“Fingerprinting” of brain tumors based on the synchrotron radiation X-ray fluorescence and x-ray absorption spectroscopy

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The X-ray absorption spectroscopy (XAS) was used to assess the forms in which iron and zinc occur in brain tumors. Based on analysis of X-ray Absorption Near Edge Structure (XANES) spectra, the average oxidation states of iron and zinc were determined in the brain tumors, whereas analysis of Extended X-ray Absorption Near Edge Structure (EXAFS) spectra was used to assess changes in the local structures of the elements studied. In our work the study of chemical forms of Fe and Zn in brain tumors was carried out on frozen samples, The samples were obtained intraoperatively from patients with glial brain tumours requiring surgical intervention. The specimens intended for histological examination were cryo-sectioned to a thickness of 5 micrometers and stained with hematoxylin-eosin. This made it possible to determine the type and grade of malignancy of the tumor studied. From the remaining tissue, samples of about 0,3 cm^3 were cut and placed in specially prepared polymer measurement containers. The front of the container, which was exposed to synchrotron radiation, was covered with X-ray-transparent Ultralene foil. The samples were then immediately frozen at -80˚C and stored in a deep freezer to minimize biological and chemical processes, e.g., oxidation. During transport to the synchrotron facility and between the measurements, the samples were stored in a container cooled with liquid nitrogen.

We analyzed tissues representing various types of brain tumors whose malignancy grades were determined in accordance with the latest (World Health Organization WHO) classification. These included glioblastoma multiforme (WHO IV), anaplastic astrocytoma (WHO III), anaplastic oligodendroglioma (WHO III) and atypical meningioma atypical (WHO II). As a control sample, brain abscess wall was used, which is non-cancerous brain tissue.

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 and Zn were recorded for the energy of the incident radiation ranging from 7.050 to 7.700 keV and from 9.60 keV to 10.40 keV, respectively. The fluorescence radiation was collected by a 7 cells SDD detector. As the reference materials for the studied elements in various oxidation states, appropriate inorganic compounds (FeO, $FeSO_4 \cdot 7H_2O$, Fe_2O_3, $Fe_2(SO_4)_3 \cdot nH_2O$, ZnO, ZnS) and metallic foil of Fe and Zn were used.

Fourier transform of Fe and Zn EXAFS data for tumor samples with various malignancy grades as a function of the radial coordinates are shown in Figures 1 and 2 The positions of the maxima of the first peaks of the analyzed spectra, which define the distance of the first coordination zone, showed no correlation with the malignancy grade. These studies have thus shown no change in the lengths of chemical bonds. The measurements showed that cryogenic methods give good results in the study of biological samples. A particular advantage is the ability to analyze samples without prior complex preparation that might affect the oxidation state and the chemical environment of the elements studied. The results offer hope that analysis of XAS spectra could be used in the future to assess tumor malignancy grade. Such data can also be useful by providing information about the change in the chemical state of the studied element in understanding processes associated with neoplasia.

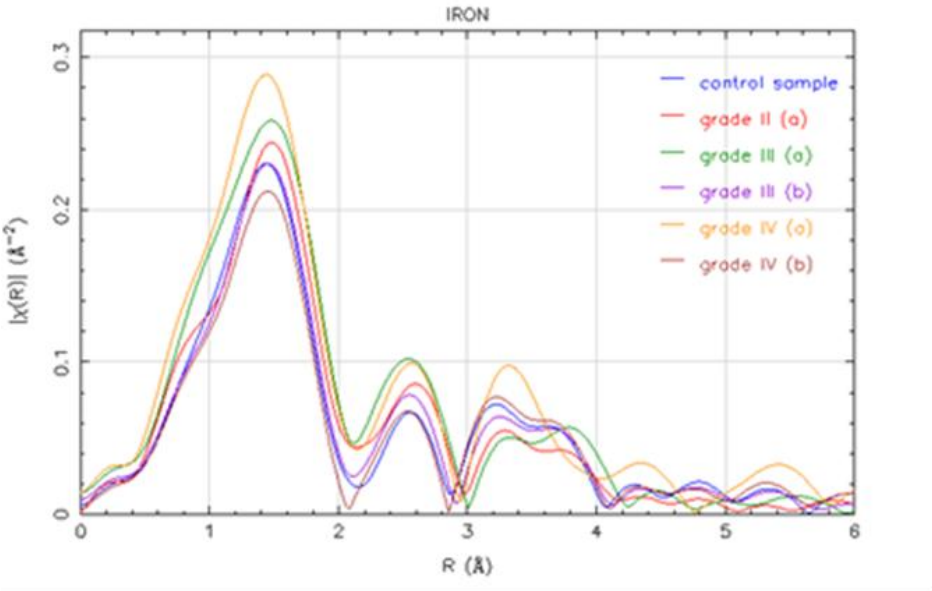

Figure 1: Fourier transform of Fe EXAFS data for tumour samples with various malignancy grades as a function of the radial coordinate

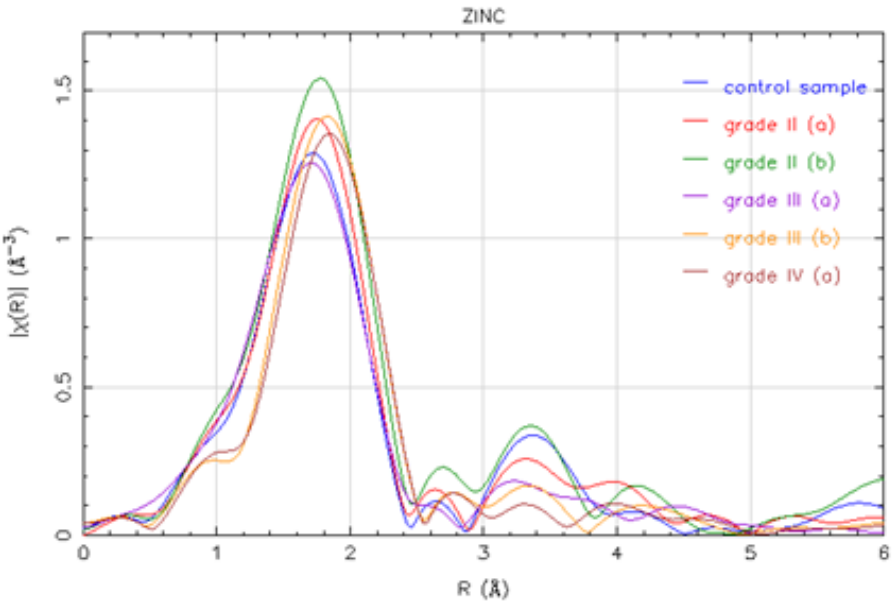

Figure 2: Fourier transform of Zn EXAFS data for tumour samples with various malignancy grades as a function of the radial coordinate

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