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The Copper–Molybdenum Antagonism in Ruminants. II [1] Interactions of Thiomolybdates with Copper(II) in Aqueous Media

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It is generally accepted that thiomolybdate ions $MoO_{4-x}S_x^{2-}$, x = 1–4, probably play a central role in the widespread molybdenum induced copper deficiency that afflicts ruminants [2–4]. We have also demonstrated that the thiomolybdate ions can readily form under conditions that simulate those in the animals' rumen [1]. Whilst there have been several reports on solids containing Cu and thiomolybdates (see *e.g.* Muller *et al.* [5]) there has been no report of a detailed study of the interaction between thiomolybdates and Cu compounds in aqueous media. We now wish to report on such a study involving several Cu(II) systems under conditions that simulate those observed in the rumen. We also include comments on previous claims made regarding properties of thiomolybdates in relation to their role in the Cu–Mo antagonism.

Experimental

N-ethylmorpholine was purified as before [1]. All reagents were of 'AnalaR' of the best grades available. $(NH_4)_2MoS_4$, Cs_2MoOS_3 and $(NH_4)_2MoO_2S_2$, were prepared and characterised as previously [1].

K_2MoS_4 was prepared as follows. Potassium molybdate (5 g) was dissolved in distilled water (10 cm^3) and 50 cm^3 of a potassium hydroxide solution (48 g in 100 cm^3 H_2O) added. Hydrogen sulphide was bubbled through the solution, maintaining the temperature between 55–60 °C, the course of the reaction being monitored by changes in the uv/vis spectrum. After four hours the solution spectrum was that expected for the MoS_4^{2-} ion (absorption maxima at 470 and 318 nm), any precipitate at this stage was filtered and the filtrate transferred to an evaporating basin. Crystals (red with a green shimmer) of the product formed after several hours at room temperature, these were filtered, washed with ethanol and dried under vacuum. The infra-red spectrum of the solid and the uv/vis spectrum of an aqueous solution of the K_2MoS_4 were identical [1] to those for $(NH_4)_2MoS_4$.

Reactions of Thiomolybdates with Fe, Cu and Zn Compounds

Stock solutions of $Cu(His)_2$, $Cu(EDTA)^{2-}$, $Fe^{III}(EDTA)^-$, $Cu(GGH)^-$ and Cu(Albumin)** were prepared by simply mixing the ligand and metal sulphate salts in an aqueous solution containing 0.15 mol dm^{-3} NaCl. In all cases a 10% excess of ligand over the stoichiometric requirement was added. Solutions containing the aqueous ions of Cu(II), Fe(II) and Zn(II) were prepared immediately prior to their use from their sulphate salts. The thiomolybdate solutions were also freshly prepared in aqueous media containing 0.1 mol dm^{-3} N-ethylmorpholine/HCl pH 7.0, and 0.15 mol dm^{-3} NaCl, prior to their use. Interactions between the thiomolybdates and metal compounds were carried out by rapidly mixing the buffered solutions of the reactants, at the appropriate temperature, to give reactant concentrations of the order 10^{-3}–10^{-5} mol dm^{-3}. Reactions were monitored by uv/vis and ESR spectroscopy. No difference in the course of the reactions was found between aerobic and anaerobic conditions so no special precautions were taken to exclude air.

Stability of the Thiomolybdates as a Function of Solution pH

Buffered solutions of different pH values were prepared as follows: pH 2.0, 0.01 mol dm^{-3} HCl and 0.15 mol dm^{-3} NaCl; pH 3–6, 0.1 mol dm^{-3} potassium hydrogen phthalate plus either HCl or NaOH to give the desired pH. Freshly prepared aqueous solutions (1 cm^3) of the thiomolybdates were rapidly mixed with buffer solution (20 cm^3) at 39 °C to give final thiomolybdate concentrations of *ca.* 1×10^{-4} mol dm^{-3}. Their stability was monitored by uv/vis spectroscopy. Uv/vis spectra were recorded on a Perkin Elmer 555 spectrophotometer and esr spectra with a Bruker ER 200D instrument using solutions rapidly frozen in liquid N_2.

Results

Reactions with $Cu^{2+}_{(aq)}$, $Cu(His)_2$, $Cu(GGH)^-$, $Cu(EDTA)^{2-}$ and Cu(Albumin)

Aqueous solutions of salts of the thiomolybdates, $MoO_{4-x}S_x^{2-}$ x = 2 to 4, on mixing with $Cu^{2+}_{(aq)}$ or $Cu(His)_2$ solutions resulted in an immediate decrease

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**Abbreviations used His, histidine anion, EDTA, ethylenediamine tetra-acetate; GGH, glycylglycyl-L-histidinate

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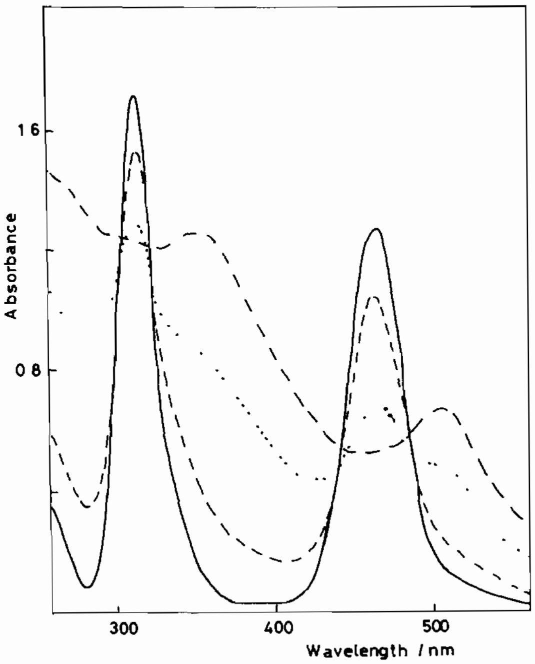

Fig. 1 Effect of the addition of $Cu(His)_2$ on the uv/vis absorption spectrum of a neutral aqueous solution of 1.0 × 10^{-4} mol dm^{-3} $(NH_4)_2MoS_4$, at 25 °C. Cu:Mo ratios 0:1 (——), 0.2:1 (– – –), 0.6:1 (.... .), 1:1 (–·– –).

in both Cu(II) and thiomolybdate concentrations. Total reduction of Cu(II) to Cu(I) (from esr measurements) and complete removal of the characteristic [1] uv/vis spectra of the thiomolybdate ions occurred at a 1:1 Cu:thiomolybdate ratio. In many of these solutions small amounts of red-brown solids formed even when the reactant concentrations were at the 10^{-4}–10^{-5} mol dm^{-3} level. The peptide complex $Cu(GGH)^-$ underwent the same reactions with the same stoichiometry but at a slower rate. Thus, for example, complete reaction at a 1:1 $Cu(GGH)^-$:MoS_4^{2-} ratio took *ca.* 20 minutes at 40 °C. With $Cu(EDTA)^{2-}$ significant changes were only observed after several hours during which time some hydrolysis of the thiomolybdate ions would have occurred. Typical spectroscopic changes are shown in Fig. 1 in which the 470 and 318 nm absorptions of MoS_4^{2-} are seen to decrease on the addition of $Cu(His)_2$. Total reduction of these absorbances occurred at a 1:1 reactant ratio. The increased background absorptions of these solutions were due to the presence of fine suspensions of the red-brown solid. The spectrum of the solution after reaction between $Cu^{2+}_{(aq)}$ and MoS_4^{2-} (λ_{max} 510 and 350 nm, see figure) is very similar to that of the known [5] compound $[(C_6H_5)_3P]_3Cu^I_2MoS_4 \cdot 0.8CH_2Cl_2$ when dissolved in dimethylsulphoxide and of $Cu^I_2MoS_4$, isolated from reaction between $Cu^I(CH_3CN)_4ClO_4$ and MoS_4^{2-} (2:1 Cu:Mo), also dissolved in dimethylsulphoxide.

Similar, well-defined, compounds of the di- and trithiomolybdates are not, as yet, available for comparison. However, from their analogous behaviour to tetrathiomolybdate with the Cu(II) species it may be concluded that Cu(I) thiomolybdate species are formed in all cases. The stoichiometry of the reactions is consistent with the two steps:

$$2Cu(II) + MoO_{4-x}S_x^{2-} + xH_2O \rightarrow 2Cu(I) + MoO_4^{2-} + S_2^{2-} + (x - 2)HS^- + (X + 2)H^+ \quad (1)$$

$$2Cu(I) + MoO_{4-x}S_x^{2-} \rightarrow Cu^I_2MoO_{4-x}S_x \quad (2)$$

(x = 2, 3 or 4) giving an overall 1:1 stoichiometry. Oxidation of inorganic and organic sulphides by metal ions to give disulphide products is well known [7].

Insufficient amounts of the red-brown solids were obtained for a full characterisation, however, their esr and ir spectra showed them to contain Cu(I) and thiomolybdate ions.

Addition of Cu(Albumin) to tri- or tetrathiomolybdate solutions resulted in a decrease in the thiomolybdate absorptions and the appearance of new peaks at 500 nm for MoS_4^{2-} reaction and at 420 nm for $MoOS_3^{2-}$ reaction. This first step was then followed by the slower reduction of Cu(II) to Cu(I) species. The stoichiometry was again 1:1 Cu:Mo. The reaction with $MoO_2S_2^{2-}$ followed a similar path but was complicated by the hydrolysis of this ion (*vide infra*).

In the animal rumen there is a large excess of sulphide over Cu and Mo. The same reactions as above were therefore studied using neutral aqueous solutions containing thiomolybdates and up to a hundredfold excess of sodium sulphide. The addition of the Cu(II) complexes gave the same products as before. Only when the Cu reactant was in excess was any copper sulphide formation observed. The results appeared to be independent of the nature of the starting Cu^{2+} reactant and of the sulphide level.

Reactions with $Zn^{2+}_{(aq)}$, $Fe^{2+}_{(aq)}$ and $Fe^{III}(EDTA)^-$

Zinc sulphate solution when added to aqueous solutions of the thiomolybdates produced no observable change to the uv/vis spectra of the thiomolybdates. Aqueous iron(II) sulphate gave no reaction with MoS_4^{2-} but reacted with $MoOS_3^{2-}$ and $MoO_2S_2^{2-}$ causing their dissociation. The stoichiometry for complete removal of the thiomolybdate spectra was *ca.* 1.5:1 and *ca.* 2.5:1 Mo:Fe for the tri- and dithiomolybdates respectively, $Fe^{III}(EDTA)^-$ had no effect on any of the thiomolybdates.

TABLE I. Times (minutes) Required for Greater than 90% Decomposition of Thiomolybdates at 37 °C and Different pH Values.

pH	MoS_4^{2-}	$MoOS_3^{2-}$	$MoO_2S_2^{2-}$
5	>60	50	~5
4	>60	4	2
3	27	<1	<1
2	9	<1	<1

Stabilities of the Thiomolybdates in Aqueous Acid Media

K_2MoS_4, prepared according to the method described in the experimental section, when dissolved in pH 7 buffered media at 25 °C was found to remain stable over a 2½ hour period. This concurs with observations [8] made using $(NH_4)_2MoS_4$ but disagrees with those of Weber *et al.* [9] who reported considerable hydrolysis with their preparation of K_2MoS_4. We note that the colour of their product, orange, differs from that normally observed for salts containing MoS_4^{2-}. The more acidic the aqueous solution, the more unstable become the thiomolybdate ions, their pH dependence is summarised in Table I. At all pH values the order of stability is $MoS_4^{2-} > MoOS_3^{2-} > MoO_2^2S_2^{2-}$.

Discussion

Strong evidence has been produced [10] for molybdenum complexation within the rumen when S and M intakes were increased within normal limits. The systemic effects of these complexes were similar to those found by adding thiomolybdates to the diets of ruminants [3, 4]. Furthermore, a strong interrelationship has been established between increased dietary Mo and S levels and a decreased absorption of Cu from the diet of ruminants [11]. Our results clearly show that any thiomolybdates formed in the rumen would readily react with Cu(II) complexes to produce Cu(I)–thiomolybdate compounds. Claims [12, 13] for the formation of a Cu(II)–thiomolybdate, *e.g.* $CuMoS_4$, can be discounted since total reduction to Cu(I) was always observed. $Cu(EDTA)_2^{2-}$ is seen to be unreactive and this could be an important fact when considering the form of Cu to be administered in overcoming the Mo induced Cu deficiency.

Finally, we would like to make some comments on claims which reflect on the stability of thiomolybdates in aqueous media and which could lead to erroneous conclusions regarding the mechanism of the Cu–Mo antagonism. First, Weber *et al.* [9] reported that K_2MoS_4 hydrolysed in neutral aqueous solutions and from this concluded that the thiomolybdate may merely be acting as a source of sulphide or molybdate or both when administered to animals. The hydrolysis is contrary to the reported [1, 8] stability of $(NH_4)_2MoS_4$ in neutral media, and contrary to our finding that K_2MoS_4 from our preparation was stable for at least 2 hours in neutral aqueous media. We can only conclude, from the nature of their [9] compound and its apparent instability in air, that their sample was impure. However, at the low pH (~2) of the gut all the thiomolybdates undergo extensive hydrolysis, the di- and trithio derivatives hydrolysing instantaneously (see Table). These observations should be borne in mind when comparing the effects of the different thiomolybdates when administered orally or when comparing ruminants and non-ruminants. For example, it has been found that the di- and trithiomolybdates, when administered to rats in their diet, caused systemic changes, but did not induce Cu deficiency [14]. This may well reflect their instant acid hydrolysis in the gut before interaction with Cu species can occur. Likewise with ruminants, orally administered thiomolybdates may differ in their eventual systemic effects when compared to the generation and reaction of thiomolybdates within the rumen.

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