The Reaction of Zinc Sulphide with Ionic Copper—the Biological Implications. Part 1. Surface Area Studies of Zinc Sulphide and its Reaction with Copper Complexes and Copper Containing Proteins

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Abstract

The role of metal sulphides vis-à-vis the availability of dietary copper in ruminant animals has been investigated using zinc sulphide as a model metal sulphide and a selection of copper complexes and copper containing proteins as models for sources of dietary copper. The extent of reactivity of zinc sulphide towards the copper complexes is dependent upon the type of donor atom co-ordinated to copper:

$$[CuL_n]^{X\pm}_{(aq)} + ZnS_{(s)} \rightleftharpoons {}'CuS'_{(s)} + [ZnL_n]^{X\pm}_{(aq)}$$

The order to reactivity is found to be Cu–O > Cu–N > Cu–S complexes and is in keeping with the reported values for the instability constant pK_n of the complexes. In contrast, no reaction is observed between zinc sulphide and the copper containing proteins studied (azurin, superoxide dismutase and cerulophasmin) and is attributed to the protection of the copper centres by the protein backbone. The results facilitate an understanding of copper metabolism in ruminants and a mechanism is proposed for the removal of dietary copper sources in such species.

Reactions between copper(II) sulphate solutions and samples of zinc sulphide having a range of specific surface areas (prepared by sintering at differing temperatures) have been studied. The fact that the reactivity is found to be highly dependent upon the specific surface area of the metal sulphide may well be of significance when considering the fate of copper in sulphur-rich biological systems.

Introduction

Interactions between copper and sulphur containing species are of particular interest in the context of the biological utilisation of copper as an essential micronutrient [1, 2] and as determinants of the mobility of copper both in geochemical systems and as a potentially toxic pollutant in ecosystems [3].

The importance of such reactions as determinants of the biological availability of copper is clear from evidence that variations in dietary content of inorganic and organic sources of sulphur, potentially reducible to sulphide (S^{2-}) or hydrosulphide (HS^-) within the digestive tract, markedly influence the susceptibility of ruminant animals to conditioned copper deficiency [4]. It is also recognised that the potency of such sulphur sources as copper antagonists is potentiated by the concurrent presence in the diet of molybdenum [5].

In this biological context as in geochemical and ecological systems, variables influencing the environmental concentrations of reactive S^{2-} or HS^- have a profound influence on the mobility of copper. While it is clear that molybdenum is particularly effective in sequestering sulphur in reactive forms potentially capable of reacting with copper [6], the possibility exists that other metals (M) capable of yielding metal sulphides (MS) may similarly modify the net mobility of $Cu^{n+}_{(aq)}$ if exchange reactions between $Cu^{n+}_{(aq)}$ and MS can occur (see Fig. 1). Such possibilities could account for growing evidence that the utilisation of copper by ruminant animals is restricted not only by molybdenum ingestion but also by high intakes of iron [7] and possibly zinc [8] and cadmium [9].

The investigations described herein form part of a programme of study investigating the role of metal sulphides upon the availability of dietary copper in ruminant animals. In the present paper zinc sulphide has been selected as a model for MS species that could possibly form in the rumen while

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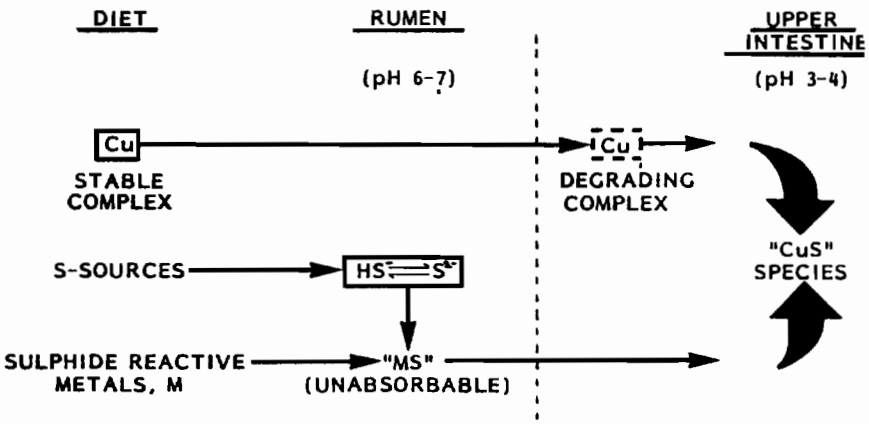

Fig. 1. Possible mechanism for the removal of dietary copper sources in ruminant animals by metal sulphide species.

a selection of copper complexes and copper containing proteins are the models used for sources of dietary copper.

Experimental

Materials

'Pure' zinc sulphide was used as supplied by Hopkin and Williams Limited, all other reagents were of 'AnalaR' quality. The copper containing proteins azurin (Az, from *Pseudomonas aeruginosa*) and superoxide dismutase (SOD, from bovine erythrocytes) were purchased from Sigma Chemical Company Limited. Ceruloplasmin, Cp (*ca.* 65% pure) was isolated from bovine plasma using a literature method [10].

Specific Surface Area Studies

Zinc sulphide samples differing in specific surface areas were produced by sintering *ca.* 1 g portions of stock material held in a platinum boat in a stream of pure dry nitrogen for exactly two hours at a range of elevated temperatures (500–1100 °C). The nitrogen gas adsorption–desorption isotherm of the stock sample and the BET specific surface area of both the stock and the sintered samples were determined at 77 K [11].

The influence of specific surface area upon the reactivity of zinc sulphide towards Cu(II) was examined by treating portions (0.0600 g, 6.16×10^{-4} mol) of the sintered material with 50.0 cm^3 aliquots of 7.87×10^{-3} mol dm^{-3} aqueous copper(II) sulphate solution. The resulting suspension was shaken for 24 h at 25.0 °C after which the solution was filtered (Whatman No. 42) and the copper concentration of the filtrate (the 'test' solution) was determined by conventional flame atomic absorbance spectrophotometry (Perkin-Elmer model 2280). A control solution containing copper(II) sulphate but no zinc sulphide was treated in an identical manner.

Reaction of Zinc Sulphide with Copper Containing Species

(i) Copper(II) complexes

Selected copper complexes containing ligands having differing types of donor atoms (oxygen, nitrogen or sulphur) were reacted with the stock zinc sulphide. Wherever possible the copper complexes were used as 8.00×10^{-3} mol dm^{-3} aqueous solutions or, for the complexes of low solubility (Table I, complexes **H** and **L**), saturated solutions were prepared. R_{50} values [2] of the copper complexes were determined by mixing 25.0 cm^3 aliquots with known quantities of stock zinc sulphide and the resultant suspension was shaken for 24 h at 25.0 °C.

The zinc complexes formed in the reaction (1) were isolated by treatment of the appropriate copper complex (5.00×10^{-4} mol dissolved in 20.0 cm^3 water) with excess stock zinc sulphide (2.44 g, 2.50×10^{-2} mol).

$$[CuL_n]^{X\pm}_{(aq)} + ZnS_{(s)} \rightleftharpoons \text{'CuS'}_{(s)} + [ZnL_n]^{X\pm}_{(aq)} \quad (1)$$

The conditions, vis-à-vis temperature and period of mixing, required for the reaction to be completed (taken to be when the concentration of the aqueous copper complex has fallen to <1% of its original value) varied considerably and in some cases (Table I, complexes **G**, **H** and **L**) complete reaction was not observed. Wherever possible the zinc complexes were isolated as solids either by evaporation of the filtrate to dryness or by trituration with ethanol (aspartate, glycinate or edta complexes). They were then compared spectroscopically (IR, KBr discs, Perkin-Elmer 683 instrument; ^1H NMR, Varian FT 80A instrument) with zinc complexes synthesised using literature methods (Table I).

TABLE I. Reaction of Zinc Sulphide with a Selection of Copper(II) Complexes

Copper complex[a]	R_{50}[b]	pK_n[c]		Preparation[d] of $[ML_n]^{X\pm}$	
		$[CuL_n]^{X\pm}$	$[ZnL_n]^{X\pm}$	M = Cu	M = Zn
Cu–O Systems[e]					
A $CuCl_2$	1.26	−0.52	−0.60		
B $CuSO_4$	1.50	2.35	2.31		
C $[Cu(ac)_2]$	1.38	3.30	unknown		
D [Cu(mal)]	1.73	5.60	3.35	Ref. 19a	Ref. 19a
Cu–N Systems					
E $[Cu(py)_4]S_2O_8$	4.40	6.54	1.93	Ref. 19b	Ref. 20a
F $[Cu(im)_4](NO_3)_2$	7.7	12.84	9.20	Ref. 19c	Ref. 20b
G $[Cu(en)_2](NO_3)_2$	13.6	19.60	10.37	Ref. 19d	not prepared
H $[Cu(dmg)_2]$	148	23.30	13.90	Ref. 19e	not prepared
Cu–S Systems[f]					
$[(n\text{-}Bu)_4N]_2[Cu(mnt)_2]$	no reaction	unknown	unknown	Ref. 19f	not prepared
$[(n\text{-}Bu)_4][Cu(tdt)_2]$	no reaction	unknown	unknown	Ref. 19g	not prepared
Cu–N Systems					
$\frac{1}{3}$N, $\frac{2}{3}$O Donation					
I $K_2[Cu(asp)_2]$	3.23	15.35	10.10	Ref. 19h	[g]
J $K_2[Cu(edta)]$	3.82	18.86	16.58	Ref. 19h	Ref. 20c
$\frac{1}{2}$N, $\frac{1}{2}$O Donation					
K $[Cu(gly)_2]$	5.68	15.25	9.96	Ref. 19i	Ref. 20d
Cu–N, S Systems					
L $[Cu(tsc)_2]Cl_2$	13.45	10.82	unknown	Ref. 19j	not prepared

[a]Abbreviations: ac = acetate anion, mal = malonate dianion, py = pyridine, im = imidazole, en = ethylenediamine, dmg = dimethylglyoximate anion, mnt = maleonitrile dithiolate dianion, tdt = toluene-3,4-dithiolate dianion, asp = aspartate dianion, edta = ethylenediaminetetraacetic acid dianion, gly = glycinate dianion, tsc = thiosemicarbazide. R_{50} Data for the Cu–X (X = O, N, S) systems have appeared in a preliminary communication [2]. [b]R_{50} given by a plot of R (*cf.* legend for Fig. 4) *versus* molar ratio of zinc sulphide to initial copper complex concentration. A linear plot is obtained for R values up to about 80% and from this R_{50} can be evaluated. [c]Ref. 21. [d]Reference for preparation of the copper and zinc complexes. The latter being used for spectroscopic comparison with the product isolated from reaction between zinc sulphide and copper complexes. [e]Aqueous solutions of $CuCl_2$ and $CuSO_4$ assumed to contain $[Cu(H_2O)_6]^{2+}$ species. [f]Reactions conducted in acetone (tdt) and 1:1 (*v*/*v*) acetone–ethanol mixture (mnt). [g]Ref. 19h was modified substituting $ZnSO_4$ for $CuSO_7$. The resulting $K_2[Zn(Asp)_2]\cdot 4H_2O$ gave satisfactory analytical results.

(ii) Copper containing proteins

4.0 cm^3 aliquots of aqueous solutions (0.50 m mol dm^{-3}) of the copper containing proteins were treated with a large molar excess of stock zinc sulphide (0.019 g, 0.20 mmol) for 24 h at 3 °C. The reaction mixture was kept in darkness and any air oxidation was minimised in all cases by gassing the reaction mixtures with nitrogen. A strictly anaerobic study of the reaction with Cp was also conducted. Control solutions containing the protein but no zinc sulphide were treated in an identical manner.

Results and Discussion

Specific Surface Area Studies

The nitrogen adsorption–desorption isotherm of the stock zinc sulphide is of type II and shows a narrow desorption hysteresis loop at high P/P° values (Fig. 2). The surface is best regarded as external surface since the pore size as judged from the application of the Kelvin equation to the desorption loop, would indicate a large pore radius and possibly interparticulate spaces. The specific surface for the stock material as determined by the BET method [11] is 61 $m^2\ g^{-1}$ but this value is significantly reduced upon sintering (Fig. 3).

The reactivity of zinc sulphide towards aqueous copper(II) sulphate is highly dependent upon the specific surface area of the sulphide (Fig. 4). This, together with the apparent external nature of the surface of the stock zinc sulphide, would suggest that the $ZnS_{(s)}/Cu^{2+}_{(aq)}$ reaction effectively occurs at the solid–liquid interface. The apparent increase

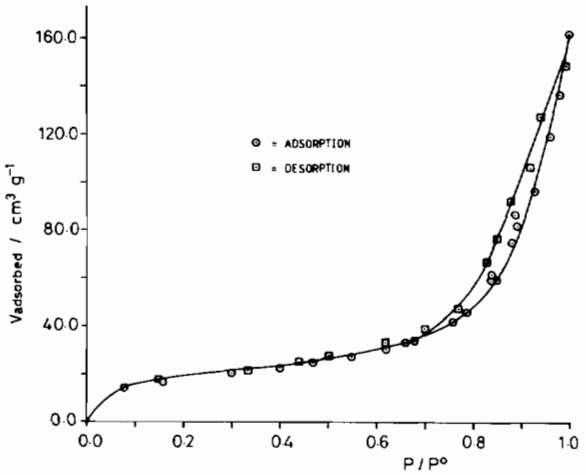

Fig. 2. Adsorption–desorption isotherm for nitrogen on stock zinc sulphide at 77 °C.

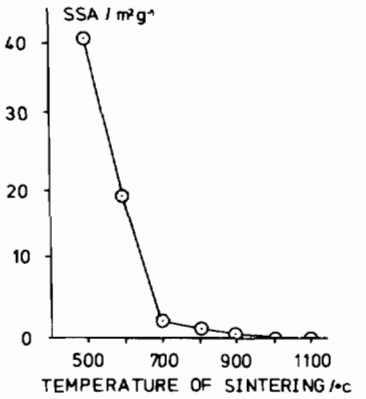

Fig. 3. Relationship between specific surface area (SSA) and sintering temperature of zinc sulphide (SSA for the unsintered stock material is 61 $m^2 g^{-1}$).

in reactivity for the samples sintered at 800 °C and 900 °C may be significant and could be associated with an increase in plasticity of zinc sulphide at its Tamman temperature [12] which, based on a melting point of 1650 °C, is 689 °C. The onset of phase transitions within the solid sulphide may also be of importance in the increased reactivity and the sphalerite → wurtzite transition is reported to occur ar 900 °C [13].

Reaction of Zinc Sulphide with Copper Containing Species

(i) Copper(II) complexes

The reactivity of zinc sulphide towards the range of copper(II) complexes studied may be quantified by evaluation of the R_{50} value [2]: the molar ratio of zinc sulphide to initial concentration of the copper complex which is required to convert 50% of the complex to copper sulphide. Thus the lower the R_{50} value the greater the extent of the reaction.

The results indicate the influence of the donor atoms co-ordinated to copper (Table I). Thus copper complexes containing ligands having oxygen-donor atoms show the greatest reactivity towards zinc sulphide while no reaction is observed for the complexes containing bidentate sulphur ligands (mnt and tdt). Consideration of ligands containing just one type of donor atom reveals, on the basis of the R_{50} data, that the order of reactivity is Cu–O > Cu–N > Cu–S complexes. This implies that the strength of the copper-donor atom interactions increases Cu–S > Cu–N > Cu–O and the observation is in keeping with the reported values for the instability constant pK_n of the copper complexes (Table I). The relationship between pK_n and R_{50}

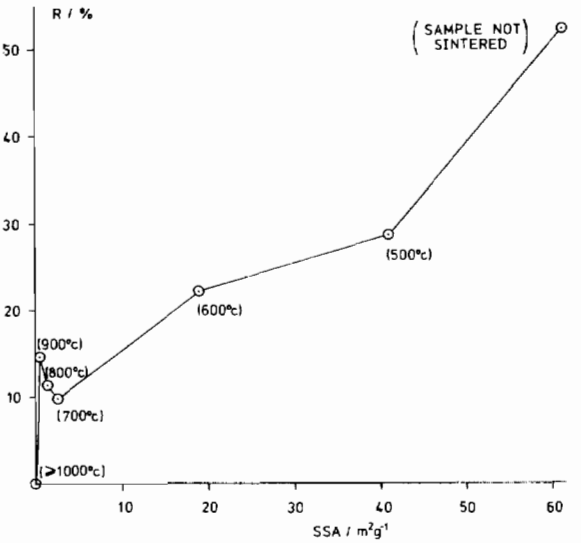

Fig. 4. Relationship between SSA of zinc sulphide and extent of its reaction (R) with aqueous copper(II) sulphate. R is defined by: $R = [(C - T)/C] \times 100\%$. C and T are the concentrations of aqueous copper(II) in control and test solutions respectively.

is well illustrated in Fig. 5 which, in addition, shows that the reactivity of the mixed donor systems is as might be anticipated. Thus in the mixed oxygen–nitrogen complexes, the higher the proportion of nitrogen donor atoms so the closer the pK_n–R_{50} value lies to the plot for the purely nitrogen donor systems. The zinc complexes formed within each of the reactions is less stable than the analogous copper complex (as predicted on the basis of the Irving–Williams [14] complex stability series and as indicated by the available pK_n data, in Table I) and the driving force for the reaction would appear to be the formation of the highly insoluble copper sulphide species.

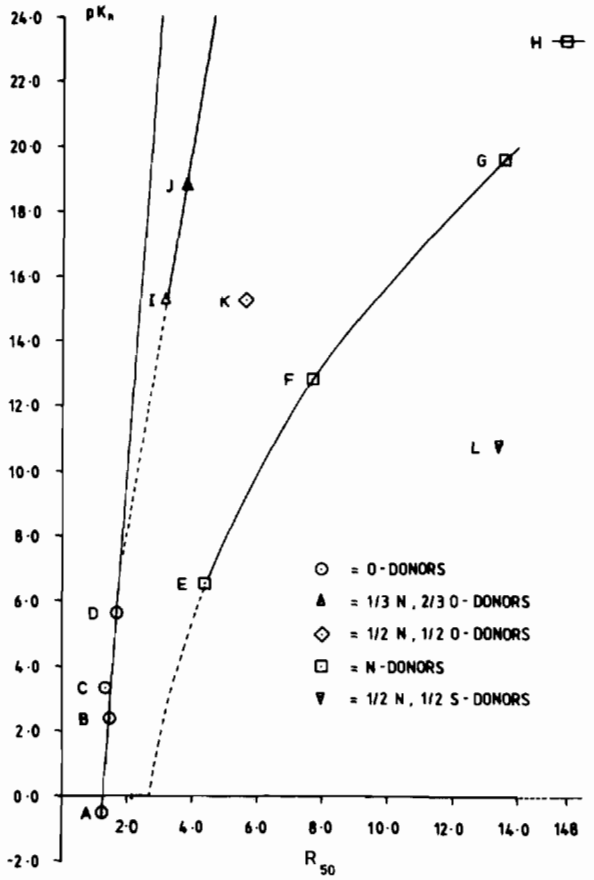

Fig. 5. Plot of pK_n of copper complexes *versus* R_{50} for a selection of copper complexes containing ligands having different types of donor atom.

(ii) Copper containing proteins

Determination of aqueous copper concentration and the monitoring of UV–Vis spectra showed no changes between the control experiment involving only SOD or Cp and those in which the zinc sulphide was also present. It is concluded that under the reaction conditions studied, these copper containing proteins do not react with zinc sulphide. Although the reaction with Az did lead to the formation of a light blue precipitate and an accompanying colourless supernatant it is tentatively proposed that the protein had been adsorbed onto the zinc sulphide surface.

Although little is known of the binding site of the copper centres in Cp [15], in SOD and Az the binding sites involve the coordination of copper to 4N and to 2N, 2S sites respectively [16, 17]. Purely on the basis of types of donor atom it might have been anticipated that zinc sulphide would react with SOD and possibly with Az. However, given the known structures of copper containing proteins (e.g. Az [18]) the lack of reactivity is not surprising in that the copper centres are likely to be hidden from the surface of the zinc sulphide by the extensive protein backbone.

The non-availability of the copper centres within such biomolecules may well be an important factor when considering the metabolism of sources of dietary copper in ruminant animals. The hitherto poorly understood feature of copper metabolism in ruminants is how any dietary copper escapes conversion to physiologically unavailable CuS or Cu_2S during its passage through the rumen in which substantial molar excesses of soluble and insoluble sulphides are always present. Our studies with copper containing proteins suggest that copper sequestered in centres remote from the surface of a copper–protein complex, as in these proteins and in the leaf copper protein, plastocyanin, could escape substitution reactions with metal sulphide species. If so, it is conceivable that the subsequent utilisation of such copper occurs after proteolytic enzyme attack in the small intestine at which site sulphide is not normally detectable [4]. In contrast, the copper of complexes containing Cu–O and Cu–N centres is readily accessible for substitution reactions at a metal sulphide surface and could well become unavailable by virtue of the reactions described herein. Our study also indicates that the fate of copper in this and other sulphide-rich biological systems could well be influenced by the specific surface area and thus the potential reactivity of other metal sulphide species that are present.

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