IRON OXIDES IN HUMAN BRAIN

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1. Introduction

Iron as a trace element plays a significant role in the human body. Total amount of this trace element in the body of the adult human is approximately 4 g. Most of the iron (more than 60%) is a part of the haemoglobin. Rest of the iron is located in the organs as liver, spleen, heart or brain in the form of the ferritin or possibly haemosiderin [1].

Ferritin is the main iron storage protein in the human body. Iron is stored in the ferritin as a trivalent and in this form it is not toxic for tissues. It consists of the spherical protein shell (with outer diameter ~12 nm and inner diameter ~8 nm) and the core in which is the iron stored [2]. The shell is composed of 24 subunits. There are two kinds of these subunits: H (heavy) and L (light) chain. H chain is capable of fast conversion of divalent iron to trivalent iron. L chain contributes to long term iron storage [3]. Haemosiderin is formed by the denaturation of ferritin and can be found in liver and spleen [4].

Iron can be possibly highly dangerous for the organism. It can participate in the production of free radicals via Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$). Free radicals cause cell damage or death. There is an assumption that the iron could be possibly linked to some of the neurodegenerative diseases such as Parkinson's disease [5].

2. Samples and methods

Samples from *Globus pallidus* were investigated. *Globus pallidus* is a part of the *Basal ganglia* which is a large subcortical structure in the human brain. This structure consists of several interconnected nuclei in the forebrain, midbrain, and diencephalon. It was agreed that *Basal ganglia* participates in the control of movement [6].

Brain tissues were collected from male donors who differed in age. Tissues were fixed in 10% formaldehyde for 24 hours. They were subsequently lyophilized and transformed to powder form. Afterwards, tablets were made from the powder to ensure better manipulation.

Samples were analysed by ^{57}Fe Mössbauer spectrometry which was carried out using a standard constant acceleration spectrometer with a ^{57}Co/Rh source. All experiments were performed in transmission geometry at room temperature and at temperature of 4.2 K using liquid helium bath cryostat. The resulting isomer shifts are quoted relative to the Mössbauer spectrum of a 12.5 μm thin bcc-Fe foil recorded at room temperature. The spectral parameters comprising isomer shift (*IS*), quadrupole splitting (*QS*), hyperfine magnetic field (*B*), line width (Γ), and area (*A*) of spectral components were refined by the CONFIT curve-fitting program [7].

X-ray diffraction experiments were performed at the High-Resolution Powder Diffraction beamline P02.1 of PETRA III electron storage ring at DESY (Hamburg,

Germany). Room temperature diffraction patterns were acquired in transmission mode. The energy of the synchrotron radiation was set to 59.81 keV which corresponds to the wavelength of 0.02073 nm. Samples were illuminated for 30 s by incident beam having a cross section of 0.6 x 0.6 mm^2. Two dimensional XRD patterns were collected using fast image plate detector Perkin Elmer 1621 (2048 x 2048 pixels, pixel size 200 x 200 μm^2) carefully mounted orthogonal to the X-ray beam. The distance between the 2D detector and the sample was adjusted to 26.3 cm in order to cover high *q*-range. CeO_2 standard was used to calibrate the sample-to-detector distance and tilt of the imaging plate relative to the beam path. The thickness of the samples was 2 mm.

3. Results and discussion

Mössbauer spectra obtained at room temperature from all investigated samples exhibit dublet like features. One of the samples was measured in a broad velocity range (± 12 mm/s) to search for possible occurrence of sextets what would indicate presence of magnetic iron oxides. No traces of any sextet were found. Subsequently, the samples were measured in a narrow velocity range (± 4 mm/s). Similar results were reported in [8, 9]. Room-temperature dublet like spectra indicate presence of very small particles which exhibit superparamagnetic behaviour. Three dublets were used for experimental data fit.

Low-temperature Mössbauer spectra from all investigated spectra are superpositions of three sextets and one dublet. Similar procedure was used in [9] where samples of human spleen were measured. It was reported in [10] that most of the iron in the human brain is ferritin-like iron. Dublet like component in spectra obtained at 4.2 K indicate presence of very small particles with blocking temperature lower than 4.2 K. Very similar spectra and dublet parameters were shown in [11] where samples of human heart tissue were measured. This dublet like component in low-temperature spectra can be due to haemosiderin. It is very likely that some of the iron in human brain is bound in such a form. It was not possible to characterize this dublet more precisely. Three sextet like components were characterized by the following parameters: $IS_{S1} \in$ <0.45, 0.52> mm/s, $IS_{S2} \in$ <0.40, 0.45> mm/s, $IS_{S3} \in$ <0.42, 0.47> mm/s, $QS_{S1} \in$ <-0.24, -0.27> mm/s, $QS_{S2} \in$ <-0.01, -0.05> mm/s, $QS_{S3} \in$ <-0.14, -0.29> mm/s, $B_{S1} \in$ <50.9, 51.9> T, $B_{S2} \in$ <48.8, 49.6> T, $B_{S3} \in$ <46.3, 46.7> T. These parameters are similar but not identical to Mössbauer parameters for goethite, akaganéit or ferrihydrite. Mössbauer spectra of human brain with their components are shown in Fig. 1.

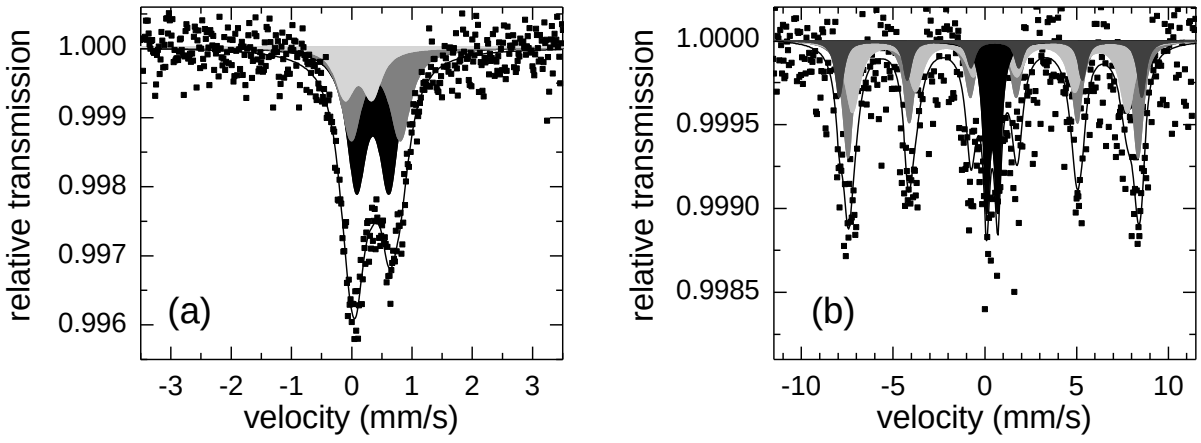

Fig. 1: *Mössbauer spectra of human brain and their spectral components obtained at room temperature (a) and at 4.2 K (b).*

The total structural factor *S(q)* and reduced pair distribution function (PDF) were obtained from integrated XRD raw data using the PDFgetX2 software [12]. *S(q)* and PDF of human brain are shown in Fig. 2. Diffused peaks of *S(q)* are characteristic for amorphous materials. Sharp peak is observed at the distance of 1.5 $Å^{-1}$ which indicates crystallinity. Significant oscillations in PDF can be observed approximately at the distance of 12 Å. It means that some atomic order can be observed within this distance. The first two PDF peaks, which correspond to the first and the second coordination shell, were analyzed. Atomic pairs with the most probable occurrences were determined and their weights were calculated. Positions of these atomic pairs correspond to bond lengths between these atoms. Atomic pairs, their bond lengths *r*, and calculated weights w_{ij} are given in Tab. 1.

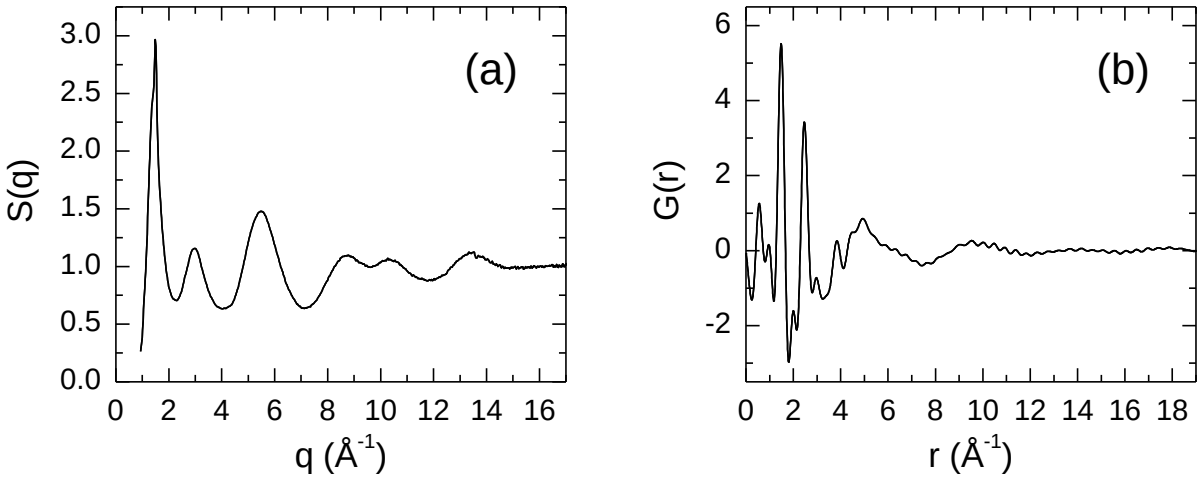

Fig. 2: *Structural factor S(q) (a) and reduced pair distribution function G(r) (b) obtained from samples of human brain.*

Tab. 1: *Atomic pairs, bond lengths (r) and weights (w_{ij}) of atomic pairs.*

atomic pair	*r* [Å]	w_{ij}
H-H	0.74	0.0243
O-H	0.96	0.0636
N-H	1.00	0.0216
C-H	1.09	0.1684
C-C	1.54	0.2917
C-O	1.42	0.2204
C-N	1.47	0.0748
O-O	1.49	0.0416

Atomic pair positions in the first and the second coordination shell are shown in Fig. 3. It can be said that the shape of the second coordination shell distribution is well described by C-C, C-O, C-N atomic pairs. Atomic pair O-O would indicate presence of peroxides which cause production of free radicals. Free radicals are very dangerous and cause cell damage. Atomic pairs H-H, O-H, N-H and C-H do not describe shape of the first coordination shell distribution completely. It can be seen that the peak of the first coordination shell is increasing approximately from the distance of 0.14 Å. It is very unlikely

that some atomic pair would be found in such a small distance because the shortest bond length is between two hydrogen atoms which is approximately 0.74 Å. Noise signal from Fourier transformation could cause some kind of deformation of the first PDF peak. Iron itself had negligible contribution to X-ray diffraction due to low concentration in samples (only 0.003 % of all atoms).

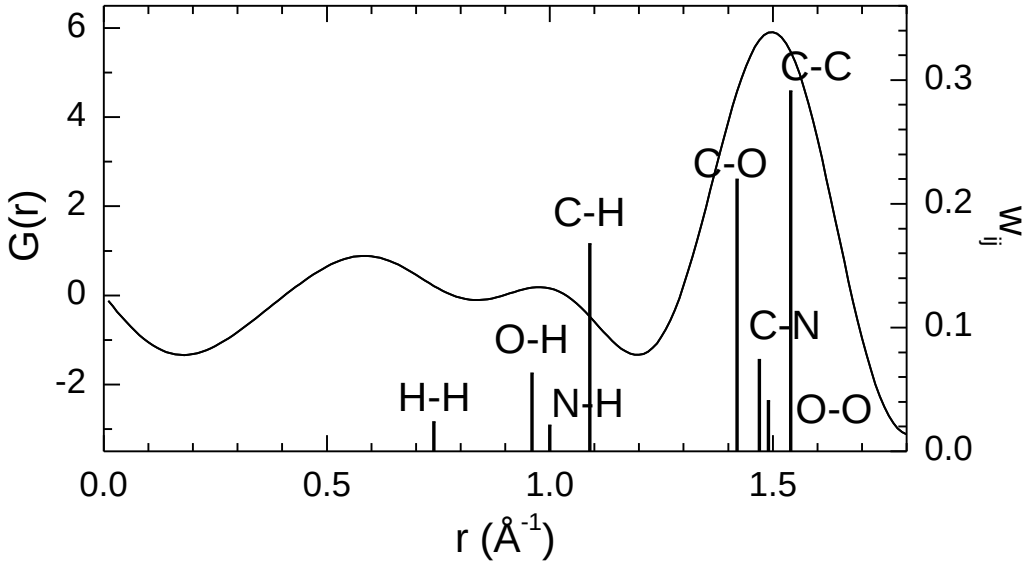

Fig. 3: *Reduced pair distribution function in the region from 0 Å to 1.8 Å and theoretical positions of the indicated atomic pairs with their weights.*

4. Conclusion

It was confirmed that Mössbauer spectroscopy is an useful tool for measurement of biological tissues even if the concentration of iron in the samples is very low. Mössbauer spectra revealed a presence of particles with non-magnetic behaviour at room temperature. At temperature 4.2 K almost all particles exhibit magnetic behaviour. The rest of the particles still exhibits superparamagnetic behaviour what indicates that their blocking temperature is lower than 4.2 K. It was suggested that those might be very small haemosiderin particles. Parameters the sextet-like components suggest possible presence of goethite, akaganéit or ferrihydrite.

Using synchrotron assisted XRD, it was not possible to reveal any iron relevant structural information due to very low concentration of iron atoms in samples. Atomic pairs with the highest contribution to PDF were revealed. All these atomic pairs are characteristic for biological materials. XRD measurement of extracted ferritin could reveal some helpful information about the iron structure.

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