ESRF	**Experiment title:** X-ray absorption spectroscopy studies of a heme-copper oxidase.	**Experiment number**: **CH-3137**
Beamline: 29	**Date of experiment**: from: 07 apr 2010 to: 13 apr 2010	**Date of report**: 03-03-2011
Shifts: 18	**Local contact(s)**: Santiago Figueroa (email: santiago.figueroa@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

E. Nazarenko*, J. Sjöhamn, L. Johansson*, P. Edlund, A. Polyakova, G. Katona*, R. Neutze*

University of Gothenburg, Department of Chemistry, Gothenburg, Sweden

A. Nasedkin*

Uppsala University, Department of Photochemistry and Molecular Science, Uppsala, Sweden

Report:

Aim:

Heme-copper oxidases are integral membrane proteins which serve as the final electron acceptor of respiratory chains across all kingdoms of life. These integral membrane proteins accept electrons so as to reduce oxygen to water and thereby exploit the chemical energy released to pump protons.

Given the complexity of these processes, it is essential to determine any effect of changes in protonation state of groups near the catalytic centre. Such groups are likely to play essential roles in these functionally important conformational changes. Most proposed proton-pumping mechanisms involve CuB site and its histidine ligands. The existence and identity of such reorganization of the CuB geometry caused by protonation/deprotonation and/or breakage of one of the Cu-N(His) bonds is a difficult matter to either prove or disprove since CuB is spectrally silent. Since the Ubiquinol oxidase consists just one Cu site and two Fe atoms it's ideal candidat to apply X-ray absorption spectroscopy to study what hapen near the Cu-site when Cu changes its oxidation state and to study pH dependence of the Cu site.

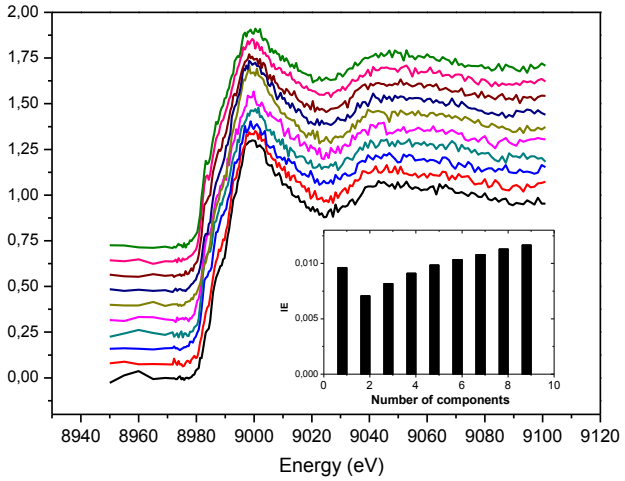
Quality of measurement/data and status / progress of evaluation:

Spectra have been mesured in solution (placed in the cappilary) in fluoresence mode using 13-element fluoresence detector. The Cu K-edge spectra have been collected in room temperature and 80 K (the cryostream has been used to cool down a sample) and in the pH range: 6.5-9.5.

Several scans of the Fe K-edge have been mesured as well. Due to the fast photoreduction of the Fe K-edge (a hundrigs seconds) we decided not continue to mesure spectra at the Fe K-edge and collect more scans for the Cu K-edge. 380 scans have been mesured in total.

Photoreduction of the Cu K-edge has been studied by collecting a set of scans (5 scans for 40 minutes) for each fresh sample in the pH range: 6.5-9.5. Then the principal component analysis (PCA) using the FitIt softwear [G. Smolentsev, Comp. Matter. Science 39, (2007), 69] has been applied to check how many intermidiates consist each brunch of spectra for the fresh sample. Results presented in the Fig. 1 indicat the presence only two components in the set of spectra. We got the same results for all pH concentrations.

Fig. 1. Experimental Cu *K-edge XANES* spectra measured during X-ray photoreduction. Insert: IE function, which was used to determine the number of observed spectral components.



Cu K-edges for oxidazed, photo-reduced and dithionite-reduced forms in the pH range 6.5-9-5 are shown in Fig.2 The energy and shape of the oxidazed edges and photo-reduced edges correspondingly remain unchanged in the whole pH range that demostrate that there is no structural change of the Cu enviroment in the pH 6.5-9.5 range.

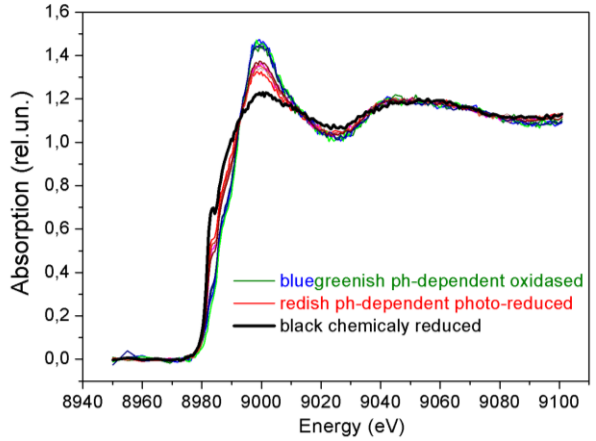


Fig. 2. Bluegreen, red – pH dependence of experimental Cu K-edge XANES spectra after Principal component analysis for each set of pH dependent data. Black – experimental chemicaly reduced sample.

The structure refinement from XANES spectra using non-muffintin simulations (FDMNES code [Y. Joly, Phys. Rev. B 63, 125120-125129 (2001)]) and FitIt softwear for fiting procedure of the spectra is currently under way. Preliminary results indicate that the Cu site is surrounded by three His for reduced state and by three histidines and a water molecule or hydroxile for oxidased state. The best fit results are shown in the Fig.3.

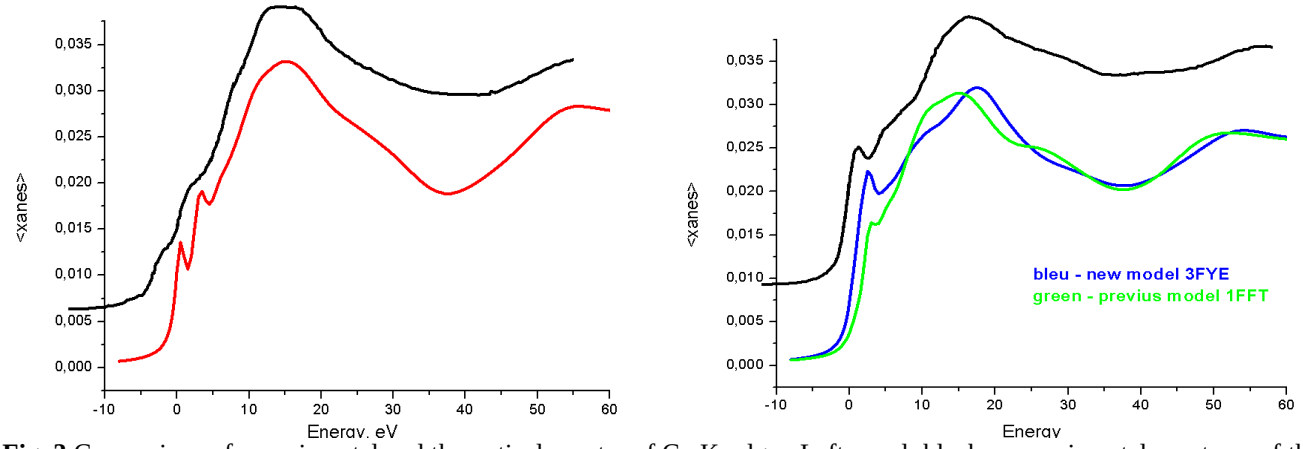


Fig. 3 Comparison of experimental and theoretical spectra of Cu K-edge. Left panel: black – experimental spectrum of the oidased state of the Cu site (Cu(I)), red – calculated spectrum using FDMNES code. Right panel: black - experimental spectrum of the reduced state of the Cu site (Cu(I)), bleu – best-fit results on the base of 3FYE PDB structure; green –best fit results on the base of 1FFT PDB structure.

Conclusion:

We report the X-ray absorption spectroscopy studies of the copper edge of the cytochrome bo3 quinol oxidase from Escherichia coli.

Our results indicate that CuB changed its associated ligands for oxidised Cu(II) and reduced Cu(I) states of the protein. However room temperature copper K-edge X-ray absorption spectra remains unchanged in the pH range 6.5-9.5 for both oxidised and reduced forms of copper correspondently, indicating that no structural changes takes place at CuB depending on pH.

Work has been presented as a poster in SBNet 2010 (Sweden) and ESRF User meeting 2011.