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The Cu–Thiolate Chromophore of Yeast Cu–Thionein

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A study on the copper binding centre of S. cerevisiae *Cu-thionein was performed. The 360 MHz ^{1}H-NMR spectra were different compared to those of vertebrate metallothionein. The single histidine residue was not involved in copper coordination. X-ray photoelectron spectrometry revealed binding energy values of Cu $2p_{3/2}$ (932.3 eV) and S $2p_{(1/2,3/2)}$ (162.0 eV) which were almost identical to those of Cu(I)(thiourea)$_3$Cl. The use of Cu-thiourea as a structural model, therefore, was encouraged especially as its tetrahedral copper-sulphur coordination is known. Extended X-ray absorption fine structure (EXAFS) measurements of Cu-thionein suggested a strikingly similar tetrahedral arrangement of 4 cysteine-sulphurs around the copper. Two sulphur atoms were 2.22 Å apart, the bond length of the other two sulphurs was 2.36 Å, respectively. The best proposal for arranging four Cu(SR)$_2$-units was a cubane-structure.*

Introduction

In the last 25 years a group of ubiquitous metal-cysteine-sulphur proteins called metallothionein became the subject of intensive investigations [1]. Apart from the mixed metallothioneins where Cu, Cd, Zn and even Hg are bound [2, 3] a homogeneous Cu–thionein has been isolated from baker's yeast [4, 5]. Although the primary structure is considerably different [6] it is continued to be termed Cu–thionein as the Cu–thiolate centres are of a striking similarity to those observed in vertebrate copper–thionein. According to the amino acid content and 4 atoms of copper a M_r of 4800 Daltons was calculated. Eight cysteine residues were found and neither methionine nor inorganic sulphur was detected.

Despite the low stoichiometry of Cu:S being close to 1:2 a tetrahedral arrangement of 4 sulphurs around the copper was proposed in 1978. The same tetrahedral arrangement for Cd and Zn was suggested for the vertebrate metallothioneins where a stoichiometry of one metal:three sulphurs is known [3, 8–10]. The soft chalcogen sulphur is well suited to act as a bridging ligand to form oligomeric metal–thiolate chromophores [11–13]. Unfortunately no X-ray diffraction data are available attributable to extreme reluctance to obtain crystalline material. Thus, an attempt was made to reveal the molecular architecture of the copper chromophore, at least, using several spectrometric methods. From ^{1}H-NMR measurements it was hoped to establish whether or not the single histidine residue of the polypeptide chain is involved in the copper coordination. X-ray photoelectron spectrometry allowed a critical summary of the charge situation on both copper and cysteine-sulphur. The technique used to collect information on the first coordination sphere around the copper was extended X-ray absorption fine structure measurements (EXAFS). Using this technique the coordination number, the type and the atomic distances of the bound first shell atoms were described.

Experimental

S. cerevisae was grown anaerobically for 48 h at 25 °C. The final copper concentration of the growth medium was 1 m*M*. The cells were washed 3 times with tap water and homogenized at 600 kg/cm^2. After centrifugation at 10 000 g the supernatant was heated to 60 °C for 3 min. The precipitates were discarded and the soluble fraction was subjected to five different preparation steps. Sequential chromatography was carried out on Biogel P6, QAE Sephadex

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A-25, Sephadex G-75 and again on QAE Sephadex A-25. The protein was desalted by passage through Biogel P-10. Crystalline $Cu(thiourea)_3Cl$ was synthesized following the procedure cited in [14]. $Cu(8\text{-mercapto-quinoline})_2$ was prepared from 8-mercapto-quinoline (Fluka, Buchs) and CuO, NH_4CuS_4, CuS and Cu-penicillamine were from the same sources as described earlier [15, 16].

Copper was assayed on a Perkin Elmer atomic absorption spectrometer (model 400 S), furnished with a HGA-76B unit. Circular dichroism spectra were run on a JASCO 20A recording spectropolarimeter and X-ray photoelectron spectra were recorded on a Varian V-IEE 15 high resolution spectrometer equipped with an 620L 8K on-line computer. 360 MHz proton nuclear magnetic resonance spectra were obtained from a Bruker Spectrospin 360 unit combined with an 8 T superconducting magnet (Oxford instruments Ltd.) and computer accessory to perform convolution difference calculations. Prior to the NMR-measurements lyophilized Cu-thionein samples were incubated for 70 h at 25 °C in 2 ml 99.6% ^2H_2O. Treatment with ^2H_2O ascertained complete ^2H replacement of exchangeable protons. After repeated lyophilization samples were dissolved in 99.6% ^2H_2O for measurements at 23 °C. The protein concentration was 20 mg/ml. The protein signal of the vacuum grease silicone served to calibrate the chemical shifts. p^2H adjustments were monitored with a meter (Knick model 645, Berlin) taking into account the solvent isotope effect. ^1H-NMR data from the apoprotein were obtained from the holoprotein which was treated with 20% ^2HCl prior to the measurement. The p^2H was lower than 0.4 and no irreversible damage of the protein was seen. At this high $^2H^+$ concentration all copper was displaced [3].

EXAFS Spectrometry

X-ray absorption and extended X-ray absorption fine structure (EXAFS) measurements were carried out at the European Molecular Biology Laboratory, Hamburg, using the Synchotron radiation facilities of the Deutsches Elektronen Synchotron (DESY). The X-ray spectrometer used for these measurements consists of two sets of horizontal and vertical precollimating slits placed at about 35 m from the tangent point of the storage ring. The first set defines the cross-section of white beam impinging on the monochromator and the second set eliminates the scattering from the first. The cross-section of the white beam allowed through by this slit system is about 1 mm vertically and 10 mm horizontally.

These slits are followed by a channel cut Silicon (III) monochromator mounted on a rotating table. The monochromatic beam thus produced passes through two more sets of horizontal and vertical slits, which further reduce the background due to scattering. These slits are followed by the reference

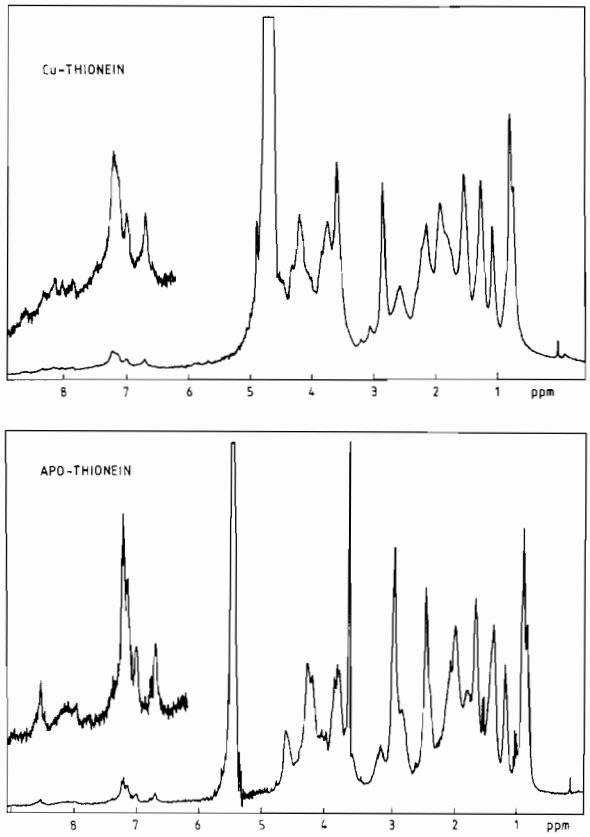


Fig. 1. 360 MHz proton nuclear resonance spectra of (a) Cu–Thionein (20 mg/ml) in ^2H_2O, pH 6.5; (b) Apo-thionein (20 mg/ml) in ^2H_2O, p^2H 0.4, adjusted with ^2HCl. Convolution difference spectra were calculated scanning the spectra 200 times at 23 °C.

ion chamber which monitors the intensity of the monochromatic beam incident on the sample whereas the transmission of X-rays through the specimen is measured by a second ion chamber. When the ion chambers, which are sealed and can be evacuated, are loaded with gas care has to be taken to adjust the pressure and composition of the gas mixture so as to minimize the effect of harmonics on the measured signal. For the present experiments a mixture of air and helium was used.

The probes, approximately 1 mm thick, were placed in a flat cell with polyacrylic windows (Mylar). Simultaneously with the absorption measurements, the fluorescence signals were recorded using eight photomultipliers distributed around the specimen so that the centre of the collected solid angle of the fluorescence signal was normal to the incoming monochromatic beam and in the plane of the polarization vector of the radiation, *i.e.* in the horizontal plane.

The signals from the ion chambers were amplified and ditigized using voltage to frequency convertors

TABLE I. Binding Energy Values of the Cu $2p_{3/2}$ and S $2p_{1/2,3/2}$ Levels.

Compound	Cu $2p_{3/2}$ (eV)		S $2p_{(1/2,3/2)}$ (eV)
	Satellite	Main peak	
Cu_2O		932.5	
CuO	943.8	934.6	
NH_4CuS_4		931.5	162.1
CuS		932.3	162.1
Cu–penicillamine		933.3	163.2
Cu(8-mercaptoquinoline)$_2$	941.2	933.4	163.1
Cu(I)–Thiourea		932.5	162.2
Cu(I)–Thionein		932.3	162.0

and scalers. The signals from the photomultipliers were amplified and submitted to a pulse height analysis to eliminate harmonic contributions and other contaminants like parasitic reflections and then counted and digitized. The experiment is integrated in the CAMAC data acquisition system of the EMBL Outstation [17] and is driven by software written in CATY.

As the signal-to-noise ratio obtained from transmission measurements is better than that of fluorescence data when the metal concentration is high, as in the case of Cu–thionein, the fluorescence data were not used for further analysis.

Results and Discussion

^{1}*H-NMR Spectrometry*

The ^{1}H-NMR spectra of both Cu–thionein and the apoprotein show differences to those reported for the vertebrate metallothioneins [18, 19]. At 0.8 ppm an intensive somewhat split signal is seen which is absent in the spectra of chicken or equine metallothionein. An identical profile is detected from 1–1.7 ppm. The discrepancies start from 1.7 ppm onwards. The band at 2.2 ppm is not present in the vertebrate metallothionein. Upon removal of copper this signal is shifted upfield to 2.35 ppm. No changes at the position of the cysteine-CH_2 protons at 2.95 ppm were seen. However, a significant sharpening is detectable upon measuring the apoprotein. Furthermore, the well pronounced resonances at 3.6 ppm which are more intense in the yeast apo-Cu–thionein should be pointed out. Unlike the case of the vertebrate metallothioneins, histidine C(4)-protons appear at 6.5–7.5 ppm, while histidine C(2)-proton resonances are detectable in the 8 ppm region. It can be clearly demonstrated that the single histidine residue of yeast copper-thionein is not involved in metal coordination because no marked changes between the resonances of the histidine C(2) and C(4)-protons of both apo- and holoprotein are seen (Fig. 1a, b). Taking into account the many other known physicochemical properties [3, 7–10] the exclusive copper to sulphur coordination is still suggested.

X-ray Photoelectron Spectrometry

Before any suggestions could be made about the oxidation state of the copper in the Cu–(thiolate)$_2$-centres of Cu–thionein it proved necessary to compare some information on simple copper–sulphur compounds [15, 16]. Cu(II)O and $Cu(I)_2O$ clearly showed different $2p_{3/2}$ binding energy values. A satellite appeared when Cu(II)O was measured. Unlike with the bound oxygen there is likely to be a distinct metal-ligand covalency in the copper sulphides. Electron delocalization takes place fairly easily with the consequence of a higher electronic conductivity. It was expected that the less electronegative sulphur would be unable to locate both electrons at the sulphur. One electron was transferred to occupy the 3d shell of copper. This phenomenon was already inferred from X-ray emission spectroscopic measurements.

The Cu $2p_{3/2}$ binding energy values of Cu_2O and Cu_2S displayed the characteristics of a $3d^{10}$ Cu. Unlike with CuO no satellites characteristic for Cu(II) were seen when CuS was measured (Table I). Furthermore, no significant spectral changes compared to CuS were noted with NH_4CuS_4, an established source for Cu(I). Cu(II) satellites were also absent using $[Cu_{14}(\text{D-penicillamine})_{12}Cl]^{5-}$. This complex was chosen as it contains a thiolate-sulphur concentration very similar to that of the metallothioneins. Originally a mixed Cu(I)/Cu(II) valence complex was deduced from X-ray diffraction studies [20, 21]. Eight out of the fourteen Cu are tetrahedrally arranged as usual for Cu(I). The remaining six Cu(II) were found to be in a square planar environment. The strong absorption in the visible region suggested

some similarity with the type 1 copper chromophores. As S → Cu charge-transfer transitions do occur in both the Cu-penicillamine complex and the type 1 copper-proteins it was expected that the actual electronic state of the copper would be considerably affected. Regardless of the molecular architecture essentially all copper in the Cu-penicillamine is found in the $3d^{10}$ electronic state. According to Jørgensen the actual oxidation state was +1 [22].

It was intriguing to note the complete absence of characteristic Cu(II) satellites in the CuS spectrum. The objection might be raised that in general sulphur binding to $3d^9$ copper may enhance an initial photoreduction of the copper during exposure to X-ray irradiation leading always to $3d^{10}$ Cu. The ease of electron delocalization of the coordinated sulphur was thought to be the cause. When there is a second electron withdrawing ligand bound to the thiolate-sulphur, no S → Cu charge-transfer transition should take place. Indeed recording of Cu(8-mercaptoquinoline)$_2$ revealed a $2p_{3/2}$ band at 933.4 eV and the characteristic satellite structure attributable to $3d^9$ copper. Thus, photoreduction of Cu(II) coordinated by sulphur ligands can clearly be excluded. When Cu-thionein was measured a large and homogeneous Cu $2p_{3/2}$ band appeared at 932.3 eV which was assigned to the exclusive presence of $3d^{10}$ Cu. Antiferromagnetic coupled $3d^9$ copper was not present as no sign of a satellite was seen. Redox titrations and the anaerobic displacement of all copper [5, 23] confirmed the existence of $3d^{10}$ copper bound to cysteine-sulphur.

In comparing the Cu $2p_{3/2}$ and S 2p-values of Cu-thionein and Cu-thiourea it was interesting to note virtually identical values. The double bonded sulphur of thiourea is probably able to withdraw electrons from the amide groups giving rise to this rather low S 2p binding energy value of 162.2 eV. In an earlier proposal of the structure of the Cu-chromophore [3, 8–10] a tetrahedral arrangement of the sulphur atoms around the copper was assumed. The known crystal structure of Cu(I)-thiourea [14] encouraged this conclusion. A useful method to examine this proposal in more detail was extended X-ray absorption fine structure (EXAFS) measurements. This technique allowed a definite conclusion on the nature of the first shell atoms, their atomic distances and the coordination number.

Extended X-ray Absorption Fine Structure

The degree of possible deterioration was examined before and after each measurement. Cu–thiourea remained colourless throughout. No detectable differences in the electronic absorption and the chir-optical properties of Cu–thionein could be seen. There was no EPR detectable copper. The optical density of the specimen was obtained by taking

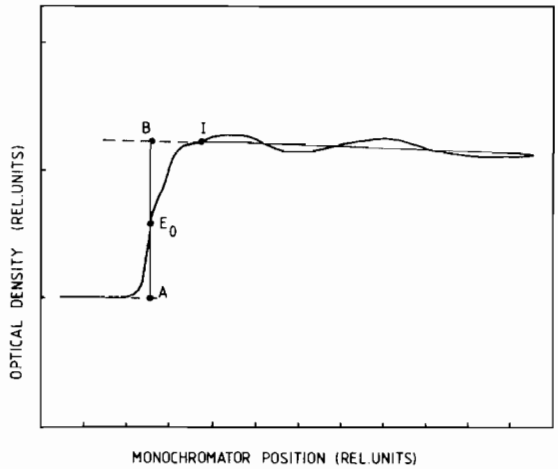


Fig. 2. Logarithm of the measured transmission for the Cu K-edge of Cu–thionein, (in relative units), *versus* monochromator position. The motor position (*i.e.* Bragg angle for the monochromator) is depicted on the abscissa. Each point represents 3.6 s of arc. The inflexion point E at the absorption edge is used to define an approximate value of the binding or ionization energy for the photoexcited K-electron. The inflexion point of the first EXAFS oscillation I is used to define a point through which the atomic absorption function in the absence of neighbours is forced to pass. The value of E_o is further refined in the theoretical fitting of the EXAFS oscillations at a later stage of the data analysis.

the logarithm of the transmission data and the EXAFS oscillations were extracted by the following procedure. The positions of the edge (E) and of the inflexion point of the first oscillation (I) shown in Fig. 2 were determined from the second derivative of the spectra. The region below the edge was first linearly extrapolated to obtain an approximate value at the edge and a fourth order polynomial was then fitted by least squares to the set of data consisting of the experimental values and the value obtained by linear extrapolation. This last point was given a weight ten times larger than the other values.

The region above the edge was smoothed by ten cycles of a 5 point moving average procedure. A fourth order polynomial was fitted to the resulting curve and the inflexion point of the first oscillation which was given ten times the weight of the other values. This polynomial was then extrapolated to the edge (B) and the edge jump indicated in Fig. 2 was scaled to the value calculated from the Viktoreen coefficients given in International Tables for X-ray Crystallography (1968).

The EXAFS which is defined as:

$$\chi(k) = (\mu(k) - \mu_o(k))/\mu_o(k)$$

where:

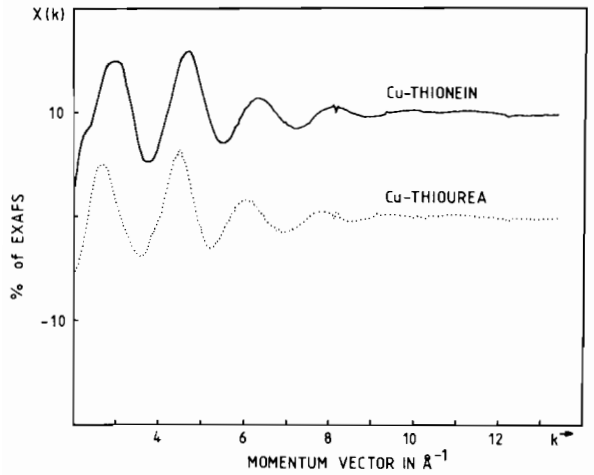


Fig. 3. EXAFS oscillations in terms of percentage of the atomic absorption *versus* momentum vector. See text for definitions. Note that the EXAFS oscillations for the protein are displaced towards higher values of k indicating that on average the bond distances for the first coordination sphere for the protein are about 5% smaller. Note also the similarity of shapes between the two spectra indicating an identical type of surrounding atoms in the protein and in the model compound.

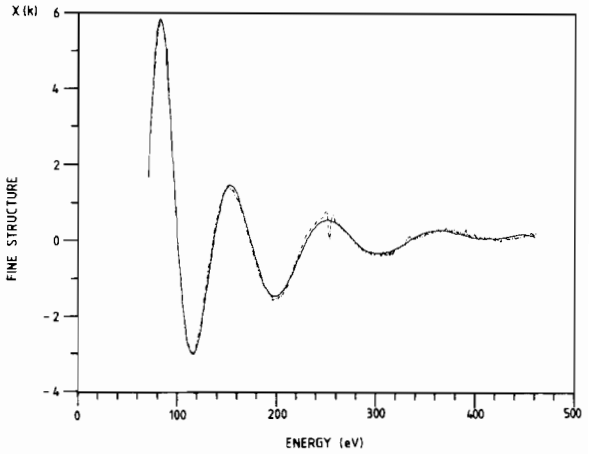


Fig. 4. First principles calculation fit to the EXAFS spectrum of Cu–thionein. (– · – · –) experimental data, (——) best theoretical fit obtained with four sulphur atoms surrounding the copper, two atoms at 2.22 Å and another two at 2.36 Å. The estimated errors in the bond distances are of the order of 0.02 Å.

$\mu(k)$ is the measured absorption coefficient and $\mu_o(k)$ is the atomic absorption in the absence of neighbours. This value, which should correspond to Viktoreen's law, was then obtained by subtraction of the values of the fourth degree polynomial above the edge from the experimental values, scaled to correspond to the edge jump, and divided by the difference given by Viktoreen's law (Fig. 3). The data reduction and EXAFS extraction programs form part of a general package produced at the EMBL Outstation (Koch *et al.*, unpublished, Pettifer and Cox, unpublished). The X-axis is calibrated in units of momentum transfer defined as:

$$k = \sqrt{2m/h^2(E_{ph} - E_o)}$$

where:

m is the electron resting mass, h is Planck's constant, E_{ph} is the photon energy, E_o is the binding or ionization energy of the core electron.

Comparison of the data in Fig. 3 reveals a very close similarity between the EXAFS spectra of Cu-thionein and the model compound Cu–thiourea.

The similarity between the EXAFS oscillations of the two spectra can be taken as an indication that the atoms surrounding the absorber are identical. Further, as the amplitudes in the two spectra, which depend on the coordination number, are identical within experimental error, one can conclude that the coordination number must also be equal.

At about 8.15 Å the spectra exhibit what is known as monochromator 'glitch', which is due to a parasitic reflection allowed through the monochromator and as such must be regarded as an artefact.

Glitches always appear at the same monochromator position *i.e.* at a well defined energy of the primary beam and although they are usually a nuisance they can occasionally be useful as wavelength markers as in the present case, where the 'glitch' was used to align the spectra of the protein and the model compound.

It should be noted that the maxima of the EXAFS oscillations for the protein appear at momentum values which are approximately 5% higher than in the model compound indicating a shorter bond length in the protein. From the known crystal structure of Cu–thiourea and the EXAFS alone one can already deduce the values of the average bond distance. The crystal structure of Cu–thiourea [14] indicates that the Cu atom is surrounded by four sulphur atoms at 2.43, 2.28, 2.35 and 2.38 Å respectively *i.e.* the first coordination shell is split. The mean value of the Cu–S distance is 2.36 Å and from the EXAFS results, the mean value for the protein should be approximately 2.29 Å.

Some small but significant differences can be observed in the shape of the EXAFS spectra of the protein and the model compounds in the region between 3 and 4 Å, where shoulders are visible in the spectrum of the model compound. These shoulders are absent in the data for the protein. One possible explanation could be that there are contributions from more distant shells in the model compound which do not show up in the spectrum of

the protein which corresponds to a more closely packed structure. The relatively large S atoms tend to screen the contribution of the more distant shells as already observed in several cases [24, 25]. This situation differs from the one observed when the first coordination shells consist of lighter atoms like nitrogen and oxygen. In this case, the contributions from the second and third shells are usually seen in the EXAFS spectra. We also carried out a first principles interpretation of the EXAFS spectrum of Cu–thionein using the theory developed by Lee and Pendry [26] as implemented in the program package written by Pettifer and Cox (unpublished). Phase shifts for the emitter atom (Cu) and the back-scatterer (S) were computed by construction of a Muffin-Tin potential [27] using the parametrized wave functions of Clementi and Roetti [28]. This program was kindly made available to us by J. B. Pendry.

It was found that in order to fit the experimental data it was necessary to split the first shell with a pair of sulphur atoms at 2.22 Å and 2.36 Å respectively. The fit obtained between the results of the theoretical calculations and the experimental data is illustrated in Fig. 4. Attempts to fit the spectrum with only one shell led to marked disagreement between theory and experiment at energies above 250 eV.

For a more comprehensive description of the effects to be considered in the interpretation of EXAFS data, and how to calculate them in the particular approach we have used, see Bordas [29]. The employed EXAFS theory cannot resolve any further splitting if it exists. Given the quality of the fit, it is more likely that further splitting would only manifest itself, if present, at much higher values of momentum, outside the range of our measurements.

The most marked differences beween the protein data and those of the model compound occur in the vicinity of the absorption edge. Figure 5 shows the absorption coefficient or rather the optical density of the Cu atom in the protein and the model compound after fitting Viktoreen's law, while Fig. 5b shows its derivatives. The inflexion point at the edge and the rise of the first oscillation appear at approximately the same photon energy. This is the most prominent peak in the derivative spectrum. At higher energies the spectra differ significantly. There is a clear spectral transition in the protein indicated by the second peak in the derivative, which is absent in the model compound.

The near edge structures, the broad oscillation corresponding to the inflexion points 3 and 4 in the derivatives, occur at different energies in the protein and model compound data and have different shapes. It is difficult to assign the edge and near edge structures unambiguously to any specific electronic transition without a very comprehensive and complex calculation which is well beyond the scope of the present work.

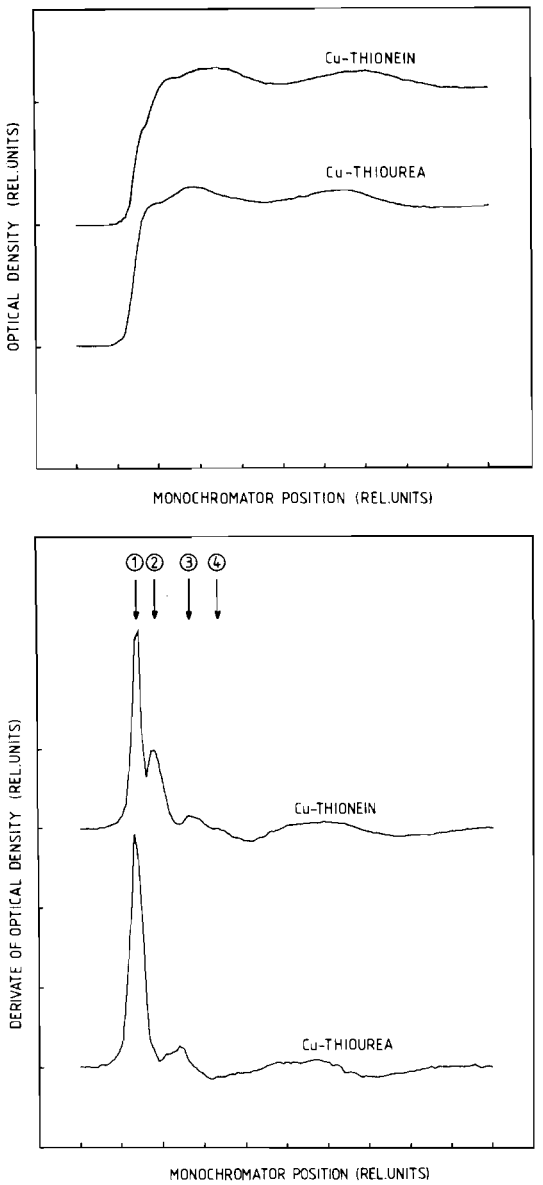


Fig. 5. Edge and near edge structure for Cu–thionein and Cu–thiourea (a) and their derivatives (b). The motor position (*i.e.* Bragg angle for the monochromator) is depicted on the abscissa. Each point represents 3.6 s of arc.

Consequently, the edge data should, in the present context, be regarded as spectroscopic indicators of differences in the electronic configuration between Cu–thionein and Cu–thiourea. The exact meaning of these differences can, however, not be extracted from the X-ray spectroscopy data alone. Despite the identical charge at the coordinate sulphurs some differences between these atoms should have been

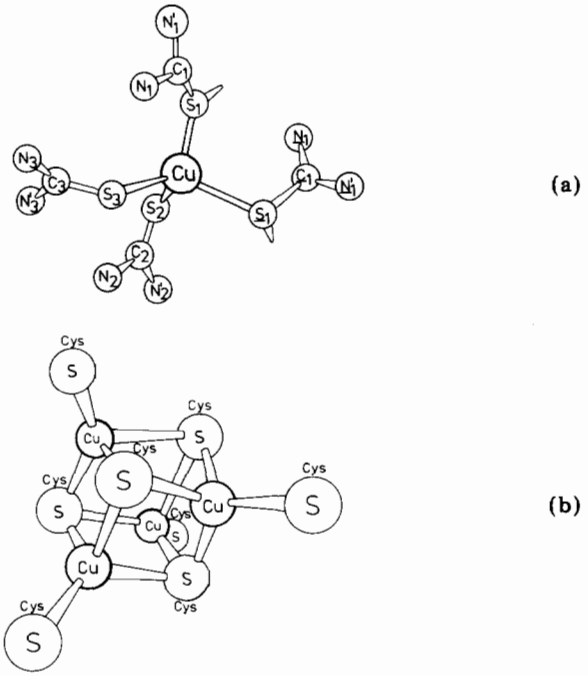


Fig. 6. X-ray structure of $Cu(thiourea)_3$ (a) and proposed structure of Cu–thionein from *S. cerevisiae* (b).

expected. In Cu–thionein the sulphur is a single bonded species while in thiourea we have to deal with a double bonded sulphur.

Conclusion

With regard to the charge and kind of first shell atoms there is a striking similarity between Cu–thiourea and Cu–thionein. The same tetrahedral arrangement of sulphur atoms around Cu(I) can be deduced. However, considerable differences in the arrangement of the Cu–S tetrahedra are expected. In Cu–thiourea each single copper–sulphur tetrahedron is bridged to the next one *via* one double bonded sulphur [14] (Fig. 6a). Endless chains are being formed. In order to obtain the same chain of Cu–thiolate tetrahedra the thiolate–sulphurs of Cu–thionein should be linked to two copper atoms each. Due to thermodynamical grounds the initial proposal of such a chain of tetrahedra must be abandoned [3, 7–9]. The strain in a four-membered ring composed of $Cu(SR)_2$-units would be enormous. Recent studies on the primary structure suggest a M_r of 4800 Daltons including 8 cysteine residues [6]. The most plausible explanation for a thermodynamically consistent arrangement would be a cubane type structure of 4 $Cu\text{-}(thiolate)_2$-units (Fig. 6b). Many known cuprous-halide and sulphur compounds occur in such a cubane arrangement [11–13, 32].

At each corner one two-coordinate cysteine–sulphur is attached to each copper separated by 2.22 Å. These cysteine–sulphurs might originate from the two isolated Cys residues and the Cys–Ser–Cys segment. The four-coordinate sulphurs located at the other four corners of the cube might be assigned to the two Cys–Cys-pairs. One Cu–S bond should be shorter at 2.22 Å leaving the other two at 2.36 Å. Thus, the two different kinds of Cu–S bonding which were elucidated from EXAFS measurements could be explained. The most striking result of all is the phenomenon that in all metallothioneins a tetrahedrally arranged mononuclear metal-$(SR)_4$ unit is present [3, 8–10, 25, 33].

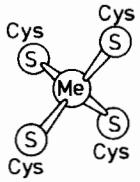
Apart from the overall structure of Cu–thionein the functional side of this type of protein remains obscure. In earlier work its possible role in controlling the intracellular copper-concentration has been extensively discussed [1, 4, 7–10]. An additional evolutionary aspect should be pointed out.

The 'soft' chalcogen sulphur is known to be a most efficient transition metal binding ligand. Incorporation of metal sulphides into proteins may have started with the iron-sulphur proteins. The next step could have been the insertion of thiolate-sulphur into both rubredoxin and metallothionein. Ferredoxin and rubredoxin have evolved to become very efficient electron carriers. A similar function of the Cu–thioneins is not known at the moment.

An attractive hypothesis might be that metallothioneins are the ancestors of the type 1 copper centres of electron transport proteins. The metal binding centres of alcoholdehydrogenase are an interesting example of the transient state. The non-catalytic $Zn(SR)_4$ complex resembles the rubredoxin iron centre and the monomeric unit of zinc– or copper–thionein. The catalytically active Zn is coordinated to two cysteine-sulphurs, one substrate binding site and one histidine-nitrogen. In other words, two thiolate-sulphurs of the metallothionein cluster have developed into somewhat more sophisticated ligands including the imidazolate moiety.

Maret *et al.* [34] succeeded in converting this catalytically active zinc-chromophore of alcohol dehydrogenase into a type 1 copper binding site thereby leaving the non-catalytic $Zn(SR)_4$ chromophore unchanged. Selective replacement of zinc by copper and substitution of the coordinated water with pyrazole led in the presence of coenzyme NAD^+ to a stable type 1 copper chromophore [34, 35]. The plastocyanin copper-chromophore [36], an established type 1 copper binding centre, consists of two sulphur ligands cysteine 84 and methioneine 92 and two histidine residues. It is conceivable to postulate the further occupation of the fourth copper binding site by a second unsaturated ring system. The two 'soft' liganded sulphurs and the somewhat 'harder' imidazolate-nitrogen of plastocyanin and azurin [37] allow electron transport reactions

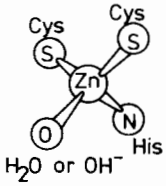
Homogeneous metal thiolate coordination



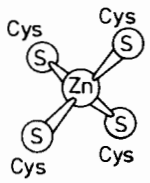
Rubredoxin, Cd-, Zn-, Cu-Thionein

'Transient state'

homogeneous thiolate coordination

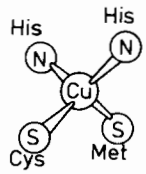


mixed ligand coordination



Liver alcohol dehydrogenase

The Type I Cu-chromophore



Plastocyanin

from the chelated copper via the protein portion efficiently.

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