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The Interaction of Copper(II) with a Lipotetrapeptide

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Abstract

The lauroyl tetrapeptide N^2-[*N*-(*N*-lauroyl-L-alanyl)-α-D-glutamyl]N^6-(glycyl-)DD, LL-2,6-diaminopimelamic acid (LTP-DAP) has been shown by potentiometry and spectroscopy to interact with Cu(II) to form the complexes [CuL], [$CuLH_{-1}$], [$CuLH_{-2}$] and, at high pH, possibly [$CuLH_{-3}$] also. Stability constants have been measured at 25 °C and an ionic strength of 0.10 mol dm^{-3} (KNO_3). Stereoselectivity between the diastereoisomers of the ligand was found to be small, probably as a result of the flexibility of the ligand molecule.

Introduction

The lauroyl tetrapeptide N^2-[*N*-(*N*-lauroyl-L-alanyl)-α-D-glutamyl]N^6-(glycyl-)DD, LL-2,6-diaminopimelamic acid (LTP-DAP shown in Fig. 1) was the first synthetic lipopeptide which has immunostimulating activity [1, 2]. Numerous analogues have been synthesised by the research laboratories of Rhone-Poulenc Santé [3] and these have been submitted to a series of physiological and biological investigations. The behaviour of such lipopeptides would be expected to be dependent on pH and on the presence of metal ions, particularly those able to deprotonate the amide nitrogen atoms of peptide bonds such as Cu^{2+}, since the chelated complexes formed would force a specific conformation on the peptide chain. Unfortunately

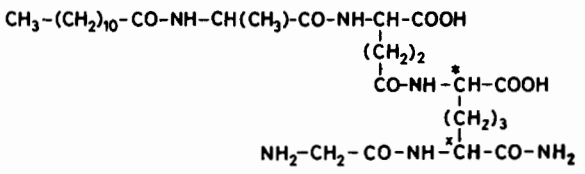

Fig. 1. Formula of LTP-DAP.

there appears to be a total absence of information on the physiochemical characteristics of lipopeptides such as ionization constants or interaction with metal ions.

We wish to report the results of a study of the three optical isomers of LTP-DAP in which the asymmetry of the carbon atoms of the pimelic acid residue, identified by * and x in Fig. 1, has been varied: LL-LTP-DAP (* = x = L), DD-LTP-DAP (* = x = D) and meso-LTP-DAP (* = L, x = D). Techniques used were micro-scale potentiometry, electron paramagnetic resonance (EPR) and circular dichroism (CD) spectroscopy.

Experimental

Lipopeptides

LTP-DAP isomers were synthesised in the laboratories of Rhone-Poulenc Santé.

Potentiometric Studies

Stability constants for H^+ and Cu^{2+} complexes were calculated from titration curves carried out at 25 °C using total volumes of 1.0–1.5 ml. Alkali was added from a 0.1 ml micrometer syringe which had been calibrated by both weight titration and the titration of standardized materials. Changes in pH were followed using a glass electrode calibrated in H^+ concentrations with $HClO_4$ [4]. All solutions were of ionic strength 0.10 mol dm^{-3} (KNO_3) and peptide concentrations of 0.001 mol dm^{-3}. Calculations were made with the aid of the SUPERQUAD computer program [5]. This allows for the refinement of total ligand concentrations and was able to confirm the purity of the peptides studied and in particular the absence of acetate (a frequent impurity in peptide samples) or other coordinating ions. In all cases duplicate or triplicate titrations were carried out at

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TABLE I. Stability Constants of Hydrogen Ion and Cu(II) Complexes of LTP-DAP at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

	Log β values		
	LL-LTP-DAP	DD-LTP-DAP	*meso*-LTP-DAP
[HL]	8.26(2)	8.28(1)	8.23(1)
[H_2L]	12.49(4)	12.36(3)	12.48(3)
stepwise constants	4.23	4.08	4.25
[H_3L]	15.43(9)	15.38(9)	15.57(8)
stepwise constants	2.94	3.02	3.09
[CuL]	6.19(5)	6.26(6)	6.12(5)
[CuLH_{-1}]	−1.30(9)	−1.06(11)	−0.72(6)
[CuLH_{-2}]	−10.65(10)	−10.48(13)	−9.44(7)
[CuLH_{-3}]	−21.60(12)		−20.10(9)

Cu:L ratios of 1:1. The 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.

Spectroscopic Studies

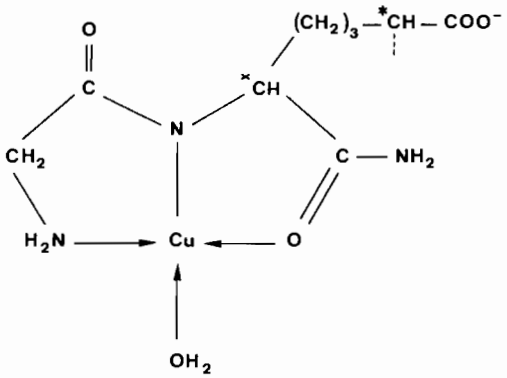
Solutions of $Cu(ClO_4)_2 \cdot 6H_2O$ and peptide (concentration range 0.0006–0.001 mol dm^{-3}) in the ratios 1:1 to 1:2 were used. Circular dichroism (CD) spectra were recorded on a Mark III Jobin-Yvon dichrograph in the 250–800 nm region. All CD spectra are expressed in terms of $\Delta\epsilon$ ($\epsilon_l - \epsilon_r$). Electron paramagnetic resonance (EPR) spectra were obtained on a Varian E 120 spectrometer at liquid nitrogen temperature and at 9.13 GHz. Diphenylpicrylhydrazine was used as a standard.

Results and Discussion

Calculated protonation constants and copper complex stability constants are given in Table I. The lipopeptides (H_2L) behave as tribasic acids. Starting in alkaline solution the first centre to protonate is the terminal amine nitrogen (log K_{HL} = 8.25, the comparable value with tetraglycine is 7.97 [6]) followed by the carboxylate oxygens (log K values 4.1 and 3.0 approximately, the comparable value with tetraglycine being 3.3). The values found are therefore close to those expected and do not show the stereoselectivity found in the protonation constants of chiral dipeptides [7], presumably because the chains separating the protonation centres in the lipopeptides are much longer and very flexible. As a result, around pH 5 the lipopeptide would exist as the zwitterionic, monoprotonated form, [HL]$^{\pm}$

In the presence of Cu(II) the first complex to form would be expected to involve chelation through the terminal $-NH_2$ nitrogen (requiring deprotonation) and the neighbouring carbonyl oxygen to give the complex [CuL] (designated as 110). This complex (log β = 6.2) is comparable to the first complex formed by tetraglycine (log β = 5.1) [4] but close comparison of the magnitudes of the values is complicated by the large hydrophobic side-chain of the lipopeptide.

Above pH 6, copper(II) is able to deprotonate the peptide nitrogen atom next to the coordinated carbonyl group to form a Cu–N$^-$ bond (the [CuLH_{-1}] (charges omitted for clarity) or 11-1 complex shown in Scheme 1). This complex is significantly less stable



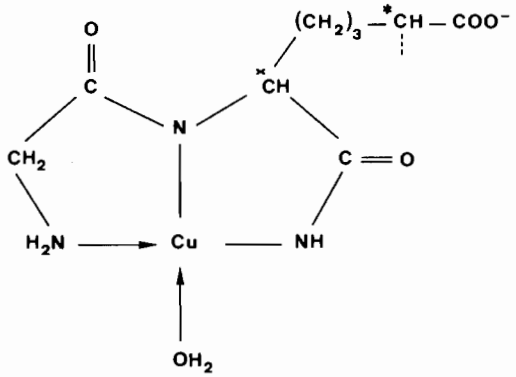
Scheme 1. Proposed structure for the [CuLH_{-1}] complex of LTP-DAP with Cu(II).

than with tetraglycine (log β = −0.3), probably as a result of the steric effect of the long side chain. The [CuLH_{-1}] complexes of the LL, DD and *meso* isomers are of almost the same stability, suggesting only small or insignificant stereoselectivity.

The third complex to form, starting around pH 8, is the [CuLH_{-2}] species with the second amido nitrogen deprotonated and coordinated to copper. The fourth coordination position in the plane of the copper ion is probably occupied by a water molecule (Scheme 2). There is the possibility that one of the carboxylate oxygen atoms could interact but this would necessitate an abnormally large chelate ring.

TABLE II. CD Data for LTP:Cu(II) Complexes

	λ_{d-d} nm ($\Delta\epsilon$)	λ_{N^-Cu} nm ($\Delta\epsilon$)	λ_{NH_2-Cu} nm ($\Delta\epsilon$)
LL-LTP-DAP			
[CuLH$_{-1}$]	660 (−0.34)	305 (+0.62)	270 (−1)
[CuLH$_{-2}$]	580 (−0.54)	305 (+0.60)	270 (−1)
[CuLH$_{-3}$]	600 (−0.9)	298 (+0.70)	260 (−2.5)
	500 (−0.7)		
DD-LTP-DAP			
[CuLH$_{-1}$]	660 (+0.30)	312 (−0.9)	270 (+0.9)
[CuLH$_{-2}$]	580 (+0.60)	308 (−0.8)	270 (+1)
[CuLH$_{-3}$]	600 (+0.56)	290 (−0.8)	260 (+2.2)
	500 (−0.30)		
meso-LTP-DAP			
[CuLH$_{-1}$]	670 (+0.40)	310 (−1)	270 (+1)
[CuLH$_{-2}$]	580 (+0.74)	310 (−1)	270 (+1)
[CuLH$_{-3}$]	600 (+0.80)	295 (−1)	260 (+2.6)
	800 (+0.40)		



Scheme 2. Proposed structure for the [CuLH$_{-2}$] complex of LTP-DAP with Cu(II).

Coordinated water molecules usually hydrolyse around pH 10–11, hence the proton ionization corresponding to [CuLH$_{-2}$] → [CuLH$_{-3}$] is almost certainly a water hydrolysis reaction.

The potentiometric results were confirmed by the spectroscopic studies. The CD spectrum of [CuLH$_{-1}$] shows a band at about 670 nm (d–d transition). This is entirely compatible with a 2N complex [8] (Table II). The sign of the Cotton effect is positive for the DD and *meso* isomers and negative for the LL isomer. This result shows unambiguously that coordination takes place from the glycine residue since the sign of the Cotton effect is not dependent on the configuration of the asymmetric carbon atom, x. In the UV region two bands are observed, at 305 and 270 nm. These can be assigned to N^-–Cu and NH_2–Cu charge transfer transitions respectively [9]. The band at 305 nm has a negative Cotton effect for the DD and *meso* isomers and a positive one for the LL isomer. The opposite is true for the band at 270 nm.

Above pH 8, the d–d band shifts to 580 nm, corresponding to a 3N species, and an isobestic point is observed at 650 nm in each isomer. At very high pH (above 11) two bands are observed in the d–d region, at 600 and 500 nm. These are probably due to the E and B transitions which overlap at lower pH. This may result from a change of symmetry around the metal ion resulting from the replacement of water in the fourth coordination site by OH^-. The two bands have the same relative intensity in the 1:1 and 1:2 metal:ligand ratios. This rules out coordination of a fourth nitrogen. At high pH the NH_2–Cu and N^-–Cu charge transfer transitions are shifted slightly to higher energies (260 and 295 nm respectively).

EPR studies confirm these results. Depending on the pH, 3 different species are clearly present. At about pH 7 the spectrum is characteristic of that for a 2N species ($A_{\parallel}$ = 165 G, $g_{\parallel}$ = 2.27) [10]. At higher pH the spectrum changes to become typical of a 3N complex ($A_{\parallel}$ = 190 G, $g_{\parallel}$ = 2.23) and, above pH 11, a new spectrum is observed ($A_{\parallel}$ = 190 G, $g_{\parallel}$ = 2.25) but still consistent with that expected for a 3N complex, as demonstrated in Fig. 2.

Stability constants of the [CuLH$_{-2}$] complexes of the LL and DD isomers do not differ significantly while the *meso* complex is significantly more stable ($\Delta \log \beta = 1.0$). A similar, but smaller, stabilization is also found in the [CuLH$_{-1}$] complex. Also, in the d–d region, the band characteristic of the 3N species appears at a slightly lower pH with the *meso* isomer than with the DD or LL isomers. The reason for this

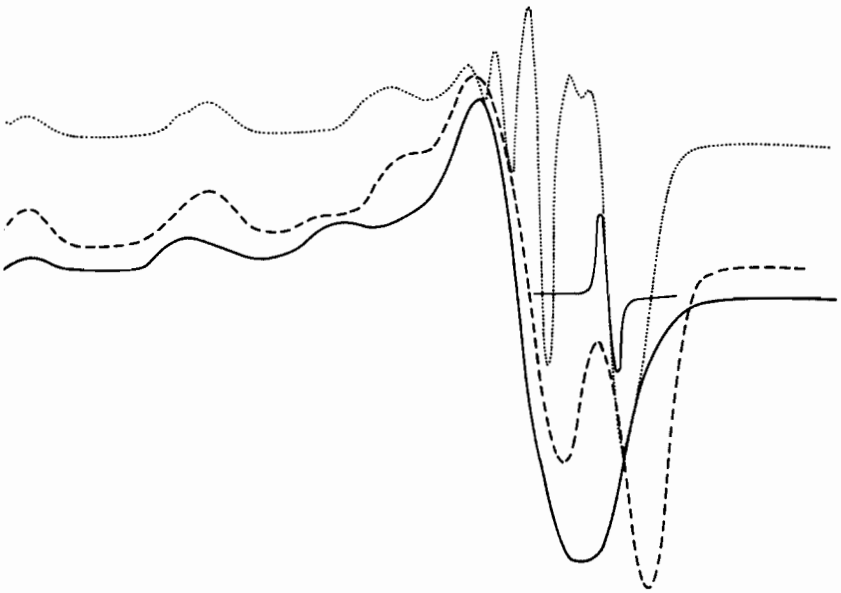


Fig. 2. EPR spectra of LTP-DAP:Cu 1:1 complexes. ——, pH = 7; – – –, pH = 9.5; ·····, pH = 11.5.

stabilization is not immediately obvious. It may be that the hydrophobic side chain can optimize hydrophobic interactions above the coordination plane when the lipopeptide has the meso chirality since stereoselectivity in Cu(II) complexes of chiral dipeptides is always greatest when the hydrophobic side chains are able to be close together, on the same side of the coordination plane. However, because of the long chain, almost any possible confirmation can be envisaged.

Acknowledgement

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