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The chemical forms of sulfur in prostate cancer tissue analyzed by means of XAS

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There is a increasing interest in the biochemistry of various elements in biosystems [1] and their role in human health and disease. The application of micro X-Ray Absorption Spectroscopy (μXAS) and the advantages of light produced by synchrotron radiation sources have made possible to determine chemical forms of different elements in complex structures such as cells and tissue. Knowledge about abnormalities of content and chemical forms of various elements is crucial in case of understanding the process of pathogenesis and molecular mechanisms underlying the etiology of many serious diseases e.g. cancer. XAS brings its own unique contribution to the list of experimental techniques used by structural biologists and starts to be complement to other spectroscopic structural methods.

Presented results were obtained during μXAS experiments performed at ANKA (Germany) and SLS (Switzerland) synchrotrons on the tissue originating from prostate cancer, one of the leading malignant disease with high risk of death among men. Prostate cancer is recognized as one of the major medical problems facing the male population and the factors that determine the risk of developing clinical symptoms are not well known [2]. Studies have shown that changes on the cellular and molecular level seems to be pathological basis of most diseases, resulting from the external factors or disrupted internal cellular processes [3]. Accurate knowledge of these mechanisms can create new opportunities to diagnose diseases at an early stage of their development and apply new, more effective therapies.

This work focused on determination of sulfur chemical species occurring in different parts of prostate cancer tissue. Sulfur was chosen as an element of interest as it plays an important role in human metabolic processes and the disruptions in homeostasis between its various forms may lead to serious pathological conditions. For instance changes in the ratio of oxidised and reduced sulfur forms may indicate changes in redox balance due to the oxidation stress, that is suspected to play significant role in carcinogenesis. Moreover, sulfur is found in one of the major low-molecular-mass thiol, essential for health - glutathione (GSH) - which has many physiological functions, including its involvement in the defence against reactive oxygen species. Therefore, changes in GSH homoeostasis are implicated in the aetiology and progression of a number of human diseases, including cancer, diseases of ageing and neurodegenerative diseases [4]. In addition, sulfenic, sulfinic and sulfonic derivatives are formed during severe oxidative stress as seen in prostate cancer and their presence in the tissue may indicate dysregulated redox balance [5].

In order to establish differences in content and distribution of various chemical form of sulfur in prostate cancer tissue two types of experiemnts were performed: 2D XAS imaging of selected areas of prostate cancer tissue and μ-XANES measurements on chosen points of interest. The prostate tissue was obtained during routine prostatectomies on patients suffering from prostate cancer. The excised gland was cut transversely with a sharp knife and a section were frozen to -20ºC, cut into 15 μm thick sections in a standard cryostat (Leica Microstystems, Germany). One section of each sample was placed on 1,5 μm thick Mylar foil (Goodfellow) and used for XAS analysis, while another adjacent section was used for histological examination. To all of the tissue sections Gleason score was assigned. The second group of sections were formalin-fixed, paraffin embedded by the routine histologic protocol.

2D XAS distribution maps were collected at the X07MB (PHOENIX I) beamline of the Swiss Light Source (Paul Scherrer Institut, Villigen, Switzerland) under high vacuum with a double crystal Si(111) monochromator to select the energy of the incoming beam. Focal spot size was about 10 μm x 10 μm on the sample. The X-ray fluorescence was detected by 4-element Si drift diode array (Vortex ME-4) placed at 90º to the incoming beam. Chosen region of the sample was scanned with the step of 10 μm and acquisition time of 0,4 s per point with four different energies corresponding to spectroscopic features in the near-edge XANES region. Chosen energies excited sulfur at different oxidation states. S K-edge μ-XANES measurements were also performed at the wiggler beamline SUL-X of the synchrotron radiation source ANKA (Karlsruhe Institute of Technology, Germany). A 7–element Si(Li) fluorescence detector (Gresham, now SGX Sensortech) was used. The dimension of the beam at sample position for this experiment was 100 μm x 100 μm. The energy was tuned with a double crystal monochromator (Si(111) crystal pair) in steps of 5 eV and 2 eV in the regions of -100 eV to -50 eV and -50 eV to -20 eV respectively before the edge and 0.2 eV in the edge region.

2D XAS distribution maps give opportunity to receive complete image of different species of the same element in the region of interest and correlate this information with histological stucture. In typical prostate tissue we can distinguish two main parts: prostatic glands and stroma composed from smooth muscle cells,

connective tissue and accompanying extracellular matrix. The example of 2D distribution of individual sulfur form fractions, calculated in accordance with the procedure proposed by Pickering et al. [6], together with the microscopic image obtained during experiment is presented in Fig. 1.

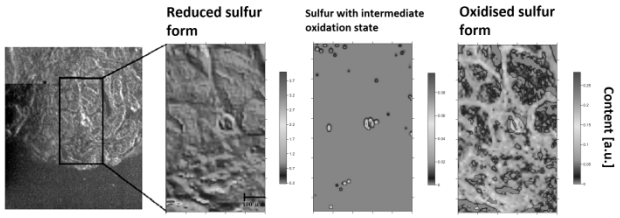

Figure 1. 2D maps of the distribution of individual chemical forms of sulfur in the selected areas of prostate cancer tissue samples together with microscopic image with marked area of scanning.

Presented sample was derived from patient diagnosed with Gleason score 6 prostate cancer. Based on the obtained results it is clearly visible that the most abundant sulfur species in examined area is the reduced form of sulfur that is present nearly homogeneously in all structures. Areas of elevated content of this S form are presented in nodular part of tissue. The sulfur with intermediate oxidation state is present in minute amounts in examined samples and it cannot be correlated with the specific histological structures. The highly oxidised forms of sulfur are minor components of glandular areas in studied prostate tissues, but their content in prostate stroma is significant [7].

In order to analyse differences in the content of chemical forms of sulfur in individual histological structures of prostate tissue, as well as in samples originating from prostate cancer with various Gleason grade, S K-edge μ-XANES measurements were performed. Point spectra were collected from samples with Gleason score 3, 4 and 5 and additionaly from samples of tissue diagnosed as benign prostatic hypeplasia (BPH). In cancerous tissue experimental points were chosen from areas of tumor cells and outside them. The representative S K-edge XANES spectra from all four sample types are presented in Fig. 2.

The shape of all spectra is similar to the one obtained for prostate cancer cell lines [8] with two strong features originatng from reduced and oxidised sulfur forms. It can be easily noticed that main differences between spectra are derived from different reduced/oxidised S forms ratio. Moreover, the structure of the second peak (around 2480 eV) indicates that it originates from the signal generated by S^{5+} and S^{6+} forms and that the content of these two forms also varies between individual samples. The detailed analysis of obtained spectra included the use of Principal Component Analysis (PCA) method to establish spectral differences and simmilarities between examined groups. Based on the evident differences in the content of oxidised sulfur species between individual samples the conclusion was drawn that the signifact role in case of prostate pathologies palys dyregulated redox balance.

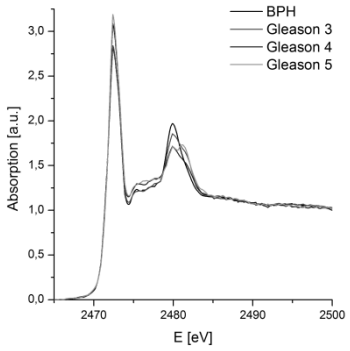

Figure 2. S K-edge μ-XANES spectra obtained on tissue samples originated from BPH and prostate cancer with various Gleson score.

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