Investigation of iron and copper oxidation state in different grades of human brain gliomas using X-ray absorption spectroscopy

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Brain tumors are still one of the main causes of death. In order to successfully combat the disease, it is extremely important to learn about the mechanism of its formation. It is believed that trace elements such as Fe, Cu and Zn play a significant role in neoplastic processes. Moreover, an important factor is the presence of Cu-Zn superoxide dismutase (CuZn SOD), which exhibits antioxidant properties. The knowledge of their forms in healthy and cancerous tissues may significantly contribute to the knowledge of biochemical reactions involved in the oncogenesis.

The samples under study were collected intraoperatively and contained brain tumors with different degrees of malignancy. The samples were prepared in two ways. For bulk analysis frozen samples were cut to a thickness of 2 mm, placed in polymer containers with a diameter of 12 mm and covered in the front with an ultralene foil. Afterwards, the samples were immediately frozen to -80°C, which prevents all the biological and chemical processes, e.g., oxidation. For local analysis the samples were cryo-cut to a thickness of 20 μm, placed on the ultralene foil, and then freeze-dried at -80°C. A non-cancerous control sample and reference materials (Fe and Cu foils, $Fe_2(SO_4)_3 \cdot nH_2O$, $FeSO_4 \cdot 7H_2O$, CuO, Cu_2O and organic reference material Cu-Zn SOD obtained from human erythrocytes) were additionally examined.

Experiments were performed at two different beamlines. The experiment on frozen samples was carried out at the beamline C in HASYLAB. Containers with samples were kept in a holder cooled with liquid nitrogen (to about -184°C) in a vacuum. The beam size ranged from 2x1 mm to 3x2 mm. Absorption spectra of Fe were recorded for the energy of the incident radiation ranging from 7.050 to 7.700 keV. The fluorescence radiation was collected by a 7 pixel Si (Li) Gresham detector.

Thin samples were measured on the bending-magnet beamline L. The beam was focused to a spot size of 15 μm with a polycapillary half-lense. The experiment was performed in air atmosphere at room temperature. Full Cu XANES spectra were collected in selected points of samples. Absorption spectra of Cu were recorded for the energy of the incident radiation ranging from 8.975 to 9.325 keV. Excited XRF spectra of secondary radiation were recorded by a Vortex SDD detector.

Cu XANES spectra of freeze-dried malignant and control samples as well as reference materials (Cu^{1+}, Cu^{2+} and CuZn SOD) are shown in Fig. 1. The edge region of the spectra has been magnified. Note, the white line for CuZn SOD lies between the white lines of reference samples present in the oxidation states of 1+ and 2+. The white line of the control (non-cancerous) sample has the shape and the position similar to the white line as that one observed for CuZn SOD. The spectra recorded for malignant samples did not differ significantly from each other, whereas the shape of the white line for the malignant sample differs from that one observed for CuZn SOD. This may suggest similar dysfunctions in metal-dependent enzymes in tumor samples.

Fe XANES spectra collected from the frozen malignant and control samples as well as reference materials ($Fe_2(SO_4)_3 \cdot nH_2O$, $FeSO_4 \cdot 7H_2O$) are shown in Fig. 2. Numerical values of the absorption edge positions were estimated as the mathematical center of the gravity of absorption curve. Results of current experiment are shown in Fig. 3 (blue dots) against the

results of previous experiments (black dots). The analysis of plots indicates that the Fe K absorption edge is shifted towards lower energy for the higher tumor grades. Further study would be required to improve the statistics of experimental results.

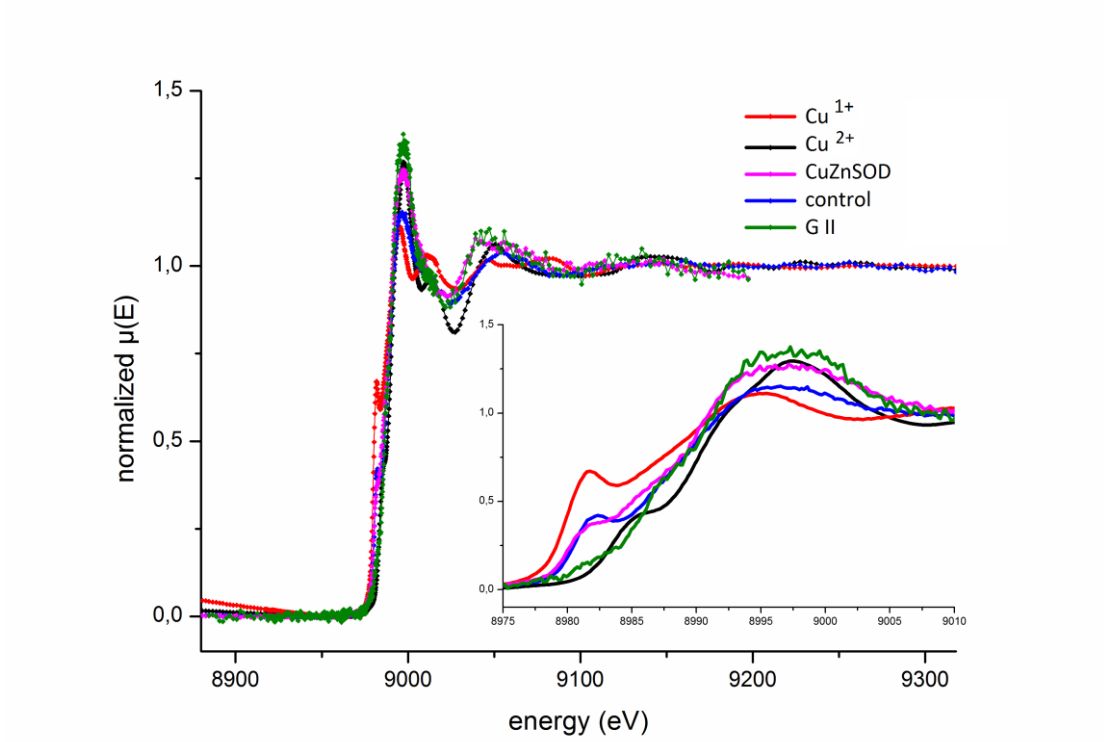

Figure 1: XANES spectra for Cu K absorption edge for selected dried brain samples (G II-glioma II grade, control) and reference materials (Cu^{1+}, Cu^{2+} and organic CuZn SOD).

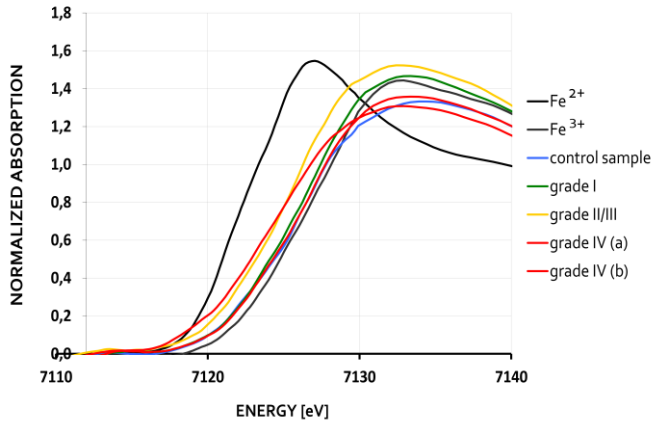

Figure 2: XANES spectra for Fe^{2+}, Fe^{3+} and brain samples (gliomas at different grades of malignancy and a non-cancerous control tissue)

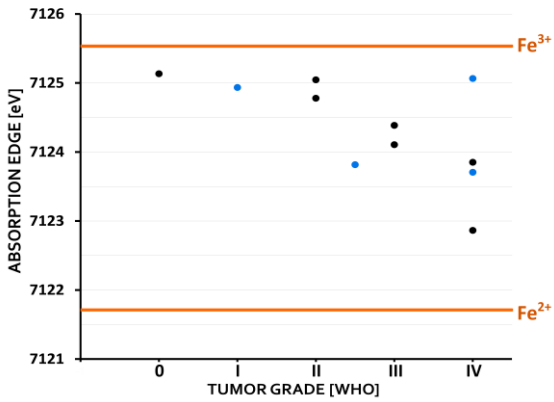

Figure 3: Absorption edge energies of Fe K for various malignancy grades. Currently measured samples (blue dots) against the results obtained for cancerous in previous experiments (black dots).

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