STUDY OF FERRITIN NANOPARTICLES

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1. Introduction

Iron is an essential element for most life on Earth, including human beings. Iron is needed for a number of highly complex processes that continuously take place on a molecular level and that are indispensable to human life, e.g. the transportation of oxygen around your body. Iron is required for the production of red blood cells (a process known as haematopoiesis), but it's also part of haemoglobin (that is the pigment of the red blood cells, see Fig.1) binding to the oxygen and thus facilitating its transport from the lungs via the arteries to all cells throughout the body. Iron is also involved in the conversion of blood sugar to energy. Metabolic energy is crucial for athletes since it allows muscles to work at their optimum during exercise or when competing.

Once the oxygen is delivered the iron (as part of haemoglobin) binds the carbon dioxide which is then transported back to the lung from where it gets exhaled. The production of enzymes (which play a vital role in the production of new cells, amino acids, hormones and neurotransmitters) also depends on iron, this aspect becomes crucial during the recovery process from illnesses or following strenuous exercise or competing. The immune system is dependent on iron for its efficient functioning and physical and mental growth require sufficient iron levels, particularly important in childhood and pregnancy, where the developing baby solely depends on its mother's iron supplies.

Iron is lost by the body through a variety of ways including urination, defecation, sweating, and exfoliating of old skin cells. Bleeding contributes to further loss of iron which is why women have a higher demand for iron than men. If iron stores are low, normal haemoglobin production slows down, which means the transport of oxygen is diminished, resulting in symptoms such as fatigue, dizziness, lowered immunity or reduced ability for athletes to keep up with their training programs.

Besides carbon, oxygen, and nitrogen, numerous other elements and their compounds are significant in the body of humans and other living beings. Among them, iron is essential element for fundamental cell functions and catalyst for chemical reaction. Iron can be found in biological tissues mainly in the form of ferritin. This primary, iron storage protein is present in the cytoplasma of the cells and in small amounts in the blood pool. It is one of the major proteins of iron metabolism. Ferritin creates spherical formations with the size of several nanometres. The core of ferritin consists of ferrihydrite – $5Fe_2O_3.9H_2O$. In excessive volume of iron in the organism, this is stored in cells in the form of hemosiderin which is considered to be a proteolytic product of ferritin.

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including algae, bacteria, higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein, but small amounts are

secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body; hence serum ferritin is used as a diagnostic test for iron deficiency anaemia.

The most important group of iron-binding proteins contain the heme molecules, all of which contain iron at their centres (see Fig. 2). Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin.

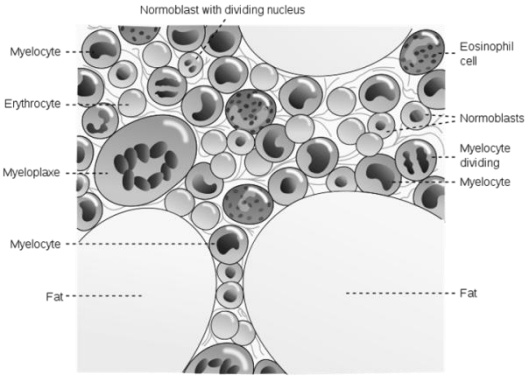

Fig. 1. *Illustration of blood cell production*

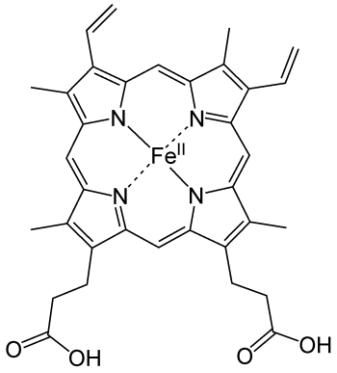

Fig. 2. *Structure of Heme b.*

Most well-nourished people in industrialized countries have 4 to 5 g of iron in their bodies. Of this, about 2.5 g is contained in the haemoglobin needed to carry oxygen through the blood, and most of the rest (approximately 2 g in adult men, and somewhat less in women of childbearing age) is contained in ferritin complexes that are present in all cells, but most common in bone marrow, liver, and spleen. The liver's stores of ferritin are the primary physiologic source of reserve iron in the body. The reserves of iron in industrialized countries tend to be lower in children and women of child-bearing age than in men and in the elderly. Women who must use their stores to compensate for iron lost through menstruation, pregnancy or lactation have lower non-haemoglobin body stores, which may consist of 500 mg, or even less.

Of the body's total iron content, about 400 mg is devoted to cellular proteins that use iron for important cellular processes like storing oxygen (myoglobin) or performing energy-producing redox reactions (cytochromes). A relatively small amount (3–4 mg) circulates through the plasma, bound to transferrin. Because of its toxicity, free soluble iron (soluble ferrous ions Fe(II)) is kept in low concentration in the body.

Iron deficiency first affects the storage iron in the body, and depletion of these stores is thought to be relatively non-symptomatic, although some vague and non-specific symptoms have been associated with it. Since iron is primarily required for haemoglobin, iron deficiency anaemia is the primary clinical manifestation of iron deficiency. Iron-deficient people will suffer or die from organ damage well before cells run out of the iron needed for intracellular processes like electron transport.

Iron is also stored as a pigment called hemosiderin which is an ill defined deposit of protein and iron, created by macrophages where excess iron is present, either locally or systemically for example among people with iron overload due to frequent blood cell destruction and transfusions. If the systemic iron overload is corrected, over time the hemosiderin is slowly resorbed by macrophages.

2. Experimental

The samples were extracted post mortem according to the Helsinki Declaration. Special attention was paid to avoid manipulations with magnetisable instruments and

environment. Fresh, soft tissues were dried in a vacuum (lyophilized) to obtain them in a form of powder.

This contribution aims in characterization of chemical states of ferritin nanoparticles of biological origin. We have studied samples prepared from human spleen. ^{57}Fe Mössbauer spectroscopy was employed as a principal method of investigation in addition to X-ray diffraction, and electron microscopy.

At room temperature, ferritin nanoparticles exhibit superparamagnetic behaviour due to their small dimensions. Corresponding Mössbauer spectra show doublet-like patterns. Consequently, experiments were performed at low temperatures (down to 5 K) that have enabled to determine the blocking temperature. Dimensions of Fe-containing species were established from detailed analyses of TEM images.

^{57}Fe Mössbauer spectra were collected at room temperature in transmission geometry using a standard constant-acceleration spectrometer equipped with a ^{57}Co/Rh source. Calibration of the spectrometer was done by an α-Fe foil (12.5 μm) at room temperature. Spectral parameters were derived by the help of the CONFIT fitting software [1].

3. Results and Discussion

3.1 *Mössbauer spectrometry*

Room temperature Mössbauer spectra of all investigated samples exhibit doublet-like features. First of all they were checked in a broad velocity range (±10.5 mm/s) to search for possible occurrence of iron oxides. No traces of sextets were revealed. Subsequently, the samples were measured in a narrow velocity range (±3 mm/s) to allow for better line resolution.

We have used three doublets to refine the experimental data. Low temperature Mössbauer effect measurements at 77 K revealed, however, no changes in the magnetic ordering of the investigated samples. Consequently, the samples were measured at 5 K. The resulting Mössbauer spectra of human spleen are shown in Fig. 3. The decomposition to individual spectral components is also shown. The obtained spectral parameters are collected in Tab. 1.

Tab. 1: *Parameters derived from Mössbauer spectra of human spleen that were recorded at the temperature T including relative area A, isomer shift IS, quadrupole splitting/shift QS, hyperfine magnetic field of the sextet components B_{hf}, and line width Γ.*

T **(K)**	**component**	***A*** **(%)**	***IS*** **(mm/s)**	***QS*** **(mm/s)**	B_{hf} **(T)**	Γ **(mm/s)**
300	hematite	28	0.37	0.96	-	0.40
	ferrihydrite	46	0.34	0.55	-	0.32
	FeIII	26	0.45	0.53	-	0.28
5	hematite	26	0.48	-0.13	51.0	0.50
	ferrifydrite (core)	20	0.47	-0.12	49.2	0.43
	ferrihydrite (surface)	20	0.45	-0.14	46.6	0.76
	FeIII	34	0.45	0.58	-	0.52

The observed Mössbauer spectra resemble those reported by Meyrick et al. [2] for set of human spleen tissues. Because of doublet-like features of room temperature Mössbauer spectra, a superparamagnetic behaviour caused by small particle sizes can be expected.

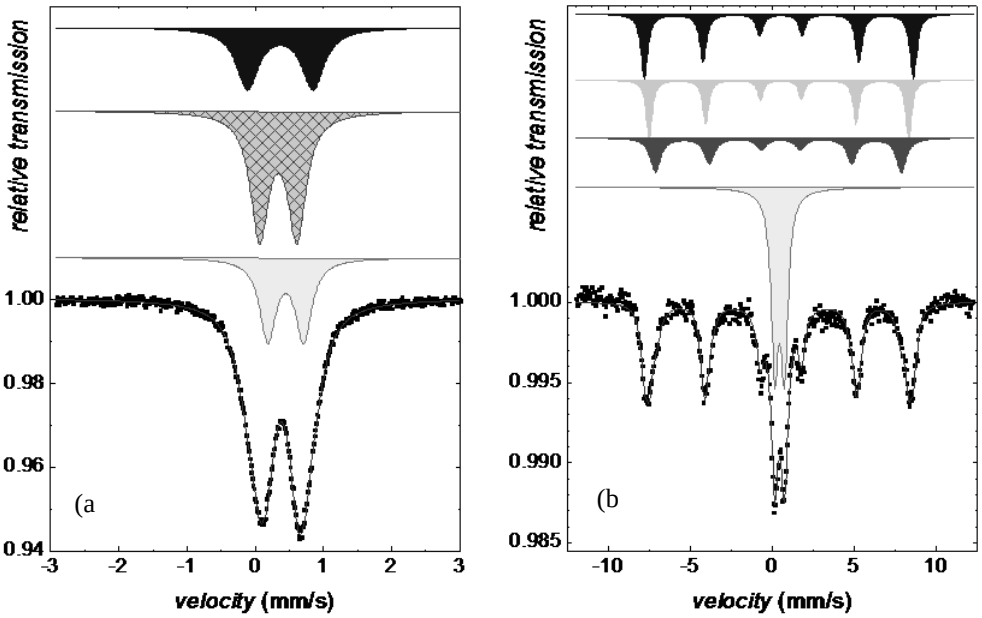

Fig. 3. *Mössbauer spectra of human spleen taken at 300 K (a) and at 5 K (b).*

We assign the doublets in room temperature spectra to hematite, ferrihydrite in haemosiderin, and ferritin [2], and to FeIII positions that presumably belong to magnetite/maghemite small particles. In the low temperature (5 K) Mössbauer spectrum, we assign one of the sextets to hematite; the obtained parameters are close to those reported by Casas et al. [3]. The following two sextets represent ferrihydrite core and surface Fe atoms, respectively [4]. It is noteworthy that the narrow lines of the former suggest relatively good crystalline arrangements whereas more than twice as broad lines of the latter sextet imply disordered (amorphous) nature of the atomic sites positioned at the surface of a core-shell ferritin model [5].

Almost one third of all Fe atoms present in human spleen exhibits paramagnetic FeIII doublet even at 5 K. They can be associated most probably with small ferrihydrite and/or magnetite/maghemite particles. In order to determine the so-called blocking temperature a series of Mössbauer spectra was recorded as shown in Fig. 4.

Blocking temperature is a technique-dependent parameter which is affected by the time window of the particular method used [4]. The positions of magnetic moments of small particles (several nanometers in size) fluctuate due to temperature driven relaxation processes. Consequently, corresponding Mössbauer spectra show doublets and/or sextets depending upon the temperature of the sample. Because we are investigating an ensemble of ferritin nanoparticles, the blocking temperature was determined as a temperature at which the areas of all magnetic sextets and doublets (non-magnetic states) are equal. The obtained values of the blocking temperatures are of 16 K for human ferritin [6]. St. Pierre et al. [7] reported blocking temperatures of about 25 to 35 K for human haemosiderin and ferritin, respectively.

3.2 *Transmission electron microscopy*

In order to determine the size of ferritin particles, we have employed TEM. Dark and light field TEM images of the human spleen tissue were analyzed. The obtained distributions of the nanograins were fitted with log normal distributions. The results of the fitting yielded average grain size of human ferritin particles of 5.4±0.1 nm. Standard deviations are almost equal within the error range giving 0.41±0.02 nm. More detailed description can be found in our recent work [8].

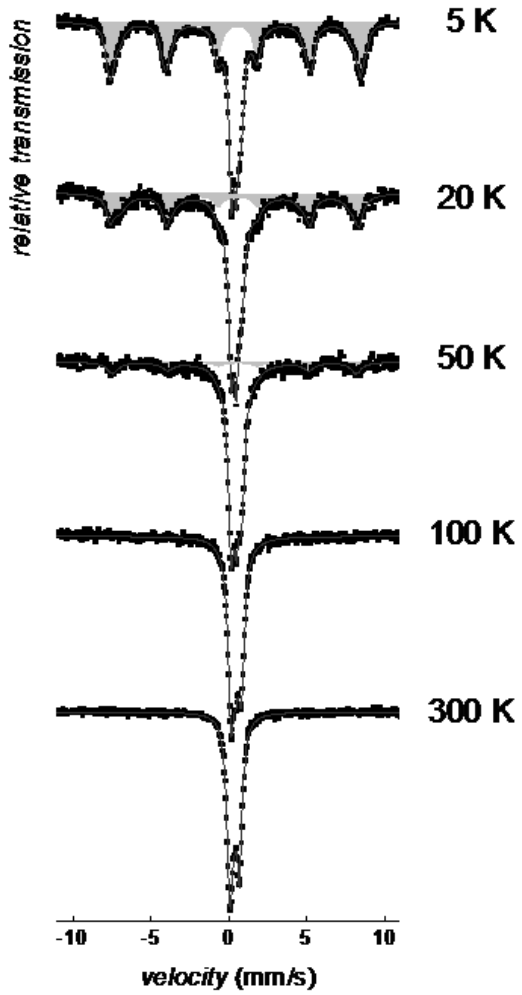

FIG. 4. Mössbauer spectra of human spleen taken at the indicated temperatures.

3.3 *X-ray diffraction*

Standard powder X-ray diffraction experiment was performed aiming at the identification of possible iron structures (hematite, maghemite, magnetite, ferrihydrite) in the spleen tissues. However, as it was confirmed by TEM the size of the ferritin particles is so small that there was only a little hope to reveal a presence of some of the minerals mentioned above.

Indeed, the obtained X-ray diffraction pattern of a human spleen [8] exhibited featureless broadened reflections. They confirm the findings from TEM and indirect indications from Mössbauer spectrometry that the structure of ferritin particles is highly disordered (amorphous) and/or that the grains are too small to provide a reasonable X-ray diffraction data analysis. Consequently, we have employed intensive X-rays obtained from a synchrotron. Detail analysis of the achieved data is presented elsewhere in these proceedings [9].

4. Conclusions

Mössbauer spectrometry confirms the presence of hematite, ferrihydrite and maghemite/magnetite in ferritin derived from human spleen tissues. The minerals are present in a form of small (about 4-5 nm in size) grains with highly disordered structure. Consequently, at room temperature all agglomerates of ferritin nanoparticles show non-magnetic behaviour. Magnetic states are revealed at low enough temperatures below the so-

called blocking temperature. Employing Mössbauer effect measurements, the latter was determined to be of 16 K for the human spleen.

Structural features of these tissues were studied by TEM technique. Employing ^{57}Fe nuclei as local probes both structural and magnetic features of the biological materials were investigated by Mössbauer spectrometry. It was possible to identify iron atoms and their neighbours.

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References:

[1] T. Zak, Y. Jiraskova: *Surface and Interface Analysis*, **38**, 710 (2006)
[2] D. Meyrick, J. Webb and C. Cole: *Inorg. Chim. Acta*, **339**, 481 (2002).
[3] L. Casas, A. Roig, E. Molins, J. M. Greneche, J. Asenjo and J. Tejada: *Appl. Phys*., **A74**, 591 (2002).
[4] G. C. Papaefthymiou: *Nano Today*, **4**, 438 (2009).
[5] R. A. Brooks, J. Vymazal, R. B. Goldfarb, J. W. Bulte and P. Aisen: *Magn. Res. Med.*, **40**, 227 (1998).
[6] M. Miglierini and A. Lancok: *Acta Phys. Pol*., **A118**, 944 (2010).
[7] T. G. St. Pierre, R. K. Pollard, D. P. E. Dickson, R. J. Ward and T. J. Peters: *Biochim. Biophys. Acta*, **952**, 158 (1988).
[8] M. Miglierini, et al.: *AIP Conf. Proc*., **149**, 107 (2012).
[9] M. Cesnek, M. Miglierini, A. Lančok: Iron-oxides in human brain, this issue.