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Co-ordination of copper(II) ions by prolyl-α,β-dehydroamino acids: comparative studies and general considerations

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Potentiometric and spectroscopic measurements and theoretical calculations have revealed that α,β-dehydroamino acid residues have a considerable effect on the co-ordination ability of an adjacent amide nitrogen towards Cu^{2+} ions. Also the side chain of such residues affects the stability constants and, in some cases, the binding mode of short peptides containing α,β-dehydroamino acid residues. The theoretical calculations showed that all dehydroamino acids except α,β-dehydroalanine tend to bend a peptide chain towards a turn conformation. This has a very strong impact on the co-ordination ability of a dehydropeptide ligand.

α,β-Dehydroamino acids (Δ-amino acids) as peptide modifiers have become important tools to provide analogues of peptide hormones with improved properties and to study structure–biological activity relationships.[1,2] Dehydroalanine operates in the catalytic site of ammonia lyses and its reactivity has recently been throughly examined.[3–7] Dehydroalanine complexes of nickel(II) were tested for the stereoselective synthesis of non-proteinogenic amino acids.[8] Our recent studies have shown that Δ-dipeptides display very unusual binding ability towards such metal ions as Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}.[9,10] The binding pattern of Cu^{2+} ions with these ligands is the same as that with the saturated parent dipeptides, however the stability constants of the complexes formed are altered. The stability of copper(II) complexes with Xaa-Δ-Ala-OH (Xaa = Gly, Phe or Val) and Gly-Δ-Xaa-OH (Xaa = Val, Leu or Phe) are, respectively, distinctly and slightly higher than those of the corresponding species with the saturated peptides.[9] These two types of unsaturated dipeptides also differ considerably in their interaction with Ni^{2+} ions: Xaa-Δ-Ala-OH form octahedral species, while Gly-Δ-Xaa-OH (Xaa = Leu or Phe) give square-planar bis complexes. The ions Zn^{2+} and Co^{2+} are able to deprotonate and bind only to the amide nitrogen of common peptides containing the histidyl residue in the position next to the terminal-N.[11] However, these ions deprotonate and co-ordinate the amide nitrogen of α,β-dehydrodipeptides at relatively low pH.[10]

The unusual structural and chemical features of Δ-amino acid residues inserted in a peptide sequence necessitates investigation of the basic properties of this family of peptides. So far, most studies have been limited to compounds containing the Δ-Phe-OH residue,[2] mainly perhaps because of its convenient chemical synthesis. This situation inspired us to undertake comparative studies on the conformational propensities of the series of model dipeptides MeCO-Pro-Δ-Xaa-NHMe, where Δ-Xaa-OH = Δ-Ala-OH, (*Z*)-Δ-Phe-OH, (*Z*)-Δ-Leu-OH, Δ-Val-OH, (*Z*)- and (*E*)-Δ-Abu-OH (Abu = 2-aminobutanoic acid) and the respective saturated compounds MeCO-Pro-Xaa-NHMe.[1,12–17] The data obtained clearly indicate (*i*) an almost perpendicular arrangement of the $C^{\alpha}=C^{\beta}$ double bond and the peptide bond, as unsaturated amino acids tend to induce a β-turn conformation of the peptide chain,[1,12–15] and (*ii*) the unique behaviour of the Δ-Ala-OH peptide; Δ-Ala-OH is the only α,β-dehydro residue with an extended conformation which allows π-electronic conjugation of its double and peptide bonds.[15–17]

In this work we present the binding ability of the series of Pro-Δ-Xaa-OH dipeptides and their saturated counterparts towards Cu^{2+} ions. The same amino acid sequence as in the conformational models investigated allowed us to analyse the general features of α,β-dehydropeptides responsible for their unusual behaviour in metal-ion binding.

Experimental

Peptides

α,β-Dehydrodipeptides were synthesized as described previously[18] by condensation of N^{α}-$PhCH_2OCO$-Pro-NH_2 with the appropriate α-oxo acid and removal of the protecting group with anhydrous trifluoroacetic acid or HBr–acetic acid. They are of 99.4–100% purity according to HPLC. The compounds Pro-Δ-Abu-OH and DL-Pro-Δ-Phe-OH·0.33 H_2O have the (*Z*) configuration. The saturated peptides Pro-Ala-OH·H_2O, Pro-Phe-OH and Pro-Val-OH·H_2O were from Sigma. The compound Pro-Abu-OH was obtained from $PhCH_2OCO$-Pro-OH and Abu-OMe·HCl by use of isobutyl chlorocarbonate, the methyl ester group was saponified and the protecting group removed. The crude product was crystallized from water–ethanol; m.p. 507–509 K (decomp.), 97.0% purity (HPLC) (Found: C, 53.5; H, 8.05; N, 14.0. Calc. for $C_9H_{16}N_2O_3$: C, 53.75; H, 8.00; N, 13.95%).

The purity and the exact concentrations of the dipeptides in solution were determined by the Gran method.[19]

Potentiometric measurements

The stability constants of complexes with H^+ and Cu^{2+} were determined by pH-metric titrations in 5 cm^3 samples in the range pH 3–9. The concentration of the $CuCl_2$ stock solution was checked gravimetrically *via* quinolin-8-olate. A peptide concentration of 4×10^{-3} mol dm^{-3} and metal : dipeptide ratios of 0 : 1, 1 : 1, 1 : 2 and 1 : 3 were used throughout the experiments. The ionic strength of the samples was adjusted to 0.2 mol dm^{-3} with KCl. The titrations were performed with a 0.2 mol dm^{-3} carbonate-free KOH solution. The pH was measured at 298 K with a Radiometer PHM 64 pH-meter equipped with a GK2301 combined electrode. The electrode system was calibrated by the

Table 1 Selected torsion angles (°) and bond lengths (Å) in optimized structures of acetylamino acids $H_3C^{\alpha}_0$–C'_0–N_1–C^{α}_1–C'_1–O″H *

	MeCO-Δ-Ala-OH	MeCO-Δ-Val-OH	MeCO-L-Ala-OH
Torsions			
$\varphi(C'_0$–N_1–C^{α}_1–$C'_1)$	180.0	85.1	120.0
$\psi(N_1$–C^{α}_1–C'_1–O″)	180.0	−29.1	−51.3
Bond lengths			
N_1–C^{α}_1	1.40	1.43	1.45
C^{α}_1–C'_1	1.48	1.49	1.51

* Formulated according to ref. 25.

method described by Irving *et al.*[20] to convert pH readings into hydrogen-ion concentrations. The stability constants of the complexes $\beta_{pqr} = [M_pH_rL_q]/[M]^p[H]^r[L]^q$ were calculated from the pH-metric titration curves with the PSEQUAD computer program.[21]

Spectroscopic measurements

Absorption spectra were recorded on a Beckman DU-650 spectrophotometer and circular dichroism spectra (CD) on an automatic JASCO J-600 spectropolarimeter in the same concentration range as that used for potentiometric studies, EPR spectra on a Bruker ESP 300E spectrometer at 120 K at X-band (9.3 GHz) with ethane-1,2-diol–water (1 : 2) as solvent. The d–d transition energies and the charge-transfer (c.t.) bands collected in Table 3 as well as the EPR parameters were used to confirm the co-ordination modes in the particular species.[9,22]

Theoretical calculations

In order to check whether the conformational features previously found for protected MeCO-Pro-Δ-Xaa-NHMe molecules could correspond to those of the dipeptides studied in this work, with a dehydroamino acid residue present, we performed *ab initio* self-consistent field (SCF) calculations on two *N*-acetylamino acids, MeCO-Δ-Ala-OH and MeCO-Δ-Val-OH, and for comparison on one saturated system MeCO-L-Ala-OH using a SUN Classic workstation and GAMESS program package.[23] The starting geometries were taken from the global minima of the conformational energy maps of the φ–ψ torsion space of the respective *N*-acetylamino acid *N*′-methylamides MeCO-(Δ)-Xaa-NHMe.[16] Geometries of the *N*-acetylamino acids *in vacuo* were fully optimized at the 3-21G level. Mulliken atomic charges[24] were calculated for the resulting structures using the 6-311G basis set.

Results and Discussion

Selected torsion angles and bond lengths in optimized MeCO-(Δ)Xaa-OH structures are given in Table 1. Fig. 1 shows these structures with total Mulliken atomic charges on selected atoms. The restricted orientation of the β substituents on the C^{α}=C^{β} in MeCO-Δ-Val-OH results in its folded conformation. The lack of these steric interactions allows MeCO-Δ-Ala-OH to be fully extended. The structural features of these simple systems are then very similar to those found in the protected dipeptides. Of the electronic interactions, most noticeable are the raised negative charge on the nitrogen atom and the increased polarization of the N–H bond as compared to those in MeCO-Ala-OH. These effects are more pronounced in planar MeCO-Δ-Ala-OH than in the folded MeCO-Δ-Val-OH molecule.

Protonation constants of the dipeptides studied are collected in Table 2. There are two steps in all cases, corresponding to the carboxylate (p$K \approx 3.0$) and amino group (p$K \approx 8.0$). Comparison of these constants of saturated and α,β-dehydro dipeptides clearly indicates that the double bond at the C-terminal α-carbon atom exerts only a slight influence on their acid–base

Table 2 Logarithms of the protonation constants of Pro-Xaa-OH peptides at 298 K and $I = 0.2$ mol dm^{-3}

Dipeptide	HL	H_2L
Pro-Ala-OH	8.83(1)	11.98(1)
Pro-Abu-OH	8.83(1)	12.11(1)
Pro-Val-OH	8.78(2)	12.14(2)
Pro-Phe-OH	8.60(2)	11.70(3)
Pro-Δ-Ala-OH	8.56(1)	11.37(1)
Pro-Δ-Abu-OH	8.76(2)	12.17(2)
Pro-Δ-Val-OH	8.91(1)	12.67(1)
Pro-Δ-Phe-OH	8.73(2)	12.06(2)

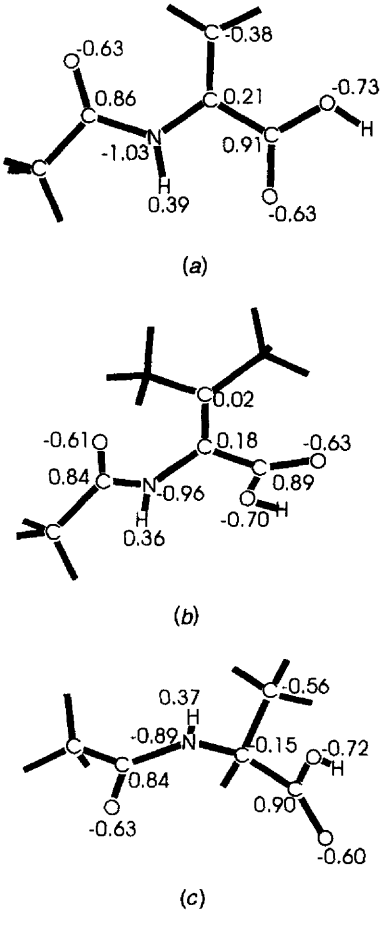

Fig. 1 Lowest minimum-energy conformation of MeCO-Δ-Ala-OH (*a*), MeCO-Δ-Val-OH (*b*) and MeCO-L-Ala-OH (*c*) along with standard Mulliken atomic charges on selected atoms as calculated with the 3-21G and 6-311G basis set, respectively

properties. It results in a small increase in the acidity of the carboxyl group of Pro-Δ-Ala-OH, while a small decrease is characteristic of all other Δ-peptides. Similar trends were observed for Gly-Δ-Xaa-OH dipeptides.[9] The stability constants and spectroscopic data for the copper(II) complexes obtained in this work are shown in Table 3. The metal-ion speciation of the simple saturated Pro-Xaa-OH dipeptides is

Table 3 Stability constants (log β) and spectroscopic data[a] for the copper(II) complexes of Pro-Xaa-OH peptides at 298 K and I = 0.2 mol dm^{-3} (G = 10^{-4} T)

			EPR		UV/VIS		CD
Dipeptide	Complex	log β	$A_{\parallel}$/G	$g_{\parallel}$	λ/nm	ε/dm^3 mol^{-1} cm^{-1}	λ/nm (Δε/dm^3 mol^{-1} cm^{-1})
Pro-Ala-OH	CuL	5.81(19)	150	2.33			
	$CuH_{-1}L$	2.73(1)	180	2.25	627	113	693 (−0.13),[b] 600 (+0.09),[b] 521 (−0.08),[b] 320 (+0.29),[c] 240 (−4.23)[d]
	$CuH_{-1}L_2$	5.37(8)					
	$CuH_{-3}L_2$	−1.84(13)					
	$CuH_{-2}L$	−6.60(1)	155	2.24	624	107	606 (+0.28),[b] 507 (−0.22),[b] 309 (+0.35),[c] 241 (−6.11)[d]
	pK^{amide} 3.08						
Pro-Abu-OH	CuL	6.26(8)	150	2.33			
	$CuH_{-1}L$	2.64(1)	182	2.24	625	112	690 (−0.18),[b] 598 (+0.11),[b] 520 (−0.11),[b] 322 (+0.35),[c] 241 (−4.88)[d]
	$CuH_{-1}L_2$	5.39(10)					
	$CuH_{-2}L$	−6.62(4)	155	2.24	622	105	605 (+0.31),[b] 505 (−0.25),[b] 309 (+0.51),[c] 241 (−6.42)[d]
	pK^{amide} 3.62						
Pro-Val-OH	CuL	6.69(5)	152	2.33			
	$CuH_{-1}L$	2.30(2)	183	2.25	627	114	660 (−0.47),[b] 318 (+0.85),[c] 250 (−4.53)[d]
	$CuH_{-1}L_2$	5.13(9)					
	$CuH_{-2}L$	−7.02(3)	168	2.23	624	106	678 (−0.36),[b] 583 (+0.23),[b] 501 (−0.33),[b] 308 (+0.95),[c] 249 (−5.64)[d]
	pK^{amide} 4.39						
Pro-Phe-OH	CuL	5.80(3)					
	$CuH_{-1}L$	3.03(1)	186	2.24	626	104	648 (−0.56),[b] 328 (−0.14),[c] 274 (−3.79)[d]
	$CuH_{-1}L_2$	5.98(8)					
	$CuH_{-2}L$	−6.05(4)	160	2.24	623	91	668 (−0.32),[b] 578 (+0.08),[b] 504 (−0.18),[b] 321 (−0.14),[c] 266 (−4.28),[d] 245 (−2.55)[d]
	pK^{amide} 2.77						
Pro-Δ-Ala-OH	CuL	6.48(7)					
	$CuH_{-1}L$	4.16(1)	188	2.25	628	112	730 (−0.13),[b] 606 (+0.23),[b] 499 (−0.04),[b] 338 (−0.16),[c] 272 (−2.33)[d]
	$CuH_{-1}L_2$	6.16(24)	178	2.24			
	$Cu_2H_{-3}L_2$	0.73(20)					
	$CuH_{-2}L$	−5.32(2)	135	2.23	619	100	655 (+0.41),[b] 500 (−0.12),[b] 300 (+0.35),[c] 275 (−1.82)[d]
	pK^{amide} 2.32						
Pro-Δ-Abu-OH	CuL	5.91(16)					
	$CuH_{-1}L$	2.54(1)	175	2.25	646	117	
	$CuH_{-1}L_2$	5.93(10)					
	$CuH_{-2}L_2$	−2.47(5)	196	2.20	534	113	
	$CuH_{-2}L$	−7.16(3)					
	pK^{amide} 3.37						
Pro-Δ-Val-OH	CuL	6.20(4)	156	2.33			
	$CuH_{-1}L$	1.90(1)	176	2.25	642	133	724 (+0.08)[b]
	$CuH_{-1}L_2$	5.03(8)					
	$CuH_{-2}L_2$	−3.66(4)	196	2.19	525	139	
	$CuH_{-2}L$	7.16(4)					
	pK^{amide} 4.30						
Pro-Δ-Phe-OH	CuL	6.39(12)					
	$CuH_{-1}L$	3.21(1)	176	2.25	640	107	
	$CuH_{-2}L_2$	−1.16(8)	200	2.20	530	138	740 (−0.07),[b] 565 (−0.02)[b]
	pK^{amide} 3.18						

[a] Assignments of the absorption and CD bands made according to refs. 9 and 22. [b] d–d Transition. [c] $N^- \rightarrow Cu^{II}$ c.t. [d] $NH_{Pro} \rightarrow Cu^{II}$ and $CO_2^- \rightarrow Cu^{II}$ c.t.

similar to that of glycylglycine.[26] The complex-formation reactions with Pro-Δ-Xaa-OH dipeptides are, however, distinctly different from those for the parent saturated dipeptides. The most unique species for peptides Pro-Δ-Xaa-OH, except Pro-Δ-Ala-OH, is the bis complex $CuH_{-2}L_2$, which as judged from spectroscopic data has four-nitrogen co-ordination, *i.e.* 2(NH,N$^-$). The compound Pro-Δ-Ala-OH forms a very strong complex $CuH_{-1}L$ instead (see below).

Copper(II) complexes with Pro-Δ-Ala-OH

Among the peptides studied Pro-Δ-Ala-OH has the lowest pK^{amide} (Table 3). Formation of the very strong complex $CuH_{-1}L$ [co-ordination mode (NH_2,N$^-$,CO_2^-)] with this peptide results from both the easy deprotonation of the amide nitrogen and the easy CO_2^- co-ordination. These are caused by two features found earlier for the dipeptide MeCO-Pro-Δ-Ala-

NHMe[15,17] and the amide MeCO-Δ-Ala-NHMe[16] and also confirmed in this work for the simple derivative MeCO-Δ-Ala-OH (Table 1, Fig. 1). That is the stable extended structure of the CONHC(=CH)CO_2H moiety (*i*) allows π-electronic conjugation of the amide bond with the $C^{\alpha}=C^{\beta}$ double bond, which acidifies the amide hydrogen making it more available for metal-ion co-ordination, and (*ii*) results in a torsion angle $\psi \approx 180°$ placing the Δ-Ala-OH carboxylate in a very favourable equatorial position to close the second chelate ring. It is noteworthy that the N-terminal residue in Xaa-Δ-Ala-OH (Xaa = Gly, Val or Phe) exerts only a minor influence on the stability of the $CuH_{-1}L$ complex.[9,10] The exception is the Pro residue which is most effective in all the peptides examined, both saturated and unsaturated ones (Table 3). The impact of the C-terminal dehydroamino acid residue on the stability of $CuH_{-1}L$ is, however, critical. The difference between the stability constants for Pro-Δ-Val-OH and Pro-Δ-Ala-OH amounts to more than two orders of magnitude, while that for the corresponding saturated peptides is much less (Table 3). The effective binding of Cu^{2+} by the amide nitrogen and the carboxylate in Pro-Δ-Ala-OH essentially obviates the formation of 4N species $CuH_{-2}L_2$ so characteristic for other Pro-Δ-Xaa-OH dipeptides (see below), but on the other hand favours the formation of the 3N complex $CuH_{-1}L_2$, which is the most stable among those investigated (Table 3).

Copper(II) complexes with Pro-Δ-Xaa-OH (Xaa = Val, Phe or Abu)

Apart from Δ-Ala-OH, all other dehydro residues induce a turn conformation in Pro-Δ-Xaa-OH dipeptides.[12-15,17] This may cause the electronic interaction between the $C^{\alpha}=C^{\beta}$ double bond and the peptide bond to be minor.[16] The pK^{amide} values for those peptides (Table 3) are indeed distinctly higher than that for Pro-Δ-Ala-OH and only slightly lower than those for the saturated analogues. The turn tendency brings about that the effect of the torsion angle on the carboxylate group positioning may be much different in these peptides from that in Pro-Δ-Ala-OH, which is clearly seen in the optimized structures of MeCO-Δ-Ala-OH and MeCO-Δ-Val-OH (Table 1, Fig. 1). This tendency destabilizes the carboxylate co-ordination and the formation of the complex $CuH_{-1}L$. The compound Pro-Δ-Val-OH for which no extended structure is accessible[16] yields the least-stable complex among these species. So, a less favourable co-ordination position of the carboxylate makes easier the formation of the 4N $CuH_{-2}L_2$ complex than is the case for Pro-Δ-Ala-OH and saturated analogues Pro-Xaa-OH (*cf.* Table 1 and Fig. 1). All saturated dipeptides give the $CuH_{-1}L_2$ complex with two ligands bound to the metal. This binding mode, however, according to spectroscopic data,[9,22] involves a 3N co-ordination (NH_2, N^-, CO_2^-) of one ligand molecule and the NH_2 group of the other. The particular behaviour of the Δ-Phe-OH residue is also interesting. This most rigid[1,14,15] and most effective hindrance-inducing dehydroamino acid, when inserted in the peptide sequence, forms the strongest $CuH_{-1}L$ and $CuH_{-2}L_2$ complexes among those with the Pro-Δ-Xaa-OH investigated.

Conclusion

Taken together, this and earlier work[1,9,10,12-17] demonstrate that at least two effects should be critical for the speciation and stabilities of the complexes formed by Δ-dipeptides. One is the electronic effect of the $C^{\alpha}=C^{\beta}$ double bond on the increase in charge on the amide nitrogen and the polarization of the N–H bond. This makes the metal-ion binding to the amide nitrogen of dehydropeptides easier when compared to that with the saturated common analogues, and is most pronounced in the extended conformation of the Δ-Ala-OH peptide system. The second effect is of steric nature and relies on the tendency of any α,β-dehydroamino acid residue, with the exception of Δ-Ala-OH, to bend the peptide chain towards a turn conformation, which affects the positioning of the carboxylate group. This may destabilize the involvement of this group in α,β-dehydropeptides in the formation of some complexes characteristic of the corresponding common peptides.

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