The glandular epithelial cells of the human prostate gland have the unique capability and function of accumulating the highest zinc levels of any soft tissue in the body. Zinc plays role in a wide range of cellular processes such as immune system function [1], antiangiogenesis [2] or apoptosis [3]. Zinc has a significant antioxidant function [4] and its presence in prostate cells is strongly connected with their metabolism [5]. All the cytosolic zinc exists as bounded form. In majority zinc is bounded to immobile macromolecules, but also to mobile ligands. XANES technique is a powerful tool for precise investigation of coordination environment of absorbing atom.

Since tissues are very complex in structure, there is a need to analyze more homogenous sample which could be made of one type of cancerous cells. Such a requirement seems to be fulfilled by culture cell lines. For this study different culture cell lines such as DU-145, PC-3, LNCaP (human prostate cancer), were used for XANES analyses.

Cells were cultured at the Dept. of Applied Spectroscopy, Institute of Nuclear Physics PAN and the Chair of Medical Biochemistry, Jagiellonian University Medical College, Kraków. Cells were grown in 25 cm² culture dishes using RPMI-1640 medium (SIGMA-Aldrich) supplemented with 10% fetal bovine serum (SIGMA-Aldrich), 100 U/ml penicillin-streptomycin solution (SIGMA-Aldrich), 10mM HEPES (SIGMA-Aldrich), 1 mM sodium pyruvate (SIGMA-Aldrich) and 4.5 g/l glucose. Culture dishes were maintained at 37°C in a humidified atmosphere of 5% CO₂ and the culture medium was replaced by a fresh one every 72 h. Cells were collected using 0.25 % trypsin (SIGMA-Aldrich) and consequently centrifuged at 1800 rpm for 4 minutes. In order to fix cells a 2% paraformaldehyde was used along with PBS and cells were kept in such a solution for 2 hours. Drops of 20µl of cells suspension were placed on 1,5 µm thick Mylar foil (Goodfellow).

XANES in fluorescent mode on K-edge of zinc experiment has been performed at beamline A1, Hasylab, DESY. Samples were measured at room temperature, in air. In the first ionization chamber the SR beam was absorbed by 10% while in the second ionization chamber by 50%. The beam size was 8 mm by 0.7 mm. Each spectrum was collected in the energy range from 9500 eV to 9750 eV and was divided into four sections with different energy steps and acquisition time (first with energy step of 5 eV from 9500 eV to 9600 eV, second with energy step of 1 eV from 9600 eV up to energy of 9620 eV, third with energy step of 0.2 eV from 9620 eV up to energy of 9700 eV and fourth with energy step of 1 eV from 9700 eV to the final energy of 9750 eV). The acquisition time was 1 second for reference materials (whole spectrum) while 10 seconds in third energy sections and 5 seconds for other regions in case of cells.

In order to check high signal-to-noise ratio, samples with different amount of DU-145 cells were measured. Relationship between amount of cells suspension (1, 2 or 3 20µl drops) and zinc K-edge step is presented in Fig.1. On the basis of that experiment the samples of 3 drops of cells suspension were analyzed only.

Zn K-edge XANES spectra for three cultured cell lines show presence of peaks of energies about 9675 eV and 9700 eV. The character of oscillations observed above the edge is very similar (Fig.2.).
Localization of Zn K-edge position in cells samples presented in Fig.3. shows, that oxidation state of zinc is the same as in case of ZnO or ZnCl₂ however the edge structure is different what can be seen on first derivative function (Fig.4), where two maximums are present.

Such observation indicates different local structure around Zn atom in cells that is observed for chemical compounds used for determination of Zn K-edge position.

References


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