Iron speciation in human cancer cells by K-edge TXRF-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 first measurements reported here were intended as a feasibility study which should i) show if the setup is sensitive enough to perform XANES measurements on the colon cell samples and ii) lead to an new and proper sample preparation method which will ensure the stability of the oxidation stage of Fe during sampling, sample treatment, storage, transport and analysis.

As total reflection geometry offers very high sensitivities for elemental determination in X-ray fluorescence analysis [1], these characteristics can be used to apply this set up for the speciation of minute amounts of metals in X-ray absorption experiments in the fluorescence mode [2,3]. The big advantage of SR-TXRF-XANES is that the sample preparation can be reduced to a minimum: the cells available in a suspension can be directly pipetted in the Si reflectors, dried, inserted in the vacuum chamber and measured.

The relation between the uptake of Fe with food and and the probability of development of colon cancer is well known. Epidemiological investigations showed a relation between development of colon cancer and Fe uptake by food as well as the oxidation state of the Fe disposal [4,5]. However, the carcinogenic impact of Fe is mostly unknown and therefore currently extensively investigated. The determination of the oxidation state of Fe seems to be an important question and the investigation of this topic should give more insight in the mechanism of cancer development.

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 of the Hamburger Synchrotronstrahlungslabor (HASYLAB) at DESY [2]. Human colon cancer cell lines (colorectal adenocarcinoma) indicated as HT-29 in the following as well as human breast cancer (adenocarcinoma) and human fibrosarcoma cell lines in different phases of the cell growth were prepared at the Department of Clinical Research, National Institute of Oncology in Budapest, Hungary. The samples have been prepared with different iron compounds (Fe2+ or Fe3+ salts) added. 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 log phase (phase of exponential growth), the cells were treated with either CoCl2, NiCl2 or metamizole sodium. 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 stationary phase.

The results of the XANES analysis of samples taken during different phases of cell growth are shown in figure 1. The XANES were found to be very similar for all phases of cell growth indicating that the chemical state of Fe remained unchanged during theses phases. In figure 2 the XANES of the sample taken during the stationary phase is compared with XANES recorded for cell samples treated with different chemical compounds during the log (exponential) phase.

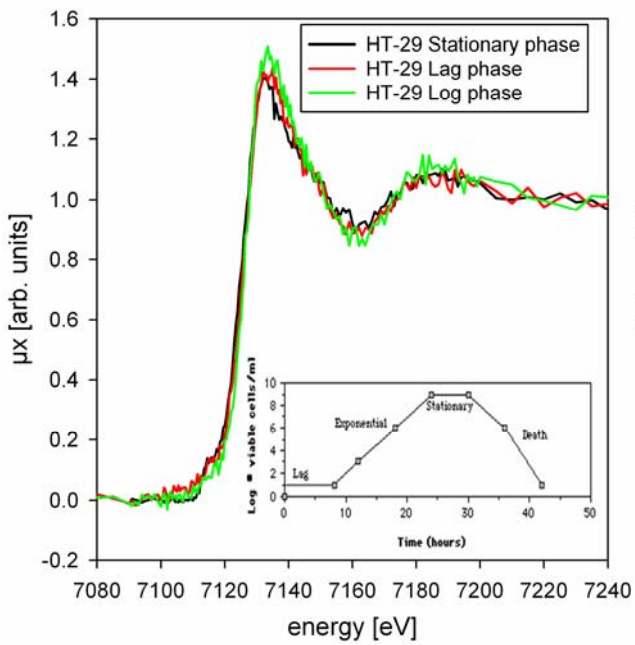

Figure 1: XANES of cell samples taken during different phases of cell growth.

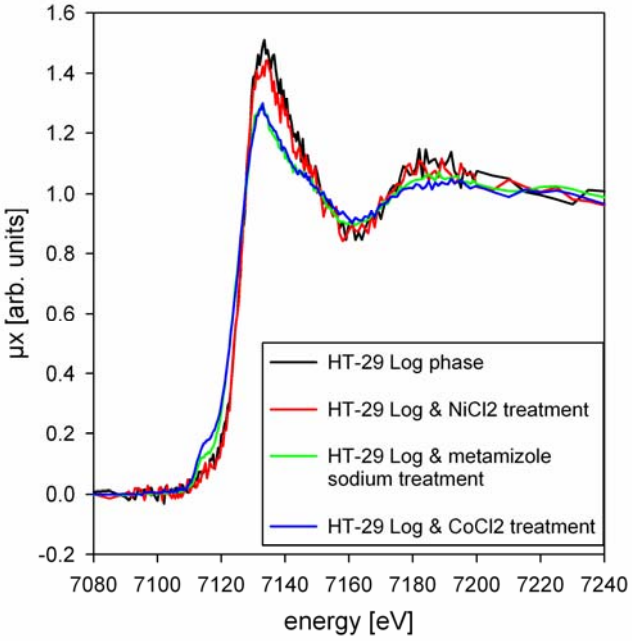

Figure 2: XANES of cells treated with different chemicals during the log phase.

The results of the measurements showed that SR-TXRF XANES analysis is feasible for the analysis of Fe in cancer cell lines. Variations in the chemical state of Iron in cell lines treated with different chemical compounds could be observed. However, further efforts have to be made to drastically increase the signal to noise ratio of the XANES spectra (e.g. by increasing the Fe concentration in the samples). At this stage a reliable evaluation of the Fe compound or oxidation state is not possible. For this evaluation an additional set of standard Fe-compounds which are expected to be present in the cells has to be analyzed. The exact knowledge about the ratio of Fe(III)/Fe(II) after administration of different type of Fe species will help to understand the mechanism of the biochemistry of Fe in the cancer cells. Moreover, the carcinogenesis of Fe in the normal cells will be better recognized.

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