Iron Speciation in human cancer cell lines by K-edge SRTXRF-XANES

Zs. Polgari[1], N.Szoboszlai[1], F.Meirer[2, 5], D,Ingerle[2], G.Pepponi[3], B.Budai[6], G.Zaray[1] ,K.Appel and C. Streli[2]

[1]Institute of Chemistry, Eötvös Lorand University, Budapest, Hungary
[2]Atominstitut, Vienna , Austria
[3]FBK-irst, Povo (Trento) Italy
[4]Hamburger Synchrotronstrahlungslabor at DESY, 22607 Hamburg, Germany
[5]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA, USA
[6]Department of Clinical Research, National Institute of Oncology, Budapest, Hungary

X-Ray Absorption Near Edge Structure (XANES) analysis in combination with Synchrotron Radiation induced Total reflection X-Ray Fluorescence (SR-TXRF) acquisition was used to determine the oxidation state of Fe in iron-overloaded human cancer cells. The Fe K-Edge XANES measurements in fluorescence mode and grazing incidence geometry were carried out using the TXRF vacuum chamber setup at the beamline L at the Hamburger Synchrotronstrahlungslabor (HASYLAB) at DESY[1-3]. The feasibility of the SR-TXRF XANES analysis is shown in detail in our previous study [4].

The excitation energy was tuned from 7015 eV to 7500 eV in varying steps (10 eV to 0.5 eV) across the iron K-edge at 7112 eV. To get reasonable peak to background ratios the acquisition time for each spectrum was set between 5 and 15 seconds depending on the Fe florescence intensity of each sample. For each specimen at least two repetitive scans were performed to increase the signal to noise ratio. During all XANES measurements the absorption of an iron foil was recorded in transmission mode simultaneously.

Human colon cancer cell lines (colorectal adenocarcinoma) as well as human breast cancer (adenocarcinoma) and human fibrosarcoma cell lines in were prepared at the Laboratory of Environmental Chemistry and Bioanalytics in Budapest, Hungary. The samples have been prepared without treatments or with treatments with different iron forms. The cellular uptake of iron was investigated recently with different analytical techniques [5].

During the sample preparation the cells were treated with Fe^{2+} or Fe^{3+} salts (i.e. $FeSO_4$, $FeCl_3$, Fe^{3+}citric) and transferrin. After washing, a cell suspension was produced and sealed avoiding air contamination. A major challenge in elemental speciation is to avoid chemical transformation during analyses. Therefore the samples were transported in argon environment. Main aim was to gain information about the iron component inside the cells after these treatments.

23 samples with different iron treatments were analyzed and the data quality, which could be improved significantly based on the results of the feasibility study [4], allowed further data processing for 20 samples.

We observed that independently of the chemical treatment all samples clearly showed a mixture of $Fe_2(SO_4)_3$ and Ferritin. As an example fig.1 shows a comparison of the spectrum recorded for the $FeSO_4$ standard (red), the standard $Fe2(SO_4)_3$ (blue) and the $FeSO_4$ treated sample. The data quality allowed linear combination (LC) fitting; fig 1 shows the result of the LC fit of the standard spectra for $Fe_2(SO_4)_3$ and Ferritin to the spectrum recorded for a sample treated with $FeSO_4$ (quality of fit parameter: R-factor = 0.000234).

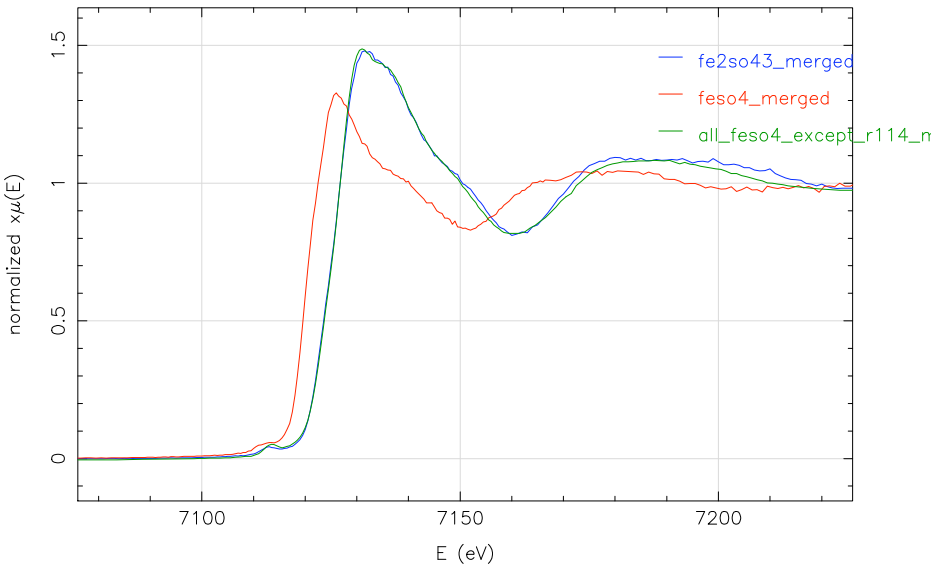

Fig 1. Comparison of $FeSO_4$ treated sample with the standards $FeSO_4$ (red) and $Fe_2(SO_4)_3$ (blue)

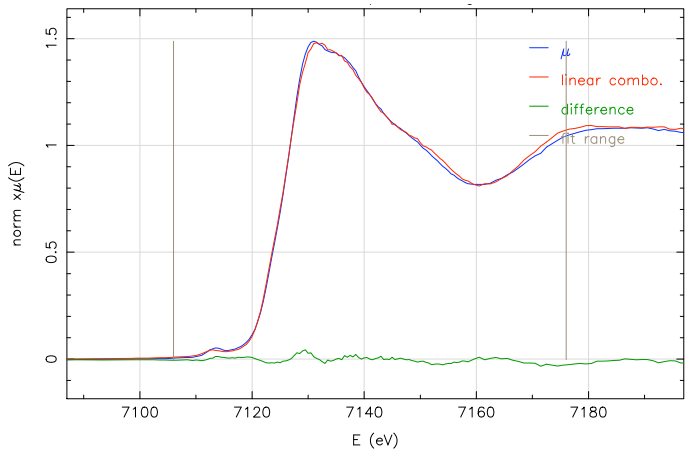

Fig.2 LC Fit of Ferritin and $Fe_2(SO_4)_3$ to $FeSO_4$ treated samples, revealed 100% $Fe_2(SO_4)_3$ and 0% Ferritin

References

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