Complexation of Divalent Metal Ions with Cyclo-L-methionyl-glycine in Aqueous Solution

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Complexation of the biologically important hard and borderline divalent metal ions with methionine and methionine-containing oligopeptides has been the object of investigations in recent years [1], and evidence has been obtained showing that it is possible to force a metal–sulfur bond formation, for example in the case of copper(II). This is of interest because thioether complexes of such metal ions are very weak, as shown by recent systematic studies on their stability in solution with a number of monodentate thioethers [2, 3]. As part of our researches on peptide-type ion carriers, we report an investigation on the coordination chemistry in water of the cyclic dipeptide cyclo-L-methionyl-glycine (CMG) with divalent first-transition series metals, with zinc(II) and calcium(II). The ligand CMG, which does not contain free α-amino and carboxylic groups, can provide useful information on the coordinative behavior of the isolated methionine sulfur, thus apparently being a simple model compound of naturally occurring thioethers. We previously reported on the stable complexes formed with CMG by soft metal ions, like palladium(II), platinum(II), mercury-(II), gold(I) and (III), many of which have been isolated in the solid state. in all cases the sulfur end of the ligand is the primary site of coordination and further coordination of the metals to deprotonated amide nitrogen atoms can occur [4].

Experimental

Cyclo-L-methionyl-glycine was prepared as previously reported [4], starting from the corresponding aminoacids. ^{1}H NMR measurements were made on a Bruker WP60 spectrometer.
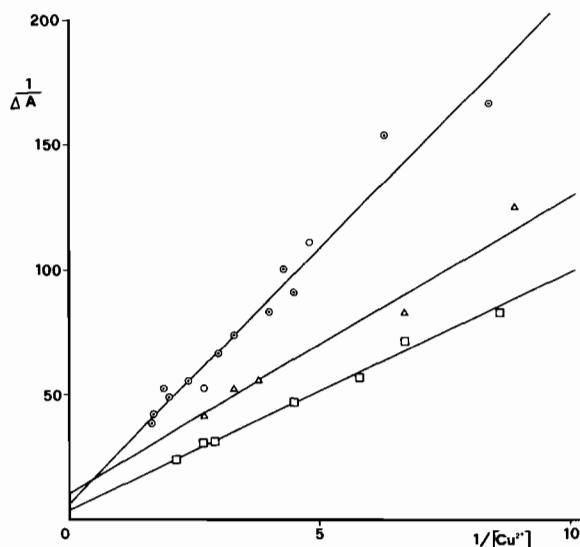
Fig. 1. Determination of the stability constant of $Cu(CMG)^{2+}$ in water, by plotting $1/\Delta A_{340nm}$ *vs.* $1/[Cu^{2+}]_{tot}$. $[Cu(ClO_4)_2]$ = 0.1120–0.5860 M; [CMG] = 0.0050 M ($\circ$), 0.0075 M ($\triangle$) and 0.0100 M ($\square$); I = 0.85–1.17 (NaClO$_4$); 25.0 °C; pH $\cong$ 3. From the intercept with the y axis, the value of ϵ_{340nm} is calculated (78 $\mp$ 47, 29 $\mp$ 12, 63 $\mp$ 36 for the three curves respectively) and from the slope the value of K is obtained (0.286 $\mp$ 0.19, 0.880 $\mp$ 0.45 and 0.382 $\mp$ 0.24). See eqn. 1.

Calculations of Stability Constants from Spectrophotometric Measurements

Absorbance spectra were recorded on a Perkin Elmer 576 spectrophotometer. Difference spectra were taken in silica tandem cells: the reference cell contained in the first compartment the metal(s) and in the other the ligand, and the sample cell contained the mixed metal(s)–ligand systems and water respectively. For the Cu^{2+}–CMG system three series of measurements were performed, with [CMG] = 0.0050, 0.0075 and 0.0100 M and $[Cu(ClO_4)_2]$ between 0.5860 and 0.1120 M. For the competition experiments, [CMG] = 0.0050 or 0.0100 M and $[Cu^{2+}]$ and $[M^{2+}]$ were adjusted so that $[Cu^{2+}]$ + $[M^{2+}]$ = 0.6 M, the metal-copper ratio ranging from 0.5 to 4. In all the experiments the ionic strength I was taken constant at I = 1.0 $\mp$ 0.2 with NaClO$_4$, and the pH was adjusted to about 3 (for the Hg^{2+}–CMG system, to about 2).

The stability constant of the $Cu(CMG)^{2+}$ species was determined by the Benesi–Hildebrand method [5], under the experimental conditions $[Cu^{2+}]_{tot} \cong [Cu^{2+}]_{free} \gg [CMG]_{tot}$, by recording the differential absorbance (ΔA) at 340 nm (eqn. 1).

$$\frac{1}{\Delta A} = \frac{1}{K\epsilon d[CMG]_{tot}} \cdot \frac{1}{[Cu^{2+}]_{tot}} + \frac{1}{\epsilon d[CMG]_{tot}} \qquad (1)$$

By plotting $1/\Delta A$ *vs.* $1/[Cu^{2+}]_{tot}$, straight lines were obtained (Fig. 1), which were calculated by a least squares procedure. The standard deviations for K and ϵ have been corrected for the number of experimental points with a 95% confidence limit. The reported value of the stability constant is a weighted mean of the three experimental data, the error given being twice the calculated standard deviation.

The calculation of the equilibrium constants for other metal ions from the competition experiments was based on eqn. 2, in the experimental conditions $[M^{2+}]_{tot} \cong [M^{2+}]_{free}$ and $[Cu^{2+}]_{tot} \cong [Cu^{2+}]_{free}$, *i.e.* providing the equilibrium constants are very low (for a comprehensive discussion of the method, see ref. [2]).

$$K_{ML}^M = \frac{\Delta A - \Delta A'}{\Delta A'} \cdot \frac{[Cu^{2+}]_{tot} \cdot K_{CuL}^{Cu} + 1}{[M^{2+}]_{tot}}, \qquad (2)$$

where ΔA and $\Delta A'$ are the differential absorptions at 340 nm, in the absence and in the presence of M^{2+} respectively.

Results and Discussion

No solid products could be isolated from the reaction between CMG and the examined hard and borderline metal ions, both in water and in alcoholic solutions. ^1H NMR and UV–visible spectroscopic evidences point to weak metal–sulfur interaction in water at pH (or pD) about 3. At higher pH values no detectable changes in the electronic spectra were observed for the copper(II) adduct, until the precipitation of copper(II) hydroxide at pH = 5–6. Potentiometric titration curves of copper(II) perchlorate solutions in the presence of increasing amounts of CMG are not appreciably different from each other, thus ruling out the possibility of deprotonation–coordination of amide nitrogen atoms of the ligand in the pH range 3–5.

The ^1H NMR resonances of CMG in D_2O (0.02 *M*), and in particular the sharp single signal relative to CH_3 protons at $\delta = 2.11$ ppm, does not apparently shift in the presence of $M(ClO_4)_2 \cdot 6H_2O$ (M = Zn, Cu, Ni, Co, Mn, Ca) in the concentration range $2 \times 10^{-4} – 4 \times 10^{-3}$ *M*. Instead, a more or less marked broadening of the resonances is observed upon addition of the salts of the paramagnetic ions. Significantly, the methyl resonance appears to be broadened to a much larger extent than the ring CH and CH_2 signals, indicating coordination of the metals to the sulfur end of the ligand. However, whereas the CH_3 peak dramatically broadened in the presence of small
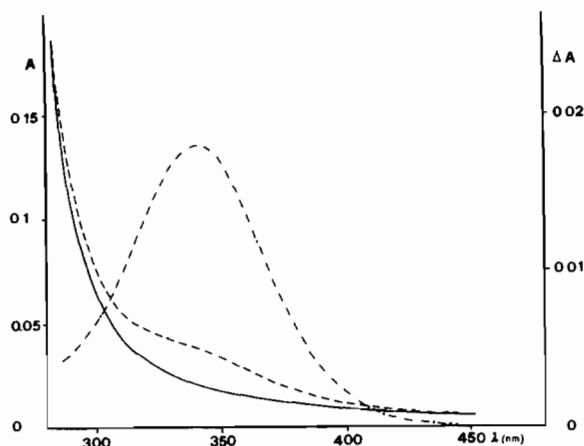

Fig. 2. Absorption spectra of: CMG (0.0075 *M*, d = 0.439 cm) and $Cu(ClO_4)_2$ (0.2620 *M*, d = 0 439 cm) in water, measured as sum spectrum (———), CMG 0.0075 *M* in the presence of $Cu(ClO_4)_2$ 0.2620 *M* (d = 0.439 cm) in water, measured as direct spectrum (– – –) and as differential spectrum (–·–·–). 25.0 °C, pH $\cong$ 3, I = 0.94 (NaClO₄).

amounts of $Cu(ClO_4)_2$ (molar ratio CMG.copper = 100 1), addition of $Co(ClO_4)_2$ or $Ni(ClO_4)_2$ only slightly affects the signal and more metal is necessary in order to obtain a detectable broadening of the resonance Finally, addition of $Mn(ClO_4)_2$ does not change the shape of the NMR spectrum of CMG, even at low CMG:metal ratio (about 5 1), indicating very weak coordination (if any) of manganese(II) to sulfur.

The relatively strong interaction of copper (II) with CMG has been quantitatively confirmed in water by electronic spectral measurements. Upon coordination of copper(II), an absorption at 340 nm ($\epsilon_M = 40 \mp 26$ M^{-1} cm^{-1}) appears, (Fig. 2) which is appropriate in energy for the typical $S(\sigma) \rightarrow Cu^{2+}(d)$ LMCT transition [6]. By measuring the intensity of the absorption (difference spectra) at various $[Cu^{2+}]$ and $[CMG]$ (see Experimental), it is possible to determine the stability constant of the complex $Cu(CMG)^{2+}$, *i.e.* log K = -0.45 ∓ 0.26.

The stability constants of other metal ions, whose $M(CMG)^{2+}$ complexes do not exhibit absorptions in the visible portion of the spectra, cannot be calculated by direct spectral measurements. However, the metal ions compete with copper(II) for the coordination to methionine sulfur and small lowerings of the intensity of the $S(\sigma) \rightarrow Cu^{2+}(d)$ transition are observed in most cases. Suitable treatment of the data (see Experimental) allows in these cases only an approximate evaluation of the stability constants for the $M(CMG)^{2+}$ complexes. log K about -0.6 for M = Mn, Ni, Zn. In the case of M = Ca, no significant changes in the intensity of the absorption at 340 nm have been observed, thus indicating a definitely

smaller stability. On the contrary, strong interaction occurs in water between CMG and the soft metal ions silver(I) and mercury(II), which leads to the complete diappearance of the 340 nm band, even with low metal.copper ratio (1:50). It should be noted that with these metals, solid adducts have been isolated of composition $HgCl_2(CMG)$ [4] and $AgNO_3(CMG)$ [7].

To conclude, the coordinating ability of the methionine sulfur of the ligand CMG appears to be rather scarce towards hard or borderline divalent metal ions, the more stable complex being formed, as expected (Irving–Williams series), with copper(II). The stability constant of $Cu(CMG)^{2+}$ in water appears to be somewhat smaller than that reported [2] for other 1:1 copper(II)–thioethers adducts in the same solvent, *e.g.* thiodiethanol (log K = 0.18). For simpler thioethers, which do not contain other potentially binding sites, there is a lack of water data, because of solubility problems. Significantly however, the stability constants of the 1.1 adducts of copper(II) with diethylsulfide and tetrahydrothiophene in 50% aqueous ethanol are very close to the value found for $Cu(CMG)^{2+}$ in water (log K = –0.47, 0.02 and –0.45 respectively).

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