Zn-K edge EXAFS study of human nails

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Abstract. Extended X-ray absorption fine structure (EXAFS) spectroscopy at the Zn - K edge is applied for the study of the bonding geometry of Zn in human nails. The studied nail clippings belong to healthy donors and donors who suffer from lung diseases. Fitting of the first nearest neighboring shell of Zn reveals that it is bonded with N and S, at distances that take values in the ranges 2.00-2.04 Å and 2.23-2.28Å, respectively. Zn is four - fold coordinated and the ratio of the number of sulfur and nitrogen atoms (\(\frac{N_S}{N_N}\)) in the first coordination shell ranges from 0.52 to 1. The sample that belongs to the donor who suffers from lung fibrosis, a condition that is related to keratinization of the lung tissue, is characterized by the highest number of \(\frac{N_S}{N_N}\). Simulation, using the FEFF8 code, of the Zn - K edge EXAFS spectra with models of tetrahedrally coordinated Zn with 1 (or 2) cysteine and 3 (or 2) histidines is satisfactory.

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 nails’ organic matrix is the \(\alpha\)-keratin, a structural insoluble protein rich in cysteine. Keratins comprise a large family of proteins that are found in epithelia while its expression in various tissues has been widely used in the fingerprinting of various carcinomas [1]. The amino acids that are found in highest amount in the nail keratin are the Glu (19mg/g), Cys (17 mg/g), Arg (17mg/g) and Ser (5 mg/g) [2]. However, the keratin composition is affected by genetic factors and diseases [3,4]. Disorders or diseases also affect some of the nails’ physical properties [5] and their visual appearance [6].

Human nails also contain small amounts of essential metallic elements like Ca, Fe, Cu, Zn and therefore they are used to monitor the essential metal concentration in the human body [7-8]. Deviations from the normal values of some metallic elements have been related to disorders or diseases as well as to environmental and nutritional factors [9,10]. Moreover, diseases or disorders may affect the bonding environment of various metallic inorganic elements in the nail, as it is observed in the case of Fe in neuron tissues [11].

Here, we apply Zn - K edge extended X - ray absorption fine structure (EXAFS) spectroscopy in order to study the bonding geometry of Zn in human nails. EXAFS spectroscopy is a well - established technique for the investigation of the bonding environment of metallic elements in metal-proteins, which serve many important biological functions [12], and in samples of biogenic nature [13]. Zn is a
significant metallic element in biological systems due to its catalytic and structural role in proteins [14].

2. Samples and experimental details
The studied samples are 6 nail clippings that were collected at the Pulmonary Clinic of the Aristotle University of Thessaloniki and they belong to donors who suffer from lung diseases. The sample names and the health condition of the donor are listed in Table 1. Prior to the measurement, the samples were cleaned with acetone, alcohol and de-ionized water in an ultrasound bath [15]. The measurements were conducted at the BESSY storage ring of the Helmholtz Center Berlin for Materials and Energy. The Zn - K EXAFS spectra were recorded at ambient conditions at the KMC II beamline which is equipped with a graded SiGe double crystal monochromator. The spectra were recorded in the fluorescence yield mode using the XFlash (ROENTEC) detector positioned in the horizontal plane at right angle to the beam. The angle of incidence was 45°. The use of an energy dispersive detector allows the discrimination of the Zn $K_{\alpha}$ fluorescence photons and thus minimizes the background in the EXAFS spectra due to the absorption of preceding edges.

3. Results and discussion
Information on the ligands of Zn is obtained after fitting the Zn - K edge EXAFS spectra in the first nearest neighboring (nn) shell using the FEFF/FEFFIT package [16]. The spectra were fitted in the R - range from 0.6 to 2.3 Å with Zn - N and Zn - S paths, since the fitting using only N, only O, only S or both N and O was of poor quality. The amplitude reduction factor, the energy origin and the Debye - Waller factors were commonly iterated for the six nail samples. Representative fitting of the filtered contribution of the first nn shell in the $\chi(k)$, is shown in figure 1(a). The fitting in R - space for the studied samples is depicted in figure 1 (b) where the Fourier transforms (FT) are shown. The fitting results are summarized in Table 1. The Zn-N and Zn-S distances range from 2.00 to 2.04Å and 2.23 to 2.28Å, respectively. The coordination number of Zn, i.e. the sum of the number of nitrogen (N_N) and sulfur (N_S) neighboring atoms, takes values in the range 4.1 ± 0.5 – 4.8 ± 1.2, indicating that Zn is four - fold coordinated. Furthermore, Zn is bonded preferably to N (since N_S/N_N < 1) except for the case of a nail that belongs to a patient who suffers from lung fibrosis, where the corresponding ratio is equal to 1.

Table 1: Sample name and EXAFS analysis results for the first coordination shell of Zn. R_N and R_S are distances to the N and S atoms, respectively and N_N and N_S are the corresponding coordination numbers. The Debye - Waller factors were commonly iterated for the N and S subshells and they were found equal to $\sigma_{N}^2=(9.2\pm4.0)\times10^{-3}\text{ Å}^2$ and $\sigma_{S}^2=(8.5\pm4.5)\times10^{-3}\text{ Å}^2$, respectively.

| Sample name | Health condition | R_N(Å) ±0.02 | R_S(Å) ±0.02 | N_N | N_S | N_S/N_N |
|-------------|------------------|-------------|-------------|-----|-----|---------|
| PD          | Healthy          | 2.04 ±0.02  | 2.23 ±0.02  | 2.9±0.7 | 1.9±1.0 | 0.66±0.38 |
| ZTH         | Healthy          | 2.02       | 2.24       | 2.9±0.5 | 1.5±0.7 | 0.52±0.26 |
| SOS         | Obstructive lung disease (clubbed nail) | 2.04 | 2.25 | 2.9±0.5 | 1.5±0.7 | 0.52±0.26 |
| KAL         | Clubbed nail     | 2.04       | 2.24       | 2.8±0.6 | 1.8±0.8 | 0.64±0.32 |
| PT          | Tuberculosis     | 2.00       | 2.28       | 2.5±0.3 | 1.6±0.7 | 0.64±0.29 |
| TD          | Fibrosis         | 2.03       | 2.27       | 2.2±0.4 | 2.2±0.7 | 1.00±0.37 |
The preferential four-fold coordination of Zn with N and S atoms is in agreement with the statistical analysis results on the bonding environment of Zn in structural proteins included in the Protein Data Bank [14]. The N and the S atoms belong mainly to histidine (His) and cysteine (Cys). The FT’s shown in figure 1(b) are characterized by structure which extends up to the radial distance of 4 Å, which depends on the amino acids bonded to Zn. Because of the significant contribution of the multiple scattering paths, instead of performing a shell-by-shell fitting, we simulated the spectra using clusters of atoms with Zn bonded to amino acids. The models were extracted from the .pdb files of proteins included in the MCISB Protein Data Bank. The simulation in k- and R-space of the spectra of the samples ZTH and PT are shown in figures 2(a) and (b), respectively. The simulations were performed using the FEFF code [16] and the electron density and the scattering potentials were computed self-consistently using a Hedin-Lundqvist exchange correlation potential. In sample ZTH,
where $N_S/N_N=0.52$, a Zn(His)$_3$(Cys)$_1$ cluster is extracted from the human carbonic anhydrase II protein [17]. In sample PT where $N_S/N_N=1$, a Zn(His)$_2$(Cys)$_2$ cluster is extracted from the Zif268 Zinc finger [18]. In order to be in agreement with the Zn - S and Zn - N distances determined from the fitting of the first $nn$ shell, the distances of Zn with its neighbors were multiplied by 0.95 and 0.99, respectively. Finally, an energy shift of -5 and -7 eV, respectively, was necessary, in order to match the EXAFS oscillations of the experimental curve and the simulation in the $k$-space.

4. Conclusions

Zn - K edge EXAFS spectra of human nail samples were successfully recorded and yield information on the bonding of Zn with the amino acids that consist the nail keratin. Fitting of the first $nn$ shell of Zn reveals that it is preferentially four - fold coordinated with N and S. The average Zn - N and Zn - S distances are equal to 2.03 and 2.25Å, respectively. The ratio of the number of S over the number of N atoms bonded to Zn is found higher in a nail that belongs to a donor who suffers from lung fibrosis, indicating a possible relation of the formation of the keratin fibrous tissue on the lung with the strong tendency of Zn to bond with S in the nails. FEFF simulation of the spectra using Zn(His)$_3$(Cys)$_1$ and Zn(His)$_2$(Cys)$_2$ clusters extracted from the structure of the human carbonic anhydrase II protein and Zif268 Zinc finger, respectively, is quite satisfactory.

Acknowledgements

The measurements at BESSY were supported by the EC - Research Infrastructure Action under the FP6 "Structuring the European Research Area" Program, through the Integrated Infrastructure Initiative “Integrating Activity on Synchrotron and Free Electron Laser Science” (Contract R II 3-CT-2004-506008).

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