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Deposits of iron oxides in the human spleen

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ABSTRACT

Scanning/transmission electron microscopy, SQUID magnetometry, and Mössbauer spectroscopy confirm presence of iron-oxide minerals of the size about 100 nm in the human spleen. Deposits of patients suffering from hereditary spherocytosis contain magnetite particles of the size above 500 nm that exhibit magnetic hysteresis even at the room temperature.

1. Introduction

There is growing information about a pathological biocrystallization of iron oxides in the human organs like brain and spleen [1]. Several minerals of the nanosize have been detected inside tissues so far, e.g. magnetite Fe_3O_4, maghemite γ-Fe_2O_3, hematite α-Fe_2O_3 and ferrihydrite $5Fe_2O_3{\cdot}9H_2O$[1]. Ferrihydrite is a physiological form of Fe(III) stored in the 6 nm cavity of ferritin. This is probably a source of iron that is found in pathological deposits of iron oxides – hemosiderin [2,3]. It is considered to be a proteolytic product of ferritin [4,5]. Changes in the shape of red blood cells (hereditary spherocytosis) affect the removal of these cells in human spleen [6]. This process leads to iron accumulation in human spleen. We assume that the form of iron deposits in hereditary spherocytosis differs from physiological form of iron – ferrihydrite.

From the view of this assumption we investigate iron deposits by scanning and transmission electron microscopy with electron diffraction, Mössbauer spectrometry and SQUID magnetometry.

2. Experimental methods

The samples were extracted post mortem at the Department of Pathological Anatomy, Faculty of Medicine, Comenius University, Bratislava, in accordance with Helsinki Declaration. During the sample preparation, special attention was paid to avoid manipulations with magnetizable instruments and environment. Fresh, soft tissues were dried in a vacuum (lyophilized). The resulting samples were obtained in a form of a powder.

Samples of spleen tissues were fixed in 10% formaldehyde for 24 h and embedded in paraffin blocks, cut by microtome to 5 μm thin sections and mounted on gelatin-coated slides. Samples without cover glass were analyzed by scanning electron microscope (JXA 840 A, JEOL) with the accelerating voltage of 20 kV. The samples for transmission electron microscopy investigation were fixed in 3% solution of glutar(di)aldehyde (SERVA, Heidelberg, Germany) for 2 h and buffered by phosphate (pH 7.2–7.4), embedded into Durcupan ACM (Fluka AG, Busch, Switzerland) as recommended by the manufacturer and cut by ultramicrotome (C. Reichert, Wien, Austria). The thickness of samples were 200 nm. Noncontrasted ultrathin sections were mounted on nickel grids and investigated by transmission electron microscope with acceleration voltage of 120 kV. To find iron-rich regions chemical analysis by EDX was applied.

^{57}Fe Mössbauer spectrometry was carried out using a standard constant acceleration spectrometer equipped with ^{57}Co source embedded in a rhodium matrix. Transmission geometry experiments were performed at 300 and 5 K. During low temperature experiments, the samples were maintained in a liquid helium bath

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[1] Under ferrihydrite an iron mineral is thought with well defined unit cell parameters (a = 2.96, c = 9.4 Å, hexagonal) but variable water content formulated as $5Fe_2O_3{\cdot}9H_2O$, $Fe_5HO_8{\cdot}4H_2O$, $Fe_5O_3(OH)_9$, $Fe_2O_3{\cdot}2FeOOH{\cdot}2.6H_2O$, $FeOOH{\cdot}0.4H_2O$, and also $Fe_2O_3{\cdot}0.5H_2O$; erroneously it is assigned as FeOOH that are polymorphs of goethite, akaganeite and lepidocrocite. The powder X-ray diffraction pattern contains either two peaks in the most disordered state or six lines in the most crystalline form.

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cryostat (SVT-400, Janis). Samples for Mössbauer effect experiments were in the powder form. For room temperature measurements they were placed into a plexiglass sample holder with a diameter of 12 mm ensuring the effective thickness of about 7 mg Fe/cm^2. Samples for low temperature measurements were sealed in aluminum foils in a form of pellets with 10 mm diameter. Isomer shifts are quoted relative to Mössbauer spectrum of a 12.5 μm thin bcc-Fe foil which was kept at room temperature. The centre of velocity scale of such Mössbauer spectrum was taken as the zero of the velocity axis. The spectral parameters comprising isomer shift, quadrupole splitting/shift, hyperfine magnetic field, line width, and area of spectral components were refined by the CONFIT curve-fitting program. Data were corrected for small absorption due to iron in the aluminium foil.

The SQUID apparatus (Quantum Design, MPMS-XL7) has been used for measurements of the magnetic moment of the specimen in the RSO mode of detection. Powder samples were weighted (10–30 mg) into gelatin-made sample holders. The centering was done at $B = 0.1$ T and $T = 5$ K. For the susceptibility measurements at the applied field $B_0 = 0.1$ T, temperature varied between 2 and 300 K. Magnetization measurements were conducted at $T_0 = 2.0$ and 4.6 K at the applied field up to $B = 7.0$ T. The measured signal has been converted to the mass magnetization and/or mass magnetic susceptibility that are presented in SI units.

3. Results and discussion

Two samples extracted from the human spleen are under investigation: a reference sample from the undiagnosed individual (**R**) and a sample with diagnosis for hereditary spherocytosis (hereafter **HS**), respectively.

Scanning electron microscopy (SEM) identified in the sample **R** clusters of iron whereas the transmission electron microscopy (TEM), after indexing, confirmed the presence of a hexagonal ferrihydrite. In addition, single crystals of magnetite were also visualized by SEM (ca 100 nm in size) and confirmed by TEM as seen in Fig. 1.

The sample **HS** exhibits deposits as large as 500 nm whose diffraction patterns correspond to the magnetite (Fig. 2).

It is very probable that the samples under investigation contain several iron-oxide minerals that show a distribution in the crystal size. Moreover, one cannot conclude that only the magnetite refers to the deposits. Notice, the magnetite is not the most stable form of iron oxides; on transforming the iron oxide minerals, the hematite represents the final form.

A detailed investigation of the sample **HS** has been done with the SQUID magnetometry. Temperature dependence of the magnetic susceptibility shows a hyperbolic-like behaviour that, however, differs from the Curie law (Fig. 3). The product function χT versus T possesses high positive slope that confirms a presence of a considerable amount of temperature-independent paramagnetism. This feature is different from behaviour of horse spleen ferritin (see ESI).

The zero-field cooled magnetisation (ZFCM) and field cooled magnetisation (FCM) data are presented in Fig. 4. It can be seen that the bifurcation point in the sample **R** is at $T_C = 50$ K that again is different from the horse ferritin where $T_C = 20$ K and the blocking temperature $T_B = 11$ K are characteristic fingerprints[2] [7]. The ZFCM curve is superimposed by a paramagnetic admixture. For the sample **HS**, however, the bifurcation point lies above 300 K, so that a search for the magnetic hysteresis could be positive.

The magnetic hysteresis has been detected for the sample **HS** at $T = 2$, 5, 10, 20, 50, 100, 200, and 300 K (Fig. 5). For the sample **R** it was detected only below 20 K (Fig. 6). To this end one can conclude that the iron-oxide deposits in the sample **HS** show a pronounced magnetoactivity (ferrimagnetism) that is present event at the room temperature.

The Mössbauer spectra of the two samples at $T = 5$ K are shown in Fig. 7. These were deconvoluted into a doublet (D) and three sextets (S1–S3) and the parameters are listed in Table 1. The sextet S1

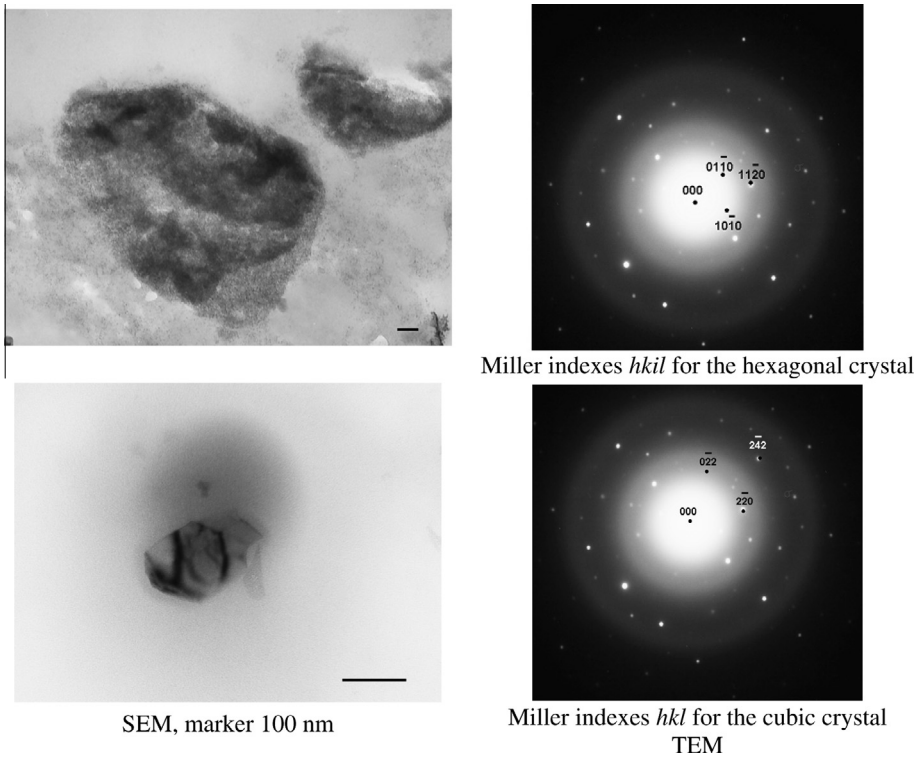

Fig. 1. SEM/TEM images of an iron deposit in the reference sample **R**. Top pair – ferrihydrite (hexagonal) from the ferritin cluster, bottom – magnetite (cubic) in the iron-oxide single crystal.