ESRF	Experiment title: **CopK and CzcE from the environmental strain *Cupriavidus metallidurans* CH34 participate in copper resistance.**	**Experiment number**: EC-327
Beamline: BM30B	**Date of experiment**: from: 20/11/2008 to: 26/11/2008	**Date of report**: 15 sept 2009
Shifts: 18	**Local contact(s)**: Jean-Louis Hazemann	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Géraldine Sarret*, CNRS & UJF-Grenoble
Florian Molton*, CNRS & UJF-Grenoble
Beate Bersch*, CNRS & IBS-Grenoble
Jacques Covès*, CNRS & IBS-Grenoble
Isabelle Petit-Haertlein*, CNRS & IBS-Grenoble

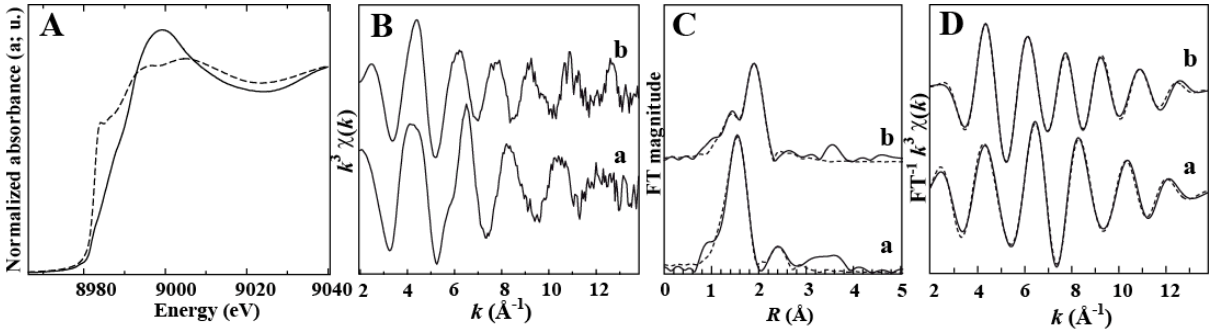
Report: CzcE and CopK are two proteins located in the periplasmic space of *Cupriavidus metallidurans* CH34, a model organism for studying the resistance to heavy-metals. Both CzcE and CopK are involved in resistance to copper but their exact role is still largely unknown [1,2]. In relation with the resolution of their 3D structures, in solution for CopK and by X-ray diffraction for CzcE, the goal of the proposal EC-327 was the identification of the copper ligands in these proteins. Copper-bound proteins were prepared as previously described [1,2].

This work led to the preparation of two manuscripts to be submitted soon to J. Mol. Biol. for CzcE and to J. Am. Chem. Soc. for CopK.

Experimental

The proteins in solution were mixed with 15% glycerol, loaded in a five-cell sample-holder with a Kapton window, and flash-frozen in liquid nitrogen. The sample-holder was loaded in a helium cryostat with temperature set to 10K during data collection. All spectra were collected in fluorescence mode by measuring the Cu Kα fluorescence with a 30-element solid-state Ge detector (Canberra). For each sample, ten to fifteen scans of 40 min each were averaged.

1- CzcE: XANES spectra for CzcE in its fully-oxidized and fully-reduced state show clear differences in the energy position and shape of the edge. The broad edge in the spectrum for oxidized CzcE is typical of Cu(II) complexes whereas the pre-edge at 8984 eV in the spectrum for reduced CzcE is characteristic of Cu(I) complexes. The Fourier transformed spectrum for CzcE in oxidized state presents an intense first peak typical of a first shell composed of O/N atoms. Correct fits were obtained with a combination of N and O atoms at an average distance of 1.97 ± 0.01 Å, with the following constraints: $N_N > 1$ and $N_O < 3$, and $N_N + N_O = 4$. A mixed O/N shell is consistent with the X-ray data showing that His and Asp are ligands for Cu(II). The second shell was fitted by 1.7 ± 0.6 C at 2.88 ± 0.02 Å. The spectrum for CzcE in reduced state presents a clearly higher frequency compared to oxidized CzcE, and the first peak of the Fourier transform is splitted in two sub-peaks. The first peak was fitted with a mixture of 1.2 ± 0.2 O/N atoms at 1.91 ± 0.02 Å and 1.8 ± 0.3 S atoms at 2.29 Å. No good fit was obtained with a total coordination number of 4. The second shell was composed of 1.4 ± 0.6 C atoms at 3.27 ± 0.05 Å. Because CzcE does not contain any cysteine, the sulfur-containing species coordinating Cu(I) atoms likely originate from the methionine residues. This is consistent with the deduced Cu-S bond length of 2.29 Å typical of weak donors such as thioether ligands. A Cu-S distance of 2.29 Å cannot be obtained without a conformational modification of the protein as judged from the positions of the methionine residues in *apo*-CzcE. For instance the shortest distance between two S atoms in *apo*-CzcE is about 10 Å considering Met47 and Met86.



CzcE experiment: Panel A, Cu-*K* edge XANES spectra for CzcE in reduced (dashed line) and oxidized (plain line) states. Panel B-D, k^3-weighted EXAFS spectra (B), Fourier transforms (non phase shift-corrected, C) and Fourier filtered contributions for the first and second shells (D) for CzcE in oxidized (a) and reduced (b) state. Solid line: experimental, dotted lines: fits.

The XAS data along with the X-ray data are compatible with the conclusion that different conformational changes occurred upon Cu(II)- or Cu(I)-binding.

2- CopK: Combined use of NMR and EXAFS data allowed the determination of the solution structure of Cu(I)-CopK with a high precision on the Cu(I)-site.

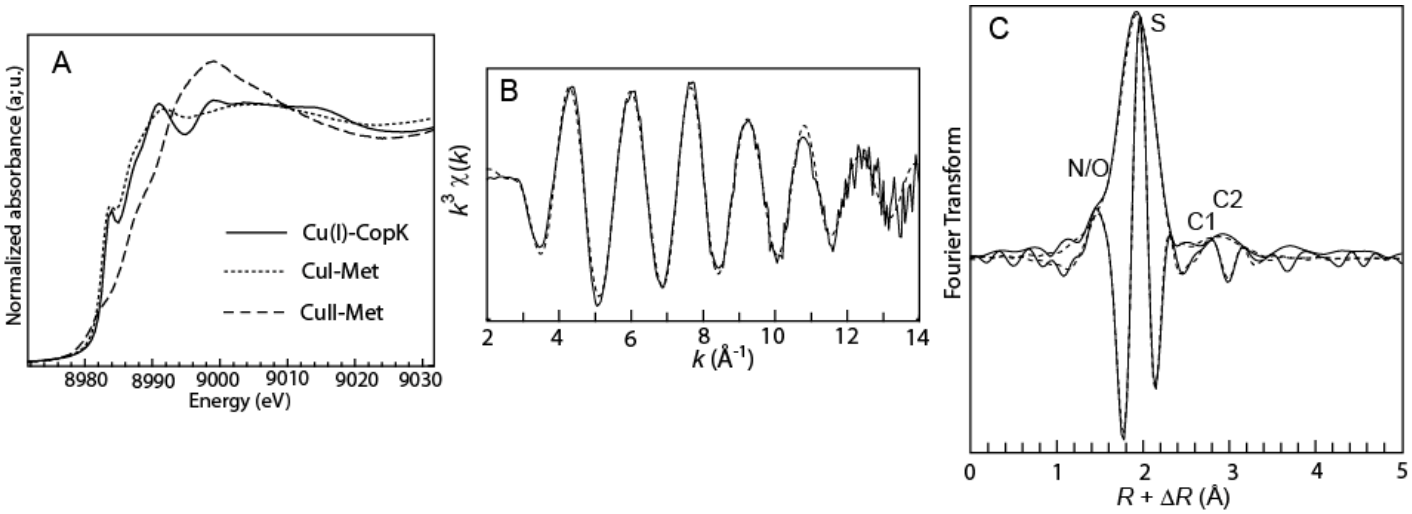


Figure 2 : A: Cu K-edge XANES spectra for Cu(I)-CopK, and Cu(I)- and Cu(II)-methionine references , B and C: k^3-weighted EXAFS spectrum and Fourier transform (modulus and imaginary part, non phase shift-corrected) for Cu(I)-CopK (experimental) and simulation (dotted lines)

From the XAS data, we concluded that the copper ion occupies two different sites. The major one is composed of an S shell at 2.31 Å and a C shell at 3.35 Å and is consistent with a Cu(I)-ion bound to the thioether group of methionine residues. The second site contains an O/N shell at 1.92 Å and a C shell at 3.12 Å and may correspond to a copper ion bound to histidine. From the coordination numbers of the first shell (0.2 O/N atoms and 3.8 S atoms), one can conclude that about 95% of copper occupies the Cu-Met site in which it is bound to four methionines.
In case of the CopK protein, the concomitant observation of the downfield shift of four methionine methyl carbons (M28, M38, M44 and M54) observed by NMR and the fit of the EXAFS data to a Cu(I) ligand shell composed of four sulfur atoms strongly suggests that the chemical shift of this methyl carbons probes the presence of a metal ion bound to the methionine sulfur atom. Combined use of the EXAFS and chemical shift data described above provided essential experimental information on the geometry of the Cu(I)-binding site.

Conclusion: In the case of CzcE, XAS data were used to further support the previous hypothesis of conformational changes linked to Cu(II) or Cu(I)-binding. For CopK, the XAS data were used to bring physical information that helped during structure resolution by NMR. In both cases, the biostructural analyses have taken advantages of the XAS data.

[1] Zoropogui *et al* (2008) Biochem. Biophys. Res. Commun. 365, 735-739.
[2] Bersch *et al* (2008) J. Mol. Biol. 380, 386-403.