ESRF	**Subcellular distribution of iron in dopaminergic neurons following exposure to Parkinson's disease inducing neurotoxins, and iron chelators.**	**Experiment number**: MD265
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Cu and Zn coordination in Cu,Zn superoxide dismutase mutants

The aim of this study was to characterize, using XANES and EXAFS, the oxidation state and coordination site of Cu in human wild type Cu,Zn superoxide dismutase (CuZnSOD) and CuZnSOD mutants involved in familial amyotrophic lateral sclerosis (fALS), a fatal neurodegenerative disease affecting motor neurons. More specifically, we would like to investigate if Cu is reduced in fALS mutants, and if a new metal binding site (sulphur-bound) exists in these mutants.

ALS results from the progressive death of few motoneurons localized in the ventral horn of the spinal cord, which causes rapid muscle degeneration and paralysis. About 10% of ALS cases are inherited; the remainder are believed to be sporadic cases. Of the inherited cases, about 20% are caused by mutations in the gene encoding CuZnSOD. CuZnSOD is an important antioxidant enzyme that catalyzes the disproportionation of superoxide anion to dioxygen and hydrogen peroxide. CuZnSOD is a dimeric enzyme with one zinc and one copper binding site per monomer. Despite the explosion of ALS research engendered by the discovery of the CuZnSOD mutations, the actual nature of the gained function by which mutant CuZnSOD kills motor neurons in ALS remains elusive.

It has been suggested that a common property of mutants is that they could exhibit weaker metal binding which renders the mutants more sensitive to disulfide reduction. Less-tightly folded enzymes could catalyse aberrant copper-mediated chemistry, owing to greater access of abnormal substrates to the active copper site. A slight modification of this idea is that mutants might handle the copper clumsily, frequently releasing it, allowing free copper to catalyse unwanted oxidative reactions.

The coupling of native IEF (isoelectric focusing) and XAS enables to study the coordination chemistry of mettalloproteins such as CuZnSOD. EXAFS experiments were performed on CuZnSOD isoforms separated according to their isoelectric point in non denaturating conditions (Fig. 1). The use of native conditions during electrophoresis is manadatory in order to avoid metal loss or redox changes. Thanks to this innovative protocol, the speciation analysis of metaloproteins on electrophoresis gels is possible which opens a large field of applications in metalloproteomics.

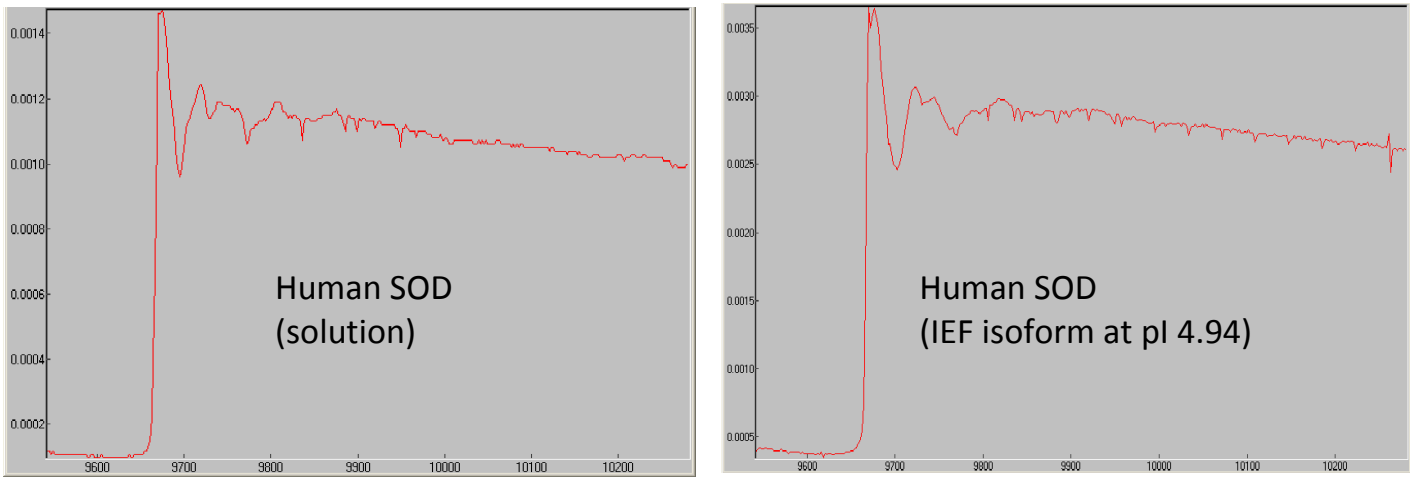

Figure 1: EXAFS spectra at Zn K-edge of human superoxide dismutase obtained on a commercial solution of SOD (left) and on the same protein after isoelectrofocusing (IEF) in non denaturatig conditions. Native IEF revealed 3 isoforms of the human SOD at isoelectric points 4.75; 4.86 and 4.94. This last isoform was analysed selectively (left). This example demonstrates the feasibility of EXAFS analysis at Zn K-edge on SOD isoforms separated by gel electrophoresis. This is to our knowledge the first example of EXAFS spectroscopy on proteins separated by gel electrophoresis. However, the background signal systematically observed during acquisition (due to electronic failure) alter the data treatment of EXAFS signal. Only the XANES region could be saved correctly.

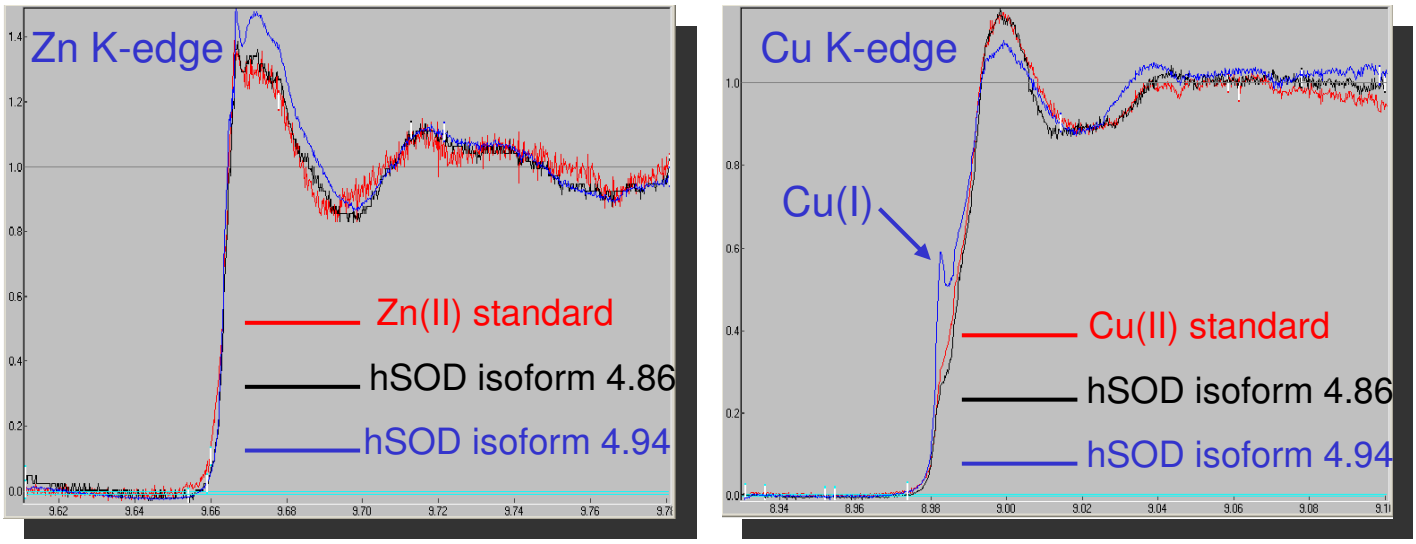

Figure 2: XANES spectra at Zn (right) and Cu K-edges (left). Spectra were numerically filtered in order to remove systematic background noise (seen in Fig. 1). As expected, Zn is it its divalent oxidation state in both isoforms of human SOD isoforms 4.86 and 4.94. Zn exists only in its Zn(II) oxidation state in biological samples. XANES at Cu K-edge revealed that the more basic isoform (4.94) presented a reduced form Cu. This is a very important result that suggests that SOD isoforms might differ in their redox state.

During this experiment we demonstrated the feasibility of XANES and EXAFS analyses on CuZnSOD separated on electrophoresis gels (Fig. 1 and 2). However, two problems occurred (1) the Cu cryostage was a source of background Cu radiation; and (2) a default in the data recording process brought systematic background noise which prevented unambiguous Zn and Cu K-edge EXAFS treatment. However, the XANES region could be treated correctly. As expected Zn appears in its Zn(II) oxidation state in all isoforms (Fig. 2). This is in agreement with the known oxidation state of Zn in CuZnSOD. Very interestingly, differences were observed in Cu oxidation states from distinct isoforms of the human CuZnSOD (Fig. 2). The more basic isoform being characterized by a reduced Cu. This result suggests that CuZnSOD mutants involved in ALS, which are characterized by a shift toward more basic pI compared to human wild type, are more prone to reduction processes. Further EXAFS experiments at Cu K-edge are necessary to conclude if the reduction processes could be due to sulphur adducts on the Cu binding site.