Complexing Properties of Phosphonodipeptides containing Aminomethylphosphonic Acid

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A series of phosphonodipeptides containing glycyl, L-alanyl, L-leucyl, or L-phenylalanyl and a terminal aminomethylphosphonic acid residue was studied pH-metrically at 25 °C and at an ionic strength of 0.1 mol dm^{-3} (KNO_3), and their protonation and complex-formation stability constants with Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} were determined. The protonation constants show the same dependence on the side chain as those of common dipeptides but the differences are not as large. The stability constants point to the formation of protonated, non-protonated and deprotonated complexes with a metal:ligand molar ratio of 1:1 and, except for zinc, to the formation of 1:2 complexes. Simultaneous deprotonation and co-ordination of the peptide amide bond was confirmed only for Cu^{2+}. The same influence of the size of the amino acid side chain as found for common dipeptides was observed but the differences are smaller due to the stronger complexing ability of the phosphonic group.

Aminoalkylphosphonic acids and their derivatives have received considerable attention because of their biological activity.[1] Phosphonodipeptides containing terminal aminoalkylphosphonic acids have exhibited bacteriostatic[2] and herbicidal[3] properties. Their synthesis and properties have been reviewed by Kafarski *et al.*[4]

In contrast to the acids, which were intensely investigated in solution, only two papers have dealt with the complexing properties of phosphonopeptides (H_2A). Hariharan *et al.*[5] synthesized glycylaminomethylphosphonic acid and glycyl-2-aminoethylphosphonic acid ($H_2NCH_2CONHCH_2CH_2PO_3H_2$) and determined their pK_2 and pK_3 values and stability constants for complex-formation with Cu^{2+}, Ni^{2+} and Co^{2+}. They observed the formation of complexes with stoichiometries of MA and M(HA) for the studied ions and ion-induced deprotonation and simultaneous co-ordination of the amide-peptide moiety only for Cu^{2+}. Kiss *et al.*[6] investigated systems of Cu^{2+} with phosphonodipeptides. In contrast to Hariharan *et al.*, they found the formation of hydroxo species and complexes with molar ratios of 1:2, of the types $[CuA_2]^{2-}$ and $[CuA_2H_{-1}]^{3-}$. The results from both these studies point to the fact that phosphonodipeptides exhibit solution properties both of common dipeptides such as the co-ordination ability of the amide-peptide moiety for copper and also of phosphonic acids such as higher stability constants and the formation of protonated complexes. The aim of the present study was to extend the number of phosphonodipeptides having the general formula $NH_2CHRCONHCH_2PO_3H_2$ {R = H[Gly-Gly-(*P*)], Me[L-Ala-Gly-(*P*)], Bu^i[L-Leu-Gly-(*P*)] or Ph[L-Phe-Gly-(*P*)]}, and also to investigate the influence of the size of the amino acid side chain on the complexing properties of phosphonodipeptides containing terminal aminomethylphosphonic acid with common divalent transition-metal ions. The complexing properties of diastereoisomeric phosphonodipeptides containing 1-aminoethylphosphonic acid form the subject of the following paper.[7]

Results and Discussion

The phosphonodipeptides were synthesized as described in our previous paper[8] by the active ester method using 1-hydroxybenzotriazole and 1,3-dicyclohexylcarbodiimide. Their purity was checked by ^{31}P and ^1H NMR spectroscopy and by chromatography. The hydration and thermal stability of the compounds were tested by TGA and the results are summarized in Table 1. All the studied phosphonodipeptides are stable up to *ca.* 170 °C. Therefore, they were dried to constant weight at the temperatures listed in Table 1, and were used as anhydrous substances for the preparation of the stock solutions, except $NH_2CH_2CONHCH_2PO_3H_2$, *i.e.* Gly-Gly-(*P*), which formed a stable hydrate and was used as the monohydrate after drying at 60–70 °C.

We had two ways to determine the concentration of the phosphonodipeptide in the stock solutions: (*a*) according to the elemental analysis, the NMR and chromatographic results and thermal stability, to calculate it from the weight of the dried compound; (*b*) to determine the actual concentration by Gran's method, see ref. 6, or to refine the concentration using the ESAB2M 87 program. We tested both possibilities. Gran's method and the ESAB2M 87 program[9] gave similar results. The found concentration of the stock solution of the dipeptide was about 4–7% higher than the concentration calculated from

Table 1 Results of TGA in the temperature range 25–280 °C

Compound	Dehydration temperature/°C	Weight loss (%)	Decomposition temperature/°C	Drying temperature/°C	Weight loss (%)
Gly-Gly-(*P*)	145–165 (1.0 H_2O)	9.66	170	60–70	—
L-Ala-Gly-(*P*)	—	—	260	60–70	—
L-Leu-Gly-(*P*)	40–150 (1.1 H_2O)	9.40	190	130–135 (1.4 H_2O)	10.69
L-Phe-Gly-(*P*)	—	—	260	60–70	—

the weight when 100% purity of the compounds was assumed. Gran's method is a very efficient method for determining the equivalence point of strong acid–strong base titrations, *i.e.* for acids with low pK_a values. This method can be used for weak electrolytes having adequate differences between pK_a values in the studied region. Our acids are weak electrolytes having smaller differences between the pK_a values as can be seen in Fig. 1 where the presence of three species in the wide region of pH *ca.* 5 to 8.5 was found in the solution. This presence of three species shifted the position of the equivalence point and, consequently, increased the apparent concentration of the compound. Some programs such as ESAB or SUPERQUAD [10] facilitate treatmcnt of concentrations as variables, however their use is clearly questionable when chemical methods can be employed. Therefore, the concentrations of the phosphonodipeptides were calculated from the masses of the pure dried compounds.

The values determined for the protonation constants are listed in Table 2 together with the pK_a values of the analogous common dipeptides and free acids. Using calibration of the glass electrode in a wide pH region from 1.7 to 12 by the method described in the Experimental section we could even determine log β_3 values accurate to three decimal places. This points to the good reproducibility and precision of the measurements under our experimental conditions. However, the real accuracy would be lower and according to ref. 13 we estimate it to be within ± 0.05 log unit.

The log β_1 values correspond to protonation of the amine group, log β_2 to protonation of $-PO_3^{2-}$ and log β_3 to protonation of the protonated phosphonic group $-PO_3H^-$. For the dipeptides, the decreasing basicity of the amine group and decreasing acidity of the terminal aminomethylphosphonic acid group (or terminal Gly in common dipeptides), in comparison with the corresponding free acids, is evident for both types of dipeptides. The trends found for log β change with the R substituents corresponding to the changes in the analogous set of common dipeptides but the differences are smaller. A comparison of peptides with $-CO_2H$ and $-PO_3H_2$ terminal groups shows the slightly higher basicity of the nitrogen atom of the phosphonodipeptides. This would point to a weak interaction between the phosphonic and amine groups.

The log β_1 and log β_2 values found for Gly-Gly-(*P*) roughly correspond to the values determined by Hariharan *et al.* [5] The observed difference may be connected with the different value of the ionic strength and probably with a different approach to the determination of the concentration of the stock solutions of the phosphonodipeptides.

The stability constants determined are listed in Table 3. The formation of complexes with ion:ligand molar ratio = 1:1 and 1:2 was observed for Cu^{2+}, Ni^{2+} and Co^{2+}. Owing to precipitation at higher pH values, for Zn^{2+} ions we observed the formation of only the 1:1 complexes. In the 1:1 series the protonated (β_{111}), non-protonated (β_{110}) and deprotonated species (β_{11-1} and β_{11-2}) were observed. The log β_{11-1} values point to the fact that deprotonation and simultaneous coordination of the amide-peptide moiety is likely only for Cu^{2+}. In addition to the non-protonated species (β_{120}), in the 1:2

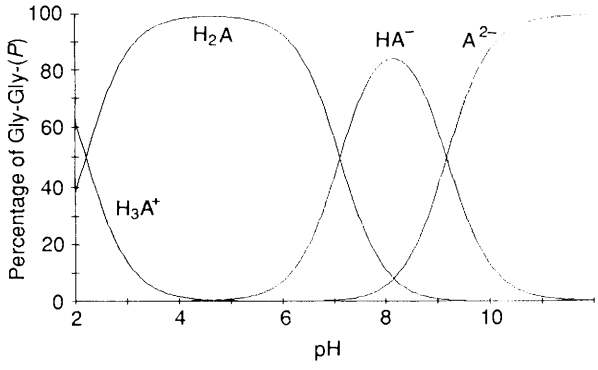


Fig. 1 Concentration distribution of Gly-Gly-(*P*) as a function of pH

Table 2 Protonation constants of the phosphonopeptides

Peptide	log β_1	log β_2	log β_3
Gly-Gly-(*P*)	8.175(2)	14.297(4)	15.512(6)
L-Ala-Gly-(*P*)	8.157(2)	14.250(7)	15.56(1)
L-Leu-Gly-(*P*)	8.070(3)	14.172(4)	15.369(7)
L-Phe-Gly-(*P*)	7.622(4)	13.744(6)	15.01(1)

Dissociation constants of $NH_2CH_2PO_3H_2$, Gly and analogous peptides. [11,12]

	pK_3	pK_2	pK_1		pK_1	pK_2
$NH_2CH_2PO_3H_2$	10.00	5.39	0.4	Gly	9.56	2.36
Gly-Gly-(*P*)	8.34	6.19		Gly-Gly	8.10	3.13
				L-Ala-Gly	8.13	3.14
				L-Leu-Gly	8.00	3.11
				L-Phe-Gly	7.46	3.12

Table 3 Complex formation stability constants of the phosphonodipeptides with divalent ions at 25 °C and I = 0.1 mol dm^{-3}; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$

Ion	Peptide	log β_{111}	log β_{110}	log β_{11-1}	log β_{11-2}	log β_{120}	log β_{12-1}
Cu^{2+}	Gly-Gly-(*P*)	11.98(1)	6.55(1)	1.644(7)	−6.71(3)	11.93(9)	4.89(4)
	L-Ala-Gly-(*P*)	11.82(2)	6.36(2)	1.586(7)	−7.09(4)	12.06(6)	4.65(6)
	L-Leu-Gly-(*P*)	11.29(3)	6.21(1)	1.464(6)	−7.46(3)	—	4.37(7)
	L-Phe-Gly-(*P*)	11.35(2)	6.12(2)	1.555(7)	−6.89(5)	11.54(8)	4.51(5)
Ni^{2+}	Gly-Gly-(*P*)	10.33(1)	4.541(2)	−3.94(3)	—	7.95(1)	−0.99(3)
	L-Ala-Gly-(*P*)	9.80(6)	3.939(8)	−4.69(2)	—	6.89(4)	—
	L-Leu-Gly-(*P*)	9.57(6)	3.786(5)	−4.68(1)	—	6.90(1)	—
	L-Phe-Gly-(*P*)	9.37(2)	3.501(4)	−5.24(5)	—	6.11(1)	−2.70(7)
Co^{2+}	Gly-Gly-(*P*)	9.2(1)*	3.458(6)	−5.41(2)	—	6.16(2)	—
	L-Ala-Gly-(*P*)	—	2.837(6)	−6.11(2)	—	4.3(1)*	—
	L-Leu-Gly-(*P*)	—	2.739(7)	−5.71(1)	—	—	—
	L-Phe-Gly-(*P*)	9.09(3)	2.651(6)	−5.874(6)	—	4.33(7)	—
Zn^{2+}	Gly-Gly-(*P*)	10.04(5)	4.17(2)	—	—		—
	L-Ala-Gly-(*P*)	—	3.817(6)	−3.79(2)	—	—	—
	L-Leu-Gly-(*P*)	—	3.627(8)	−4.12(5)	—	—	—

* Low abundance.

Table 4 Derived constants of the phosphonodipeptides

Ion	Peptides	$\log K_{M(HA)}$	$pK_{M(HA)}^{-H}$	pK_1^{-1}	pK_1^{-2}	$\log K_2$	$\log (K_1/K_2)$	pK_2^{1}	$\log K_{2A}$
Cu^{2+}	Gly-Gly-(*P*)	5.86	5.43	4.91	8.35	5.38	1.17	7.04	3.25
	L-Ala-Gly-(*P*)	5.73	5.46	4.77	8.68	5.70	0.66	7.41	3.06
	L-Leu-Gly-(*P*)	5.19	5.08	4.75	8.98	—	—	—	2.91
	L-Phe-Gly-(*P*)	5.23	5.23	4.56	8.45	5.42	0.70	7.03	2.95
		$\log K_{M(HA)}$	$pK_{M(HA)}^{-H}$	pK_1^{-1}	$\log K_2$	$\log (K_1/K_2)$	pK_2^{1}		
Ni^{2+}	Gly-Gly-(*P*)	4.21	5.79	8.48	3.41	1.13	8.94		
	L-Ala-Gly-(*P*)	3.71	5.86	8.63	2.91	0.99	—		
	L-Leu-Gly-(*P*)	3.47	5.78	8.47	3.11	0.68	—		
	L-Phe-Gly-(*P*)	3.25	5.87	8.74	2.61	0.89	8.81		
Co^{2+}	Gly-Gly-(*P*)	—	—	8.87	2.70	0.76	—		
	L-Ala-Gly-(*P*)	—	—	8.95	—	—	—		
	L-Leu-Gly-(*P*)	—	—	8.45	—	—	—		
	L-Phe-Gly-(*P*)	2.97	6.44	8.52	1.68	0.97	—		

Table 5 Derived stability constants of the common dipeptides from the literature[11,12]

Metal ion	Peptide	$\log K_1$	pK_1^{-1}	pK_1^{-2}	$\log K_{2A}$
Cu^{2+}	Gly-Gly	5.55	4.15	9.31	3.14
	L-Ala-Gly	5.27	3.80	9.54	2.62
	L-Leu-Gly	4.79	3.31	—	2.05
	L-Phe-Gly	4.93	3.67	9.26	2.78
		$\log K_1$	$\log K_2$	$\log (K_1/K_2)$	pK_2^{-1}
Ni^{2+}	Gly-Gly	4.05	3.20	0.85	9.0
	L-Ala-Gly	3.60	2.81	0.79	8.6
	L-Leu-Gly	3.44	2.96	0.48	8.6
Co^{2+}	Gly-Gly	3.04	2.34	0.67	—
	L-Leu-Gly	2.42	2.0	0.4	—

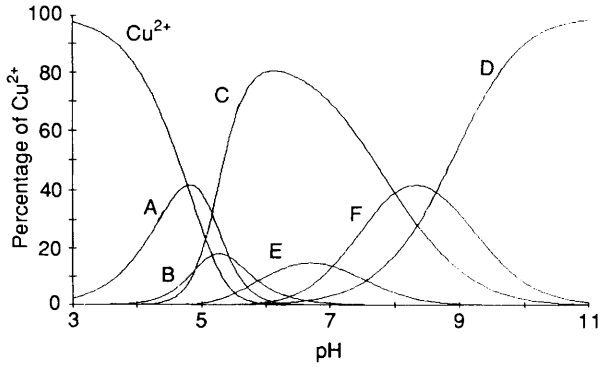


Fig. 2 Concentration distribution of the complexes formed in the Cu^{2+}–Gly-Gly-(*P*) system as a function of pH (c_{Cu} = 0.0025, c_L = 0.005 mol dm^{-3}). Species: A = $[Cu(HA)]^+$, B = $[CuA]$, C = $[CuAH_{-1}]^-$, D = $[CuAH_{-2}]^{2-}$, E = $[CuA_2]^{2-}$ and F = $[CuA_2H_{-1}]^{3-}$

series the deprotonated species (β_{12-1}) were observed in the Cu^{2+} and Ni^{2+} systems.

The log β_{pqr} values determined for the studied ions with phosphonodipeptides are not convenient for comparison with the analogous systems of common dipeptides. Therefore, the derived constants for both phosphonodipeptides and common dipeptides are listed in Tables 4 and 5. The values for the common peptides are taken from refs. 11 and 12. The derived constants correspond to the reactions (1)–(7), where $\log K_1 = \log \beta_{110}$.

$$M^{2+} + HA^- \longrightarrow [M(HA)]^+ \qquad \log K_{M(HA)} = \log \beta_{111} - pK_2 \quad (1)$$

$$[M(HA)]^+ \longrightarrow [MA] + H^+ \qquad pK_{M(HA)}^{-H} = \log \beta_{111} - \log \beta_{110} \quad (2)$$

$$[MA] \longrightarrow [MAH_{-1}]^- + H^+ \qquad pK_1^{1} = \log \beta_{110} - \log \beta_{11-1} \quad (3)$$

$$[MAH_{-1}]^- \longrightarrow [MAH_{-2}]^{2-} + H^+ \qquad pK_1^{2} = \log \beta_{11-1} - \log \beta_{11-2} \quad (4)$$

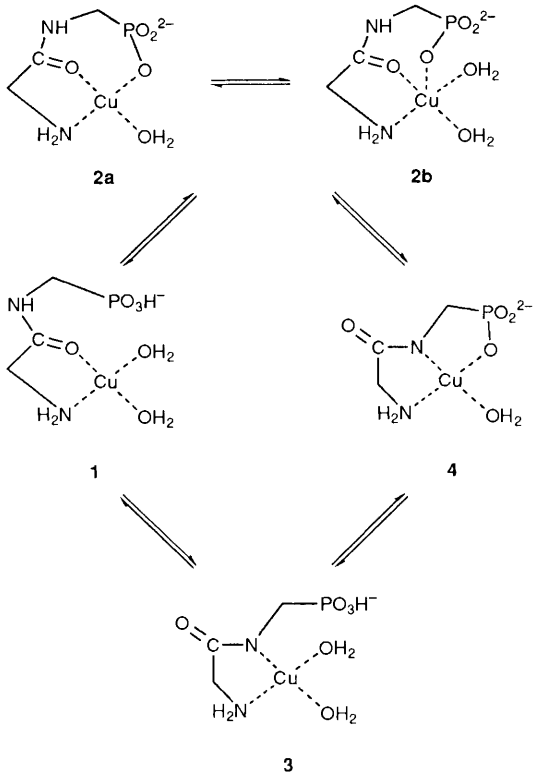
$$[MA] + A^{2-} \longrightarrow [MA_2]^{2-} \qquad \log K_2 = \log \beta_{120} - \log \beta_{110} \quad (5)$$

$$[MA_2]^{2-} \longrightarrow [MA_2H_{-1}]^{3-} + H^+ \qquad pK_2^{-1} = \log \beta_{120} - \log \beta_{12-1} \quad (6)$$

$$[MAH_{-1}]^- + A^{2-} \longrightarrow [MA_2H_{-1}]^{3-} \qquad \log K_{2A} = \log \beta_{12-1} - \log \beta_{11-1} \quad (7)$$

The complexing properties of Cu^{2+} ions differ from those of the other ions studied, and therefore the discussion will first focus on the systems with copper. The species and complex-formation constants found correspond to those determined by Kiss *et al.*[6] In contrast to Hariharan *et al.*,[5] the formation of 1:2 complexes was observed. A typical distribution diagram for the system with Gly-Gly-(*P*) is depicted in Fig. 2. In the acidic region at pH about 3 the protonated species $[Cu(HA)]^+$ is formed. The dissociation constants of the protonated complexes $pK_{M(HA)}^{-H}$ are very similar to the pK_2 values of the free phosphonodipeptides and indicate that the proton is bonded to the phosphonic group, which is or is not co-ordinated, and the carbonyl and amine moieties are bonded to the copper ion and form five-membered rings (structure **1** in Scheme 1). The same kind of co-ordination is assumed for the common dipeptides where carboxyl is not co-ordinated.[14] Owing to the suggested similar co-ordination, it is possible to compare the log $K_{M(HA)}$ values of the phosphonodipeptides and the log K_1 values of common dipeptides (see Tables 4 and 5). We can see that the values of the two constants are comparable and changes in the side chains show the same trend.

The further deprotonation of the common dipeptides corresponds to the deprotonation and simultaneous co-ordination of the amide peptide bond to Cu^{2+}. In the phosphonodipeptide systems, deprotonation of $[Cu(HA)]^+$ and formation of the [CuA] species begins at pH *ca.* 4 and the next step in deprotonation and formation of the dominant species $[CuAH_{-1}]^-$ occurs in the same pH region. The two processes overlap. The $[CuAH_{-1}]^-$ species corresponds to the



Scheme 1

proposed structure **4** in Scheme 1. The amine, amide peptide and terminal phosphonic groups are co-ordinated to Cu^{2+} and form two five-membered rings. As is shown in Scheme 1, we can assume two alternative co-ordination modes, structures **2** and **3**, for the [CuA] species. Structure **2** involves a deprotonated phosphonic group and co-ordination *via* the amine, carbonyl and phosphonic groups. These groups form five-membered and disadvantageous seven-membered rings with the phosphonic group in the equatorial or axial position (**2a** or **2b**). In structure **3**, deprotonation and simultaneous co-ordination of the amide peptide group is assumed. The phosphonic group is protonated and can be co-ordinated or non-co-ordinated. Comparison of the log β_{110} values for the [CuA] and [NiA] species (Table 3) would point to structure **2**. On the other hand, our experience with the analogous platinum(II) and palladium(II) systems and copper systems with diastereoisomers of phosphonodipeptides [7] indicates that a mechanism of deprotonation through structure **3** is probable.

If we compare the values of log β_{110} for phosphonodipeptides with the log K_1 values of common dipeptides (Tables 3 and 5), it is evident that phosphonodipeptides form more stable complexes and the decrease in the stability from Gly-Gly-(*P*) to Phe-Gly-(*P*) is smaller.

According to Scheme 1, the deprotonation and simultaneous co-ordination can be described by the pK_1^{-1} value (Table 4). The pK_1^{-1} values are lower than those of $pK_{M(HA)}^{-H}$ and therefore [CuA] should be a stronger acid than $[Cu(HA)]^+$. This does not seem to be logical. However, when the [CuA] species was not included in the model the goodness-of-fit tests were much worse. An explanation follows from the distribution diagram. Both deprotonation processes occur virtually simultaneously and the abundance of [CuA] is low and overlapped by the dominant $[CuAH_{-1}]^-$ species (Fig. 2). Nevertheless, these values (pK_1^{-1}) are relatively similar for each phosphonodipeptide system, similarly to Kiss's systems,[6] and indicate that this process [equation (3)] occurs at a pH value of about 1 log unit higher than for the common dipeptides. Kiss *et al.*[6] explained this effect by electrostatic effects, since $[CuAH_{-1}]^-$ is neutral for common dipeptides, while it has a negative charge in the monophosphono complexes. Another explanation follows from comparison of the pK_a values of the amino groups in aminoalkylphosphonic acids [15] and in common amino acids.[11] The differences found are the same, about 1 unit. Thus, the increase in the pH value for deprotonation of the amide peptide bond could be explained as for aminoalkylphosphonic acids, *i.e.* by repulsion of the negative charge in the phosphonate part of the molecule and consequently higher electron density on the nitrogen atom. The decrease of the pK_1^{-1} values in the phosphonodipeptide series is not significant as in corresponding series of the common dipeptides, probably due to higher values of log β_{110}.

The deprotonation of the equatorially bonded molecule of water and the formation of hydroxo-complexes starts in the region pH 7–8, lower than for the common dipeptides (see the pK_1^{2} values in Tables 4 and 5). It seems to be easier than for the common dipeptide complexes, probably due to the influence of the neighbouring deprotonated phosphonic group which is probably able to anchor a hydrogen atom through a hydrogen bond and then to transfer it to a hydration sphere.

In contrast to the Cu^{2+} systems with the common dipeptides, $[CuA_2]^{2-}$ species were found in the phosphonodipeptide systems. In agreement with Kiss *et al.*[6] we assume equatorial co-ordination of the amine and carbonyl peptide groups of both ligands and probably only one phosphonic group co-ordinated axially. This mode of co-ordination corresponds to the fact that the formation processes of [CuA] and $[CuA_2]^{2-}$ partially overlap each other (Fig. 2) and is also consistent with the assumed mechanism of deprotonation *via* structure **2**. The log (K_1/K_2) values (Table 4) show decreasing ability to form the $[CuA_2]^{2-}$ species as the size of side chain increases. For Leu-Gly-(*P*) the formation of 1:2 complexes was not observed. Deprotonation of the $[CuA_2]^{2-}$ species and formation of $[CuA_2H_{-1}]^{3-}$ begins in the region pH 6–7 and this process is overlapped by formation of [CuA], $[CuA_2]^{2-}$ and $[CuAH_{-1}]^-$. No influence of the side chain on the deprotonation (pK_2^{-1} values in Table 4) was observed. In contrast to $[CuA_2]^{2-}$, $[CuA_2H_{-1}]^{3-}$ was observed in systems of common dipeptides. A study of the analogous complexes of the common dipeptides by EPR and UV/VIS spectroscopy by Szabó-Plánka *et al.*[16] points to the formation of the $[Cu(ZH_{-1})Z]^-$ species when a molecule of the common dipeptide (Z) is deprotonated and co-ordinated in a tridentate manner in the equatorial positions and the next molecule is co-ordinated equatorially only through the carbonyl group and axially through the amine group. The terminal carboxyl group is non-co-ordinated. A similar co-ordination sphere is assumed for $[CuA_2H_{-1}]^{3-}$. In systems with Leu-Gly-(*P*) the formation of 1:2 complexes was not observed, probably due to the bulky isobutyl side chain. In the 1:2 series we can compare only the log K_{2A} values. These values indicate that addition of a ligand to the $[CuAH_{-1}]^-$ species is a little more advantageous for phosphonodipeptides, probably due to the higher basicity of the amine group. The formation of 1:2 complexes seems to be easier for phosphonodipeptides than for aminoalkylphosphonic acids, where the co-ordination of both phosphonic groups is supposed.[15] This corresponds to the smaller steric hindrance for phosphonodipeptides where one phosphonic group should not be co-ordinated in $[CuA_2]^{2-}$ and one or both should not be co-ordinated in $[CuA_2H_{-1}]^{3-}$.

Systems containing Ni^{2+}, Co^{2+} and Zn^{2+} exhibited different behaviour and a typical distribution diagram is shown in Fig. 3. Formation of complexes starts at about pH 5 and the precipitation of nickel or cobalt hydroxide occurs in the region pH 8.5–9. In the zinc systems the precipitation of insoluble complexes starts in the region pH 5.5–8.0 depending on the hydrophobicity of the ligand. Nickel(II) forms protonated complexes in amounts of 10–20%. The major species in the system is the non-protonated species [NiA]. The calculated stability constants are lower than in the Cu^{2+} systems. However, the same type of co-ordination as in structure **1** of the

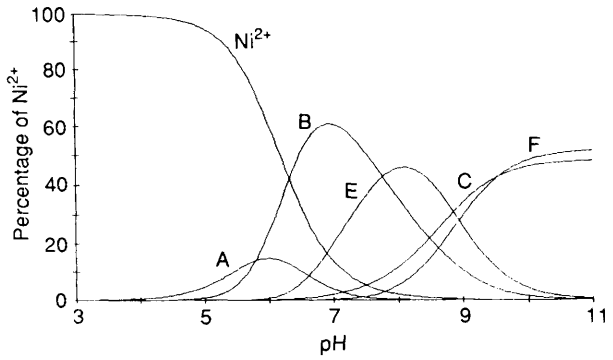


Fig. 3 Concentration distribution of the complexes formed in the Ni^{2+}–Gly-Gly-(*P*) system as a function of pH (c_{Ni} = 0.0025, c_L = 0.005 mol dm^{-3}). Species: A = $[Ni(HA)]^+$, B = [NiA], C = $[NiAH_{-1}]^-$, E = $[NiA_2]^{2-}$ and F = $[NiA_2H_{-1}]^{3-}$

Cu^{2+} systems is assumed for the protonated species $[Ni(HA)]^+$ and as in structure **2** for the non-protonated species [NiA]. The log $K_{M(HA)}$ values decrease with decreasing basicity of the amine groups, and as the steric hindrance increases. The $pK_{M(HA)}{}^{H}$ values roughly correspond to the pK_2 values of the free phosphonodipeptides and, therefore, the phosphonic group should not participate in the co-ordination.

Deprotonation and simultaneous co-ordination of the peptide amide moiety to nickel was observed for common dipeptides at pH *ca* 9.[14] For phosphonodipeptides the same reaction should occur at higher pH, about 10. However, nickel hydroxide precipitated in this region. In the Ni^{2+} systems, as with Cu^{2+}, the formation of $[NiAH_{-2}]^{2-}$ species was not observed. We found only the $[NiAH_{-1}]^-$ species, the $pK_1{}^1$ values of which correspond to $pK_1{}^2$ in the Cu^{2+} systems (Table 4). Therefore, the log β_{11-1} values point to the formation of hydroxo complexes. The phosphonodipeptides form $[NiA_2]$ complexes and the log K_1/K_2 values decrease with increasing chain size.

In the Co^{2+} systems the [CoA] and $[CoAH_{-1}]^-$ species were found. The protonated $[Co(HA)]^+$ and $[CoA_2]^{2-}$ species were observed in small amounts but not for L-Leu-Gly-(*P*) (Table 3). Owing to precipitation in the neutral pH region, only 1:1 complexes could be found in the Zn^{2+} systems. For L-Phe-Gly-(*P*) precipitation started in the acidic region and therefore the system could not be investigated. We assume the same stereochemistry for the cobalt and zinc complexes as in the corresponding nickel complexes.

Conclusion

Comparison of the acid–base and complexing properties of the series of common dipeptides and the studied phosphonodipeptides results in the following conclusions. (1) The trends found for pK_A with the side chain corresponding to those for common dipeptides but the differences are smaller. The observed slightly higher basicity of the amine groups indicates their weak interaction with the phosphonic groups. (2) In contrast to the common dipeptides, deprotonation and simultaneous co-ordination of the peptide amide group was observed only in systems with Cu^{2+}. (3) The formation constants of the studied ions with phosphonodipeptides are higher than those with common dipeptides. This increase is in the order $Cu^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+}$, as in corresponding systems with aminoalkylphosphonic acids. (4) Similar to the common dipeptide series, the formation constants decrease in the order Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}.

Experimental

The TGA measurements were carried out on a TG-750 Stanton Redcroft instrument in the temperature range 25–280 °C in the presence of air.

Preparation of the Phosphonodipeptides and Chemicals.—The phosphonodipeptides were prepared, isolated and their purity checked according to the procedure described in our previous paper.[8] The stock solutions of the individual metal cations were acidified solutions of the nitrates, recrystallized from aqueous solutions. The metal content was determined by titration with ethylenediaminetetraacetate solution and excess of nitric acid was determined by pH-metric acid–base titration using Gran's method. The nitric acid was prepared from recrystallized potassium nitrate on a column with Dowex 50 (H^+ form).

Potentiometric Titrations.—Potentiometric measurements were carried out using a PHM 84 pH-meter, ABU 80 autoburette and a GK 2401 B combination electrode (Radiometer) in a glass vessel (10 cm^3) thermostatted at 25 ± 0.1 °C at an ionic strength of $I(KNO_3)$ = 0.1 mol dm^{-3}. An inert atmosphere was ensured by constant passage of argon saturated with the solvent vapour. The initial solution volume was 5 cm^{-3} and the phosphonodipeptide concentration was 0.005 mol dm^{-3} for determination of protonation constants and in the range 0.004–0.007 mol dm^{-3} for determination of the stability constants. The metal:ligand ratio was 1:1, 1:2 or 1:4. The total number of data points was more than 200. After calibration using two buffers, precision calibration was carried out by titration of 0.015 mol dm^{-3} HNO_3 with 0.2 mol dm^{-3} KOH at 25 °C and at an ionic stregth of 0.1 mol dm^{-3} (KNO_3) in the region pH 1.8–12.0, with the pH-meter yielding *E* values. The relation between *E* and $-\log[H^+]$ is expressed by equation (8) where the term E_o contains the standard potentials of the

$$E = E_o - S(-\log[H^+]) + j_a[H^+] + j_b(pK_w/[H^+]) \quad (8)$$

electrodes used and the contribution of inert ions to the liquid-junction potential, *S* corresponds to the Nernstian slope the value of which should be close to the theoretical value, and $j_a[H^+]$ and $j_b[OH^-]$ are the contributions of H^+ and OH^- ions respectively, to the liquid-junction potential. It is clear that j_a and j_b cause deviation from a linear dependence between *E* and $-\log[H^+]$ only in the strongly acidic and strongly basic pH regions. The equation is used in the ESAB 2M 87 program.[9] The values of E_o, *S*, j_a and j_b in equation (8) at pK_w = 13.75 were calculated for each calibration and were then used for calculation of the $-\log[H^+]$ values from the *E* titration values of each series of measurements by our program.

The stability and protonation constants β_{pqr} are concentration constants and are defined by $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$. The constants were calculated using the MINIQUAD 82 program.[17] Our procedure was tested by the 'glycine test'.[18]

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References

1 *The Role of Phosphonates in Living Systems*, ed. R. L. Hildebrand, CRC Press, Boca Raton, FL, 1983; J. S. Thayer, *Appl. Organomet. Chem.*, 1989, **3**, 203; V. P. Kukhar and N. M. Solodenko, *Ukr. Biokhim. Zh.*, 1988, **60**, 95; P. Kafarski and B. Lejczak, *Phosphorus Sulfur Silicon Relat. Elem.*, 1991, **63**, 193.
2 F. R. Atherton, C. H. Hasal and R. W. Lambert, *J. Med. Chem.*, 1986, **29**, 29 and refs. therein.
3 P. Wieczorek, B. Lejczak, M. Kaczanowska and P. Kafarski, *Pestic. Sci.*, 1990, **30**, 43.

4 P. Kafarski, B. Lejczak and P. Mastalerz, *Beitr. Wirkstofforschung*, 1985, **25**, 1; P. Kafarski, 12 Polskie Sympozjum Peptydowe, Karpacz, 1993.
5 M. Hariharan, R. J. Motekaitis and A. E. Martell, *J. Org. Chem.*, 1975, **40**, 470.
6 T. Kiss, E. Farkas, H. Kozlowski, Z. Siatecki, P. Kafarski and B. Lejczak, *J. Chem. Soc., Dalton Trans.*, 1989, 1053.
7 P. Hermann, I. Lukeš, P. Vojtíšek and I. Císařová, following paper.
8 P. Hermann, I. Lukeš, B. Máca and M. Budešinský, *Phosphorus Sulfur Silicon Relat. Elem.*, 1993, **79**, 43.
9 G. Arena, E. Rizzarelli, S. Sammartano and C. Rigano, *Talanta*, 1979, **26**, 1; C. Rigano, M. Grasso and S. Sammartano; *Ann. Chim. (Rome)*, 1984, **74**, 537; C. De Stefano, P. Princi, C. Rigano and S. Sammartano, *Ann. Chim. (Rome)*, 1987, **77**, 643.
10 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
11 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974–1989, vols. 1–6.
12 V. Cucionatta, R. Purrello and E. Rizzarelli, *Comments Inorg. Chem.*, 1990, **11**, 85.
13 W. A. E. McBryde, *IUPAC Chemical Data Series No. 17*, Pergamon, Oxford, 1978; G. Anderegg, *IUPAC Chemical Data Series No. 14*, Pergamon, Oxford, 1977.
14 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385; I. Sóvágó in *Biocoordination Chemistry: Coordination Equilibria in Biological Systems*, ed. K. Burger, Ellis Horwood, Chichester, 1990, p.135.
15 T. Kiss, J. Balla, G. Nagy, H. Kozlowski and J. Kowalik, *Inorg. Chim. Acta*, 1987, **138**, 25.
16 T. Szabó-Plánka, G. Peintler, A. Rockenbauer, M. Gyor, M. Varga-Fabian, L. Institorisz and L. Balaszpiri, *J. Chem. Soc., Dalton Trans.*, 1989, 1925.
17 A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.
18 A. Braibanti, G. Ostacoli, P. Paoletti, L. D. Pettit and S. Sammartano, *Pure Appl. Chem.*, 1987, **59**, 1721.

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