the overall stabilities of the (NH_2,N$^-$,COO$^-$) coordination modes. The large differences in these values, however, suggest that the relative stability of the linked chelate systems follow the order: GlyGly (5,5) ∼ Gly–β-Ala (5,6) > β-AlaGly (6,5) > β-Ala–β-Ala (6,6). At the same time this stability order of the ligands correlates with the metal binding strength of the various ligands, which is reflected in the enhanced stability of both (NH_2,CO) and (NH_2,N$^-$,COO$^-$) coordination modes of GlyGly and Gly–β-Ala.

3.2. Nickel(II) complexes of tripeptides

The stability constants obtained for the nickel(II) complexes of tripeptides are collected in Table 2 and the data reveal significant similarities in the complex formation processes of the six ligands. The metal ion coordination starts via the binding of the N-termini in the form of the (NH_2,CO) chelates in the species $[NiL]^+$.

Table 5
Stability constants ($\log \beta_{pqr}$) of the palladium(II) complexes of tripeptides

Species	GlyGlyGly [a]	GlyGly–β-Ala	Gly–β-AlaGly	β-AlaGlyGly	Gly–β-Ala–β-Ala	β-AlaGly–β-Ala
[HL]	7.93	7.93	8.06	9.29	8.12	9.29
$[H_2L]^+$	11.25	12.02	11.42	12.55	12.21	13.40
$[PdL]^+$	15.92	16.79(2)	16.26(5)	14.40(20)	16.66(3)	
$[PdH_{-1}L]$	12.65	10.97(12)	12.06(10)	8.76(10)	13.24(5)	12.64(4)
$[PdH_{-2}L]^-$	9.07	10.98(2)	11.79(2)	9.03(3)	10.12(3)	9.58(3)
$[PdL_2]$	23.00					
$[PdH_{-1}L_2]^-$	19.81					
$[PdH_{-2}L_2]^{2-}$	13.40					
$\log K_0$	7.99	8.86	8.20	5.11	8.54	
$\log K_{-1}$	4.72	3.04	4.00	−0.53	5.12	3.35
$\log K_{-2}$	1.14	3.05	3.73	−0.26	2.00	0.29

$T = 298$ K, $I = 0.2$ mol dm^{-3} ($KCl+KNO_3 = 1:1$), standard deviations are in parenthesis.
[a] From Ref. [18].

The bis(ligand) complexes $[NiL_2]$ are always formed in relatively low concentration and in the case of tripeptides containing N-terminal β-alanyl residues these species cannot be even detected. The deprotonation of the two amide functions takes place in a cooperative manner and the mono-deprotonated complexes $[NiH_{-1}L]$ are generally minor species.

In addition to the above mentioned similarities there are, however, some major differences in the complex formation processes of triglycine and the tripeptides containing β-alanine. These differences are best represented by the comparison of the speciation curves of the nickel(II)–GlyGly–β-Ala nickel(II)–β-AlaGlyGly systems in Fig. 2 and by some calculated equilibrium parameters in Table 2.

The $\log K_0$ values unambiguously prove again that the five-membered (NH_2,CO) chelates are favoured over the six-membered ones. As a consequence, the complex formation processes of the tripeptides containing N-terminal β-alanyl residues are shifted to a slightly alkaline pH range and the suppression of hydrolytic reactions requires the application of excess ligand. In the case of β-Ala–β-Ala–β-Ala, which can form only six-membered chelate rings, the precipitation of nickel(II) hydroxide cannot be avoided even at high ligand to metal ion ratios and stability constants were not determined for this ligand. The cooperative deprotonation of the two amide functions rules out the calculation of the successive pK values of the amide groups. A comparison of the $\log K_{-2}$ values in Table 2, however, makes it possible to give the stability order for the (NH_2,N^-,N^-,COO^-) coordination mode of the nickel(II) complexes: GlyGly–β-Ala (5,5,6) ≥ Gly–β-AlaGly (5,6,5) ≥ GlyGlyGly (5,5,5) > β-AlaGlyGly (6,5,5) > Gly–β-Ala–β-Ala (5,6,6) > β-AlaGly–β-Ala (6,5,6). This stability order is rather similar to those reported for the corresponding copper(II) complexes and reflects that the six-membered rings in C-terminal or internal positions slightly enhance the thermodynamic stability of the linked chelate systems. On the other hand, the six-membered chelates at the N-termini or the presence of two or more six-membered chelates are especially unfavoured in the coordination sphere of square planar nickel(II) complexes.

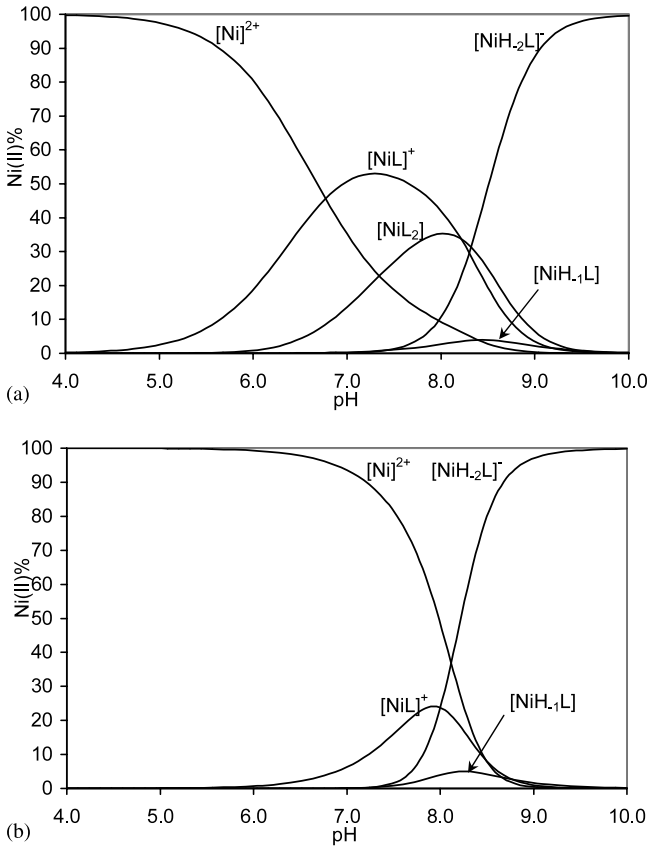


Fig. 2. Concentration distribution of the species formed in the nickel(II)–GlyGly–β-Ala (a) and nickel(II)–β-AlaGlyGly (b) systems as a function of pH. ($c_{Ni(II)} = 2 \times 10^{-3}$ mol dm^{-3}, $c_L = 4 \times 10^{-3}$ mol dm^{-3}).

The (NH_2,CO)-coordinated $[NiL]^+$ complexes are paramagnetic and octahedral species with all tripeptide

ligands, but the formation of the (NH_2,N^-,N^-,COO^-)-coordinated $[NiH_{-2}L]^-$ species was always accompanied with the appearence of the characteristic yellow colour of the square planar nickel(II) complexes. The visible absorption spectra of these species have been recorded and the absorption maxima (λ_{max}) and molar absorptivities (ε) are listed in Table 3.

It is a common feature of the tripeptide complexes of nickel(II) that the spectral parameters are not much affected by the presence of non-coordinating side chain residues. For example $\lambda_{max} = 429 \pm 2$ nm were reported for the nickel(II) complexes of tripeptides containing methionine in all possible locations [22]. However, it is clear from Table 3 that the modification of the chelate ring sizes slightly affects the spectral parameters, although the coordinating donor atoms are the same. A comparison of λ_{max} values reveals that the inclusion of the six-membered rings into the linked chelate systems shifts the absorption maxima towards the low energies. This effect is especially pronounced if more β-alanyl residues are present in the coordination sphere of the metal ion and this observation seems to be in agreement with the reduced thermodynamic stability of the corresponding complexes.

3.3. Palladium(II) complexes of dipeptides

Our previous studies [18] on the palladium(II) complexes of the most common di- and tri-peptides reveal that palladium(II) ions form very stable complexes with peptide ligands. In the absence of chloride ions the deprotonation and coordination of both terminal amino and amide groups occur in strongly acidic solution (pH < 1). Chloride ion can be used as a competitive ligand in equilibrium studies and the stability constants of palladium(II) complexes of dipeptides were determined in the presence of a high excess of chloride ions. Similar experimental conditions were used in the case of dipeptides of β-alanine and the stability constants are reported in Table 4.

A comparison of the data in Table 4 reveals that there are some differences in the complex formation processes of GlyGly and the other three dipeptides with palladium(II). The successive formation of the species $[PdL]^+$, $[PdH_{-1}L]$ and $[PdH_{-2}L]^-$ was reported in the equimolar solution of palladium(II) and GlyGly and their metal binding sites were described by [NH_2,N^-](COOH), [NH_2,N^-,COO^-] and [NH_2,N^-,COO^-,OH^-] coordinations, respectively. This speciation is very similar to that of the corresponding copper(II) containing systems. However, it is important to note that the species $[PdL]^+$ is not a [NH_2,CO] chelate, but the ligand is coordinated via the terminal amino and deprotonated amide functions, while the carboxylic group remains protonated under the strongly acidic conditions. As a consequence, the comparison of log K_0 values in Table 4 is misleading, although it reflects the reduced metal binding ability of dipeptides containing N-terminal β-alanyl residues. In the case of β-Ala–β-Ala the log β_{101} values cannot even be determined, because the complex formation processes are shifted to a less acidic pH range where the carboxylate groups are deprotonated.

The effect of the chelate ring size on the thermodynamic stability of palladium(II) complexes is very well reflected in the log K_{-1} values which express the relative stability of the $[PdH_{-1}L]$ complexes: Gly–β-Ala (5,6) > GlyGly (5,5) > β-Ala–β-Ala (6,6) ~ β-Ala–Gly (6,5). This stability order is different from those reported for copper(II) and nickel(II) complexes and it requires further explanation. The difference in the stability constants of Gly–β-Ala and GlyGly is much higher than the standard deviations of the equilibrium data supporting that the (5,6)-membered linked chelates are favoured over the pure five-membered ones. This observation may be explained by the high ionic radii of palladium(II) ions. However, it is also obvious from these data that the presence of the N-terminal β-alanyl residues has the opposite effect and significantly reduces the thermodynamic stability of palladium(II)–peptide complexes.

The species $[PdH_{-2}L]^-$ corresponds to the formation of mixed hydroxo complexes and the pK values of coordinated water molecules (pK(OH) in Table 4) are very similar to each other. This suggests that the different chelate ring sizes do not have significant influence on the possibility of hydrolytic reactions of peptide complexes. The same statement is held for the formation of the $[PdH_{-1}L_2]^-$ bis(ligand) complexes. The binding sites of this species were interpreted by the tridentate [NH_2,N^-,COO^-]-coordination of one ligand and monodentate NH_2-coordination of the other one. The values log K([$PdH_{-1}L_2$]/[$PdH_{-1}L$]) in Table 4 express the binding constant of the second ligand in the species $[PdH_{-1}L_2]^-$. The differences in these values correspond well to the differences in the basicities of the ligands suggesting the same binding modes for the tripeptides of β-alanine.

The most striking difference between the complex formation processes of GlyGly and the other three dipeptides is connected to the existence of the species $[PdH_{-2}L_2]^{2-}$. Previous studies on the palladium(II) complexes of dipeptides came to the conclusion that the formation of the 4N-coordinated bis(ligand) complexes takes place in a slightly acidic or neutral pH range for the X-Gly type dipeptides (X = Gly, Ala, Phe), while this process is shifted to a strongly alkaline pH range (pH > 11) if the amino acid sequence is reversed (Gly-X). On the basis of these results the formation of $[PdH_{-2}L_2]^{2-}$ would be expected in the palladium(II)–β-Ala–Gly system, but it is clear from Table 4 that this species was not formed with any of the ligands contain-

ing β-alanyl residues. This contradiction, however, can be easily explained if one takes into account the stability order outlined above for the $[PdH_{-1}L]$ species. The complex $[PdH_{-2}L_2]^{2-}$ should contain two bidentately coordinated ligands, but according to the stability order the (NH_2,N^-) coordination mode is much less favoured with the six-membered chelates.

The absence of the species $[PdH_{-2}L_2]^{2-}$ in the palladium(II)–β-Ala–Gly and palladium(II)–Gly–β-Ala systems was also proved by ^1H NMR measurements. The NMR spectra of the free ligands and the 1:1 and 1:2 palladium(II):peptide ratios were recorded as a function of pH. Three sets of proton resonances were identified at 1:2 ratios at pH 9 with both ligands and they can be assigned to: (i) the free ligand (in low concentration), (ii) the tridentately coordinated ligand in the species $[PdH_{-1}L]$ and (iii) the monodentate amino-coordinated ligand with free amide and carboxylate residues in the species $[PdH_{-1}L_2]^-$.

3.4. Palladium(II) complexes of tripeptides

A comparison of the equilibrium data of the palladium(II) complexes of tripeptides in Table 5 reveals two major differences in the complex formation reactions of triglycine and the tripeptides containing β-alanyl residues. These differences are reflected in the very low concentration of the species $[PdH_{-1}L]$ and the absence of bis(ligand) complexes with the ligands containing β-alanine. Complex formation processes between palladium(II) ions and triglycine (or GlyGlyAla) were described by the successive deprotonation and metal ion coordination of the amino and amide nitrogen donor atoms, and the existence of the species $[PdL]^+$, $[PdH_{-1}L]$ and $[PdH_{-2}L]^-$ was proved by potentiometric and NMR measurements [18]. However, in the case of tripeptides containing one or two β-alanyl residues $[PdH_{-1}L]$ is always a minor species and these systems are characterized by the formation of $[PdL]^+$ and $[PdH_{-2}L]^-$ as the major species at any metal ion to ligand ratios. There are two possibilities for the metal ion coordination modes of the complex $[PdL]^+$:(NH_2,CO) with free carboxylate or (NH_2,N^-) with protonated carboxylic functions.

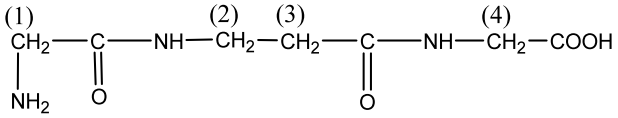
^1H NMR measurements have been performed in the palladium(II)–Gly–β-AlaGly system for the clarification of the metal binding sites in the various complexes. Proton resonances of all methylene groups of Gly–β-AlaGly (see Table 6) can be easily assigned and their chemical shifts are very sensitive to protonation or metal ion coordination of the neighbouring donor atoms. The spectra recorded in equimolar solution of palladium(II) and Gly–β-AlaGly clearly indicate the existence of two major species in the pH range 2–4.5. A very significant upfield shift of the C(2) methylene protons (~ 0.4 ppm as compared to the free ligand) can be observed at low pH values, while the resonances of the C(3) and C(4) protons are slightly shifted downfield. These data suggest the deprotonation and metal ion coordination of the first amide function (from the N-termini) in the species $[PdL]^+$ and its most reasonable coordination mode can be described by the metal binding of the terminal amino group, one deprotonated amide nitrogen atom and the oxygen atom of the second amide function, while the carboxylic residue remains protonated at these low pH values. The increase of pH results in the upfield shift of the C(3) proton resonances too, indicating that both amide functions are deprotonated and coordinated in the species $[PdH_{-2}L]^-$.

The $\log K_0$ values can be used to estimate the metal binding ability of the ligands in the species $[PdL]^+$. These values clearly indicate that the tripeptides containing N-terminal β-alanyl residues are much weaker complexing agents than the tripeptides with N-terminal glycyl residues. In other words it means that the five-membered chelates of both (NH_2,CO) and (NH_2,N^-) coordination modes are favoured over the six-membered ones.

In agreement with the complex formation reactions of triglycine the major species is $[PdH_{-2}L]^-$ in all systems and it is described by the common (NH_2,N^-,N^-,COO^-) coordination mode. The stability order of this binding mode can be easily established on the basis of the $\log K_{-2}$ values: Gly–β-AlaGly (5,6,5) > GlyGly–β-Ala (5,5,6) > Gly–β-Ala–β-Ala (5,6,6) > GlyGlyGly (5,5,5) > β-AlaGly–β-Ala (6,5,6) ≥ β-AlaGlyGly (6,5,5). This stability order is significantly different from those reported for nickel(II) in Section 3.2 or in copper(II) in our previous study [1]. The major conclusion from this stability order is that the presence of a six-membered chelate ring in fused chelate systems significantly enhances the palladium(II) binding ability of the ligands. This is especially true, however, valid only if the six-membered chelates are built up from (N^-,N^-) and (N^-,COO^-) trigonal donor functions, while the (NH_2,N^-) six-membered chelate has a destabilizing effect. The increased thermodynamic stability of the peptide complexes with (5,6,5) and (5,5,6) linked chelate rings has already been reported for the corresponding copper(II) complexes, too. In the case of palladium(II) the enhancement of stability is, however, much higher than it was for copper(II) and it probably can be explained by the difference in the ionic radii of the two metal ions.

The absence of $[PdH_{-2}L_2]^{2-}$ bis(ligand) complexes represent the major difference of β-alanine peptides from triglycine. The formation of three different bis(ligand) complexes was described in the palladium(II) triglycine system. $[PdL_2]$ and $[PdH_{-1}L_2]^-$ were detected only in low concentration and their coordination modes were interpreted by the mixed, tridentate and monodentate coordinations of the two ligands. The complex

Table 6
The ^{1}H NMR chemical shifts (ppm) of the various species measured in the palladium(II)–Gly–β-AlaGly system



Ratio (Pd(II):L)	p*D*	Species	(1)	(2)	(3)	(4)
Free ligand	1.91	$[H_2L]^+$	3.781	3.538	2.564	3.998
Free ligand	4.89	[HL]	3.775	3.536	2.534	3.770
1:1	2.00	$[PdL]^{2+}$	3.505	3.020	2.583	4.136
1:1	4.95	$[PdH_{-2}L]^-$	3.456	3.086	2.314	3.970
1:2	4.82	[HL]	3.775	3.536	2.533	3.767
		$[PdH_{-2}L]^-$	3.463	3.095	2.320	3.979

$[PdH_{-2}L_2]^{2-}$, however, was identified as the major species above pH 5 in the presence of excess of ligand. Its binding mode was described by bis(bidentate) (NH_2,N^-)-coordination with free carboxylate residues. None of these complexes were detected in the palladium(II)–tripeptide systems containing β-alanyl residues. The absence of $[PdL_2]$ and $[PdH_{-1}L_2]^-$ can be easily explained because these species should contain the tridentate coordination mode of the ligands, but it is very much suppressed as it was discussed in the previous paragraph. For the tripeptides containing N-terminal β-alanyl residues the destabilizing effect of the six-membered (NH_2,N^-) chelate, as in the case of palladium(II) complexes of Gly–β-AlaGly and GlyGly–β-Ala, and the outstanding thermodynamic stability of the (NH_2,N^-,N^-,COO^-) coordination mode can be responsible for the absence of the species $[PdH_{-2}L_2]^{2-}$. The NMR spectra obtained in the solutions containing palladium(II) ions and Gly–β-AlaGly at a 1:2 ratio correspond very well to the co-existence of the 1:1 species $[PdH_{-2}L]^-$ and the free ligand at pH 4.5, supporting the absence of any bis(ligand) complexes in this system.

4. Conclusion

The stability constants obtained for the nickel(II) and palladium(II) complexes of di- and tri-peptides of β-alanine reveal that the inclusion of six-membered chelate rings into the linked chelate systems may significantly influence both stoichiometry and thermodynamic stability of the complexes. Coordination geometry of the nickel(II) complexes are not much affected by the chelate ring size, but the stability constants or the metal binding ability largely depend on the number and location of β-alanyl residues in the amino acid sequence. Nickel(II) complexes of the dipeptides are characterized by the formation of $[NiH_{-2}L_2]^{2-}$ as the major species containing an octahedral metal ion in the (NH_2,N^-,COO^-) coordination environment. The stability order of the ligands in these complexes is: GlyGly (5,5) ~ Gly–β-Ala (5,6) > β-AlaGly (6,5) > β-Ala–β-Ala (6,6). This stability order unambiguously shows the destabilizing effect of the N-terminal β-alanyl residues and it was observed in all other cases including the copper(II), nickel(II) and palladium(II) complexes of di- and tri-peptides.

All tripeptides formed diamagnetic square planar complexes with nickel(II) and the following stability order was obtained for the (NH_2,N^-,N^-,COO^-) coordination mode: GlyGly–β-Ala (5,5,6) ≥ Gly–β-AlaGly (5,6,5) ≥ GlyGlyGly (5,5,5) > β-AlaGlyGly (6,5,5) > Gly–β-Ala–β-Ala (5,6,6) > β-AlaGly–β-Ala (6,5,6) ≫ β-Ala–β-Ala–β-Ala. A very similar stability order was reported for the corresponding copper(II) complexes and the data reflect that the presence of two or more six-membered chelate rings is unfavoured in the equatorial coordination sphere of the two metal ions. The UV–Vis spectral parameters of these complexes revealed that the inclusion of the six-membered chelate rings into the linked chelate systems of peptide complexes shifts the absorption bands towards the lower energies.

In the case of palladium(II) complexes not only the stability constants, but the metal ion speciation of the various systems is influenced by the replacement of five-membered chelates with six-membered ones. Formation of bis(ligand) complexes was not observed with any di- and tri-peptides, while the bis(NH_2,N^-) coordinated $[PdH_{-2}L_2]^{2-}$ complexes were formed in both the palladium(II)–GlyGly and palladium(II)–GlyGlyGly systems. The stability order for the [NH_2,N^-,COO^-] coordinated $[PdH_{-1}L]$ complexes is: Gly–β-Ala (5,6) > GlyGly (5,5) > β-Ala–β-Ala (6,6) ~ β-Ala–Gly (6,5) and this reflects the destabilizing effect of the N-terminal and the stabilizing effect of the C-terminal β-alanyl residues.

The following stability order was obtained for the quadridentate (NH_2,N^-,N^-,COO^-) coordination mode in the $[PdH_{-2}L]^-$ complexes: Gly–β-AlaGly

(5,6,5) > GlyGly–β-Ala (5,5,6) > Gly–β-Ala–β-Ala (5,6,6) > GlyGlyGly (5,5,5) > β-AlaGly–β-Ala (6,5,6) ≥ β-AlaGlyGly (6,5,5). This stability order is significantly different from those reported for copper(II) and nickel(II) complexes and suggests that the inclusion of β-alanyl residues into the amino acid sequence in internal or C-terminal positions significantly enhances the palladium binding strength of the ligands.

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (OTKA T034361 and TS 040685).

References

[1] D. Sanna, C.G. Ágoston, G. Micera, I. Sóvágó, Polyhedron 20 (2001) 3079.
[2] O. Yamauchi, Y. Hirano, Y. Nakao, A. Nakahara, Can. J. Chem. 47 (1969) 3441.
[3] O. Yamauchi, Y. Nakao, A. Nakahara, Bull. Chem. Soc. Jpn 46 (1973) 2119.
[4] H. Sigel, B. Prijs, R.B. Martin, Inorg. Chim. Acta 56 (1981) 45.
[5] R. Barbucci, L. Fabbrizzi, P. Paoletti, Inorg. Chim. Acta 7 (1973) 157.
[6] R. Barbucci, L. Fabbrizzi, P. Paoletti, A. Vacca, J. Chem. Soc., Dalton Trans. (1973) 1763.
[7] G.R. Hedwig, H.K.J. Powell, J. Chem. Soc., Dalton Trans. (1973) 793.
[8] A. Hanaki, T. Kawashima, T. Konishi, T. Takano, D. Mabuchi, A. Odani, O. Yamauchi, J. Inorg. Biochem. 77 (1999) 147.
[9] N.N. Greenwood, A. Earnshaw, Chemistry of the Elements, Butterworth-Heinemann, Oxford, 1997.
[10] H. Sigel, R.B. Martin, Chem. Rev. 82 (1982) 385.
[11] I. Sóvágó, in: K. Burger (Ed.), Biocoordination Chemistry, Ellis Horwood, New York, 1990.
[12] R.B. Martin, M. Chamberlin, J.T. Edsall, J. Am. Chem. Soc. 82 (1960) 495.
[13] A. Kaneda, A.E. Martell, J. Am. Chem. Soc. 99 (1977) 1586.
[14] G. Brookes, L.D. Pettit, J. Chem. Soc., Dalton Trans. (1975) 2106.
[15] H.C. Freeman, J.M. Guss, Acta Crystallogr. B 34 (1978) 2451.
[16] E.W. Wilson, Jr., R.B. Martin, Inorg. Chem. 9 (1970) 528.
[17] A. Iakovidis, N. Hadjiliadis, Coord. Chem. Rev. 135/136 (1994) 17.
[18] C.G. Ágoston, T. Kowalik-Jankowska, I. Sóvágó, J. Chem. Soc., Dalton Trans. (1999) 3295.
[19] L.I. Elding, Inorg. Chim. Acta 6 (1972) 647.
[20] H. Irving, G. Miles, L.D. Pettit, Anal. Chim. Acta 38 (1967) 475.
[21] L. Zékány, I. Nagypál, in: D. Leggett (Ed.), Computational Methods for the Determination of Stability Constants, Plenum Press, New York, 1985.
[22] K. Várnagy, B. Bóka, I. Sóvágó, D. Sanna, P. Marras, G. Micera, Inorg. Chim. Acta 275–276 (1998) 440.