

XANES micro-imaging of Zn speciation in human brain tumors

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The samples designed to micro-imaging of Zn oxidation states were taken intraoperatively from brain gliomas of different types and various grades of malignancy. The investigation included also tissue apparently without malignant infiltration as a control. XANES analysis was performed in thin freeze-dried cryosections.

The Zn XANES measurements were performed at the bending magnet beamline L at HASYLAB. Synchrotron radiation from the storage ring was monochromated with a Si(111) double crystal monochromator. To reduce the beam size the polycapillary half-lenses for monochromatic applications was used. The beam was focused to a size of 15 μm in diameter. The data were measured in fluorescence mode for the biological specimens and the reference samples. The measurements were carried out in air. The characteristic X-ray lines were measured by the Vortex SDD detector from SII Nano Technology USA Inc. The full XANES spectra were collected in selected points for samples of brain gliomas as well as for the reference materials (Zn foil, ZnO and ZnS as powders). The absorption spectra near Zn K-edge were measured for the energy range from 9.55 to 10.0 keV. The measurement time for each analyzed energy point was 1 s for reference materials and 5 s for tissue samples. Micro-imaging of Zn oxidation states involved two-dimensional scanning of the tissue samples with the step size equal to 20 μm both horizontally and vertically. Typically the areas of tissue slices selected for scanning were few hundred micrometers by few hundred micrometers. Mapping of reduced form of Zn was performed at the energy equal to 9.653 keV. The energy of 9.659 keV was applied to detect Zn 2+. The time of acquisition was equal to 30 s per one measurement point for tissue samples. To perform mapping of chemical forms of Zn, the position of the samples, was changed with respect to the incident beam. The full XANES spectra at the Zn K α edge were collected in selected points of neoplastic and control samples. For the analysis the "homogeneous" areas of cancerous (G) or control tissue (C1) were selected. Moreover the calcification (G_Ca) or other Zn-rich structures (C2) were investigated. The fluorescence intensities of Zn K α lines were normalized (point by point) to the incident photon flux and to the mean number of counts from the energy range 9.80–10.0 keV.

The Zn XANES profiles for tissue samples as well as the reference materials were presented in Figure 1. Additionally the edge regions of the spectra were focused. The location of the points analyzed was shown in Figure 2. It was found that position of the edge in XANES spectra collected for all the tissue structures is the same as for the reference materials in which zinc is present on +2 oxidation state. The results of Zn chemical state imaging obtained for neoplastic tissue (anaplastic oligodendroglioma classified as III grade of malignancy) and control sample were illustrated in Figure 2. The presence of metallic Zn was not found. Distribution of oxidized form of Zn in tissue structures was determined. It was noticed that the areas of calcification reveal high level of Zn 2+.

Acknowledgments:

"The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 226716 and the Ministry of Science and Higher Education (Warsaw, Poland) grant no N N518 377 537"

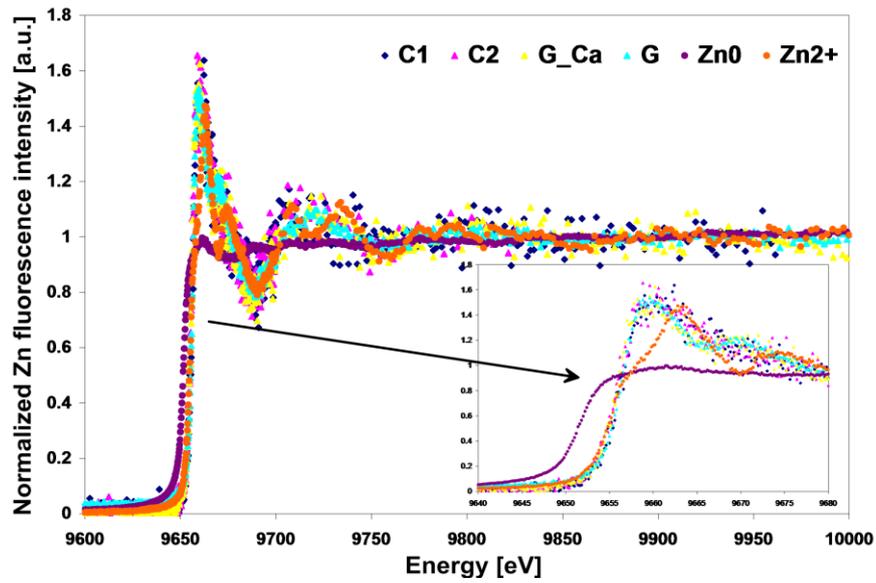


Figure 1. The Zn XANES profiles for brain glioma (G – tissue, G_Ca – calcification) and control (C1, C2) tissue samples in comparison with reference materials of Zn0 and Zn2+.

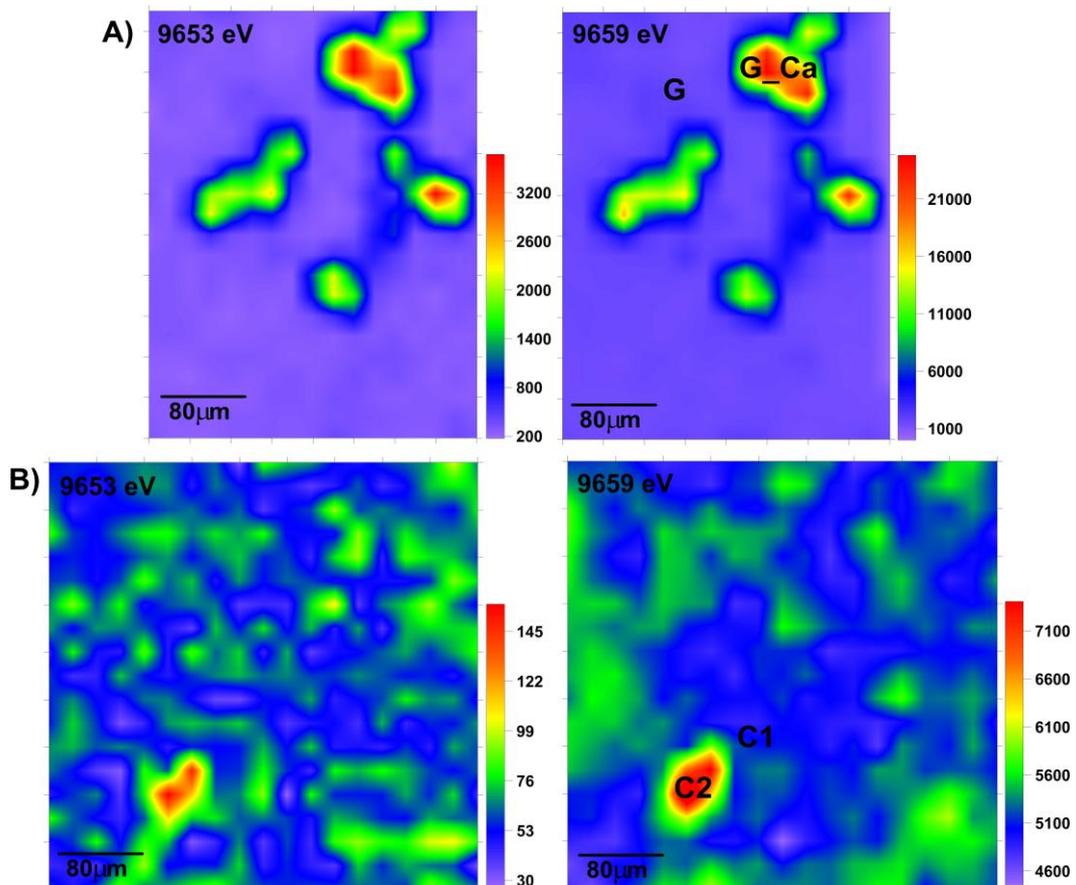


Figure 2. Scanning x-ray microprobe determination of zinc oxidation states in oligodendroglioma anaplasticum (A) and control sample (B). The energy of excitation was shown in upper, left corner of each map. G – homogeneous neoplastic tissue, G_Ca calcification, C1 – homogeneous control tissue, C2 – Zn-rich area. Data presented in arbitrary units.