Low temperature magnetic analysis in the identification of iron compounds from human brain tumour tissue

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Abstract: In the brain, iron plays an important role, but also is potentially toxic if iron metabolism is disrupted. Excess iron accumulation in the brain has been shown to be associated with neurodegenerative diseases. However, identification of iron compounds in human tissue is difficult because concentrations are very low. Three types of magnetic methods were used to characterize iron compounds in tumour tissue from epileptic patients. Isothermal Remanent Magnetization (IRM) was measured at 77 K and 300 K and reveals a low-coercivity phase with the properties of magnetite or maghemite. Induced magnetization was measured between 2 K and 300 K after cooling in zero-field and in a 50 mT field. These curves reveal an average blocking temperature of 11 K, which is compatible with ferritin. The results of this study show that the combination of different magnetic methods provides a useful and sensitive tool for the characterisation of magnetic iron compounds in human tissue.

1. Introduction

Iron is an essential component of physiological processes in virtually all organisms. However, it can also be toxic by acting as a catalyst for the production of free radicals. It has long been known that a disruption in iron metabolism is associated with neurodegenerative diseases (e.g. [1],[2]). Iron-induced epileptic activity as a result of intracranial bleeding and electrophysiological responses are described in several studies, which suggest that iron overload may lead to a predisposition to epilepsy [3, 4].

The primary form of iron storage in most living organisms is in the core of the protein ferritin. Ferritin retains iron in a soluble, non-toxic form. It consists of a protein shell composed of 24 polypeptide subunits that form a spherical cage of approximately 12 nm outer diameter and 8 nm inner diameter. The interior of the shell is occupied by the iron biomineral ferrihydrite (5Fe$_2$O$_3$·9H$_2$O), which undergoes antiferromagnetic ordering at low temperature and has a net magnetic moment that arises from uncompensated surface spins and defects in the crystal structure. The magnetic properties of horse spleen ferritin are well characterized, but those of ferritin in the human brain are not well studied. Biogenic magnetite (Fe$_3$O$_4$) is another iron compound that was first identified by Kirschvink et al. [5] in human brain tissue obtained during autopsies. Dobson and Grassi [6] determined the magnetite concentrations in tissue removed from the hippocampi of epileptic patients. The source of biogenic magnetite in brain tissue is still unknown, but since ferrous iron (Fe$^{2+}$) is toxic, the fact that magnetite, which contains both ferrous and ferric (Fe$^{3+}$) iron, is present in the tissue raises important questions regarding its possible role in neurodegenerative diseases. We have investigated the usefulness of low temperature magnetic measurements in identifying the different iron phases that are found in brain and epilepsy/tumour tissue. The ability to identify different iron phases both
qualitatively and quantitatively will be necessary to further examine the relationship between iron oxide particles and neurodegenerative disease.

2. Material and methods
Samples consist of tumour- and hippocampal tissues that were resected from patients with Mesial Temporal Lobe (MTLE) epilepsy. The tissue was immediately frozen at -80° C to prevent any chemical changes in the iron mineralogy. Isothermal Remanent Magnetization (IRM) acquisition was imparted with an ASC Impulse Magnetizer and measured both on the frozen tissue at 77 K and on freeze-dried tissue at room temperature on a 3-axis 2G SQUID magnetometer. All measurements were made 60 seconds after the pulse, so only the stable remanent magnetization was measured. Measurements of induced magnetization (DC susceptibility) as a function of temperature were made during warming from low temperature after zero-field cooling (ZFC) and after cooling in a 50 mT field (FC) with a Quantum Design MPMS SQUID magnetometer. Induced magnetization as a function of field (hysteresis curves) was measured at 5 K, 25 K, 77 K and 300 K, also on a Quantum Design MPMS SQUID magnetometer. For these MPMS measurements the samples were first freeze-dried.

3. Results and discussion
3.1 Isothermal Remanent Magnetization (IRM)
Acquisition of IRM at 77 K and at room temperature (300 K) shows a low-coercivity phase that is saturated by 200-300 mT, which is consistent with the presence of the ferromagnetic iron oxides magnetite and/or maghemite (\(\gamma\)-Fe\(_2\)O\(_3\)) – an oxidation product of magnetite (figures 1,2). Ferritin is not magnetically ordered above 65 K and therefore does not contribute to the remanent magnetic signal. The difference in the intensity of the saturation IRM between 300 K and 77 K is due to the magnetic ordering of superparamagnetic particles of the low-coercivity phase.

![Figure 1. IRM acquisition in a sample of tumour tissue at 300 K and 77 K.](image)

The coercivity of remanence (\(H_c\)) was obtained by giving the samples a saturation magnetization in one direction and then acquiring the IRM in the antipodal direction. The values for \(H_c\) were consistently between 20 mT and 40 mT for all samples (figure 2).

3.2 Induced DC-magnetization versus temperature
Induced magnetizations were measured in a field of 50 mT between 2 K and 300 K after cooling in zero field (ZFC) as well as in a 50 mT field (FC) (figure 3). The curves superimpose above the bifurcation point at 25 K; this indicates the onset of magnetic ordering. The ZFC curve exhibits two local maxima at 11 K and around 50 K. The maximum at 11 K indicates the average blocking
temperature of ferritin in the human brain. The second maximum is believed to be due to pure magnetite [7]; this will be the focus of further investigations.

**Figure 2.** IRM acquisition at 77 K on different samples of tumour (T) and hippocampus (H) tissue from several MTLE patients, indicated by the sample names.

There is a strong increase in the ZFC induced magnetization below 6 K (figure 3), which can be attributed to a paramagnetic phase, such as heme-iron from blood in the tissue. Analyses of blood [8] show no peaks due to ordering or phase transitions in the ZFC curve, meaning that the ZFC and FC curves showed identical paramagnetic-like decay from 2 to 300 K. ZFC and FC induced magnetizations were measured on pure blood samples under the same conditions as for the tumour tissue (figure 4). The subtraction of the 1.7 mg blood signal, which approximates the blood content in the sample, from the measured data reveals that at low temperatures two magnetic contributions can be decomposed: one from heme-iron and the other from ferritin.

**Figure 3.** FC and ZFC magnetizations induced in a DC-field of 50 mT in a tumour tissue sample.

**Figure 4.** ZFC magnetizations induced in a DC-field of 50 mT in tumour tissue, before and after subtraction of the blood signal.
3.3 Induced magnetization versus field (Hysteresis)

Hysteresis measurements at different temperatures show the presence of both a low- and high-coercivity phase, which are attributed to magnetite and ferritin, respectively. A strong diamagnetic signal from the tissue as well as the contribution from paramagnetic phases was subtracted, revealing the presence of a low-coercivity phase at 300 K and 77 K. In the hysteresis curves at 25 K and 5 K, the gradual ordering with temperature of the high-coercivity ferritin phase is seen. Hysteresis measurements show an open loop at 5 K with a coercive field of 50 mT. The loop remains open up to high fields, which is typical for antiferromagnetic nanoparticles. More detailed results from these experiments are published elsewhere [9].

4. Conclusions

Low temperature magnetic methods were successful in identifying a low-coercivity phase that may be attributed to magnetite and/or maghemite and a high-coercivity phase, ferritin. In addition a paramagnetic signal that is consistent with heme-iron from blood was identified in the tissue sample from the measurement of induced magnetization as a function of temperature. At present it has not been possible to distinguish if magnetite or maghemite is responsible for the low-coercivity phase. It was not possible to identify a Verwey transition in the induced DC magnetization curves due to the weakness of the signal; however, the peak in magnetization found around 50 K has only been identified in magnetite [7]. Magnetic methods provide a sensitive tool to examine the role of iron and iron oxide particles in brain tissue, even for very small concentrations.

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