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Fe distribution and speciation in human nails

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ABSTRACT

Micro-X-ray Fluorescence (μ-XRF) and Fe–K edge micro-X-ray Absorption Near Edge Structure (μ-XANES) spectroscopies are applied for the study of the distribution and the spatially-resolved bonding environment of Fe in human nails. The μ-XRF maps reveal that Fe forms islands where its concentration is up to six times higher than in the rest of the sample. Comparison of the map characteristics of two nails that belong to healthy and ill donors (affected by lung cancer), reveals that there is no significant variation in the size distribution of the Fe-rich inclusions contrary to their spatial density which is found higher in the nail of the ill donor. However, a relation between the variations of the spatial density and the illness can not be established due to the small number of the studied samples. The analysis of the Fe–K μ-XANES spectra reveals that the bonding environment of Fe varies between the samples and between different spots of the same sample with different Fe concentration. The characteristics of the pre-edge peak in the Fe–K XANES spectra indicate the presence of both Fe^{+3} and Fe^{+2} that participate in the formation of distorted octahedra. Finally, the area under the pre-edge peak depends linearly on the position of the absorption edge indicating variation of the Fe ligation between the samples and/or between different spots in the same sample.

1. Introduction

The human nail is a modified type of epidermis that consists of compact layers of dead cells of epithelium. The main constituent of the nail is the organic matrix which is formed by keratin, a sulphur-rich structural insoluble protein. Keratins comprise a large family of proteins that are found in epithelia. Amino acid mutation in keratins has been related to development of neoplasms and other diseases [1]. The amino acids that are present in large amounts in the nail keratin are the Glu (19 mg/g), Cys (17 mg/g), Arg (17 mg/g) and Ser (5 mg/g) [2]. However, the amino acid content of the human nails shows genetic variations [3] and is affected by the donor's disease [4]. Structurally, the keratinized matrix consists of filament-like (low sulphur) proteins which are embedded in a non-filamentous matrix comprised of high-sulphur proteins [3]. Not only the amino acid content and sequence in the keratin but also some of the physical properties and the visual appearance of the nails are affected by disorders or diseases [5]. For example, osteoporosis, hypothyroidism, and tuberculosis enhance the nail brittleness [6]. The physical properties of the nails may also be affected by medication [7] while, due to their small growth rate, they provide a history of drug intake and toxin exposure [8].

Human nails contain small amounts of essential metallic elements like Ca, Fe, Cu, Zn and for this reason they are used to monitor their concentration in the human body [9–11]. Lack or excess of some of these elements has been related to disorders or diseases as well as to environmental and nutritional factors. For example in patients with cystic fibrosis and in asthmatic patients Fe and Zn deficiency has been detected, respectively [12,13]. Deviation from the normal values of Fe has been detected in cancerous tissues of penis and testis [14], breast [15], stomach and kidney [16], and thyroid [17]. Mapping of the Fe concentration in the arteries of rabbits with atherosclerosis has shown that Fe is present in early lesions at high concentrations compared to the artery wall, suggesting that the local elevated Fe concentration may provide an accelerated process of atherosclerosis in specific regions of the artery [18]. The local bonding environment of Fe in human tissues is also affected by diseases. Yoshida et al. applied synchrotron radiation (SR) assisted microbeam X-ray Absorption Near Edge Structure (XANES) spectroscopy to detect differences in the bonding environment of Fe in neurons from a patient suffering from Parkinson's disease [19].

Here we apply X-ray Fluorescence (XRF) and XANES spectroscopies for the study of the distribution and speciation of Fe in human nails. XRF spectroscopy is well suited for the quantitative analysis of elements with $Z > 11$ which are present in human body tissues and fluids [20–23]. On the other hand, XANES spectroscopy is a well-established technique for the investigation of the bonding

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environment of both metallic and non-metallic elements in samples of biogenic nature [24–26]. The XANES spectra of the Fe containing compounds are often characterized by a peak before the absorption edge (pre-edge peak) which is attributed to forbidden 1s → 3d electronic transitions [27–30]. When the bonding environment of Fe is centrosymmetric, such as octahedral, the transition is quadrupole allowed and the intensity of the pre-edge peak is weak. However, the loss of inversion symmetry (as in planar tetrahedral or bipyramidal coordination of Fe) or the distortion of the Fe-centered octahedron increases the mixing of the 3d Fe orbitals with the p orbitals of the ligand and the transition becomes dipole allowed. In this latter case the intensity of the pre-edge peak is 20–100× stronger [29]. Therefore, the pre-edge peak is expected to be sensitive to the local geometry changes in the coordination of Fe in the human nail. Additional information can be extracted from the position of the absorption edge (E_{abs}) which is strongly related to changes in the valence and ligation of the Fe atom [31].

Recent developments in focusing devices, e.g. capillary optics, which reduce the SR beam size down to the micrometer scale, permit the 2-dimensional mapping of the distribution of the metals [23]. Additionally, micro-XRF and micro-XANES (hereafter designated μ-XRF and μ-XANES, respectively) spectra that are recorded using the focused beam, provide spatially resolved information and can be used in order to reveal concentration – dependent changes in the bonding environment of the metals in the nail [32]. Therefore, combined analysis of the μ-XRF and μ-XANES spectra can reveal whether the metals form metallic clusters or if they are bonded to amino acids as in the case of metalloproteins which serve many important biological functions [33].

2. Experimental details

The studied samples are nail clippings that were collected at the Pulmonary Clinic of the Aristotle University of Thessaloniki with the donor's consensus. Sample H belongs to a healthy 33-year old female donor, while samples C1 and C2 belong to male donors 79 and 55 years old, respectively, affected by lung cancer. Prior to the measurement the samples were cleaned with acetone, alcohol and deionised water in an ultrasound bath [34].

The μ-XRF maps and μ-XANES spectra were recorded at the μXAS beamline of the Swiss Light Source (SLS) Synchrotron Radiation Facility of the Paul Scherer Institute. The beamline has an undulator source and is equipped with a double Si(1 1 1) crystal monochromator. A Kirkpatrick – Baez mirror system focuses the beam to the 2 × 2 μm size. The flux of the impinging X-rays is in the range of 10^{12} ph/s/400 mA. The single element Si fluorescence detector (KETEK) was positioned on the horizontal plane at right angle to the beam. The sample was positioned at 45° to the beam on a stage that is moved micrometrically with proper step motors. The μ-XRF maps were recorded using 7150 eV photons with 2 or 1 μm step and they are normalized to the maximum intensity. The μ-XANES spectra were recorded at the Fe–K-edge from sample spots with different iron concentration, after setting proper detector energy windows in order to electronically discriminate the Fe K_α fluorescence signal and eliminate the contribution of preceding absorption edges. The spectra of hematite (α-Fe_2O_3) and magnetite (Fe_3O_4) powder reference samples, were recorded in the transmission mode.

3. Results and discussion

3.1. μ-XRF spectra and mapping

Representative μ-XRF spectra recorded from two positions of a nail sample are shown in Fig. 1. The S, Ca and Fe fluorescence peaks can be clearly distinguished along with the peak due to scattering at energy E_o, and the peak due to Ar that is present in the air-path before and after the sample. The spectra were normalized to the intensity of the Ar peak since the acquisition geometry was retained. Comparison of the intensity of the Fe K_α peak in the two spectra, reveals that they correspond to sample spots with significantly different concentration of Fe. The energy window that was used for the construction of the fluorescence maps and the acquisition of the XANES spectra is also indicated in Fig. 1.

Typical 400 × 400 μm XRF maps from the H and C1 samples, recorded with 2 μm step using the Fe K_α fluorescence, are shown in Fig. 2. The maps are normalized to the maximum Fe K_α fluorescence intensity and they reveal the tendency of Fe to segregate. A more detailed 25 × 25 μm map, which is recorded with a 1 μm step around a local maximum of the Fe concentration, is also included in Fig. 2 and reveals that this particular Fe-rich inclusion is about 15 μm large. The mean value and the standard deviation of the normalized intensity for the 400 × 400 μm maps shown in Fig. 2 are listed in Table 1. In order to obtain information on the size distribution and density of the Fe-rich inclusions, "binary" maps were constructed. In the binary maps the value 1 is assigned to points with intensity higher than 130% of the mean intensity while the value 0 is assigned to the rest of the points with intensity lower than 130% of the mean. The binary maps that correspond to the 400 × 400 μm intensity maps of Fig. 2 are shown in the insets of Fig. 3. In the same Figure the size distribution of the Fe-rich inclusions, as it is determined from the binary maps, is shown for the samples H and C1. The density of the Fe-rich inclusions (number of regions that show segregation of spots with value 1) as well as the percentage of the binary-map area they occupy (A^{130}) are listed in Table 1. These results indicate that the Fe concentration shows a slightly larger variation in sample C1 compared to sample H. Additionally, although the number of the Fe-rich inclusions is higher in the C1 sample there is no strong evidence, within the available spatial resolution, of size variations among the C1 and H samples. However, in order to draw safe conclusions

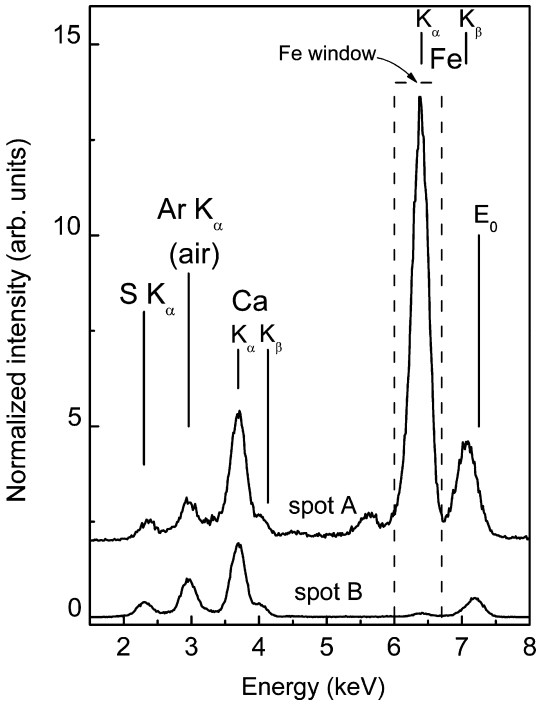

Fig. 1. Representative μ-XRF spectra recorded from two different spots of a human nail using excitation beam with energy 7.25 keV. The energy window used to discriminate the K_α fluorescence photons is also shown.