Co-ordination Abilities of Thioamide Analogues of Amino Acids and Peptides. Copper(II) and Nickel(II) Complexes†

Teresa Kowalik and Henryk Kozłowski*
Institute of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland
Imre Sóvágó and Katalin Varnagy
Institute of Inorganic and Analytical Chemistry, Kossuth-University, H-4010 Debrecen 10, Hungary
Godfryd Kupryszewski and Krzysztof Rolka
Institute of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

Potentiometric and spectroscopic results for Cu^{II} and Ni^{II} complexes with leucine-*N*-methylamide, methionine-*N*-methylamide, their thiocarbonyl analogues, and for (phenylalanyl)methionine–*N*-methylamide are reported. For solubility reasons only spectroscopic results in ethanol solutions are presented for Cu^{II} complexes with the last of these. Thiocarbonyl was found to be much more effective in metal ion binding than carbonyl donor and the complexes formed with metal–sulphur bonds are several orders of magnitude more stable than the respective species with carbonyl binding. The sulphur donation, however, does not prevent thioamide nitrogen deprotonation and co-ordination at high pH.

Recent studies on the thioamide analogues of natural peptides have shown that the modification of a peptide bond, –CO–NH– to –CS–NH–, has a considerable effect on the biological activity of a biomolecule.[1,2] In metallopeptide systems the amide linkage is usually a critical binding site of many metal ions and it decides structural and thermodynamic features of the complexes formed.[3] Thus it was of biological as well as thermodynamic and structural interest to follow the co-ordination ability of the thioamide linkage and to compare its characteristic features with those of normal amide bonding.

The carbonyl group of a peptide bond appears to be a weak donor as far as Cu^{II} or Ni^{II} ions are concerned though it usually bonds with a metal ion by closing a chelate ring.[3] The thiocarbonyl group is quite a specific donor, *e.g.* in thiourea,[4] and its introduction to a peptide linkage might change dramatically the binding capability of the peptide (*e.g.* complex formation constants).

In this work we present a spectroscopic and potentiometric study of Cu^{II} and Ni^{II} complexes with methylthioamides of amino acids and dipeptides.

Experimental

In the pH-metric studies the ligand concentration varied between 1 and 4 × 10^{-3} mol dm^{-3}, while the metal ion to ligand ratio ranged from 1:1 to 1:4. Argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide, and for stirring the solutions during the titration with carbonate-free potassium hydroxide. Measurements were made with a Radiometer pHM64 pH-meter with G202B glass and K4Cl calomel electrodes and an ABU13 autoburette. The calculation of H^+ ion concentration from the pH measured and the other details of the pH-metric procedure have been described previously.[5] All pH-metric studies were carried out at 25 °C, at a constant ionic strength of 0.2 mol dm^{-3} (KCl). Stability constants were calculated with a general computational program published recently.[6] The standard deviations of the stability constants were ±0.02—0.04 log unit. E.s.r. spectra were recorded on a JEOL JES-ME-3X spectrometer at 120 K and 9.14 GHz. Absorption spectra were recorded on a Beckman UV5240 spectrophotometer. C.d. spectra were recorded on an automatic JASCO-J-20 spectropolarimeter in the 200—800 nm region. Results are expressed in terms of $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$.

Methylthioamides and dipeptides were prepared with Lawesson's reagent[1,7,8] using the classical procedure in solution. All were in hydrochloric form. The detailed description of the synthesis and elemental analysis of amides and thioamides used in this work is given in ref. 1. The purity of the ligands has been checked by pH-metric titrations.

Results and Discussion

The pK values of the ligands and their stability constants for the copper(II) and nickel(II) complexes are summarized in Table 1.

As can be seen from Table 1, the replacement of oxygen by sulphur in the amide group does not have a significant effect on the protonation constant of the amine group. The slight increase in basicity might be explained by the lower electronegativity of the sulphur atom.

As regards the complex formation reactions the species formed with copper(II) are the same for both amino acid amides and their thio derivatives, this implies that the species present are similar to those formed in the copper(II)—glycinamide systems.[9,10] However, the stability constants of the various complexes and consequently the concentration distribution curves are significantly different for amides and thioamides. In the case of thioamides, complexes of composition MA and MA_2 (overall charges are omitted throughout for clarity) have much higher stability constants compared to the amino acid amides, due to the higher donor strength of sulphur donor atoms. Figures 1 and 2 represent the corresponding concentration distribution curves for leucine-*N*-methylamide (leu-NHMe) and for thioleucine-*N*-methylamide (tleu-NHMe).

Comparison of Figures 1 and 2 reveals that CuA and CuA_2 type complexes of amino acid thioamides are formed at much lower pH than those of amino acid amides. For example, at pH *ca.* 4 almost 100% of a copper(II) ion is in the ligand-free solvated form, Cu^{2+}, *e.g.* in Cu^{II}–leu-NHMe system, while in the case of tleu-NHMe the formation of CuA_2 is almost completed by this pH value. The spectroscopic data for Cu^{II}–met-NHMe (methionine–*N*–methylamide) and leu-NHMe solution clearly indicate the formation of CuA and CuA_2 species below pH 7 (Table 2). The spectroscopic assignment of the respective species was made as described earlier.[11-14] The spectroscopic

† *Non-S.I. unit employed:* G = 10^{-4} T.

Table 1. Stability constants for copper(II) and nickel(II) complexes of various amides and thioamides (HA); T = 298 K, I = 0.2 mol dm^{-3} (KCl)*

Species	met-NHMe	tmet-NHMe	leu-NHMe	tleu-NHMe	phe-met-NHMe
HA	7.31	7.35	7.72	7.75	7.15
CuA	4.39	7.66	4.78	7.48	4.23
CuA_2	8.16	15.26	8.89	15.14	
CuA_2H_{-1}	1.12	7.10	1.73	6.72	2.50
CuA_2H_{-2}	−7.56	−2.13	−7.01	−2.46	
$CuAH_{-1}$					−0.24
$CuAH_{-2}$					−8.64
log K_1/K_2	0.62	0.06	0.67	−0.18	
Species					
NiA	2.79	4.23			
NiA_2		8.40			
NiA_2H_{-1}		−0.49			
NiA_2H_{-2}		−9.98			

* Charges for the various species are omitted throughout for clarity. $pM + qA + rH \rightleftharpoons M_pA_qH_r$; $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$

Table 2. Spectroscopic data for the copper(II) complexes with amides and thioamides of met, leu, and phe-met-NHMe in aqueous solutions[a]

	Absorption spectra (d–d transition)		C.d. spectra		E.s.r. spectra	
Ligand Species	λ/nm	ε/dm^3 mol^{-1} cm^{-1}	λ/nm	Δε/dm^3 mol^{-1} cm^{-1}	$g_{\|}$	$A_{\|}$/G
met-NHMe						
CuA	730	30	735	−0.18 (B + E)	2.288	150
CuA_2	655	60	635	−0.57 (B + E)	2.285	160
CuA_2H_{-2}	550	60	630	−0.475 (B)	2.211	187
			505	−0.214 (E)		
tmet-NHMe						
CuA					2.238[b]	151[b]
CuA_2	650	400	670	−1.44 (B)	2.223	164
	350	6 000	557	+0.33 (E)		
			340	+4.5 S→Cu^{II}		
CuA_2H_{-2}	540	400	530	−0.22 (B + E)	2.161	174
			317	+1.6 N^-→Cu^{II}		
tleu-NHMe						
CuA					2.236[b]	153[b]
CuA_2	650	400			2.169	161
	361	3 500				
CuA_2H_{-2}	515	200			2.159	190
	310	1 800				
phe-met-NHMe						
$CuAH_{-1}$	620	140	640	+0.018 (B)		
			560	−0.139 (E)		
			325	+0.6 N→Cu^{II}		
$CuAH_{-2}$	576	140	616	+0.40 (B)	2.217	176
			505	−0.145 (E)		
			323	+0.394 N→Cu^{II}		

[a] See Table 1 for conditions. [b] Seen in e.s.r. spectra in ethanol solutions without addition of base.

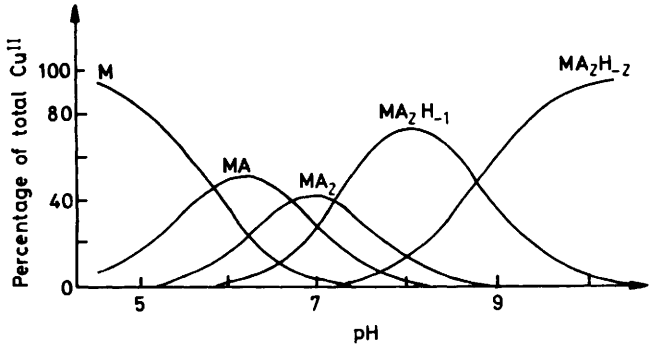

Figure 1. Species distribution curves for Cu^{II}–leu-NHMe (1:2) in aqueous solution

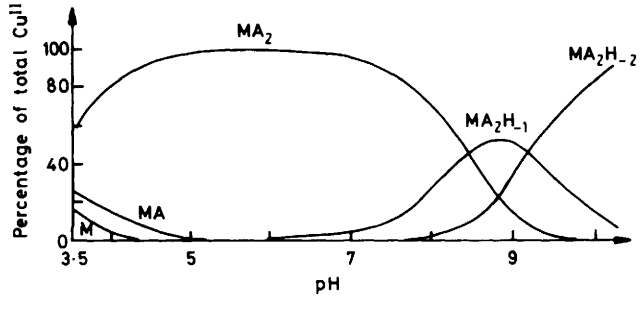

Figure 2. Species distribution curves for Cu^{II}–tleu-NHMe (1:2) in aqueous solution

Table 3. Absorption, c.d., and e.s.r. spectral data for Cu^{II}–phe-tmet-NHMe (1:2 molar ratio) in ethanol solution (x = mol of KOH per mol of Cu)

x	λ/nm	ε/dm³ mol⁻¹ cm⁻¹	Transition	λ/nm	Δε/dm³ mol⁻¹ cm⁻¹	Transition	$A_{\parallel}$(G)	$g_{\parallel}$
0	600	231	*d–d*	690	−0.373		169	2.211
	314	2 361	$N^-\rightarrow Cu^{II}$ (c.t.)	590	+0.425	*d–d*		
			+ intraligand	508	−0.344			
				366	+2.120	$S\rightarrow Cu^{II}$ (c.t.)		
				323	−2.666	$N^-\rightarrow Cu^{II}$ (c.t.)		
4	595	268	*d–d*	687	−0.186		178	2.208
				588	+0.501	*d–d*		
				503	−0.320			
				365	+2.800	$S\rightarrow Cu^{II}$ (c.t.)		
				322	−3.567	$N^-\rightarrow Cu^{II}$ (c.t.)		
8	582	272		687	−0.373		184	2.210
	307	5 640		575	+1.026	*d–d*		
				486	−1.294			
				362	+1.380	$S\rightarrow Cu^{II}$ (c.t.)		
				306	−3.150	$N^-\rightarrow Cu^{II}$ (c.t.)		
10	567	271		676	−0.466		unresolved spectrum	
	303	5 430		570	+0.548	*d–d*		
				485	−0.583			
				362	+1.300	$S\rightarrow Cu^{II}$ (c.t.)		
				306	−3.333	$N^-\rightarrow Cu^{II}$ (c.t.)		

Table 4. Absorption, c.d., and e.s.r. spectral data for Cu^{II}–phe-tmet-NHMe (1:2 molar ratio) in ethanol solution (x = mol of KOH per mol of Cu)

x	λ/nm	ε/dm³ mol⁻¹ cm⁻¹	Transition	λ/nm	Δε/dm³ mol⁻¹ cm⁻¹	Transition	$A_{\parallel}$(G)	$g_{\parallel}$
0	605	87	*d–d*	705	−0.142			
				596	+0.233	*d–d*		
				512	−0.206			
				370	+0.760	$S\rightarrow Cu^{II}$ (c.t.)		
				325	−1.180	$N^-\rightarrow Cu^{II}$ (c.t.)		
2	607	120	*d–d*	700	−0.176		unresolved spectrum	
				596	+0.250	*d–d*		
				517	−0.224			
				368	−0.880	$S\rightarrow Cu^{II}$ (c.t.)		
				321	−1.270	$N^-\rightarrow Cu^{II}$ (c.t.)		
4	580	150	*d–d*	600	+0.073		192	2.191
				595	−0.261	*d–d*		
				524	−0.297			
				365	+0.980	$S\rightarrow Cu^{II}$ (c.t.)		
				315	−1.212	$N^-\rightarrow Cu^{II}$ (c.t.)		
6	537	121	*d–d*	676	−0.600			
				591	+0.079	*d–d*		
				516	−0.582			
				351	+1.330	$S\rightarrow Cu^{II}$ (c.t.)		
				308	−1.450	$N^-\rightarrow Cu^{II}$ (c.t.)		
10	532	99	*d–d*	652	−0.288	*d–d*	198	2.185
				513	−0.312			
				360	−0.360	$S\rightarrow Cu^{II}$ (c.t.)		

absorption and c.d. spectral data for thioamides of methionine (tmet-NHMe) and leucine (tleu-NHMe) are considerably different than those of amino acid amides (Table 2).

In the absorption spectra at pH 3—8 the *d–d* band at 650 nm ($\varepsilon \simeq 400$ dm³ mol⁻¹ cm⁻¹ at pH 5.5) indicates that the major species is MA_2 with two nitrogens bound to Cu^{II} *i.e.* with two (NH_2, CS) donor sets involved.[11–14] The binding of the sulphur donor is also demonstrated by very high values of ε for the *d–d* transitions as well as by a strong absorption at 350 nm ($\varepsilon \simeq 6\,000$ dm³ mol⁻¹ cm⁻¹ at pH 6) which corresponds to the $S\rightarrow Cu^{II}$ charge-transfer (c.t.) transition.[15,16] Above pH 7 a new base-consuming process begins in both cases which, similarly to glycinamide,[9,10] can be attributed to deprotonation and co-ordination of amide nitrogen. Corresponding pH values are slightly lower in the case of amino acid amides. These results mean that the co-ordination of sulphur atoms in CuA and CuA_2 complexes results in a higher stability, which is especially significant for CuA_2 type complexes. This is reflected in the decrease of the log K_1/K_2 values. The formation of species with composition of CuA_2H_{-1} and CuA_2H_{-2}, however, suggests that sulphur donation cannot prevent deprotonation and co-ordination of amide nitrogen.

The co-ordination of thioamide nitrogen in MA_2H_{-1} and MA_2H_{-2} species is seen also in the spectroscopic data. At pH > 8 in both Cu^{II}–thioamide systems the *d–d* band shifts to higher energy (Table 2). The $S\rightarrow Cu^{II}$ c.t. band vanishes and the

new band is observed around 310 nm (ε = 1 800 dm^3 mol^{-1} cm^{-1}) which is usually assigned as a N→Cu^{II} c.t. transition (see also c.d. spectra in Table 2).

The complex formation reactions of (phenylalanyl)-methionine-*N*-methylamide (phe-met-NHMe) are very similar to that of glycylglycinamide.[9] Namely, deprotonation and co-ordination of two amide nitrogens occurs in two separate steps (with p*K* values of 4.47 and 8.40, respectively), with the formation of $CuAH_{-1}$ and $CuAH_{-2}$ complexes. Because of the low solubility of the thio derivative a pH-metric study could not be performed for the Cu^{II}–phe-tmet-NHMe [(phenylalanyl)thiomethionine-*N*-methylamide] and Cu^{II}–phe-tleu-NHMe systems. The Cu^{II} complexes with phe-tmet-NHMe and (phenylalanyl)thioleucine-*N*-methylamide (phe-tleu-NHMe) could be studied, however, in ethanol solution by c.d. absorption, and e.s.r. spectra. The spectroscopic data for Cu^{II}–phe-tmet-NHMe and Cu^{II}–phe-tleu-NHMe in ethanol solution are given in Tables 3 and 4.

These results clearly indicate that, without base addition, the major species in the complex with a (NH_2,N^-,S) donor set is $CuAH_{-1}$. The *d*–*d* transition at *ca.* 600 nm suggests that at least two nitrogen donors are involved in Cu^{II} co-ordination.[11–14] The transitions at *ca.* 310 nm in the absorption spectra and at *ca.* 320 nm in the c.d. spectra support the N^- donor binding of a peptide linkage and a band at *ca.* 360 nm (c.d. spectra) indicates binding of thiocarbonyl sulphur to the metal ion (see above).[15,16] The addition of a large amount of base leads to a considerable increase in the *d*–*d* transition energy in both cases and to the intensity decrease of the S→Cu^{II} charge transfer band (Tables 3 and 4). Thus, in the basic solutions the dipeptide thioamide forms MAH_{-2} species with a (NH_2,N^-,N^-) donor set, as was found in the dipeptide amides described above.

In the case of nickel(II) ion the complex formation with methionine-*N*-methylamide (met-NHMe) and with thiomethionine-*N*-methylamide (tmet-NHMe) was studied. Because of precipitation the stability constants can be calculated only for species NiA in the Ni^{II}–met-NHMe amide system. Similarly to copper(II), the thio derivative forms more stable complexes with nickel(II) and formation of NiA and NiA_2 complexes prevents precipitation. In basic solution a new base-consuming process is seen which leads to the formation of species NiA_2H_{-1} and NiA_2H_{-2} having square-planar geometry, similarly to nickel(II)–glycinamide.[17]

Summarizing the results of the equilibrium studies it can be stated that the presence of a thiocarbonyl group increases very distinctly the stability of copper(II) and nickel(II) complexes of type MA and MA_2. However, it is also obvious from the results that the thiocarbonyl group is not able to prevent an amide nitrogen deprotonation and its co-ordination in basic solutions. It means that complexes of MA_2H_{-1} and MA_2H_{-2} are not hydroxo complexes but they contain the deprotonated amide nitrogen in the co-ordination sphere of the metal.

Acknowledgements

This work was partly supported by the Polish Academy of Sciences.

References

1 K. Rolka, M. Kruszyński, G. Kupryszewski, U. Ragnarsson, K. Kolasa, W. Turski, and Z. Kleinrok, *Acta Pharm. Suec.*, 1984, **21**, 173.
2 K. Klausen, A. F. Spatola, C. Lemieux, P. Schiller, and S. O. Lawesson, *Biochem. Biophys. Res. Commun.*, 1984, **120**, 305.
3 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385 and refs. therein.
4 E. A. H. Griffith, W. A. Spofford, and E. L. Amma, *Inorg. Chem.*, 1978, **17**, 1913.
5 A. Gergely and I. Nagypál, *J. Chem. Soc., Dalton Trans.*, 1977, 1104.
6 L. Zékány and I. Nagypál, in 'Computational Methods for the Determination of Stability Constants,' ed. D. Leggett, Plenum Press, New York, 1985.
7 S. Scheibye, B. S. Pedersen, and S. O. Lawesson, *Bull. Soc. Chim. Belg.*, 1978, **87**, 229.
8 K. Clausen, M. Thorsen, and S. O. Lawesson, *Tetrahedron*, 1981, **37**, 3635.
9 I. Sóvágó, B. Harman, B. Radomska, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1986, 235.
10 H. Sigel, *Angew. Chem., Int. Ed. Engl.*, 1968, **7**, 137.
11 H. Kozłowski, M. Bezer, L. D. Pettit, M. Bataille, and B. Hecquet, *J. Inorg. Biochem.*, 1983, **18**, 231.
12 L. D. Pettit, I. Steel, G. Formicka-Kozłowska, T. Tatarowski, and M. Bataille, *J. Chem. Soc., Dalton Trans.*, 1985, 535.
13 L. D. Pettit, I. Steel, T. Kowalik, H. Kozłowski, and M. Bataille, *J. Chem. Soc., Dalton Trans.*, 1985, 1201.
14 G. Formicka-Kozłowska, H. Kozłowski, and B. Jeżowska-Trzebiatowska, *Inorg. Chim. Acta*, 1977, **25**, 1.
15 H. J. Schugar, in 'Copper Coordination Chemistry. Biochemical and Inorganic Perspectives,' eds. K. D. Karlin and J. Zubieta, Academic Press, New York, 1982, p. 43 and refs. therein.
16 H. Kozłowski and T. Kowalik, *Inorg. Chim. Acta*, 1979, **34**, L231.
17 T. F. Dorigatti and E. J. Billo, *J. Inorg. Nucl. Chem.*, 1975, **37**, 1515.

Received 16*th September* 1985; *Paper* 5/1596