Studies on Transition-metal–Peptide Complexes. Part 13.* Copper(II) and Nickel(II) Complexes of Amino Acids and Peptides containing a Thioether Group

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The stability constants of the copper(II) and nickel(II) complexes of amino acids [*S*-methyl-L-cysteine and methionine (Met)] and peptides [Met-Gly, Gly-Met, Met-His, and His-Met (Gly = glycine, His = histidine)] containing thioether groups have been determined. It was found that, as for the amino acids, the presence of the thioether group in the peptides (HA) has only a slight influence on the co-ordination of the ligands. For the peptides containing an N-terminal methionine residue (Met-Gly and Met-His), the axial interaction of the thioether group may be appreciable, which inhibits the formation of complexes $[MA_2H_{-1}]^-$.

Recent research results have clearly demonstrated that certain transition-metal ions play a basic role in directing a number of biochemical processes. This revealed the need for comprehensive studies of metal ion–bioligand interactions, as model systems; within this, the investigation of the complex-forming properties of amino acids and peptides is of particular importance. Appreciable results have been achieved in this field during the past 20 years, and the main conclusions have been reviewed.[1,2] A survey of the relevant literature data shows that unsolved problems are generally encountered only in connection with those ligands where other complex-forming donor groups are present besides the functional groups characteristic of amino acids and peptides (amino and carboxyl groups). Our earlier results and other literature data clearly indicated, for instance, that primarily peptides containing imidazole-N or sulphur donor atoms exhibit co-ordination chemical behaviour essentially different from that of simple aliphatic dipeptides (*e.g.* glycylglycine).[3–6] The special role of the sulphur donor atom in the co-ordination of peptides containing thiol or thiocarbonyl groups can be explained relatively easily;[5,6] the available information on ligands containing the thioether group, however, is much sparser and much more contradictory.

Earlier studies on the complexes of *S*-methylcysteine, methionine, and their peptides quite clearly demonstrated that the thioether group can undergo a significant interaction with a metal ion only in *S*-methylcysteine.[7,8] In the case of methionine the corresponding interaction is negligible for steric reasons.[1,9–11] This difference between the complex-forming properties of the two amino acids is likewise observed in the copper(II) complexes of their dipeptides with glycine.[12] A direct interaction with the methionine thioether sulphur atom can naturally be detected with metal ions that are softer in character (*e.g.* Ag^+).[13] At the same time, it is well known that the borderline 3*d* transition-metal ions are often bonded to the thioether group of methionine in certain metalloproteins. One of the most familiar examples of this is provided by the blue copper proteins, where further co-ordination sites of the copper(II) ion are occupied by imidazole-N or thiol sulphur atoms.[14]

For a fuller understanding of the complex-forming properties of peptides containing the thioether group, therefore, it is necessary to extend the investigations to methionine peptides which contain other stable complex-forming groups in their side-chains. The present publication reports results on the equilibrium conditions relating to the copper(II) and nickel(II) complexes of histidylmethionine (His-Met) and methionylhistidine (Met-His). To facilitate comparability of the data, equilibrium studies were also performed on the copper(II) and nickel(II) complexes of glycylmethionine (Gly-Met), methionylglycine (Met-Gly), and simple amino acids containing a thioether group, *S*-methyl-L-cysteine (smc) and methionine (Met).

Experimental

S-Methyl-L-cysteine and L-methionine were obtained from Reanal, the glycine-containing peptides from Sigma, and the histidine-containing methionine peptides from Serva. The concentrations of the metal chloride stock solutions were measured gravimetrically *via* precipitation of the quinolin-8-olates.

In the pH-metric measurements, the metal-ion concentration varied in the range 1×10^{-3}—5×10^{-3} mol dm^{-3}, and the ligand concentration in the range 2×10^{-3}—1×10^{-3} mol dm^{-3}. Measurements were made at metal ion:ligand ratios between 1:1 and 1:4. During titration of the 10-cm^3 samples with CO_2-free KOH, argon was bubbled through to ensure the absence of atmospheric O_2 and CO_2 and to mix the solution. The ionic strength was adjusted to 0.2 mol dm^{-3} with KCl. All measurements were made at 25 ± 0.1 °C. A Radiometer pHM 64 pH-meter, G202B glass and K401 calomel electrodes, and ABU 13 automatic burettes were employed. The method of calculating the hydrogen-ion concentration from the measured pH, and the other details of the pH-metric procedure, were reported earlier.[15] The stability constants were calculated by means of a general equilibrium evaluation program (PSEQUAD).[16] In all systems, the error in the stability constants was within ±0.02—0.04 log units.

Spectrophotometric studies on the histidine-containing peptides were carried out with a Beckman Acta MIV spectrophotometer under conditions analogous to those employed for pH-metry.

Results and Discussion

The protonation constants of smc and Met, and the stability constants of the corresponding copper(II) and nickel(II) complexes, are given in Table 1. The data are in good agreement with earlier results relating to ligands containing the thioether group,[10] and are indicative of complex-formation processes similar to those of the simple aliphatic amino acids. The $pK_2 - \log K_1$ data, which express the relative stabilities of the complexes, are systematically lower for smc, however, than for Met. This means that the copper(II) and nickel(II) complexes of smc are the more stable, thereby pointing to the participation of the thioether group in the co-ordination. Nevertheless, the equilibrium data for the nickel(II) complexes also demonstrate

* Part 12, T. Kiss and Z. Szücs, *J. Chem. Soc., Dalton Trans.*, 1986, 2443.

that the presence of the thioether group cannot prevent the formation of the complexes NiA_3 (HA = dipeptide), *i.e.* both smc and Met can act only as bidentate ligands in the the tris complexes.

The equilibrium data for the aliphatic dipeptides containing the thioether group (Gly-Met and Met-Gly) are listed in Table 2. For comparison, this Table also contains the earlier stability constants determined for the simplest dipeptide, Gly-Gly.[15] In connection with the amino acids, our previously detailed results demonstrated that the thioether group of Met does not have an appreciable influence on the co-ordination conditions determined by the amino and carboxyl groups. The data in Table 2 permit a similar conclusion concerning the role of the thioether group in the peptides. For all three dipeptides, the formation of a species of type $[CuA]^+$ can be observed as the first step in the process of copper(II) complex formation; in this species, as for Gly-Gly, co-ordination may be achieved through the amino-N and the carbonyl-O donor atoms. In all three cases, the formation of $[CuAH_{-1}]$ is determining in the physiological pH range; in this species, the amino group, the deprotonated peptide N, and the carboxyl group take part in the co-ordination. The species $[CuAH_{-2}]^-$ and $[Cu_2A_2H_{-3}]^-$ are mixed hydroxo complexes formed in basic solution. The three dipeptides therefore undergo completely identical complex-formation processes with the copper(II) ion; however, there are minor differences in the absolute values of the stability constants. These differences are primarily in the constants $\log K^{MA_2H_{-1}}_{MAH_{-1}}$, which relate to the binding of the second ligand. The formation of the species $[CuA_2H_{-1}]^-$ can be described by the process $[CuAH_{-1}] + A^- \rightleftharpoons [CuA_2H_{-1}]^-$ and the second ligand is bound to the metal ion *via* the amino and carboxyl groups in axial–equatorial co-ordination.[17] The equilibrium constant ascribed to this process is smallest in the case of Met-Gly, which indicates that, depending on its steric arrangement, the thioether group may influence the accessibility to the free co-ordination sites of the complex of type $[CuAH_{-1}]$. The data allow the assumption that, following the co-ordination of Met-Gly *via* its amino-N, amide-N^-, and carboxyl-O^- donor atoms, it becomes possible for the thioether group in the N-terminal side-chain to enter into a weak axial interaction with the central metal ion. This can clearly cause only a slight increase in the stability of the complex $[CuAH_{-1}]$, but it can appreciably inhibit the possibility of axial–equatorial bonding of a second peptide molecule. The data show that the same effect is not expressed if the thioether group is in the C-terminal side-chain, *i.e.* the complex-formation processes for Gly-Met can be regarded as identical in all respects with those for Gly-Gly.

Analysis of the equilibrium data for the nickel(II) complexes leads to similar conclusions. The pK values relating to deprotonation of the amide group (pK^{NH}) vary in a similar way as with the copper(II) ion, and the formation of the complexes $[NiA_2H_{-1}]^-$ and $[NiA_2]$ is likewise inhibited. The inhibitory effect is particularly marked in the case of Met-Gly, where $[NiA_2H_{-1}]^-$ is not formed in measurable concentration.

The equilibrium data for the histidine-containing dipeptides are listed in Table 3, which also gives the previously determined stability constants for glycyl-L-histidine (Gly-His) and L-histidylglycine (His-Gly).[3,4] Before interpreting the data, it is necessary to repeat that the presence of imidazole-N atoms in the peptide chain mainly influences the complex-formation conditions compared to those for Gly-Gly. For both copper(II) and nickel(II) the determining process in the case of Gly-His is the formation of $[MAH_{-1}]$, in which, however, the co-

Table 1. Equilibrium data for copper(II) and nickel(II) complexes of *S*-methyl-L-cysteine and methionine at 298 K and I = 0.2 mol dm^{-3} KCl: $pM + qA + rH \rightleftharpoons M_pA_qH_r$, where $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$

	Proton complexes		Copper(II) complexes		Nickel(II) complexes	
Species	smc	Met	smc	Met	smc	Met
$[HA]$	8.72	9.12				
$[H_2A]^+$	10.92	11.34				
$[MA]^+$			7.65	7.76	5.14	5.23
$[MA_2]$			14.13	14.29	9.78	9.71
$[MA_3]^-$					12.76	12.60
$\log K_1/K_2$			1.17	1.23	0.50	0.75
$pK_2 - \log K_1$			1.07	1.36	3.58	3.89

Table 2. Equilibrium data for copper(II) and nickel(II) complexes of Gly-Gly, Gly-Met, and Met-Gly at 298 K and I = 0.2 mol dm^{-3} KCl

Species	Gly-Gly*		Gly-Met		Met-Gly	
$[HA]$	8.13		8.29		7.63	
$[H_2A]^+$	11.30		11.33		10.82	
	Cu^{II}	Ni^{II}	Cu^{II}	Ni^{II}	Cu^{II}	Ni^{II}
$[MA]^+$	5.56	4.04	5.75	4.14	5.04	3.33
$[MAH_{-1}]$	1.33	−4.74	1.60	−4.77	1.24	−4.92
$[MA_2H_{-1}]^-$	4.46	−1.63	4.97	−2.82	3.78	
$[MAH_{-2}]^-$	−8.04		−8.04		−8.29	
$[M_2A_2H_{-3}]^-$	−4.51		−3.57		−4.45	
$[MA_2]$		7.50		7.56		5.88
$[MA_2H_{-2}]^{2-}$		−11.50		−12.40		−12.64
pK^{NH}	4.23	8.78	4.15	8.91	3.80	8.25
$\log K^{MA_2H_{-1}}_{MAH_{-1}}$	3.13	3.11	3.37	1.95	2.54	

* log β values from ref. 15.

Table 3. Equilibrium data for copper(II) and nickel(II) complexes of histidine-containing peptides at 298 K and I = 0.2 mol dm^{-3}

Species	Gly-His[a]		Met-His		His-Gly[a]		His-Met	
$[HA]$	8.22		7.59		7.59		7.50	
$[H_2A]^+$	14.99		14.16		13.53		13.50	
$[H_3A]^{2+}$	17.50		17.12		16.49		16.08	
	Cu^{II}[a]	Ni^{II}[b]	Cu^{II}	Ni^{II}	Cu^{II}[a]	Ni^{II}[b]	Cu^{II}	Ni^{II}
$[MAH]^{2+}$	12.45	11.34						
$[MA]^+$	9.06	4.68	8.75	4.22	8.85	6.81	8.55	6.70
$[MAH_{-1}]$	4.91	−1.35	4.50	−1.54				
$[MA_2]$	15.96	9.64			15.06	12.30	14.60	12.13
$[MA_2H_{-1}]^-$	8.02	2.07						
$[M_2A_2H_{-2}]$					8.20		7.46	
pK^{NH}	4.15	6.03	4.25	6.76				

[a] log β values from ref. 3. [b] log β values from ref. 4.

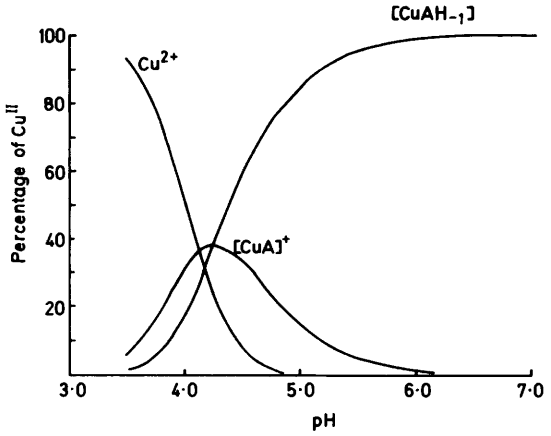

Figure 1. Concentration distribution of complexes formed in the copper(II)–Met-His system, as a function of pH. $c_A = 2.37 \times 10^{-3}$, $c_M = 1.19 \times 10^{-3}$ mol dm^{-3}

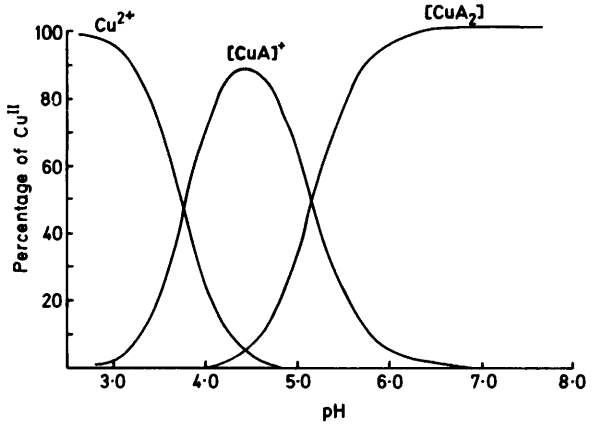

Figure 3. Concentration distribution of complexes formed in the copper(II)–His-Met system, as a function of pH, at a ligand excess. $c_A = 3.27 \times 10^{-3}$, $c_M = 1.11 \times 10^{-3}$ mol dm^{-3}

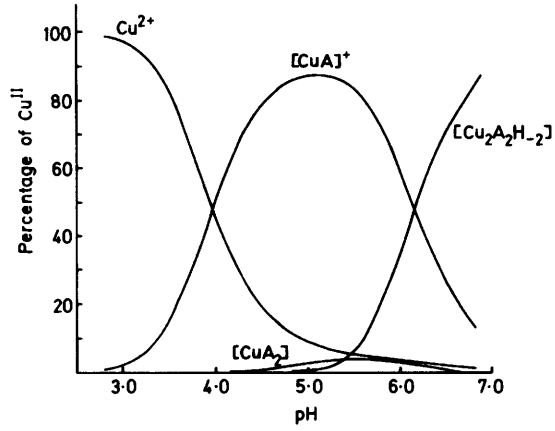

Figure 2. Concentration distribution of complexes formed in the copper(II)–His-Met system, as a function of pH, in equimolar solutions. $c_A = c_M = 2.38 \times 10^{-3}$ mol dm^{-3}

ordination takes place *via* the amino-N, the deprotonated peptide-N$^-$, and the imidazole-N donor atoms. In contrast, at a metal ion:ligand ratio of 1:1 in the copper(II)–His-Gly system, the dimeric complex [Cu$_2$A$_2$H$_{-2}$] is formed through Gly-Gly-like co-ordination, and the imidazole N acts as a bridging ligand. A ligand excess completely suppresses the deprotonation of the amide group, and a bis complex [CuA$_2$] is formed through His-like co-ordination. With nickel(II) as the central metal ion, only the latter process need be taken into consideration at all metal ion-to-ligand ratios.[3,4]

The data in Table 3 clearly reveal that the presence of His is determining even in the methionine-containing peptides. It emerges from a comparison of the data that the equilibrium conditions for the Met-His and the His-Met complexes differ considerably, not only from one another, but also from those for the complex-formation processes for Met-Gly and Gly-Met. At the same time, they are in good agreement with the equilibrium conditions for Gly-His and His-Gly. This is to be seen in Figures 1—3, where the concentration distributions in the systems copper(II)–Met-His and –His-Met are depicted as functions of the pH.

From Table 3 and Figure 1, it may be stated that for the copper(II) complexes of both Gly-His and Met-His the determining process is the formation of [CuAH$_{-1}$]. As regards the possibility of formation of the bis complexes [CuA$_2$H$_{-1}$]$^-$, however, there are appreciable differences between the two ligands. This complex is not formed in the copper(II)–Met-His system, *i.e.* the inhibitory effect of the thioether group on the co-ordination of the second ligand is manifested more strongly here than in the case of Met-Gly. Evidence of this effect is also provided by the results of the spectrophotometric studies. In the copper(II)–Gly-His system, it was found[5] that the absorption band at 600 nm assigned to the species [CuAH$_{-1}$] is shifted to 575 nm as a consequence of the equatorial co-ordination of the fourth N atom and the formation of [CuA$_2$H$_{-1}$]$^-$. However, the absorption band at 590 nm in the copper(II)–Met-His system does not display a spectral change under the same experimental conditions.

From the data for the nickel(II) complexes in Table 3, it may also be concluded that the inhibitory effect of the thioether group discussed above is likewise observed in the nickel(II)–Met-His system. For both metal ions, therefore, the complex-forming properties of Gly-His and Met-His are similar in many respects, but the presence of the thioether group also results in differences as concerns the bis complexes.

At the same time, the complex-forming properties of His-Gly and His-Met are identical as regards both the compositions of the complexes formed and the possible co-ordination sites. In the case of His-Met, similarly to His-Gly, it is necessary to take into consideration the deprotonation and co-ordination of the peptide-NH group only in the copper(II)–His-Met systems with a composition of 1:1, through the formation of the dimeric species [Cu$_2$A$_2$H$_{-2}$]. When there is a ligand excess, only the complexes [CuA$_2$] are formed, in which the co-ordination may take place in a similar manner as the free histidine. The good agreement between the equilibrium data for His-Gly and His-Met indicates that the thioether group obviously plays a negligible role in the co-ordination of His-Met.

To summarize the findings concerning the effects of the thioether group in the methionine-containing peptides, it may be stated that the presence of this donor group did not result in the appearance of basically new co-ordination conditions in any of the cases examined. However, the data also clearly demonstrate that the thioether sulphur atom of Met-Gly and Met-His may enter into a weak axial interaction with the central metal ion in the complexes of type [MAH$_{-1}$]. This interaction is primarily manifested in inhibition of the formation of the complexes [MA$_2$H$_{-1}$]$^-$, and always only if the N-terminal amino acid is methionine.

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