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An EXAFS study of the copper accumulated by yeast cells

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Summary. X-ray absorption spectroscopy has been applied to the in vivo examination of copper-resistant yeast cells. The in vivo structure of the metal-binding site of the accumulated copper has been compared to that of the purified yeast thionein. Analysis of the EXAFS spectra performed on intact yeast cells indicates that the accumulated copper is univalent and is exclusively coordinated to sulfur atoms at a distance of 219 pm with an average coordination number of 2. In contrast, the purified protein indicates a univalent copper trigonally coordinated to sulfur at a distance of 221 pm. These discrepancies are discussed in terms of copper location in the resistant yeast cells.

Key words: EXAFS - Cu(I)-thionein - Copper-enriched yeast cells

Introduction

The molecular side of copper transport in biological systems is unknown. The intriguing role of this highly reactive transition metal requires a finely tuned regulation of cellular copper levels. Intracellularly there is a protein called 'metallothionein' where copper is firmly bound in stable Cu(I)-thiolate centres. It is increasingly considered to be required for controlling copper metabolism (Weser and Hartmann 1984; Felix et al. 1989).

Due to the multiplicity of the *CUP1* gene, yeast cells are able to enrich copper from the growth media where this metal can be present up to millimolar concentrations (Ecker et al. 1986; Wright et al. 1988). In the intact protein isolated by gel filtration, 8 Cu(I) atoms are associated with 12 cysteine residues. The biosynthesis of Cu_8-thionein is highly induced in these resistant cells in proportion to the concentration of added copper.

EXAFS measurements on the holoprotein revealed each Cu(I) atom to be surrounded by three thiolate sulfur atoms. The Cu-S distance was 223 pm (George et al. 1988). In an earlier EXAFS study (Bordas et al. 1982), four sulfur atoms were assigned to be the first shell atoms around Cu(I) at two different distances, i.e. 222 and 236 pm. These discrepancies in both coordination number of first shell atoms and bond lengths could be attributed to the two different isolation techniques. Structural changes or deterioration might have been occurred. A restricted accuracy of the theoretical analysis available at that time could alternatively have been the cause. Thus, it was of interest to repeat the EXAFS measurements on a Cu_8-thionein which was isolated exclusively by gel filtration (Weser and Hartmann 1988). The same measurements were carried out using intact copper-loaded yeast cells. It was attractive to compare the EXAFS data of both isolated Cu-thionein and the protein still remaining inside the cells.

Materials and methods

The copper-resistant strain *Saccharomyces cerevisiae* X2180-1Aa was grown in a medium containing 1 mM $CuSO_4$ for 48 h at 25° C. The cells were harvested, washed three times with 50 vol. distilled water and lyophilized. No cell damage during the washing procedure was observed. Yeast Cu-thionein was isolated from ruptured cells using a method described elsewhere (Winge et al. 1985; Weser and Hartmann 1988). A metal content of 8 mol copper atoms/mol protein with a 1:1.5 Cu/S ratio was obtained.

X-ray absorption spectroscopy was performed on freeze-dried samples disposed into aluminium sample holders with cello-tape windows. EXAFS spectra were recorded in the transmission mode for the purified protein and in the fluorescence mode for the yeast cells at the Daresbury Synchrotron radiation source. Data analysis utilized the single scattering spherical-wave method for calculating EXAFS with phase shifts derived from *ab initio* calculations as previously described (Lee and Pendry 1975). The quality of fits was assessed by using criteria previously described (Abrahams et al. 1986).

Results and discussion

The X-ray absorption edges of purified metallothionein and of the copper-resistant yeast are reported in Fig. 1.

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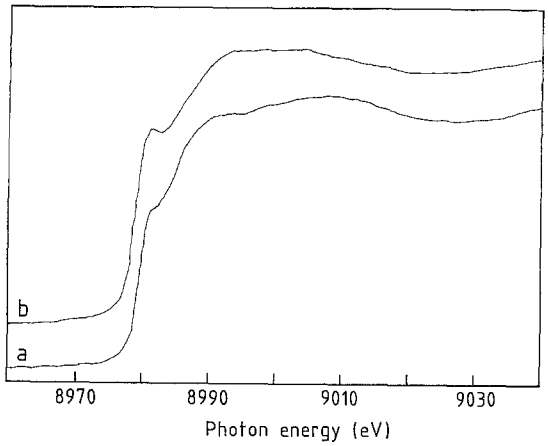

Fig. 1. Copper K absorption edge of yeast copper-metallothionein *(a)* and copper-resistant yeast cell *(b)*

The edge structures are very similar and both lack the 1s-3d transition near 8980 eV confirming the presence of a reduced copper in both cases. The K^3-weighted ex-

Table 1. EXAFS parameters for purified metallothionein and copper-resistant yeast cells

Substance	n	r(Å) [pm]	σ^2(Å^2) [pm^2]
Protein	3.0	2.21 [221]	0.018 [1.8]
Cell	2.0	2.19 [219]	0.024 [2.4]

perimental EXAFS spectrum of the purified copper protein and its Fourier transform are shown in Fig. 2A (full line). The Fourier transform indicates that the EXAFS data are dominated by a strong Cu-S interaction, giving rise to a major peak at $R = 0.2$ nm. In order to determine an accurate Cu-S distance and coordination number, both the EXAFS and its Fourier transform were simulated. The simulations are also represented in Fig. 2 (broken lines) and the best-fit parameters are reported in Table 1. Indication for a copper trigonally coordinated to sulfur atoms with a Cu-S distance of 221 pm was obtained in line with recent results (George et al. 1988) and in contrast with a previous re-

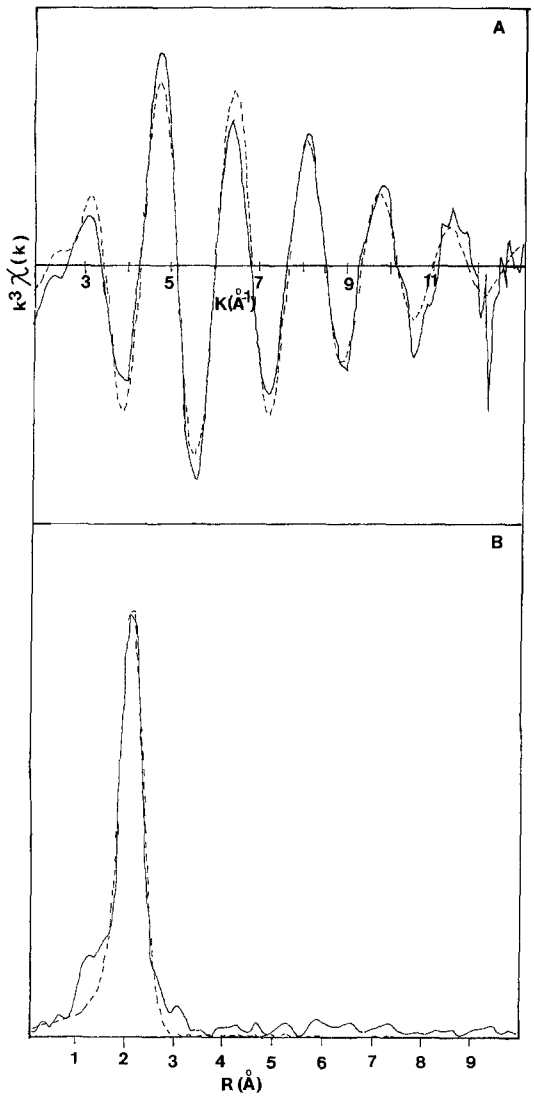

Fig. 2. Copper EXAFS **(A)** and EXAFS Fourier transform **(B)** of yeast copper-metallothionein. 1 Å^{-1} ≡ 10 nm^{-1}; 1 Å ≡ 0.1 nm

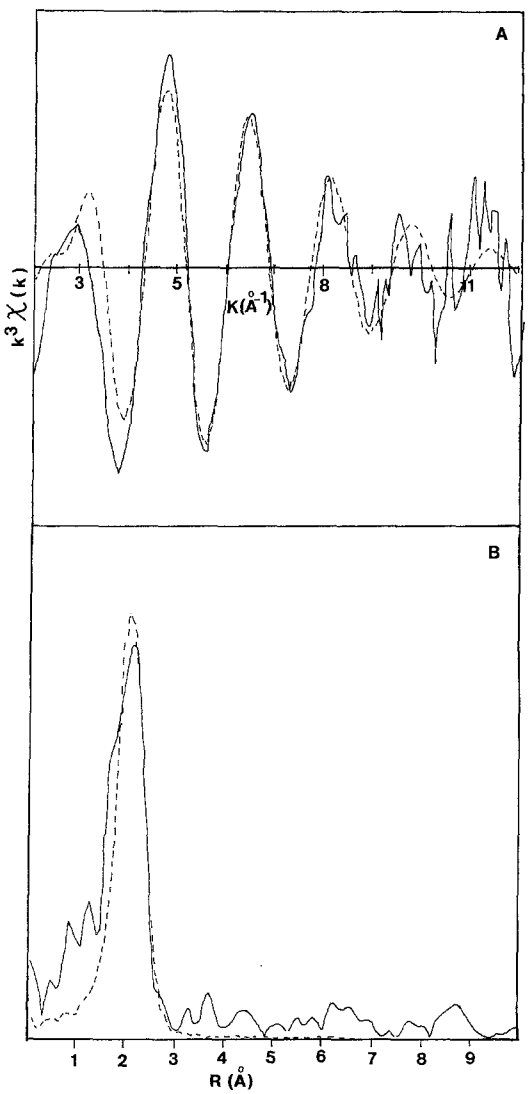

Fig. 3. Copper EXAFS **(A)** and EXAFS Fourier transform **(B)** of copper-resistant yeast cell. 1 Å^{-1} ≡ 10 nm^{-1}; 1 Å ≡ 0.1 nm

port (Bordas et al. 1982). This discrepancy with the previous results can probably be explained by the lower accuracy of the theoretical analysis available at the time of the earlier paper.

The K^3-weighted experimental EXAFS spectrum of the cells and its Fourier transform is reported in Fig. 3. The spectrum displays a lower signal/noise ratio with respect to the purified holoprotein but it is still of sufficient quality to allow an accurate analysis. The simulated spectra are also represented in Fig. 3 (broken lines) and the corresponding best-fit parameters are reported in Table 1. In this case a Cu–S distance of 219 pm and a coordination number of 2 was obtained.

It was intriguing that the copper sulfur bonding distances close to 220 pm were virtually identical. A difference in the coordination number between the homogeneous Cu-thionein and the yeast cells was observed: the calculated numbers dropped from 3 to 2. Independent studies on intracellular and extracellular Cu_8-thionein have demonstrated the chemical and structural identity of the two proteins, including copper content, molecular mass, luminescence emission and electronic absorption (Felix et al. 1989). Thus, structural differences for this phenomenon cannot be taken into account. One or more additional intracellular Cu compounds must have obscured the initial coordination number of 3. It was striking that essentially all the copper was in the reduced state as there is no evidence for the presence of Cu(II) in the copper K-edge spectrum (see Fig. 1). The most likely candidate is assumed to be a Cu(I)-glutathione complex. In copper-resistant rat hepatoma cells, significant concentrations of cytoplasmic copper were detected in a Cu(I)-glutathione complex (Freedman et al. 1989). The present observation suggests that this phenomenon may also be the case for the copper-resistant yeast.

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References

Abrahams IL, Bremner I, Diakun GP, Garner DC, Hasnain SS, Ross I, Vasak M (1986) Structural study of the copper and zinc sites in metallothioneins by using extended X-ray-absorption fine structure. Biochem J 236:585–589

Bordas J, Koch HJ, Hartmann HJ, Weser U (1982) Tetrahedral copper–sulphur coordination in yeast Cu-thionein an EXAFS study. FEBS Lett 140:19–21

Ecker DJ, Butt TR, Sternberg EJ, Neeper MP, Debouck C, Gorman JA, Crooke ST (1986) Yeast metallothionein function in metal detoxification. J Biol Chem 261:16895–16900

Felix K, Hartmann HJ, Weser U (1989) Cu(I)-thionein release from copper-loaded yeast cells. Biol Metals 2:50–54

Freedman HJ, Ciriolo MR, Peisach J (1989) The role of glutathione in copper metabolism and toxicity. J Biol Chem 264:5598–5605

George NG, Byrd J, Winge DR (1988) X-ray absorption studies of yeast copper metallothionein. J Biol Chem 263:8199–8203

Lee PA, Pendry JB (1975) Theory of the extended X-ray absorption fine structure. Phys Rev B 11:2795–2811

Weser U, Hartmann HJ (1984) In: Lontie R (ed) Copper proteins and copper enzymes, vol 3. CRC Press, Boca Raton, FL, pp 151–173

Weser U, Hartmann HJ (1988) Differently bound copper(I) in yeast Cu_8-thionein. Biochim Biophys Acta 953:1–5

Winge DR, Nielson KB, Gray WR, Hamer DH (1985) Yeast metallothionein. Sequence and metal-binding properties. J Biol Chem 260:14464–14470

Wright CF, Hamer DH, McKenney K (1988) Autoregulation of yeast copper-metallothionein gene depends on metal binding. J Biol Chem 263:1570–1574