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THE INFLUENCE OF ASPARTIC OR GLUTAMIC ACID RESIDUES IN TETRAPEPTIDES ON THE FORMATION OF COMPLEXES WITH NICKEL(II) AND ZINC(II)

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Abstract—The formation of the complexes formed by Ni^{II} and Zn^{II} with Asp-Asp-Asp and a series of tetrapeptides containing one or two Asp residues or one Glu residue are reported. Stability constants were measured pH-metrically. The particular species and the metal ion binding sites were determined using ^1H NMR, UV–vis and CD spectroscopy. The β-carboxylate group of the Asp residue stabilizes the complexes significantly, particularly when present as the N-terminal residue. As a result the tendency for Ni^{II} to deprotonate and bind to amide-nitrogen atoms, forming planar diamagnetic complexes, is reduced and their formation delayed to a significantly higher pH when compared to other peptides. The side chain of the Glu residue has a much smaller effect. As anticipated, Zn^{II} was unable to deprotonate and bind to peptide nitrogens.

With simple tetrapeptides, e.g. tetraglycine, Ni^{II} initially coordinates to the N-terminal amino-group to form a 1N complex (binding via $\{NH_2, C{=}O\}$) and, as the pH is raised, is able to deprotonate and bind to three successive peptide nitrogens to eventually form a 4N complex. In the presence of a peptide ligand the d^8 Ni^{II} ion can exist in two distinct coordination geometries, paramagnetic octahedral (green) and diamagnetic square planar (yellow). The 1N complexes, NiL^+, are generally octahedral, while those with two or three deprotonated peptide donors (i.e. Ni—N^- bonds) tend to be planar. Hence an abrupt change in coordination from octahedral to planar is to be expected as the pH is raised. This leads to 'cooperative coordination' with complex formation changing very rapidly from 1N to 4N coordination around pH 8.5.[1] Consequently, with simple tetrapeptides, the species $NiH_{-1}L^+$ and $NiH_{-2}L$ are very difficult to detect, solutions changing rapidly with pH from green to the yellow ($\lambda_{max} \sim 410$–450 nm) characteristic of planar Ni^{II} in the 4N complex, $[NiH_{-3}L]^-$.

Recent studies of the complexes of tetrapeptides that contain aspartyl residues [Asp or D, —NHCH(CH_2COOH)CO—] have shown that the

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lateral β-carboxylate group can have a critical impact on the binding ability of peptides to Cu^{II} ions, especially when the residue involved is -Asp^{2}- or -Asp^{3}-.[2] The lateral carboxylate-O^{-} interferes with the sequence of peptide nitrogen deprotonation and bonding to form Cu—N^{-} bonds because it can form stable, six-membered chelate rings. As a result, the $CuH_{-1}L$ species is stabilized dramatically when an -Asp^{2}- is present, in contrast to $CuH_{-2}L$, which is stabilized by -Asp^{3}-. As a result of this stabilization, coordination of the fourth nitrogen with Ala-Ala-Asp-Ala is completely obstructed. With Cu^{II}, chelation through the β-carboxylate-O^{-} is preferentially equatorially and in the coordination plane, while apical coordination has a much less significant effect. With paramagnetic Ni^{II}, all octahedral coordination sites are equally accessible and, as a result, the effect of coordination through lateral carboxylate groups on the speciation may be very different. In particular, apical coordination could have a major influence on the switch from paramagnetic to diamagnetic Ni^{II}, leading to a very different species distribution as a function of pH, and the stabilization of 2N and 3N complexes.

Studies of biologically active peptides (or their N-terminal fragments) such as thymopotoietin (Arg-Lys-Asp-...),[3,4] fibrinopeptide (Ala-Ala-Ser-...)[5,6] and angiotensin II (Asp-Arg-Val-...)[7,8] and of a range of model tetrapeptides containing Asp or Glu as one of the amino acid residues[2] have demonstrated the effect which the Asp residue can have on coordination to Cu^{II}, while the Glu residue (which, if chelated, would form a 7-membered chelate ring) has a very small, almost insignificant effect. We wish to report an extension of these studies to the Ni^{II} complexes of a range of tetrapeptides containing one or two Asp residues or one Glu residue, using potentiometry, spectroscopy (visible and CD) and NMR. In addition, complexes with Gly-Asp have been studied for comparison.

With simple peptides, zinc is unable to deprotonate and bind to peptide nitrogens, although this can take place when a His residue is present in the peptide chain.[9] We have therefore studied the Zn^{II} complexes of some of the ligands containing Asp residues in an attempt to identify any influence which coordination through the lateral carboxylate group may have.

EXPERIMENTAL

Peptides

The peptides were synthesized by standard methods as described elsewhere.[2] Tetra-alanine and Gly-Asp were purchased from Sigma Chemicals.

Spectroscopic studies

Solutions of $Ni(ClO_4)_2$ (Fluka) with metal ion concentrations between 0.004 and 0.008 mol dm^{-3} and metal to ligand ratios of 1 : 4 were used. Absorption spectra were recorded on a Cary 219 spectrophotometer and CD spectra on a Jobin-Yvon Mark III dichrograph in the 200–800 nm region. All CD spectra are expressed in terms of $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$. NMR spectra were recorded on a Bruker 400 MHz spectrometer with peptide concentration 0.005 mol dm^{-3} at 300 K using Ni^{II}: L ratios of 1 : 1.25. Analysis and simulation of the proton ABC spectra were carried out on a Hewlett–Packard HP 9826 computer.

Potentiometric studies

Stability constants for complexes of H^{+}, Ni^{II} and Zn^{II} were calculated from titration curves carried out using total volumes of 1.5 cm^3. Alkali was added from a 0.1 cm^3 micrometer syringe which had been calibrated by both weight titration and titration standardized materials. Peptide concentration was 0.003 mol dm^{-3} and the metal to ligand molar ratios were 1 : 4 to 1 : 6 with Ni^{II} and 1 : 3 with Zn^{II}, and the ionic strength was adjusted to 0.10 mol dm^{-3} (KNO_3). The titrations were performed with a micro combined glass-calomel electrode (Russel) calibrated in hydrogen ion concentrations using $HClO_4$,[10] using the Molspin automatic titration system, with two or three titrations per determination. The pH was measured at 298 K in the 4.5–10.5 range. The stability constants were calculated from the pH-metric titration curves with a SUPERQUAD computer program.[11]

Standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species in the equilibrium.

RESULTS AND DISCUSSION

Complexes of the following 13 peptides were studied: Asp-Ala-Ala-Ala (DAAA), Ala-Asp-Ala-Ala (ADAA), Ala-Ala-Asp-Ala (AADA), Ala-Ala-Ala-Asp (AAAD), Asp-Ala-Ala-Asp (DAAD), Ala-Asp-Asp-Ala (ADDA), Ala-Asp-Ala-Asp (ADAD), Ala-Ala-Asp-Asp (AADD), Asp-Asp-Asp (DDD), Glu-Ala-Ala-Ala (EAAA), Ala-Glu-Ala-Ala (AEAA), Ala-Ala-Glu-Ala (AADA) and Gly-Asp. Since all carboxylate groups are fully ionized in the pH regions at which coordination to Ni^{II} or Zn^{II} takes place, the ionized ligands will differ in charge depending on the number of carboxyls

present. Charges on the complexed species will therefore be omitted for simplicity.

Proton complexes

Protonation constants were determined and checked against those reported earlier.[2] In all cases good agreement was found with K_{HL} values agreeing to better than 0.02 log units. In all cases the first constant (around pH 8) refers to protonation of the amino nitrogen and this is the only protonation to have any significant effect on coordination to Ni^{II} or Zn^{II}. The other stepwise protonation constants (pH 2–4) all refer to carboxylate protonations and are macro-constants with contributions from the two or more carboxyl protonations. Values for the protonation constant, log K_{HL}, are given in Table 1. Values for Gly-Ala agree well with those reported in the literature, determined at $I = 0.2$ mol dm^{-3} (log $K_{HL} = 8.35$, log $\beta_{H2L} = 12.66$).[12]

Ni^{II} complexes

The kinetics of formation of complexes of Ni^{II} with peptides are frequently very slow, particularly in the region of the switch from paramagnetic to diamagnetic nickel. As a result, equilibria are difficult to study potentiometrically over the pH range of 7–9. A further complication was added by β-carboxylate coordination in peptides containing the Asp residue, which appeared to slow the equilibria even more than usual. This made the precise measurement of pH in this region even more difficult and time consuming. This was particularly noticeable when the peptides contained two β-carboxylate groups, but was less significant with the peptides containing Glu residues. As a result, stability constants for species which are present in the region of the paramagnetic–diamagnetic ($NiH_{-1}L$, 2N and $NiH_{-2}L$, 3N) change have a lower precision. Equilibria are established somewhat more rapidly in the presence of excess ligand (e.g. Ni: L of 1:4 or above), conditions which favoured formation of NiL_2 when the peptides contained Asp^1 residues. Stability constants for the complexes with Ni^{II} and, where measured, with Zn^{II} are given in Table 1. Spectroscopic data for complexes with Ni^{II} are found in Table 2 and selected NMR data

Table 1. Formation constants for complexes of peptides with H^+, Ni^{II} and Zn^{II} at 25 °C and $I = 0.10$ mol dm^{-3} (KNO_3)

	log β values H^+ and Ni^{II} complexes					
	HL	NiL	NiL_2	$NiH_{-1}L$	$NiH_{-2}L$	$NiH_{-3}L$
DAAA	7.55	4.84(2)	8.48(2)	−3.58(3)		20.17(3)
ADAA	7.91	3.51(1)		−4.08(2)		21.41(2)
AADA	8.29	3.91(2)			−10.29(3)	−18.58(8)
AAAD	7.44	3.80(2)		−3.80(2)		20.80(6)
DAAD	8.01	5.78(3)	9.73(5)			−20.52(5)
ADDA	8.30	4.21(2)			−10.93(2)	−20.63(3)
ADAD	8.34	4.03(2)			−14.30(3)	−24.00(4)
AADD	8.32	5.44(4)		−2.33(7)	−10.30(5)	−19.95(6)
DDD	8.11	5.70(2)	9.47(7)	−2.33(4)	−11.09(3)	
EAAA	7.79	3.70(2)		−4.39(1)		20.39(3)
AEAA	7.96	4.01(3)		−3.87(2)		20.29(3)
AAEA	8.11	3.69(2)		−4.97(3)		21.29(4)
AAAA	8.13	3.06		−4.97		−21.29
Gly-Asp	8.42	4.52(1)	7.28(3)	−4.52(3)		

	Zn^{II} complexes			
	ZnL	ZnL_2	$ZnH_{-1}L$	$ZnH_{-2}L$
DAAD	4.88(3)		−4.00(4)	−12.57(6)
ADDA[a]	3.69(7)		−4.50(6)	−13.10(5)
ADAD	3.84(4)		−4.73(5)	−13.77(5)
AADD[a]	3.77(8)		−4.77(8)	−14.42(9)
DDD	4.55(2)		−4.58(4)	−13.46(3)
Gly-Asp	3.96(1)	6.64(4)	?	−13.48(2)

[a] Sufficient material for one complete titration only.

Table 2. Spectroscopic data for Ni^{II} complexes with single Asp- or Glu-containing peptides

Peptide	Species	Absorption bands λ, nm (ε)	CD bands λ, nm ($\Delta\varepsilon$)
DAAA	NiL, $NiL_2(O_h)$	390 (18)[a]*	
		650 (<5)[a]*	
	$NiH_{-3}L$(SP)	414 (145)[a]	458 (−0.47)[a]
			257 (+1.98)[b]
ADAA	NiL,$NiH_{-1}L(O_h)$	390 (22)[a]	
	$NiH_{-3}L$(SP)	413 (168)[a]	460 (−0.52)[a]
			260 (+5.1)[b]
AADA	$NiL(O_h)$	392 (<5)[a]	
	$NiH_{-2}L$(SP)	420 (56)[a]	
	$NiH_{-3}L$(SP)	436 (210)[a]	490 (−0.52)[a]
			420 (+0.29)[a]
			272 (+0.15)[b]
AAAD	NiL,$NiH_{-1}L(O_h)$	390 (sh)[a]*	
	$NiH_{-3}L$(SP)	412 (195)[a]	448 (−0.54)[a]
			258 (+1.79)[b]
EAAA	NiL,$NiH_{-1}L(O_h)$	650 (<5)[a]*	
		390 (10)[a]*	
	$NiH_{-3}L$(SP)	412 (162)[a]	458 (−0.65)[a]
			257 (+2.32)[b]
AEAA	$NiL(O_h)$	400 (sh,16)[a]	
	$NiH_{-1}L(O_h)$	400 (sh,40)[a]	
	$NiH_{-3}L$	416 (142)[a]	458 (−0.65)[a]
			258 (+2.34)[b]
AAEA	$NiL(O_h)$	650 (<5)[a]	
		390 (20)[a]	
	$NiH_{-1}L$	650 (<5)[a]	
		390 (37)[a]	
	$NiH_{-3}L$	414 (194)[a]	454 (−1.95)[a]
			258 (+6.6)[b]

[a] *d–d* transitions.

[b] $N^- \rightarrow Ni^{II}$ charge transfer transition.

SP, square-planar; O_h, octahedral.

*Overlapping of transitions for two species, suggesting O_h geometry.

in Table 3. Selected species distribution curves are shown in Figs 1 and 2. Coordination of AAAA with Ni^{II} is similar to that found with tetraglycine (log K_{NiL} = 3.64).

Peptides with N*-terminal Asp residues (DAAA, DAAD and DDD)*

The position of the Asp residue in these peptides would be expected to give it a major influence on coordination, particularly at lower pH. In all cases the NiL species is much more stable (by a factor of up to 2 log units) and, as a result, much more significant than with tetra-alanine itself, and is the dominant species to above pH 7, as shown in Figs 1 and 2. The bis-complex, NiL_2, also forms when Asp is in position 1 of the peptide chain, whereas it was not detected unambiguously in equilibria with peptides which do not have an N-terminal Asp residue. The bis-complex is particularly important in the presence of excess ligand, and has the effect of delaying deprotonation and binding of peptide nitrogens to the region of pH 9. This species was also found in complexes with Asp-Arg-Val-Tyr, the N-terminal tetrapeptide fragment of Angiotensin II.[7] Absorption spectra show both the NiL and

Table 3. NMR chemical shifts (in ppm from TMS) for protons of metal-free and coordinated AAAD in 4N complexes with Ni^{II} at pH 11.8; rotamer populations of the Asp^1 residue as defined in Fig. 3

Residue	Ala^1		Ala^2		Ala^3		Asp^4	
proton	CH_α	CH_3	CH_α	CH_3	CH_α	CH_3	CH_α	$CH_{2\beta}$
Free ligand	3.53	1.28	4.35	1.43	4.43	1.44	4.39	2.58, 2.68
Bound ligand	3.34	1.21	3.54	1.23	4.60	1.25	3.56	2.16, 2.49
$\Delta\delta$	0.19	0.07	0.81	0.20	0.83	0.19	0.83	0.42, 0.19

	Rotamer populations		
	rotamer I	rotamer II	rotamer III
Free ligand	0.15	0.57	0.28
Bound ligand	0.32	0.57	0.11

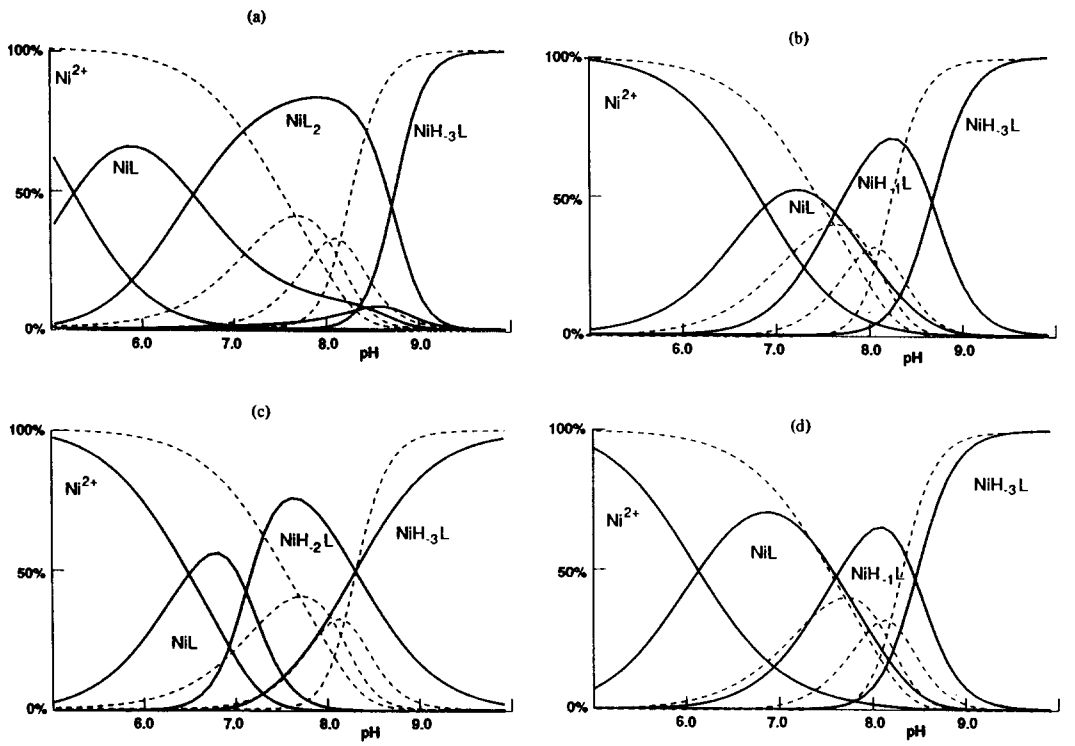

Fig. 1. Species distribution curves for Ni^{II} (0.001 mol dm^{-3}) in Ni^{II}:L ratio 1:4 with DAAA (a), ADAA (b), AADA (c) and AAAD (d). Dotted lines are for complexes with AAAA.

NiL_2 complexes to contain octahedral Ni^{II}, and it is interesting to note that the bis-complex is not formed with Cu^{II},[2] which prefers coordination via equatorial positions. It can therefore be assumed that both NiL and NiL_2 involve the β-carboxylate of the Asp^1 residue in facial, tridentate binding *via* the amine-N, carbonyl-O and carboxylate-O^- donors.[13] The enhanced stability resulting from this tridentate coordination makes the ligands more able to bind Ni^{II} below pH 6 (see Figs 1 and 2). For example, with tetra-alanine only about 5% of the metal ion is bound at this pH, whereas with DAAA the figure is 85%. The coordination equilibria of DAAA and DAAD are very similar indeed, as shown in Figs 1 and 2. The only significant difference is slightly greater delay (about 0.4 pH units) in formation of the diamagnetic, 4N, $NiH_{-3}L$ complex with DAAD, suggesting that the second carboxylate also participates in coordination to the Ni^{II}.

The high stability of the NiL and NiL_2 complexes means that $NiH_{-1}L$ is only a very minor species which could not even be detected with DAAD, making it even less significant than with tetra-alanine, and $NiH_{-2}L$ could not be identified with either ligand. The switch to diamagnetic Ni^{II} can

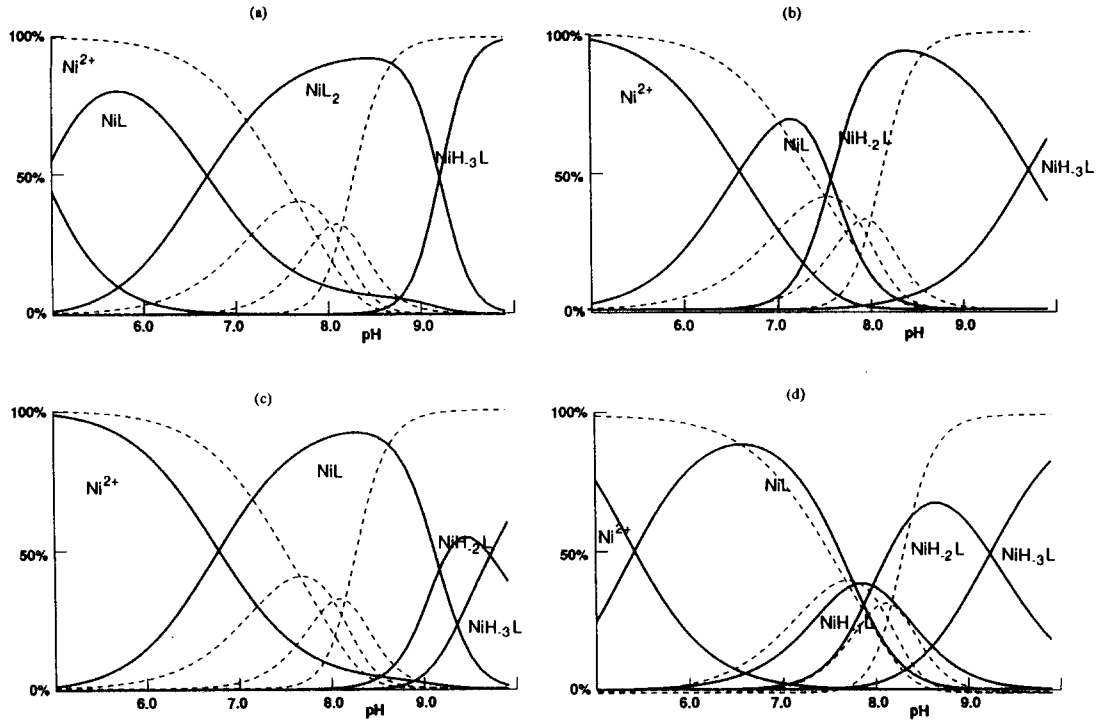

Fig. 2. Species distribution curves for Ni^{II} (0.001 mol dm^{-3}) in Ni^{II}:L ratio of 1:4 with DAAD (a), ADDA (b), ADAD (c) and AADD (d). Dotted lines are for complexes with AAAA.

take place only after breaking the nickel–carboxylate bonds, which would coordinate octahedrally and, as a result, takes place even more abruptly, and at a higher pH, than with tetra-alanine.

The tripeptide DDD contains four carboxylate donors and should not be compared directly with the tetrapeptides because it cannot form a 4N complex. However, from the evidence of the tetrapeptides with Asp^1 residues, the NiL and NiL_2 species should be even more important. This was found to be the case; the complexes were very stable and significant peptide-N deprotonation did not commence until above pH 8, as shown in Figs 1 and 2. From spectroscopy, the 3N $NiH_{-2}L$ species was clearly a diamagnetic complex.

Peptides with -Asp^2-residues (ADAA, ADDA, ADAD). The influence of -Asp^2- residues is less specific than that discussed above. The NiL complex is more important than with tetra-alanine, particularly with ADAD, but the more significant difference is the greater importance of the $NiH_{-1}L$ and/or $NiH_{-2}L$ complexes around pH 8, which have the effect of delaying formation of $NiH_{-3}L$ significantly. Similar behaviour was found in the coordination of Ni^{II} to Ala-Asp-Ser-Gly, the N-terminal fragment of fibrinopeptide A.[5] When the peptide contains two Asp residues (ADDA and ADAD) it was impossible to fit experimental pH-metric data to a model including the $NiH_{-1}L$ species, but a very good fit was obtained by including $NiH_{-2}L$ when the $NiH_{-1}L$ species was rejected. With both of these ligands, formation of the $NiH_{-3}L$ complex was delayed, when compared to tetra-alanine, by more than 1 log unit demonstrating the importance of bonding from the β-carboxylates.

The influence of -Asp^2- on stabilization of the $NiH_{-1}L$ species with Ni^{II} is less dramatic than with Cu^{II} when equatorial binding of the β-carboxylate causes the $CuH_{-1}L$ complex to be the predominant species in the pH range of 4.5–9.5.[2] This may be because, with Ni^{II}, formation of a 2N complex with one peptide-N bond allows the β-carboxylate to bind octahedrally, forming a paramagnetic $NiH_{-1}L$ complex. In this species, the two unpaired electrons of Ni^{II} ion are near the switch point to a singlet, planar configuration. Hence the facile breaking of the carboxylate bond to give the expected 4N, $NiH_{-3}L$ complex, behaviour which would not be expected with the d^9 Cu^{II} ion.

Peptides with -Asp^3- residues (AADA, AADD, ADDA). The influence of β-carboxylate coordination is comparable to that found with ADAA, only now the 2N $NiH_{-1}L$ complex could not be identified whereas, from potentiometry, the $NiH_{-2}L$ complex (3N coordination) could. The NiL species forms at a somewhat lower pH than

with tetra-alanine and, with AADD, is a very important species below pH 7.5. The importance of an O-terminal β-carboxylate in metal binding by tetrapeptides is discussed below. With both AADD and ADDA, formation of the 3N ($NiH_{-3}L$) complex is delayed significantly as a result of this interaction, which reaches a maximum concentration around pH 7.5, but this readily deprotonates to form $NiH_{-3}L$. Spectroscopic evidence shows this 4N ($NiH_{-3}L$) species to be planar and diamagnetic as expected, and also suggests that the $NiH_{-2}L$ complex is also planar (Table 2).

Peptides with -Asp[4]-residues (AAAD, DAAD, ADAD and AADD). With all of these systems, apart from DAAD, which has an N-terminal Asp residue, the NiL species is more significant than may be expected. The effect is real, and was also found in the complexes with Cu^{II}.[2] Chelation spanning the four amino acid residues would not be expected unless conformational factors encouraged it.

Complexes with Gly-Asp. Complexes of Ni^{II} with Gly-Asp were also studied pH-metrically, and results are given in Table 1. Diamagnetic complexes were not expected and the species identified were NiL, NiL_2 and $NiH_{-1}L$. An earlier study found very similar values for log K_{NiL} (4.44) and log β_{NiL2} (7.02), but did not report the $NiH_{-1}L$ complex, which is the predominant species at high pH.[14]

Peptides with Glu residues (EAAA, AEAA, AAEA). The results obtained for the Ni^{II}:EAAA system are closer to those with tetra-alanine than those for the Asp analogue, confirming the assumption that a γ-carboxylate is much less effective in metal binding than a β-carboxylate, presumably as a result of the larger chelate ring which would result. Significant enhancement in the stability of the 1N complex is found, however (demonstrated in Figs 1 and 2), showing that limited γ-carboxylate coordination is taking place. The extent is a little larger than with Cu^{II},[2] presumably because with Ni^{II} coordination is octahedral. NiL_2 could not be identified, and γ-carboxylate chelation does not significantly influence the subsequent coordination scheme. Complexes with AEAA and AAEA are even closer to those with tetra-alanine. Again, the most significant difference is found in the slightly higher stability of the NiL species, suggesting octahedral coordination from the β-carboxylate oxygens.

^1H *NMR spectra of* 4N *complexes*

To confirm the binding sites of tetrapeptides in the diamagnetic 4N complexes, ^1H NMR spectra at pH 11 were recorded for AAAD. The chemical shifts of metal-free and complexed tetrapeptides are given in Table 3. The large chemical shifts variations of all α-CH protons on binding to Ni^{II} confirm that all four nitrogens take part in coordination. For the Asp residue, the metal complexation leads to a decrease in the mole fraction of rotamer III in favour of rotamer I. The destabilization of rotamer III (removal of β-carboxylate group from over the complex plane; Fig. 3), indicates the formation of 4N planar complexes.

Zn^{II} *complexes*

Zinc is usually not able to promote amide nitrogen deprotonation, hence coordinating side chains are potentially even more important in the effective binding of peptides. Amongst these, the β-carboxylate of an Asp residue would be expected to be one of the more important, especially in systems which do not contain donor groups such as an imidazole ring or thiols. The peptides containing two or more Asp residues were therefore studied using pH-metric titrations and NMR to identify the extent of β-carboxylate binding. For the pH-metric studies, Zn^{II}: peptide ratios of 1:3 were used and, under these conditions, ZnL_2 complexes could not be detected convincingly; calculated stability constants are shown in Table 1.

Peptides with N-terminal Asp residues. Compared to complexes with simple peptides, these form more stable ZnL complexes as a result of β-carboxylate bonding. Equilibria of Zn^{II} with peptides above pH 7 are normally almost impossible to treat quantitatively with the precision expected. Polymeric complexes (e.g. $Zn_2H_{-2}L_2$, $Zn_3H_{-4}L_2$ etc.) have to be included, making it difficult to identify, unambiguously, the species which really exist (calculated stability constants usually have large standard deviations than usual[15]). These species probably involve hydroxide bridges linking Zn^{II} ions and these polymerize further to give $Zn(OH)_2$ precipitates. With Asp^1 peptides this behaviour is suppressed below pH 8 and, to a large extent, by the other peptides with Asp^1 residues. The experimental data can be fitted within experimental error to pH 10 with the complexes $ZnH_{-1}L$ and $ZnH_{-2}L$ [e.g. ZnL(OH) and $ZnL(OH)_2$] only; polymeric species are not

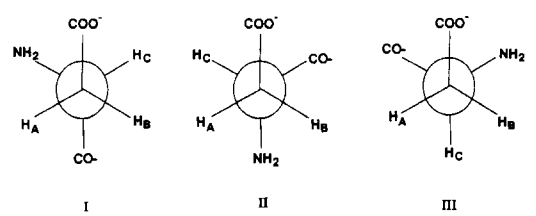

Fig. 3. Notation for rotamer isomers around the Asp^1 residue.

required. Again, this suggests that the peptides are acting as tridentate ligands.

^{1}H NMR spectra, recorded at pD 7.5, confirm that the β-carboxylate groups are coordinated. The chemical shift of the α-CH of the Asp^1 residues shows little change in the presence of zinc ions (0.04 ppm), while the β-CH_2 protons show shifts of up to 0.13 ppm. There are also clear changes in the rotamer populations of the Asp^1 residues (calculated as described previously[8] and using the rotamer notation shown in Fig. 2), where coordination to zinc stabilizes rotamer III (increase from 0.22 to 0.59) at the expense of rotamer I (decrease from 0.58 to 0.22). This indicates the coordination of three donors of the Asp^1 residue (NH_2, CO and β-COO^-) since only in rotamer III are they in a position for simultaneous binding.

Peptides with Asp residues in other positions. When the Asp residue is in second or subsequent positions stabilization through carboxylate bonding is less significant and partially hydrolysed species have to be assumed from pH 7. Again, goodness-of-fit with pH-metric data up to pH 10 are obtained by including only the monomeric species $ZnH_{-1}L$ and $ZnH_{-2}L$. The stability constants for the ZnL complexes are comparable to, but a little higher than, those for simple peptides (by NMR, the log K_{ZnL} value for triglycine is 3.18 and for tetraglycine 2.96[16]); chemical shifts for the β-CH_2 protons and in rotamer populations on addition of zinc ions are very small, suggesting no significant changes in conformation, as would be expected if β-carboxylate binding is insignificant. The titrations with Gly-Asp gave very consistent results with a high precision right up to pH 10. The ZnL_2 complex was a minor species and $ZnH_{-1}L$ could not be detected. It is interesting to note that, in this group of peptides, the $ZnH_{-2}L$ complexes appear to be almost identical.

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