TETRAHEDRAL COPPER–SULPHUR COORDINATION IN YEAST Cu-THIONEIN

An EXAFS study

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1. Introduction

A copper containing thiolate-rich protein occurs in yeast [1,2]. Due to many similarities of the metal-thiolate chromophore to that of the metallothioneins [3] it is presently termed Cu-thionein. During gel filtration the protein migrates with an apparent relative M_r of 10 000 [2]. On the basis of amino acid analyses the relative M_r value is 4800 [4] including 4 g-atoms of copper/molecule. The stoichiometry of copper to cysteine-sulphur was 1:2.

At present the structure elucidation of the copper chromophore remains obscure. Copper is in the 3 d^{10} state [2] and bound with cysteine-sulphur [5]. Taking into account the many physicochemical properties a tetrahedral structure of the copper-thiolate centre was proposed in 1978 involving three-coordinate sulphur throughout [5–8]. A suitable method to shed more light on the precise copper–sulphur coordination promised to be the extended X-ray absorption fine structure (EXAFS) of Cu-thionein. Cu(thiourea)$_3$Cl was used as a convenient reference compound attributable to both its tetrahedral copper–sulphur arrangement and its known X-ray structure [9].

Virtually identical EXAFS spectra of Cu(thiourea)$_3$Cl and Cu-thionein were obtained suggesting the same tetrahedral arrangement of the sulphurs around the copper. A slight distortion of the copper-thiolate tetrahedra in Cu-thionein is assumed due to the somewhat shorter distances between each of the two sulphur pairs bound at 2.22 Å and 2.36 Å, respectively. Unlike with Cu(thiourea)$_3$Cl a more closely packed structure was considered for Cu-thionein. The best guess for the arrangement of four copper-thiolate tetrahedra fitted the cubane type of many known Cu(I) compounds.

2. Experimental

Cu(thiourea)$_3$Cl was prepared and recrystallized from hot water following the procedure in [9]. Cu-thionein was isolated from Cu-supplemented baker's yeast [2]. The washed cells were homogenized at 600 kg/cm^2 and the 10 000 × g supernatant was heated to 60°C for 3 min. After removal of precipitates the soluble fraction was chromatographed on Biogel P6, QAE Sephadex A-25, Sephadex G-75 and again on QAE Sephadex A-25. The protein was desalted on Biogel P10 and lyophilized. Copper was quantitated on a Perkin Elmer atomic absorption spectrometer, model 400 S, equipped with a HGA-76B graphite furnace. Circular dichroism was measured on a JASCO 20A recording spectropolarimeter.

Extended X-ray absorption fine structure data were collected at the European Molecular Biology Laboratory (EMBL), Hamburg Outstation, using the Synchotron radiation facilities of the Deutsches Elektronen Synchotron (DESY). Lyophilized specimen, ~1 mm thick, was placed in a flat cell between polyacrylic windows (Mylar). The employed X-ray spectrometer was situated at ~35 m of the tangent point of the storage ring. X-ray absorption spectra were recorded on the white beam line using channel cut silicon (III) monochromators. The logarithm of the transmission data were further used for conversion to EXAFS modulation spectra employing the data reduction and EXAFS extraction programs at the

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EMBL outstation. For detailed description of the techniques see [10,11].

3. Results and discussion

Upon recording the X-ray absorption spectra the degree of possible deterioration was examined. The ϵ-values and the circular dichroism of Cu-thionein remained unchanged before and after irradiation. Neither was there an observable decomposition of Cu-thiourea. The EXAFS oscillations of either copper compound revealed a striking similarity in both shape and amplitude (fig.1). It can be deduced that the atoms surrounding the copper are identical i.e., sulphur, and, that the coordination number must also be equal.

The maxima of the EXAFS oscillations for Cu-thionein appear at momentum values which are apparently 5% higher compared to those observed for Cu-thiourea. This suggests a somewhat shorter copper–sulphur bond length in the protein. Taking into account the known mean value of the copper–sulphur distance of Cu-thiourea (2.36 Å) [9] the corresponding mean value for Cu-thionein should be ~2.29 Å. The small shoulders near 4 Å in the EXAFS spectrum of Cu-thiourea are absent using the protein. These small but significant differences may be attributed to contributions of more distant shells. Due to the absence of these contributions a more compact structure could be assumed for the metal-thiolate centre in Cu-thionein.

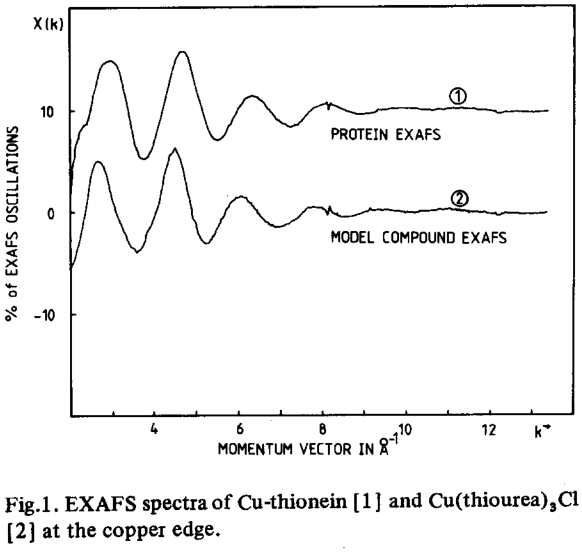

Fig.1. EXAFS spectra of Cu-thionein [1] and Cu(thiourea)$_3$Cl [2] at the copper edge.

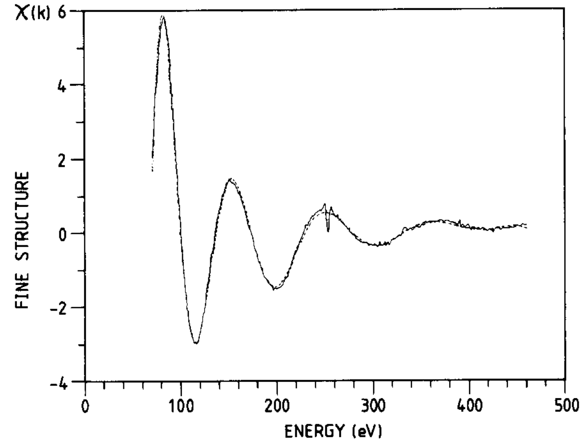

Fig.2. First principle calculation fit to the EXAFS spectrum of Cu-thionein. The full line corresponds to the experimental data, the broken line corresponds to the best theoretical fit obtained with four sulphur atoms surrounding the copper, two atoms at 2.22 Å and another two at 2.36 Å. The estimated errors in the bond distances are ~0.02 Å.

A first principle interpretation of the EXAFS spectrum of Cu-thionein was carried out employing the methods in [10,12–14]. 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 (fig.2). It is needless to discuss the high quality of the fit.

There are considerable difficulties in maintaining our original proposal of the chain-type tetrahedral copper-thiolate centres in Cu-thionein [5–8]. By way of contrast Cu-thiourea is well known to form polymeric corner-linked tetrahedra. One double-bonded sulphur serves as the bridging ligand. In Cu-thionein only thiolate sulphur is present. The stoichiometry is 8 S:4 Cu. An extraordinary strain of a possible ring containing four Cu(SR)$_2$-units could be expected. Such an arrangement should be, thermodynamically rather unlikely.

Many known Cu(I) compounds tend to form fairly easily cubane centres [15–18]. In the light of the present data the best guess for smoothly arranging the four Cu(SR)$_2$ species could well be successful in a cubane-type structure (fig.3). Four close thiolate-sulphurs sticking 2.22 Å out of the distorted cube might originate from the Cys–X–Cys and the two isolated Cys-residues from the protein backbone [4]. The soft single-bonded sulphurs forming part of the cube tend to be well-suited to become four-coordinate

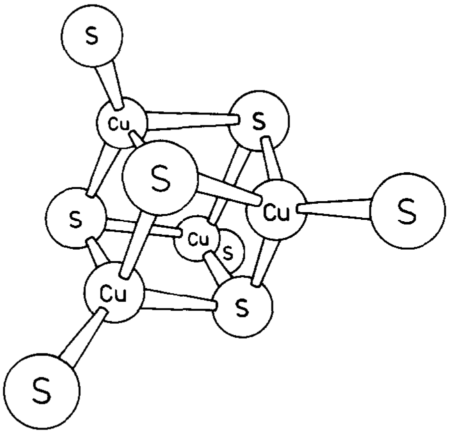

Fig.3. Proposed arrangement of Cu-thiolate tetrahedra in yeast Cu-thionein.

[15–18]. A minor dislocation of this four-coordinate sulphur fulfils the requirement of another shorter Cu–S-bonding each at 2.22 Å leaving the other two Cu–S-bondings at 2.36 Å.

The four cysteine sulphur forming parts of the cube may be assigned to the two Cys–Cys pairs of the primary protein structure. Of course, such a cubane-type metal binding centre similar to that of the 4 Fe–4 S-centres should demand a possible role in electron transport reactions.

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