Iron Oxides Particles in Globus Pallidus of Human Brain

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Abstract. *Iron is essential element used for fundamental cell functions, catalyst for chemical reaction. We used scanning and transmission electron microscopy, energy-dispersive microanalysis, electron diffraction, UV and EPR measurements for investigation of iron particles in globus pallidus of human brain. Scanning electron microscopy revealed iron rich complexes with Na, Si, P, S, Cl, Ca and Cu. Transmission electron microscopy revealed bumpy, solid particles of platy and sometimes rounded shape with the size around 3 µm. These ones were identified as hematite. Spectral measurements showed the presence of Fe(III), Cu(II) and some amount of Fe(II). We consider low-temperature process of hematite formation in human globus pallidus by aggregation-dehydration-rearragement in aqueous environment through ferrihydrite influenced by organic and inorganic factors.*

Keywords: iron, copper, brain, hematite

1. Introduction

Bio-mineralization process in organism is results of the interaction between metabolic processes products and the surrounding environment. Iron can be found in human body mainly in the form of ferritin. The size of ferritin core is 8 nm and has polyphasic structure consisting of ferrihydrite, hematite and magnetite. Pathological bio-mineralization may happen under some circumstances. The result of this process depends on many other factors such as chemical elements, temperature/pH and time. Iron induces reactive oxygen species (ROS), which react with iron resulting in iron oxides, hydroxides and oxide hydroxides.

2. Subject and Methods

Samples

Postmortem tissues were taken from globus pallidus externa of nine human brains without any clinico-pathological findings on a disease of central nervous system.

Scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX)

We used 3% fixation solution of glutar(di)aldehyde buffered by phosphate for scanning electron microscopy. Samples at autopsy were dehydrated in graded acetone, subjected to critical point drying of CO_2 (CPD 030, BAL-TEC, BG PRŰFZERT). Specimens were mounted on carbon stubs and coated with layer of carbon in ion sputtering apparatus (SCD 050, BALZERS, Lichtenstein). They were examined JXA 840 A (JEOL, Japan) with the accelerating voltage of 15 kV. Simultaneous EDX analysis was performed with the aid of

KEVEX 3205-1200 (Kevex, Valencia, Ca). The time period of spectrum collection was 200 s with the energy range 0.160 to 9 keV.

Transmission electron microscopy (TEM) and electron diffraction
The samples at autopsy for transmission electron microscopy investigation were fixed in glutar(di)aldehyde solution (SERVA, Heidelberg, Germany) for two hours. After dehydration the tissue by alcohol, samples were embedded into Durcupan ACM (Fluka AG, Busch, Switzerland) and cut by ultramicrotome with thickness 200 nm (C. Reichert, Wien, Austria). Noncontrasted ultrathin sections were investigated by selective electron diffraction in transmission electron microscope CM 100 (Philips, Eindhoven, Netherlands) with acceleration voltage of 80 kV.

Spectral measurements
Electronic spectra of the powdered samples in nujol mulls were recorded at room temperature on Specord 200. EPR spectra were measured using eitjer a Bruker ER 200E SRC with an internal marker for determining the frequency on a Bruker EMX series with an ER 035 NMR gaussmeter and System Donner EMX frequency counter. The EPR spectra were measured in polycrystalline form at room temperature.

3. Results

SEM examination of globus pallidus samples shows iron-oxygen rich particles (see Fig. 1). The size of them with organic envelope was in the range of 0.5 – 3 µm. The shape of the complex is irregular. The chemical composition of iron rich complex is Na, P, S, Cl, and Ca. Some complexes contain also various amount of Cu (Cu Kα 8,04 keV).

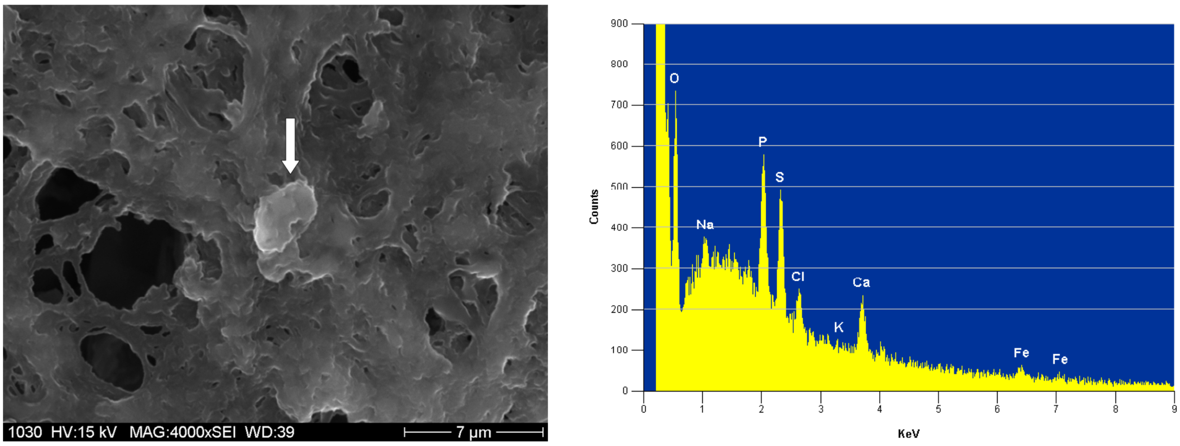

Fig. 1. Iron-oxide rich complex in globus pallidus of human brain (arrow) in SEM. EDX spectrum reveals the presence of Na, P, S, Cl, Ca and Fe. Range 0.160 to 9 keV, spectrum time collection was 200 s.

TEM investigation reveals bumpy, solid particles of irregular shape (see Fig. 2). Their size is 1-4 µm.

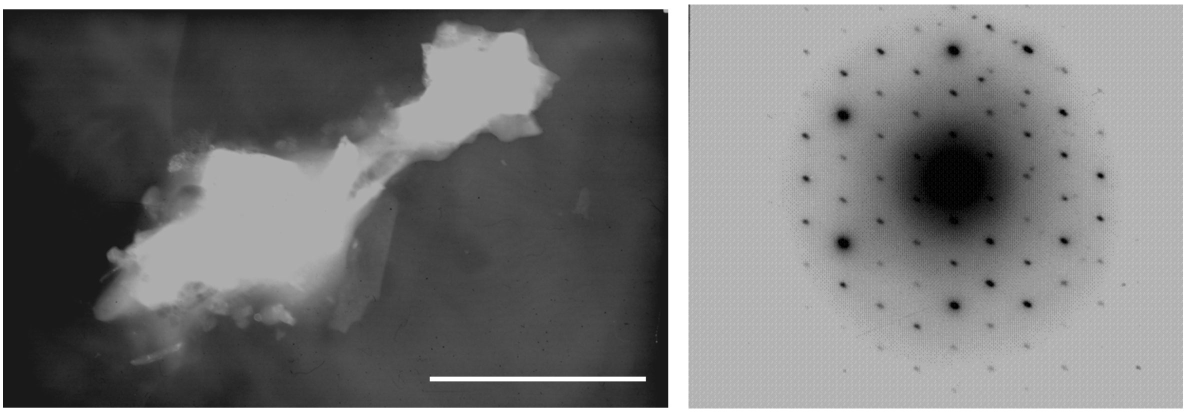

Fig. 2. Iron-oxide rich particle in TEM from globus pallidus of human brain (left) and its diffraction pattern corresponding to α-Fe_2O_3 (hematite). Scale bar = 3 µm.

Selective electron diffraction in TEM shows well crystalline α-Fe_2O_3 with hexagonal structure with lattice parameters a = 0.503 nm, c = 1.375 nm corresponding to hematite (see Fig. 2).

The absorption features in the spectra are primarily charge transfer in origin with various weak features in are assigned as d-d transitions. Because of the oxidizing power of Fe(III), ligand to metal charge transfer bands often observe the very low intensity d-d absorption. However, it can be present some amount of Fe(II) as well. The solid state electronic spectra of the samples are almost identical with the band approximately at 398(±2), 278(±2), 230(±2) and 214(±2) nm. The EPR spectra of the samples are similar and complex. The pseudo rhombic spectra indicate the presence of Fe(III). The spectra show the additional bands at about 300(4) and 230(5) mT, which indicate the presence of Cu(II).

4. Discussion

Hematite is the end product of phase transformation of various iron oxides. The water/OH is fundamental for non-thermal conversion of ferrihydrite to hematite (α-Fe_2O_3). The most important chemical elements influencing iron oxides formation are phosphorus and sulphur. The higher concentration of phosphate is associated with non-crystallinity of iron biominerals. The iron overload leads to ROS induction and subsequently to the regulation of antioxidant defenses involving thiol (-SH) metabolism. Cysteine, amino acid with –SH group, effect ferrihydrite transformation into hematite, goethite and lepidocrocite at pH 6 – 8 [1]. It was observed spontaneous ferrihydrite transformation to well crystalline hematite with little goethite at room temperature for 20,4 years in closed vessels in aqueous system [2]. In globus pallidus of human brain there are conditions that favor hematite formation [3]. EPR spectra of the samples indicate the presence of Fe(III) iron, what is in good agreement with electron diffraction measurements. But the presence of Fe(II) can not be ruled out. Copper like iron has crucial role as redox active metal in biological reactions. But its elevated level is involved in the pathogenesis of many neurological diseases. ROS are scavenged by antioxidant enzymes Cu-Zn superoxide dismutase (Cu-Zn-SOD). Copper-iron interactions in the body have significant physiological and pathological relevance [4].

5. Conclusions

Iron is essential element used for fundamental cell functions, catalyst for chemical reaction in the brain. Its bio-mineralization process in the brain is the interaction between metabolic processes products, surrounding environment, chemical elements and compounds. Hematite is stable phase and is the end product of transformation of other iron oxides. In globus pallidus of human brain there are conditions that favor hematite formation. From this point of view copper-iron interaction may play significant role.

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