Impact of α,β-dehydroamino acid residues on the binding abilities of di-, tri- and tetra-peptides

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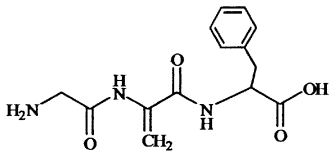
Insertion of a dehydroamino acid residue into a sequence of di-, tri- or tetra-peptide changed considerably the binding abilities of peptide ligands towards copper(II) ions. Potentiometric and spectroscopic (EPR, UV-VIS and CD) data have shown that the amide nitrogen of the dehydroamino acid residue is more effective in co-ordination than its parent analogue. In the case of the bulky ΔPhe residue also the (*Z*–*E*) isomerisation has a critical impact on the co-ordination equilibria in the system studied.

Introduction

Our previous works on the co-ordination abilities of α,β-dehydroamino acids (Δ-amino acids) have shown that a double bond placed between the α and β carbons in an amino acid molecule may have a critical impact on metal ion binding.[1–3] The reason for binding enhancement in the presence of a double bond in metal–dipeptide complexes seems to originate from its effect on the adjacent peptide nitrogen. Even metal ions like Zn^{II} and Co^{II} are able to deprotonate such an amide bond nitrogen, at relatively low pH, forming very strong complex species. Theoretical calculations showed that all dehydroamino acids except α,β-dehydroalanine tend to bend a dipeptide chain towards a turn conformation. This also has a strong effect on the co-ordination ability of dehydropeptides.[3]

Δ-Amino acids as peptide modifiers have become an important tool to provide derivatives of natural peptides (*e.g.* hormones[4,5]) with interesting biological activities. It should be also mentioned that the (*Z*)–(*E*) isomerism of Δ-amino acids could be critical for their biological activity. The (*Z*) isomers are much more common than the (*E*) isomers and are found *e.g.* in antibiotics.[1,6]

In this work we have performed studies on tri- and tetra-peptides having Δ-amino acid residues at different positions to complete our knowledge about the impact of a dehydroamino acid residue on metal–peptide interactions. To evaluate the effect of (*Z*) and (*E*) isomerism on complex formation we have performed also a study on the Cu^{II}–Gly-(*Z*)ΔPhe and Cu^{II}–Gly-(*E*)ΔPhe systems.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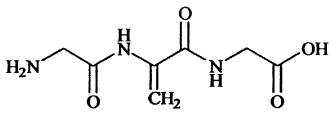
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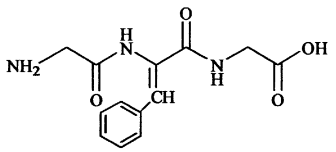
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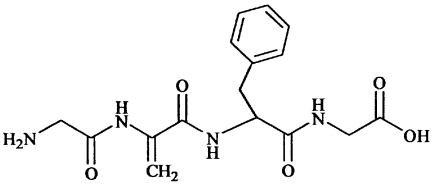
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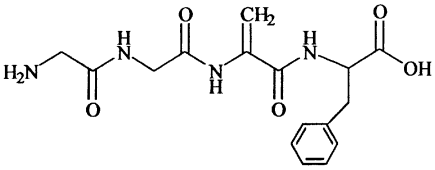
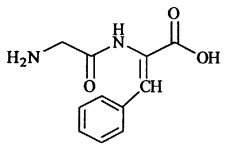

Gly-Gly-ΔAla-Phe

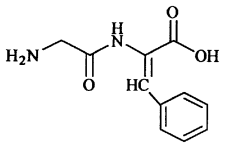
Experimental

Potentiometric studies

The titrations were performed at 25 °C using total volumes of 2.0 cm^3 and a MOLSPIN automatic titration system. Changes of pH were followed by using a glass calomel elec-



Gly-(*Z*)ΔPhe



Gly-(*E*)ΔPhe

trode (Russell CMAWL), calibrated for hydrogen-ion activity.[7] The relationship between activity and concentration was calculated daily by titration with HNO_3. All titrations were carried out under argon and at constant ionic strength of 0.1 M (KNO_3). A 0.05 M solution of $Cu(NO_3)_2$ was used as the stock for metal ion, and the metal to ligand molar ratios were 1 : 1 and 1 : 2. All titrations were performed over the pH range 2–10.5.

The calculations of the stability constants ($\beta_{pqr} = [M_p H_r L_q]/[M]^p[H]^r[L]^q$) were performed with the SUPERQUAD computer program.[8] The standard deviations quoted refer to random errors only.

Spectroscopic studies

Absorption spectra were recorded on a Beckman DU 650 spectrophotometer. Circular dichroism (CD) spectra on a JASCO J 715 spectropolarimeter in the 900–200 nm range and EPR spectra on a Bruker ESP 300E spectrometer at X-band (9.3 GHz) at 120 K. The metal concentration in spectroscopic measurements was adjusted to CD 1.5×10^{-3}, EPR 3×10^{-3} and UV–VIS 1×10^{-3}, mol dm^{-3}, and metal to ligand ratios were 1 : 2. The spectroscopic parameters were obtained at the maximum concentration of the particular species from the potentiometric calculations.

Peptide synthesis

Dipeptides were synthesized as described earlier.[9] Tripeptides were obtained according to the following scheme: Boc-dipeptides containing a C-terminal dehydroamino acid were coupled with dipeptide (for tetrapeptide) or amino acid (for tripeptide) *p*-nitrophenylanilides by means of the mixed carboxylic–carbonic anhydride method, using isobutyl chloroformate. Blocking groups were removed by a standard method with trifluoroacetic acid. The ligand purity was better than 98%, as checked with potentiometry, NMR and HPLC methods.

Results and discussion

Copper(II) complexes with Gly-(*Z*)ΔPhe and Gly-(*E*)ΔPhe

H^+ and copper(II) complex stability constants are collected in Table 1. Both isomers have very similar protonation constants for NH_2. However, different values of log *K* ($\Delta \log K = 0.72$) are observed for the CO_2^- function. This difference in p*K* indicates that the aromatic ring position may influence the acidity of carboxylate quite distinctly.

Potentiometric data suggest the formation of two different sets of species for both isomers (Table 1, Fig. 1). The Gly-(*Z*)-ΔPhe forms four complexes, two minor species CuL and $CuH_{-1}L_2$ and two major complexes $CuH_{-1}L$ and $CuH_{-2}L_2$. The same model was also found for other dehydrodipeptides, except X-ΔAla, for which a $CuH_{-2}L_2$ species could not be detected.[1,3] Spectroscopic data (Table 2) clearly show the $\{NH_2, N^-, CO_2^-\}$ and $2 \times \{NH_2, N^-\}$ binding modes for $CuH_{-1}L$ and $CuH_{-2}L_2$, respectively.[1,10] For the other isomer, Gly-(*E*)ΔPhe, only three species could be detected, $CuH_{-1}L$, $CuH_{-2}L$ and $CuH_{-1}L_2$. The $CuH_{-1}L$ complex with 2N co-ordination is of similar stability to that found for the (*Z*) isomer. However, the formation of the $CuH_{-2}L_2$ complex with the (*E*) isomer could not be seen. It seems that conjugation of double bonds in the CONHC=C fragment in the (*Z*) isomer is higher than that in the (*E*) isomer, which enhances the stability of $CuH_{-2}L_2$.[1] The formation of the 3N complex, $CuH_{-1}L_2$, with the $\{NH_2, ^-, CO_2^-, NH_2\}$ binding mode, by Gly-(*E*)ΔPhe is clearly supported by the spectroscopic data. The EPR spectra indicate the increase of $A_{\parallel}$ from 180 to 189 G, while $g_{\parallel}$ decreases from 2.248 to 2.220 above pH 9 (Table 2). This is a typical behavior of the EPR parameters when consecutive nitrogens are involved in copper(II) ion co-ordination.[1,10] It is interesting that the $CuH_{-1}L_2$ complex

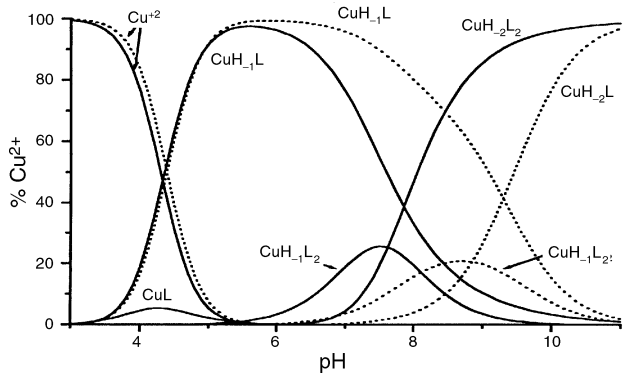


Fig. 1 Species distribution curves for Cu^{II}–Gly-(*Z*)ΔPhe (solid line) and –Gly-(*E*)ΔPhe (dotted line) for 1 : 2 Cu^{II} : L molar ratio and copper(II) concentration of 1×10^{-3} mol dm^{-3}.

Table 1 Stability constants of copper(II) complexes of dehydropeptides at 25 °C, $I = 0.10$ mol dm^{-3} (KNO_3)

	Dehydrodipeptides			Dehydrotripeptides				Dehydrotetrapeptides		
	Gly-(*Z*)ΔPhe	Gly-(*E*)ΔPhe	Gly-Phe[1]	Gly-ΔAla-Phe	Gly-ΔAla-Gly	Gly-(*Z*)ΔPhe-Gly	Gly-Ala-Gly[12]	Gly-Gly-ΔAla-Phe	Gly-ΔAla-Phe-Gly	tetraGly[13]
HL	7.99 ± 0.01	8.09 ± 0.01	8.09	7.74 ± 0.01	7.75 ± 0.01	7.82 ± 0.02	8.09	7.95 ± 0.01	7.99 ± 0.03	7.97
H_2L	11.15 ± 0.01	10.43 ± 0.02	11.08	10.72 ± 0.01	10.83 ± 0.01	11.09 ± 0.03	11.43	11.10 ± 0.01	11.68 ± 0.03	11.15
p*K*(NH_2)	7.99	8.09	8.09	7.74	7.75	7.82	8.09	7.95	7.99	7.97
p*K*(CO_2)	3.16	2.34	2.99	2.98	3.08	3.27	3.34	3.15	3.69	3.18
CuL	557 ± 0.09	—	5.58	—	—	5.19 ± 0.06	5.60	—	6.62 ± 0.02	5.07
$CuH_{-1}L$	2.19 ± 0.01	2.14 ± 0.01	1.76	1.58 ± 0.01	1.56 ± 0.01	0.85 ± 0.01	0.24	−0.05 ± 0.01	1.92 ± 0.02	−0.54
$CuH_{-2}L$	—	−7.15 ± 0.01	—	−3.58 ± 0.01	−3.44 ± 0.01	−5.20 ± 0.06	−5.50	−5.49 ± 0.01	−4.06 ± 0.02	−7.47
$CuH_{-3}L$	—	—	—	−15.54 ± 0.09	−14.88 ± 0.02	−13.77 ± 0.06	—	−14.04 ± 0.01	−13.44 ± 0.03	−16.78
$CuH_{-1}L_2$	5.74 ± 0.02	4.88 ± 0.04	4.94	—	—	—	—	—	—	—
$CuH_{-2}L_2$	−1.87 ± 0.01	—	—	—	—	0.68 ± 0.01	—	—	—	—
pK_1^{amide}	3.38	—	3.82	5.16	5.00	4.34	5.36	—	4.70	5.61
pK_2^{amide}	7.61	—	—	—	—	6.05	5.74	5.44	5.98	6.93
pK_3^{amide}	—	—	—	—	—	—	—	8.55	9.38	9.31
pK_4	—	9.29	—	11.96	11.44	8.57	—	—	—	—

Table 2 Absorption, CD and EPR parameters for copper(II) complexes with dehydropeptides

Ligand	Species	λ/ nm	ε/ $dm^3\ mol^{-1}\ cm^{-1}$	λ/ nm	$\Delta\varepsilon$/ $dm^3\ mol^{-1}\ cm^{-1}$	$A_{\parallel}$/ $cm^{-1} \times 10^4$	$g_{\parallel}$
Gly-(*E*)ΔPhe	$CuH_{-1}L$	631	97			180	2.248
	$CuH_{-1}L_2$	604	142			189	2.220
Gly-(*Z*)ΔPhe	$CuH_{-1}L$	644	91			175	2.252
	$CuH_{-2}L_2$	[a]				194	2.196
Gly-(*Z*)ΔPhe-Gly	$CuH_{-1}L$	640	73			171	2.275
	$CuH_{-2}L_2$	[a]				195	2.194
Gly-ΔAla-Gly	$CuH_{-1}L$	683	17			174	2.257
	$CuH_{-2}L$	543	157			198	2.194
Gly-ΔAla-Phe	$CuH_{-1}L$	635	55	273	−1.690	179	2.256
				309	+0.033		
				553	−0.660		
	$CuH_{-2}L$	543	149	273	−1.190	198	2.196
				304	+0.160		
				553	−1.120		
	$CuH_{-3}L$	543	133	273	−0.980	197	2.203
				300	+0.264		
				553	−1.040		
Gly-ΔAla-Phe-Gly	$CuH_{-1}L$	628	60			172	2.252
	$CuH_{-2}L$	558	96	271	−0.373	192	2.214
				300	+0.326		
				556	−0.294		
	$CuH_{-3}L$	507	168	275	−0.566	203	2.180
				301	+0.546		
				460	+0.139		
				538	−0.549		
Gly-Gly-ΔAla-Phe	$CuH_{-1}L$	611	41				
	$CuH_{-2}L$	561	147	258	−2.536	188	2.217
				308	−1.072		
				553	+0.234		
				664	−0.183		
	$CuH_{-3}L$	523	181	277	−0.454	206	2.176
				303	+0.099		
				494	−0.294		
				571	+0.315		

[a] Unresolved broad shoulder around 450–500 nm.

of the (*E*) isomer is distinctly weaker than that of the (*Z*) isomer.

The variations of spectroscopic parameters in the case of the (*Z*) isomer are much more distinct and occur at pH above 6. The $A_{\parallel}$ changes from 175 to 194 G, while $g_{\parallel}$ decreases from 2.252 to 2.196. This indicates the formation of the major 4N species above pH 8 and it agrees with potentiometric data (Fig. 1) showing the formation of the $CuH_{-2}L_2$ species.

Copper(II) complexes with Gly-ΔAla-Gly, Gly-ΔAla-Phe and Gly-(*Z*)ΔPhe-Gly

Gly-ΔAla-Gly and Gly-ΔAla-Phe form identical sets of complexes with similar stabilities with Cu^{II} (Fig. 2a, Table 1). The presence of the dehydroamino acid residue in the second position enhances the binding ability of the first amide nitrogen. Thus, the CuL complex, which is usually a minor species in normal tripeptides, with the $\{NH_2, C{=}O\}$ binding mode, is not formed. Two other species, $CuH_{-1}L$ and $CuH_{-2}L$, with $\{NH_2, N^-\}$ and $\{NH_2, 2N^-, CO_2^-\}$ binding modes, respectively, are distinctly more stable than those of the parent tripeptide (Table 1, Fig. 2b). Of interest is the formation of the $CuH_{-3}L$ species. The pK values for the reaction $CuH_{-2}L \rightarrow CuH_{-3}L + H^+$ above 11 strongly indicate hydrolysis of the metal–carboxylate bond.[1,11]

The insertion of the (*Z*)ΔPhe residue into the second position changes considerably the binding ability of the tripeptide ligand. The three species $CuH_{-1}L$, $CuH_{-2}L$ and $CuH_{-3}L$ are the same as those obtained for two tripeptides discussed above. The stability constants of the complexes of the latter

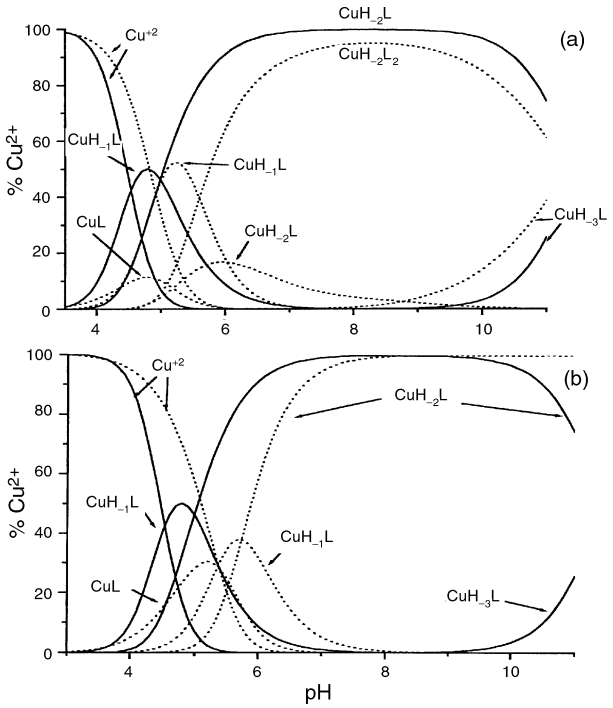


Fig. 2 Species distribution curves for (a) Cu^{II}–Gly-ΔAla-Gly (solid line) and Cu^{II}–Gly(*Z*)ΔPhe-Gly (dotted line) and (b) Cu^{II}–Gly-ΔAla-Gly (solid line) and Cu^{II}–Gly-Ala-Gly (dotted line). Conditions as in Fig. 1.

tripeptide are, however, considerably different from those obtained for the two peptides with Δ-Ala inserted into the second position. The $CuH_{-1}L$ complex of Gly-(*Z*)ΔPhe-Gly is about 0.7 log unit weaker than that of Gly-ΔAla-Gly or Gly-ΔAla-Phe and the $CuH_{-2}L$ complex is about 1.6 log unit weaker, respectively (Table 1). These results may indicate that in the $CuH_{-1}L$ and $CuH_{-2}L$ complexes with the $\{NH_2, N^-\}$ and $\{NH_2, 2N^-, CO_2^-\}$ co-ordination modes, respectively, the (*Z*)ΔPhe residue decreases considerably the binding ability of the amide nitrogens. On the other hand, log *K* of the hydrolysis reaction $CuH_{-2}L \rightarrow CuH_{-3}L + H^+$ for the latter peptide is much lower (8.57) than for the other two peptides (11.5–11.9, Table 1). This may indicate that the (*Z*) isomer of ΔPhe has a very strong impact on the metal–carboxylate bond making it weak. The position of the aromatic ring of Phe in the (*Z*) isomer induces strong steric hindrance on the carboxylate, making its binding to Cu^{II} difficult. This type of steric effect was predicted earlier by theoretical calculations performed for Cu^{II}–dipeptide systems.[3] This weak metal–carboxylate binding allows the second molecule of the tripeptide to enter the co-ordination, and formation of the $CuH_{-2}L_2$ species is observed. The latter complex is a major species above pH 6 (Fig. 2a). The $CuH_{-2}L$ complex, which is a major species above pH 5 for both Gly-ΔAla-Xaa ligands and for the parent Gly-Ala-Gly (Fig. 2b), is only a minor complex in the case of the Cu^{II}–Gly-(*Z*)ΔPhe-Gly system (Fig. 2a). The EPR spectra exhibit ^{14}N hyperfine splitting (HFS) in their perpendicular segments (Fig. 3). There is a rather clear difference in the number of HFS lines, which is 7 for Gly-ΔAla-Gly and 9 for the (*Z*)ΔPhe analogue. These results support the formation of the 3N species, $CuH_{-2}L$, as a major complex for the former tripeptide and 4N $CuH_{-2}L_2$ complex for the latter ligand.

Copper(II) complexes with Gly-ΔAla-Phe-Gly and Gly-Gly-ΔAla-Phe

The stabilities of the proton and copper(II) complexes obtained from potentiometric calculations are collected in Table 1. The data for tetraGly are given for comparison. Both peptides give similar sets of species as tetraGly, although the concentration of the CuL complex for Gly-Gly-ΔAla-Phe is too low to be recorded by potentiometric measurements. The stability constants obtained for both dehydropeptides are distinctly different from those of tetraGly and they also differ from each other (Table 1). The species distribution curves clearly show that insertion of Δ-Ala into the second position makes the tetrapeptide a much more efficient ligand for Cu^{II} in acidic solutions (Fig. 4). The decrease of the copper(II) aquaion concentration for this ligand is much more rapid than that of the two other peptides indicated in Table 1. However, above pH 5, when the 3N species ($CuH_{-2}L$) and then the 4N ($CuH_{-3}L$) species are formed their formation is easier for Gly-

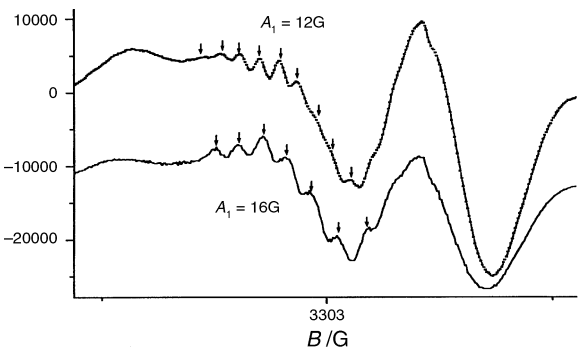


Fig. 3 EPR spectra of $CuH_{-2}L$ (below) and $CuH_{-2}L_2$ (above) for L = Gly-ΔAla-Gly and Gly-(*Z*)ΔPhe-Gly, respectively. Arrows indicate ^{14}N hyperfine splitting.

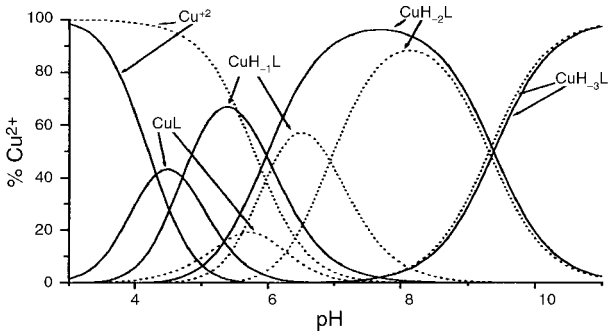


Fig. 4 Species distribution curves for Cu^{II}–Gly-ΔAla-Phe-Gly (solid line) and Cu^{II}–tetraGly (dotted line). Conditions as in Fig. 1.

Gly-ΔAla-Phe. This is clear when pK^{amide} values (dissociation of amide nitrogen in the presence of metal ion) are taken into account (Table 1). The lowest pK^{amide} is observed for the amide nitrogen of the dehydroamino acid residue. Thus, the second ΔAla favors the formation of $CuH_{-1}L$ (2N) species, while insertion of this residue into the third position causes easier formation of the $CuH_{-2}L$ (3N) complex.

Conclusion

The data discussed above clearly indicate that a Δ-amino acid residue may have a critical impact not only on the stabilities of the complexes formed but also favor the formation of very specific complex species not found for parent ligands. The $CuH_{-2}L_2$ complexes formed in the case of di- and tri-peptides are unique for ligands having a Δ-amino acid residue in their sequences. The presence of the α,β-double bond in a Δ-amino acid residue has a very distinct impact on the amide nitrogen binding ability, due to the coupling of this bond with the π-system of the amide moiety. The insertion of the Δ-amino acid may also induce steric hindrance on the C-terminal carboxylate function allowing easier co-ordination of the second ligand in short dehydropeptides (see also refs. 1–3). It is also worthy of note that the (*E*) and (*Z*) isomers are critical for the structures and stabilities of the complexes formed.

Acknowledgements

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