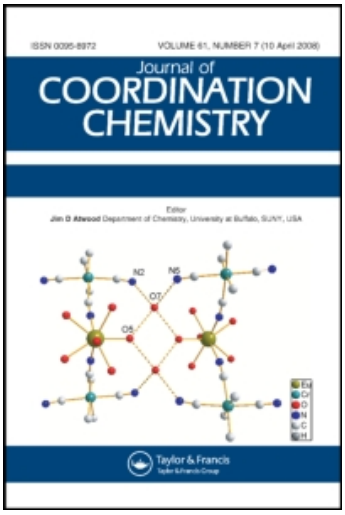

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COORDINATION OF COPPER(II) TO CYCLIC PEPTIDES WITH A CYSTEINIC DISULFIDE BRIDGE: COMPLEX STABILITY AND VISIBLE ABSORPTION SPECTRA

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COORDINATION OF COPPER(II) TO CYCLIC PEPTIDES WITH A CYSTEINIC DISULFIDE BRIDGE: COMPLEX STABILITY AND VISIBLE ABSORPTION SPECTRA

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Solutions containing copper(II) and an −S−S− bridged cyclic oligopeptide (general formula $\overline{\text{Cys-(Gly)}_n\text{-Cys}}$, CG_nC, $0 \leq n \leq 4$) in aqueous KCl (0.1 mol dm^{-3}) were studied at 25°C by potentiometric titration methods and absorption spectrometry. The ligands studied were found to give rise to a number of complexes; besides several (3 to 5) monoligand complexes, the formation of at least one (up to 5) bis-ligand species was detected for each ligand. Stability constants of all detected complex species (4 to 11 per ligand) were determined. From the acidity constants of individual complexes (computed from stability constants) the hapticities of the ligands were inferred. Visible absorption spectra of all examined solutions contain one peak whose position shifts hypsochromically (≈740 nm ... 540 nm) with increasing deprotonation and hapticity, indicating changes in the coordination geometry of the copper(II) ion, probably from tetragonal towards distorted tetrahedral. With CG_2C, and additional absorption peak (588 nm) was osbserved.

KEYWORDS: copper(II)-peptides, cyclic peptides, peptide complexes, stability constants

INTRODUCTION

The disulfide (−S−S−) bridge is a very frequent structural fragment in peptides and proteins (*e.g.*, oxytocin, vasopressin, insulin). Owing to their well-defined structure, synthetic cyclic disulfides have aroused considerable interest in recent years, serving as useful models for explaining structure-activity relationships,[1] as well as for studying conformational properties of peptides.[2,3] However, interactions

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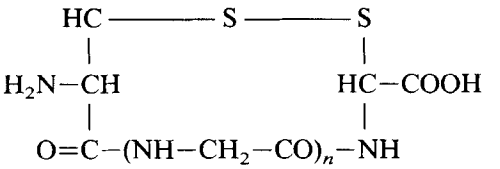
of these peptides with metal ions has not yet been systematically studied, in spite of the fact that metal ions may appreciably influence their physiological activity. A recent study of salt-dependent structural changes of neurohormones has shown that some metal ions (*e.g.*, lithium) may induce conformational rearrangements of the peptides with resulting changes in binding domains.[4]

Coordination compounds of −S−S− bridged cyclopeptide ligands should be of interest from the theoretical point of view because, besides the common peptide-metal bonding modes (*via* carboxyl oxygen, amino and imide nitrogens), interaction with the disulfide bridge is also possible. If present, such interaction should give rise to additional stabilisation while steric strain in the new ring thus formed should have an opposite influence on complex stability. Compounds from this class have not yet been systematically studied, except for the paper by Pettit *et al.*[5] on complexes of copper(II) and nickel(II) with vasopressin and its derivatives, and our report[6] on the synthesis and protonation constants of a series of compounds having the general formula shown below.



($0 \leq n \leq 4$, abbreviations: CC, CGC, . . ., CG_4C).

The aim of the present paper was to study copper complexes formed by these compounds, by using potentiometric titration methods and visible absorption spectrometry. Because, with the aforementioned ligands, not only mono-ligand species (as in the case studied by Pettit *et al.*[5]) but also bis-ligand complexes could be expected, two ligand/metal mole ratios (1:1 and 2:1) were used in the titrations.

EXPERIMENTAL

The ligands were synthesised according to previously described procedures.[6] All other chemicals were of analytical grade. Water was first de-ionised and then distilled twice in an all-glass still.

Potentiometry

Stability constants of copper(II) complexes with cyclic dipeptides were determined at 25 ± 0.05°C in an aqueous KCl (0.1 mol dm^{-3}) solution by potentiometric titration. This ionic medium was chosen instead of (much more common) KNO_3 in order to provide simpler UV spectra (see the next section) and for compatibility with our earlier work.[6] The formation of comparatively unstable chlorocopper complexes[7] was estimated to be negligible under the experimental conditions chosen.

The apparatus used was described elsewhere.[6] An acidified (HCl) peptide solution (10 cm^3, ligand concentration *ca* 0.002 mol dm^{-3}, $[CuCl_2]$ *ca* 0.002 or 0.001 mol dm^{-3}) was titrated in a thermostatted double-walled glass vessel with aqueous KOH

(0.1 mol dm^{-3} with the same KCl background). Purified and pre-equilibrated nitrogen gas was bubbled through the solution during the titration.

The measuring system was calibrated in terms of hydron* *concentration*. Therefore, a calibration titration of an HCl solution (in aq. KCl) was performed before and after each experiment. Initial estimates of stability constants were refined by using the two programmes, BEST[8] (mostly for exploratory computations) and SUPERQUAD[9] (for final estimation). Acidity constants reported earlier[6] were used in all computations. Species whose concentrations never exceeded *ca* 2 per cent of total metal concentration were considered to be uncertain and were omitted in final computations because the uncertainty range of p[H] measurement was estimated to be ± 0.005.

Electronic Spectrophotometry

Since only limited amounts of ligands were available, electronic spectra had to be recorded *during* the titrations. A Hewlett-Packard 8452A diode array spectrophotometer (equipped with a flow-through cell, $l = 1$ cm, and a peristaltic pump) was used for this purpose. As the total constituent concentrations were chosen so as to maximise the accuracy of potentiometric measurements the optimum conditions for spectroscopic measurements (especially in the UV region) could not be met.

RESULTS

Both methods[8,9] of analysis of potentiometric data led to identical sets of complexes, with closely similar estimates of cumulative stability constants† of copper(II) complexes with the studied ligands. The final estimates of β_{qnp} are summarised in Table 1, together with their standard errors which are typically of the order of ± 0.05 log units. For several species the constants are somewhat less precise and this could be explained by poor stability of the ligand, low concentration of the species in question or neglect of a marginal species. Having in the view the rather rich speciation in most cases, the precision of the constants was deemed satisfactory.

Most of the complexes that have been inferred from the potentiometric data are weak Brønsted acids and/or bases. Their acid/base properties are best seen from the acidity constants (see Table 2) which were computed from the relevant cumulative stability constants, (*1*)

$$pK^{a}_{qnp} = \lg(\beta_{qnp-1}/\beta_{qnp}). \qquad (1)$$

* In compliance with present IUPAC recommendations, the terms *hydron* and *hydronation* are used instead of the more usual *proton* and *protonation*, respectively, in the following text.

† Although not in accordance with IUPAC recommendations, it is advantageous to define the stoichiometric constants as *dimensionless quantities, viz*,

$$\beta_{qnp} = \frac{[M_qL_nH_p]\cdot(c^\circ)^{q+n+p+1}}{[M]^q[L^n[H]^p}$$

(c° = mol/L), whereby any possible subsequent calculation (*e.g.* taking logarithms) becomes both simple and dimensionally consistent.

Table 1 Cumulative stability constants* for copper(II) complexes, $[Cu_qL_nH_p]$, with cyclic oligopeptides, HL, at T = 298.2 K, in aqueous KCl (0.1 mol dm^{-3}).

Ligand	Complex	lg β_{qnp}	p[H] range	max. α_{qnp}¶ 1:1	2:1
CC	CuL	4.66(4)	3.0 ... 7.0	30	35
	$CuLH_{-1}$	−0.75(2)	4.3 ... 10.1	90	75
	$CuLH_{-2}$	−9.77(4)	7.2 ... 10.1	85	100
	CuL_2H_{-1}†	[2.3]	5.6 ... 10.6	30	30
CGC	CuL	3.40(11)	3.1 ... 6.5	15	15
	$CuLH_{-1}$	−1.01(3)	3.7 ... 8.5	80	65
	$CuLH_{-2}$	−8.34(4)	5.4 ... 11.1	90	45
	$CuLH_{-3}$	−19.19(6)	8.3 ... 11.1	60	60
	CuL_2H_{-1}	1.85(10)	4.4 ... 8.7	20	20
	CuL_2H_{-2}	−4.67(6)	5.4 ... 11.5	60	60
CG_2C	CuL	3.60(16)	3.5 ... 6.9	15	20
	$CuLH_{-1}$	−1.45(4)	4.3 ... 8.3	80	70
	$CuLH_{-2}$	−8.91(8)	5.7 ... 8.4	30	35
	$CuLH_{-3}$	−16.40(6)	6.6 ... 11.1	90	95
	$CuLH_{-4}$	−27.23(11)	8.7 ... 11.1	40	55
	CuL_2H_{-1}†	[1.6]	5.0 ... 8.5	25	25
CG_3C	CuL	3.88(18)	3.1 ... 6.0	20	20
	$CuLH_{-1}$	−0.82(4)	3.8 ... 7.8	70	50
	$CuLH_{-2}$	−7.27(7)	4.9 ... 8.7	60	25
	$CuLH_{-3}$	−14.58(7)	5.9 ... 11.0	30	50
	$CuLH_{-4}$	−24.64(11)	8.1 ... 11.0	80	10
	CuL_2H_{-1}	2.72(9)	4.4 ... 7.8	40	40
	CuL_2H_{-2}	−3.91(13)	5.3 ... 9.0	30	30
	CuL_2H_{-3}	−11.57(21)	6.4 ... 10.5	30	30
	CuL_2H_{-4}	−20.21(11)	7.3 ... 11.0	90	90
CG_4C	CuL	4.09(3)	3.1 ... 6.2	20	20
	$CuLH_{-1}$	−0.88(6)	4.1 ... 7.9	50	40
	$CuLH_{-2}$	−7.23(2)	5.0 ... 9.3	60	38
	$CuLH_{-3}$	−14.84(2)	6.2 ... 11.0	100	90
	$CuLH_{-4}$	−26.61(7)	8.6 ... 11.0	10	10
	CuL_2	8.13(12)	4.3 ... 6.9	12	12
	CuL_2H_{-1}	2.83(4)	4.6 ... 8.3	40	40
	CuL_2H_{-2}	−4.32(12)	5.7 ... 9.3	22	22
	CuL_2H_{-3}†	[−12.5]	6.9 ... 10.4	12	12
	CuL_2H_{-4}†	[−23.0]	9.3 ... 10.9	30	30

* Standard error is given in parentheses. † This species was not confirmed in all experiments.
¶ $\alpha_{qnp} = [Cu_qL_nH_p]/C_{Cu}$.

Table 2 Acidity constants (expressed as pK^a_{qnp}) for copper(II) complexes, $[Cu_qL_nH_p]$, with cyclic oligopeptides, HL, at T = 298.2 K, in aqueous KCl (0.1 mol dm^{-3})

Complex	CC	CGC	CG_2C	CG_3C	CG_4C
CuL	5.41	4.41	5.05	4.70	4.97
$CuLH_{-1}$	9.02	7.33	7.46	6.45	6.35
$CuLH_{-2}$		10.85	7.49	7.31	7.61
$CuLH_{-3}$			10.83	10.06	11.77
CuL_2					5.3
CuL_2H_{-1}		6.52		5.44	7.15
CuL_2H_{-2}				7.66	8.2
CuL_2H_{-3}				8.64	10.5

Visible absorption spectra of the (acid) solutions to be titrated contained one or two peaks located above 700 nm. With the addition of alkali, the peaks gradually shifted towards shorter wavelengths and, at sufficiently high p[H], reached characteristic stable positions (λ_f). The general pattern of the p[H] dependence of the peak wavelength can be seen from the example shown in Figure 1 for (Cu^{+2} + CG_2C).

Unfortunately, because of the performance of the spectrometer and the sub-optimal design of the experiments (see Experimental section), the precision of the spectrophotometric data was not sufficient for resolving the recorded spectra into contributions from individual species. Therefore, only the final positions of the absorption peaks in each titration are given in Table 3.

DICUSSION

Visible absorption spectra

In each examined system, the position of the main LF peak gradually shifted towards shorter wavelengths with increasing p[H], that is with increasing dehydronation and increasing ligand hapticity. It can be seen from Table 3 that the difference in the c_M/c_L ratio (1:1 or 1:2) did not change the final peak positions and this fact may be taken as an indication, though not conclusive proof, of the similarity in the coordination patterns in dehydronated mono- and bis-ligand complexes.

It is well known that the position of the LF absorption peak in the spectra of copper(II) complexes with peptides depends on the number of nitrogen atoms bound to the central atom.[10–12] The numbers of bound nitrogens, estimated by using Pettit's tabulation,[12] are also shown in Table 3 where it can be seen that only CC complexes contain bidentate copper, while the other ligands are either tri- or tetradentate, at least in their dehydronated complexes. These estimates of ligand hapticities can be seen to be consistent with the stability data (*vide infra*). The

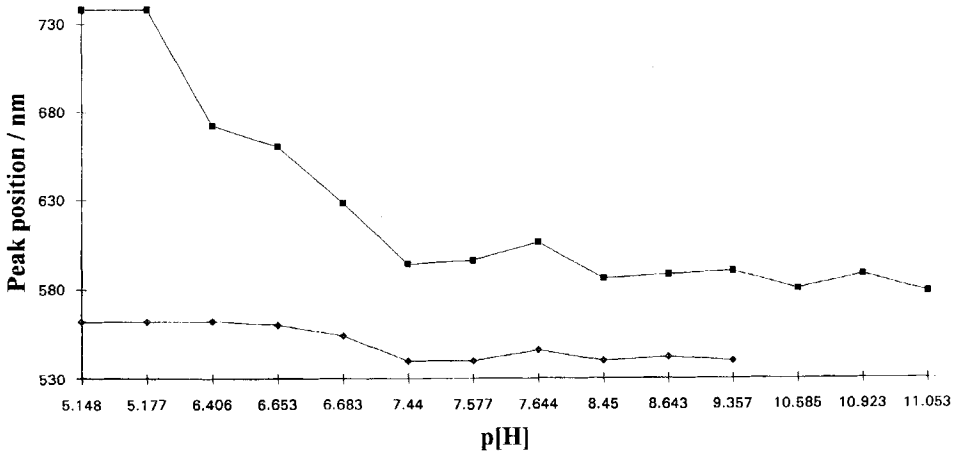


Figure 1 Positions of absorption peaks as a function of p[H] during the alkali titration of an acidified solution of CG_2C and Cu^{+2}.

Table 3 Final (high p[H]) positions (λ_f/nm) of absorption maxima; initial (low p[H]) wavelength: $\lambda_i > 700$ nm

Ligand	c_M/c_L		Coordination
	1:1	1:2	
CC	660	660	2N
CGC	542	541	3N
CG_2C	541	540	3N
	588	588	3N
CG_3C	508	508	4N
CG_4C	507	507	4N

presence of an additional peak in the spectrum of the Cu^{+2} + CG_2C system might possibly indicate the coexistence of two different 3N complexes but conclusive interpretation has to await more experimental data.

Mono-ligand complexes

With all ligands, HL, the existence of simple CuL complexes was detected by analysing the potentiometric data. These complexes are not only much less stable (by 2 to 5 log units) than the corresponding aminoacidatocopper(II) complexes (*cf.* for example β_{110} values of 8.27 and 7.00 for glycine[13] and cysteine,[14] respectively) but they are also less stable (by 1 to 2 log units) than, *e.g.*, glycylglycinatocopper(II).[15] However, it should be kept in mind that the basicities of the amino groups in both amino acids, as well as in glycylglycine, are considerably greater than the basicities of the ligands studied here. The stability constants of CuL complexes of the cyclic peptides are well correlated with the ligand basicities, *i.e.*, with the pK^a of the amino group; the correlation coefficient amounts to 0.943 ($\alpha < 0.01$), the correlation about the origin being as high as 0.9986 ($\alpha < 0.0001$). This correlation indicates that the ligating sites in the CuL complexes are probably the same, irrespective of the ligand.

Like most peptide complexes, CuL species were found to be polyhydric weak acids liberating 2 (CC), 3 (CGC) or 4 (CG_2C, CG_3C, CG_4C) hydrons at sufficiently high p[H] values. It can be seen in Table 2 that, for each ligand, the last pK^a_{qnp} value is much higher (range: 9 ... 12) than the preceding ones. The last pK^a_{qnp} value may be ascribed to the dehydronation of a coordinated water molecule[7b] while the preceding pK^a_{qnp} values reflect the displacement of peptide $-NH-$ hydrons upon formation of an additional coordination bond. The first pK^a_{qnp} values are clustered around 5 ± 0.5 and, with the exception of the $[Cu(CC)]^+$ complex, the penultimate pK^a_{qnp} values are all near 7.5. As the amino group is in all probability also coordinated the hapticities of the ligands are 2 (CC), 3 (CGC) and 4 (CG_2C, CG_3C, CG_4C).

Although pK^a_{qnp} values do give some information on the energy of the copper-imide bond, they can hardly be given a precise interpretation without knowledge of the acidity constants of $-NH-$ groups in the uncomplexed ligand. The same holds for β_{2np} values.

Bis-ligand complexes

The simplest bis-ligand species, CuL_2, was detected only in the $Cu^{+2} + CG_4C$ system. In all other systems it was either found to be negligible ($\alpha < 2\%$) or it was discarded by the computer programme as meaningless. Nevertheless, some of the dehydronated 'daughter species' have been detected; their pK^a_{qnp} values can be found in Table 2.

An uncertainty remains as to whether *any* bis-ligand complexes are formed with CC and CG_2C because the existence of CuL_2H_{-1} species was not confirmed in all experiments with these two ligands. Only two dehydronated bis-ligand species were found in the Cu^{+2} + CGC system. Surprisingly enough, the two bulkiest ligands, CG_3C and CG_4C, were found to form many bis-ligand species (4 and 5, respectively). Because of the smaller number of data for bis-ligand complexes, the measured β_{qnp} and pK^a_{qnp} values can hardly be given a sound interpretation.

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