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研 究

Studies of Inorganic Crystals in Biological Tissue: Magnetite in Human Tumor

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SYNOPSIS

Ferromagnetic materials, magnetite crystals, have been extracted and identified from magnetotactic bacteria, polyplacophoran mollusks (chitons), salmons, tuna and recently tissues of the human brain. Ferritin, the iron storage nonheme-protein contains hydrated iron oxide ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$) in its core. It is suggested that this hydrated oxide in ferritin would be the precursor of magnetite in both magnetotactic bacteria and the chitons. Many studies have described that ferritin were related with cell proliferation, specially tumor cell growth. In this study, we attempted to investigate the presence of magnetite in tumor tissues. We report the first detection of magnetic materials in various human tumor tissues (melanoma, breast, ovary, testicle, sarcoma, meningioma, glioblastoma, astrocytoma, glioma, metastasis) with the use of SQUID magnetometry. The magnetometry data for the magnetic materials are consistent with magnetite (Fe_3O_4). Its concentration of various tissue types were measured via SIRM. Tumor tissues were also stained blue with the Perls staining method for detecting ferritin. Our experiments show that the distribution of magnetite and ferritin in tumor tissues might depend on the etiology of the tumor. Ferritin distribution as indicated through staining suggest an irregular pattern with distinctive foci. However, a spacial covariance between ferritin and magnetite was unable to demonstrate in this study.

KEY WORDS

Magnetite, ferritin, tumor cell, Perls staining, SQUID magnetometry.

1 Introduction

Formation of iron-oxides in biosystem is of interest to the material and biological sciences. The ferric oxide, ferrihydrite ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$) and the ferromagnetic material, magnetite (Fe_3O_4) can be listed as examples. Ferritin, the iron storage nonheme-protein) comprises a protein shell, called apoferritin, which contains 24 polypeptide subunits surrounding a ferrihydrite single crystal of about 8 nm diameter. The compound is paramagnetic at room temperature and its crystal size is controlled by the amount of iron in the cell. Several studies suggest that the bindings of Fe(III) occur at nucleation sites on the interfaces of apoferritin subunits, as crystal growth can favor oxidation at pH7 through redox and kinetic reactions. The biological materials in which magnetite crystals are extracted and

identified include chitons (Mollucsan class: Polyplacophora) whose teeth consist of magnetite^{3,4}, magnetic bacteria⁵ (*Magneto sprillum*) which contain a chain of magnetite and salmon in whose ethmoid a chain of magnetite crystals is contained⁶. The color of teeth of chiton gradually changes with growth stage'. In the early growth stage, the teeth filled with protein and chitin do not contain iron. In the second stage, they get non-crystalline ferrihydrite. As protein, ferritin, are deposited into vacuoles, the teeth start to turn reddish. Finally, it changes to black color of magnetite. Similarly, magnetotactic bacteria produce noncrystalline ferrihydrite in early growth stage within a phospholipid vacuole. Later, it is converted to magnetite shaped in linear chains. From these observations, ferrihydrite seems to be the precursor of magnetite^{8,9}. The presence

of ferritin in the cell as Fe suppliers, or the presence of substances of precursor imply possibility of formation of magnetite. In a previous study, we reported the first detection of magnetite in human brain tissues. Compared with ferritin and magnetite distribution in human brain, magnetite is distributed homogeneously over the whole brain except meninges, but not for ferritin¹⁰. This result suggests that magnetite in the human brain does not form spontaneously from ferritin.

In general, both MRI imaging and Perls staining methods^{11,12} are used routinely for determining the relative amounts of Fe(+III) distribution in the whole brain^{13,14}, which is dominated by ferritin. Ferritin molecules are degraded in lysosomes¹⁵ and synthesized in free polysome in various tissue cells such as hepatocytes, erythroblasts, Kupffer cells, and many kinds of neoplastic cells^{16,17}. Various malignant cells have increased levels of ferritin in their tissues. Increased concentrations of ferritin have been demonstrated in myeloblastic leukaemia. Immunohistochemical study¹⁸ also showed increase of serum ferritin levels in various tumor cells¹⁹. In this study, we attempted to investigate the presence of magnetite in tumor tissues. We report the first detection of magnetite in the various human tumor tissues through the use of SQUID magnetometry. Characterization was carried out from the observed magnetic properties. Whereas, the relation between Fe(+III) distribution and magnetite concentration in tumor tissues were confirmed by Perls staining method.

2 Material and Experimental Procedure

The presence of magnetite in tumor materials and the relationship between its distribution and that of the bulk iron, most commonly present as ferritin, were determined in this study. The concentrations of magnetite in various kinds of tumor tissue samples were estimated from isothermal remanent magnetization (IRM) curves, whereas Perls staining method was used for the relative distribution of Fe(+III) in ferritin. All experiments were conducted in the Caltech magnetically shielded clean room (4.2 x 3.3 x 2.8,

approximate interior field 300 nT)²⁰.

2.1 Material

The 11 pathological specimens from different kinds of human tumors comprising melanoma (5 patients), breast (2 patients), ovary (3 patients), testicle (1 patient), sarcoma (1 patient), meningioma (7 patients), glioblastoma (3 patients), astrocytoma (1 patient), glioma (1 patient), metastasis (1 patient) and a part of the frontal lobe from human brain were obtained. Tumor tissues removed during surgical treatment for cancer were obtained from the pathologists at the City of Hope National Cancer Institute, Duarte California. Human brain samples were obtained from the Alzheimer's Disease Research Center Consortium of Southern California. All tissues were handled through the permission of Institute Review Board (IRB) established by the U.S. National Health Service.

2.1.1 Magnetometry

Samples were prepared from the specimens with suitable size (about 5 ~ 20 g) for the magnetometry. All tissue samples were washed with tripple distilled deionized water, and frozen by dipping directly into liquid nitrogen. Samples less than 5 g were placed into an icecube mold and frozen into an ice block with a small amount of water. Ice cubes of 15 ~ 18g were used as a test of background noise level.

2.1.2 Glassware

Non-magnetic materials such as ceramic knives, glass pipets, teflon forceps, pyrex glass containers, and monofilament string were used. These materials were stored in 2 N HCl for at least 2 days, followed by rising in water and drying. These conditions will solubilize any iron compounds on the surface of the objects, minimizing the possibility of contamination²¹.

2.1.3 Staining

Modified Perls staining method¹¹ was carried out for detecting Fe(III) in the tissue samples. Five fragments from tumor samples (melanoma, ovary, kidney, breast) and frontal lobe (cerebral) were stained with potassium ferrocyanide. The procedure is as follows.

- (1). Slice with about 5 mm thick from each tissue sample.
- (2). Wash with water and incubate for 5 minutes

with 4-5% HCl solution at 37.

(3). Rinse in 4~5 % HCl solution at about 24°C followed by incubating in 4 ~ 5 % HCl solution containing 2% potassium ferrocyanide $K_4[Fe(CN)_6]$.

(4). View and photograph through an optical dissecting microscope.

2.2 Instrumentation

2.2.1 Device

All material were measured using SQUID magnetometer (2-G Enterprises model 760) utilizing radio-frequency (Rf) biasing²⁰. A computer-controlled pulse coil (Caltech), capable of generating a variable, unipolar peak transient magnetic field for IRM acquisition in fields of up to 1.9 T is located on-line with the system, as is a demagnetization solenoid capable of producing sinusoidally-decreasing alternating field (2-G Enterprises) of 200mT.

2.2.2 Measurement

Fig.1 shows the schematic diagram for magnetic measurements of biological samples. Individual tissue samples (frozen) were fastened to thin monofilament string, and the computercontrolled stepping motor moved them vertically between IRM coil, Af coil and Helmholtz-coil pickup loop (measurement region) of the SQUID. First, a sample was demagnetized completely in the peak field (200mT) of the Af coil, then magnetized at 100 mT pulse from the IRM coil. Then a sample was demagnetized (0~200 mT, 25 steps) in Af coil. When the moment of the sample dropped below instrument noise level, the sample was subjected to be magnetized (0~631 mT, 25steps) in IRM coil, progressively. Remanent magnetization moment of a sample was measured after each step for plotting the progressive remanence curve of IRM and Af. During a measurement, the temperature inside of measurement region was kept at around -15°C by cold nitrogen gas flow from the bottom of SQUID magnetometer. Cold nitrogen gas steam was originated by heating liquid nitrogen in a Dewar with a heat resistor.

Ice cube samples were measured as a reference of background noise level before and after each measurement.

In a clean room

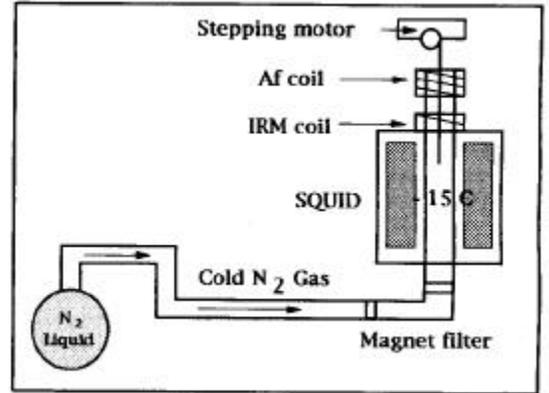


Fig. 1 Schematic diagram for magnetic measurements in the magnetically shielded clean room. IRM: isothermal remanent magnetization. Af of sIRM: the progressive alternating-field demagnetization of the saturation IRM.

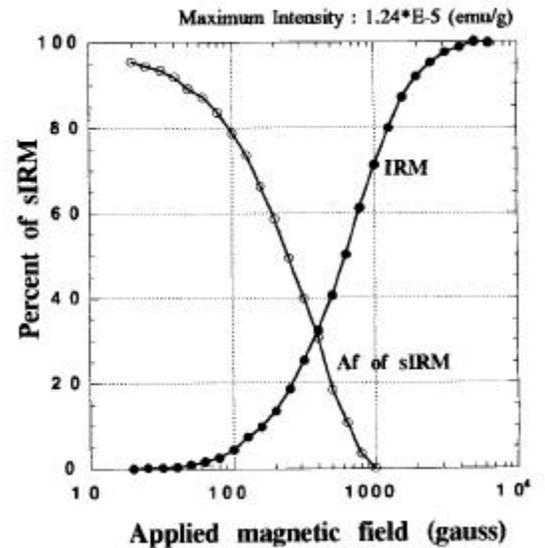


Fig.2 Magnetic property of a sample meningioma tumor tissue.

3 Result

3.1 Distribution of magnetite

Fig.2 and Fig.3 show the remanence curves of IRM and Af of two tumor tissues, meningioma and melanoma. The vertical axis indicates the relative remanence remaining in the samples (Af or IRM/sIRM,%).

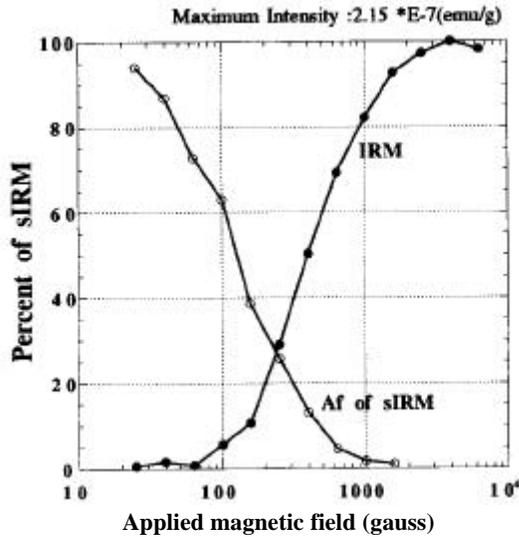


Fig.3. Magnetic property of a sample melanoma tumor tissue.

The horizontal axis is a strength of an applied field (0 ~ 631 mT). The both curves labelled IRM are saturated in applied field before 300 mT. IRM and Af curves become symmetric at the field 25mT (Fig.2) and at the field 40 mT (Fig.3). Both line slopes at the cross point of IRM are not steep.

Fig.4 shows the progressive remanence curve of IRM and Af for ice cube as background noise level. The sIRM per gram of ice cubes are within the range of 0.01~0.04($\mu A \cdot m^2/kg$) in 631 mT, and an average value is 0.03($\mu A \cdot m^2/kg$). These results imply that there are a small amount of ferromagnetic materials in the tumor tissues.

Table 1 shows mean values of sIRM and coercivity values of tissue according to the location of the tumor. All measured tissues reached saturation before 300mT, and the mean coercivity values are between 10 ~ 50 mT. However, data show that sIRM vary with a location of the tumor. The highest sIRM per gram of tumor tissues is 84.52 ($\mu A \cdot m^2/kg$) for glioblastoma and lowest sIRM per gram is 0.015 ($\mu A \cdot m^2/kg$) from breast. The difference sIRM between breast and glioblastoma samples is nearly 6000 times, and an average value is 0.03($\mu A \cdot m^2/kg$). These results imply that there are a small amount of ferromagnetic materials in the tumor tissues.

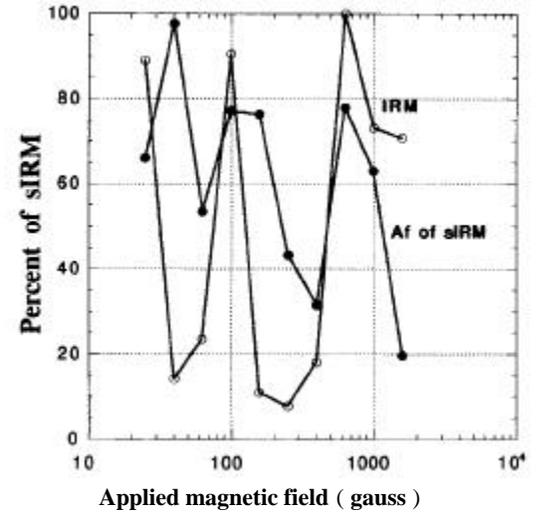


Fig.4 Magnetic property of a sample ice cube as background noise level.

Table 1 shows mean values of sIRM and coercivity values of tissue according to the location of the tumor. All measured tissues reached saturation before 300mT, and the mean coercivity values are between 10 ~ 50 mT. However, data show that sIRM vary with a location of the tumor. The highest sIRM per gram of tumor tissues is 84.52 ($\mu A \cdot m^2/kg$) for glioblastoma and lowest sIRM per gram is 0.015 ($\mu A \cdot m^2/kg$) from breast. The difference sIRM between breast and glioblastoma samples is nearly 6000 times.

3.2 Distribution of Fe(III) - ferritin

Moderated blue stain was noted in all of the samples stained. Fig.5(A) (breast), Fig.5(B) (melanoma), Fig.5(C) (kidney) are the photos which show the ferritin distribution. All showed the presence of Fe(III) as very distinctive blue dot. On the contrary, Fig.5(D) showed that frontal lobe was stained blue more uniformly within the areas.

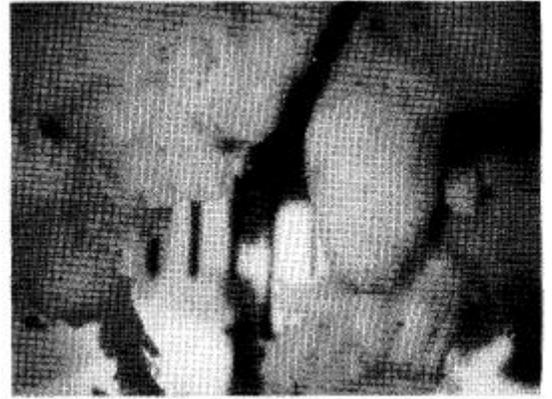
4 Discussion

Iron is the only major transition metal used in human biology that can form ferrimagnetic compounds.

Of the ferrimagnetic iron oxide compounds, beside magnetite, the sulfide minerals have beside magnetite, the sulfide minerals have only reported from bacterial activity.

Table 1 Mean sIRM and coercivity values of tissues according to the location of the tumor.

Location I.D.No.	Weight g	sIRM/weight $\mu A \cdot m^2/kg$	Coercivity mT	No. of subsamples
Malenoma				
6255	12.7	0.98	22	3
3855	12.0	0.22	44	3
4453	8.3	0.09	24	1
3473	18.4	0.38	27	4
Breast				
875	16.5	0.014	28	2
3824	4.1	0.038	24	1
Sarcoma				
4445	24.1	0.037	27	4
Testicle				
5546	21.2	0.11	26	3
Ovary				
5380	16.4	0.042	27	2
Kedney				
4766	19.1	0.23	35	1
Metastasis				
159	0.7	2.12	20	1
Meningioma				
181	0.6	9.74	36	1
182	0.4	2.82	28	1
186	0.64	5.92	28	1
213	1.8	0.49	20	1
224	2.2	5.09	24	1
253	1.3	13.17	40	1
258	1.0	3.23	20	1
Glioma				
254	2.21	0.71	26	1
Glioblastoma				
207	0.5	84.52	15	1
208	1.3	2.25	23	1
209	1.7	3.06	17	1
Astrocytoma				
218	9.79	4.08	20	1
Glioma				
254	2.1	1.52	26	1



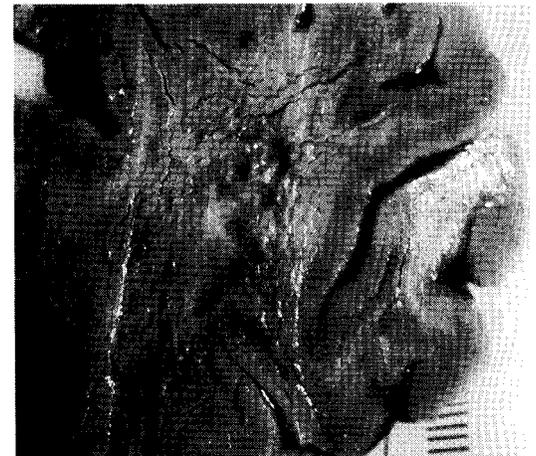
(B) mela noma tumor



(C) kedney tumor



(A) breast tumor



(D) frontal lobe in brain

Fig.5 Photos which show the ferritin distribution by Perls staining. (A) breast tumor

(B)melanoma tumor (C) kidney tumor (D) frontal lobe in brain.

Of the ferrimagnetic iron oxide compounds, beside magnetite, the sulfide minerals have beside magnetite, the sulfide minerals have only reported from bacterial activity. Ferrimagnetic minerals can be distinguished by the lower range of coercivities than the antiferromagnetic ones as the natural distribution²²⁾ and its saturation field.

In the past, SQUID magnetometry study of biological tissues such as magnetotactic bacteria, salmon and human brain suggested that ferrimagnetic material was magnetite. All of the IRM curves were saturated in applied field by 300mT and the cross points of IRM and Af curves, measured of the average coercivity value, were between 10 to 50 mT. Crystals were extracted and identified as a single domain magnetite through the use of EPA, TEM and electron diffraction.

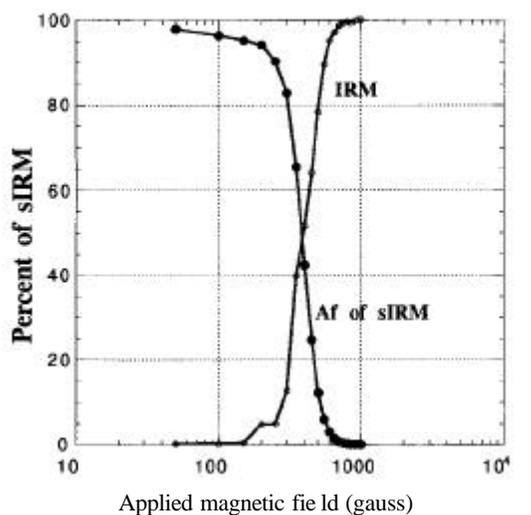


Fig.6 Magnetic property of a sample magnetotactic bacteria.

In this study, ferrimagnetic material in human tumor tissues was detected by SQUID magnetometry. Although we have not shown the extraction and identification of crystals yet, the data are consistent with the presence of single domain magnetite particles²²⁾ in the tumor tissues. The slope of the IRM curve indicate relative size distribution²²⁾. Fig.6 is the progressive remanence curves of IRM and Af of magnetotactic bacteria (strain MS-1, *M.magnetotacticum*) cultured in the Caltech

laboratory. The slope of the IRM is 8.2 (% of sIRM/logarithmic scale of mT). Fig.2 and Fig.3 show that slopes of the IRM curves in tumor tissues are around 3.0 (% of sIRM/logarithmic scale of mT). Table 1 Mean sIRM and coercivity values of tissues according to the location of the tumor. Fig.7 shows the photo of extracted magnetite particles from magnetotactic bacteria whose particle sizes range between 30 ~ 100nm. From these results and previous human studies, the size range of magnetite particle in tumor

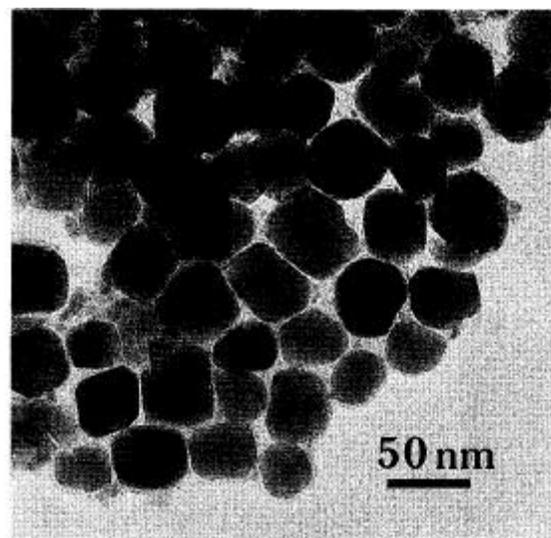


Fig.7 Photo of extracted magnetite particles from magnetotactic bacteria.

tissues seems to be relatively larger than magnetite particles from magnetotactic bacteria and similar to that in human brain tissue.

Perls staining showed the nonheme ferritin iron in tumor tissues. The direct relationship between sIRM values and the location of blue stain could not be found. Examples are Fig.5(A) with relatively large stain areas but a low sIRM value ($0.014 \text{ u A} \cdot \text{m}^2/\text{kg}$), Fig.5(B) with small stain areas but a high sIRM value ($1.31 \text{ u A} \cdot \text{m}^2/\text{kg}$) and Fig.5(C) with small stain areas and a medium sIRM value ($0.23 \text{ u A} \cdot \text{m}^2/\text{kg}$). However, it was observed that ferritin in tumor tissues is distributed randomly as distinctive granular dots. Highest value of sIRM is $84.52 \text{ (u A} \cdot \text{m}^2/\text{kg)}$ in glioblastoma and lowest value of sIRM is $0.015 \text{ (u A} \cdot \text{m}^2/\text{kg)}$ in breast. There is almost 6000

times different in sIRM depending samples, which implies that the presence of magnetic materials might be related to the origin of tumor materials.

We have previously reported that the distribution of ferritin is not correlated to the distribution of magnetite in the human brain; this implies that magnetite does not form spontaneously from ferritin. However, magnetite in tumor tissues might form from ferritin in a biochemically controlled process, as it does in bacteria and chitons. There are reports from an immunohistochemical study that ferritin might be a useful marker in some of testicular tumors¹⁶⁾. Ferritin can be synthesized not only in specific cells, but also in many kinds of neoplastic cells¹⁷⁾. If ferritin plays the role of ontogenesois for malignant cell growths, the increase of serum ferritin levels are associated with several conditions including malignancies and hepatocellular carcinoma^{18,19)}.

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