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

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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 human cancer cells. The second measurement campaign reported here was intended to be an extended feasibility study. Measurements of adequate standard samples and cell samples treated with different chemical compounds have been performed.

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 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 and merged 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 different phases of the cell growth were prepared at the Laboratory of Environmental Chemistry and Bioanalytics in Budapest, Hungary. The samples have been prepared with and without different treatments. 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. When the cell growth was in its stationary phase, the cells were treated with either CoCl2, NiCl2, antimycin or 5-fluorouracil (5FU). Main aim was to gain information about the influence of these treatments on the cells relating to the Fe species. For comparison samples have also been taken during other characteristic phases of cell growth, namely the Lag phase (phase of no growth) and the Exponential phase (phase of exponential growth).

Untreated and treated cell samples and iron reference compounds have been compared to find a possible correlation between treatments and the oxidation state of Fe. The comparison was made between the untreated samples, and Co-treated or Ni-treated samples. All XANES spectra were found to be very similar and the edge position of untreated sample is centered between the edge positions of treated samples ($\Delta \sim 0.5$ eV). It can be concluded that no significant changes took place due to the CoCl2 or NiCl2 treatments. However, it has to be noted that the treatments were only 20 minutes long.

New XANES spectra of Fe^{2+}-citrate, Fe^{2+}-α,,α'-dipiridil, $K_4Fe(CN)_6$ as Fe^{2+} standards,Fe^{3+}-H_3PO_4, Fe^{3+}-citrate, Fe^{3+}-EDTA and $K_3Fe(CN)_6$, and ferritin as Fe^{3+} standards have been recorded. The best agreement between the majority of samples and the measured standards was found for the ferritin reference. Small differences cold be seen between samples treated with 5FU and antimycin

and the ferritin standard: the energy of the iron K-edge of samples treated with 5FU was shifted towards higher energies, while the samples treated with antimycin had similar XANES spectra like the Fe2+-a,a'-dipyridyl standard and also shifted towards higher energies.
We could show that SR-TXRF XANES analysis is feasible for the analysis of Fe in cancer cell lines. Small differences in XANES of cells prepared with different treatments have been found and will be further investigated. Furthermore, the method enables the acquisition of TXRF spectra along with the XANES spectra on the same samples. Without having any quantification performed so far, but based on the present results we suggest that the internal standardization method will be useful for direct elemental analysis with small cell numbers.

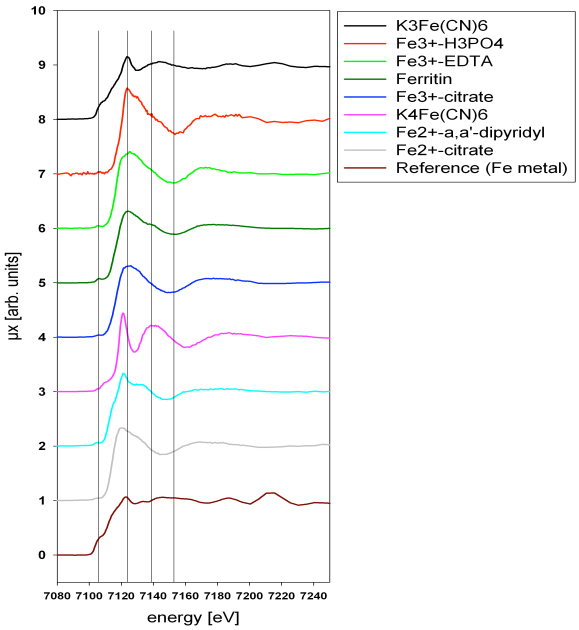

Figure 1: XANES of all new standard compaounds measured for comparison with the cell lines spectra.

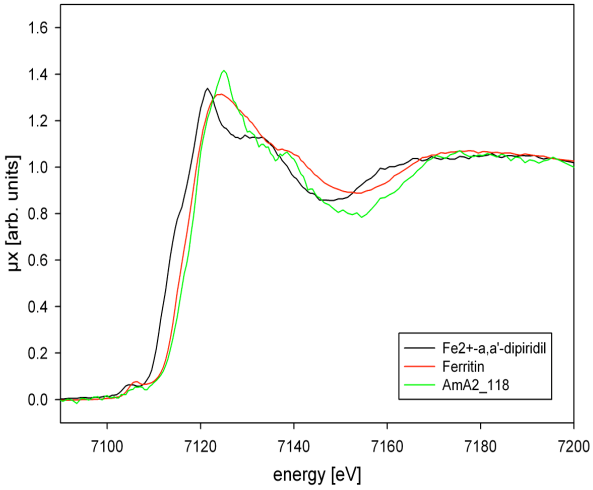

Fig.2 XANES spectra of a HT-29 sample comparing to samples spectra treated with antimycin (AmA2_118) and ferritin (the most similar standard compound)

References

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