A STUDY OF MAGNETIC PROPERTIES OF MAGNETOTACTIC BACTERIA

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ABSTRACT The first direct measurements of magnetic properties of magnetotactic bacteria from natural samples are presented. Measurements were made at 4.2 K, using a Superconducting Quantum Interfering Device (SQUID) magnetometer. From the magnetization results an anisotropy is obtained that is typical of magnetized ferro- or ferri-magnetic materials. The average magnetic moment of the bacteria determined from the results is in good agreement with the estimated moment from electron microscopy.

INTRODUCTION

Magnetotactic microorganisms, found in sediments from marine and fresh waters, have recently been the subject of several studies that focused on different aspects of the magnetotaxis (Blakemore, 1975; Frankel and Blakemore, 1980; Towe and Moench, 1981; Esquivel et al., 1983; Frankel et al., 1983; Kirschvink, 1983; Lins de Barros and Esquivel, 1985). These microorganisms show a peculiar behavior: they orient and swim in the direction of a magnetic field. This orientation is caused by the interaction of the microorganism's magnetic moment in a magnetic field, and it is pronounced even in fields of the order of the geomagnetic field. Magnetic properties of magnetotactic bacteria are important for a basic understanding of these microorganisms.

Natural samples have a low concentration of bacteria, making it difficult to measure directly their magnetic moment. Indirect estimates of the moment were made from U-turn analysis or from electron microscopy measurements (Kalmijn, 1981; Esquivel et al., 1983; Lins de Barros and Esquivel, 1985; Esquivel and Lins de Barros, 1986). To be able to measure directly the magnetic moment of the samples we developed a simple magnetic method for sample enrichment without adding impurities; the samples are purified and the concentration of the bacteria is increased magnetically. This technique is suitable for studies of the adaptability of the bacteria to their environment.

Here we present the first direct measurements of the average magnetic moment and the anisotropy in the magnetization of natural samples. The measurements were made with a Superconducting Quantum Interfering Device (SQUID) magnetometer at 4.2 K. The results agree well with estimates from electron microscopy measurements.

TECHNIQUES

Sample Preparation

The samples were collected in a small freshwater river at a depth of ~50 cm. They had a great number of magnetotactic bacteria that swam to the South magnetic pole. After standing in the laboratory for 2 or 3 wk, the population of magnetotactic bacteria in these samples increased significantly, even without any chemical enrichment. The samples were magnetically concentrated using a special vessel which ends in a micropipet (Fig. 1). This procedure permits us to obtain a very pure sample containing a high concentration of magnetotactic bacteria. Their magnetotactic behavior has been checked by optical microscopy. The bacteria have been fixed in glutaraldehyde 2.5% in 0.1 M phosphate buffer. Transmission electron microscopy of the sample assured us that we were observing exclusively only one morphological type of magnetotactic bacterium, which is that shown in Fig. 2. We have counted the number of bacteria using an improved Neubauer chamber (hematometer, Max Levy, Philadelphia, PA) to evaluate their concentration.

Experimental Procedure

Measurements of the magnetization were made using a SQUID magnetometer (model 130; SHE Corp., San Diego, CA) where the sample flux is coupled to the detector by a flux transformer in a gradient configuration. The magnetometer output is connected to a XY plotter and the sample is linearly moved through both flux transformer coils by a device whose output drives the X-axis of the plotter. The SQUID and the flux transformer are mounted in a dewar with liquid helium at 4.2 K. A steady field, H_0, is trapped in a cylindrical lead shield. The maximum H_0 value allowed by the magnetometer coils is ~150 G. For the magnetization M measurements, the samples have been oriented in external magnetic fields, H_{ext}, ranging from 2 G to 6 kG. The oriented samples were frozen to liquid nitrogen temperature by simply dropping them with the sample holder into a liquid nitrogen bath. During this entire process the samples were kept in the external field, H_{ext}. This procedure gave a cooling rate of ~180°/min. The samples were kept frozen at liquid N_2 temperature until they were ready to be placed in the helium dewar. Fig. 3 shows the spherical sample holder whose internal volume is 0.38 cm^3 and its accessories. It is made from an araldite block AV-8 CIBA. A cotton thread (dental thread) passing through the inox tube supports the sample holder. Such a set-up makes it possible to change the orientation of the sample inside the dewar simply by rotating an external pulley. The steady

FIGURE 1 The apparatus, described in the text, to magnetically concentrate samples of magnetotactic microorganisms.

field, H_0, is in the direction of the sample holder axis. The samples are oriented in a magnetic field, H_{ext}, normal to H_0 in the plane of the pulley (Fig. 3).

RESULTS

Figs. 4 *a* and *b* show the magnetic flux variation of samples oriented and frozen in magnetic fields, H_{ext}, of 6 kG and 2 kG, respectively, as a function of its position relative to the flux transformer coils. These signals were obtained in the absence of the magnetic field H_0 and they are related to the

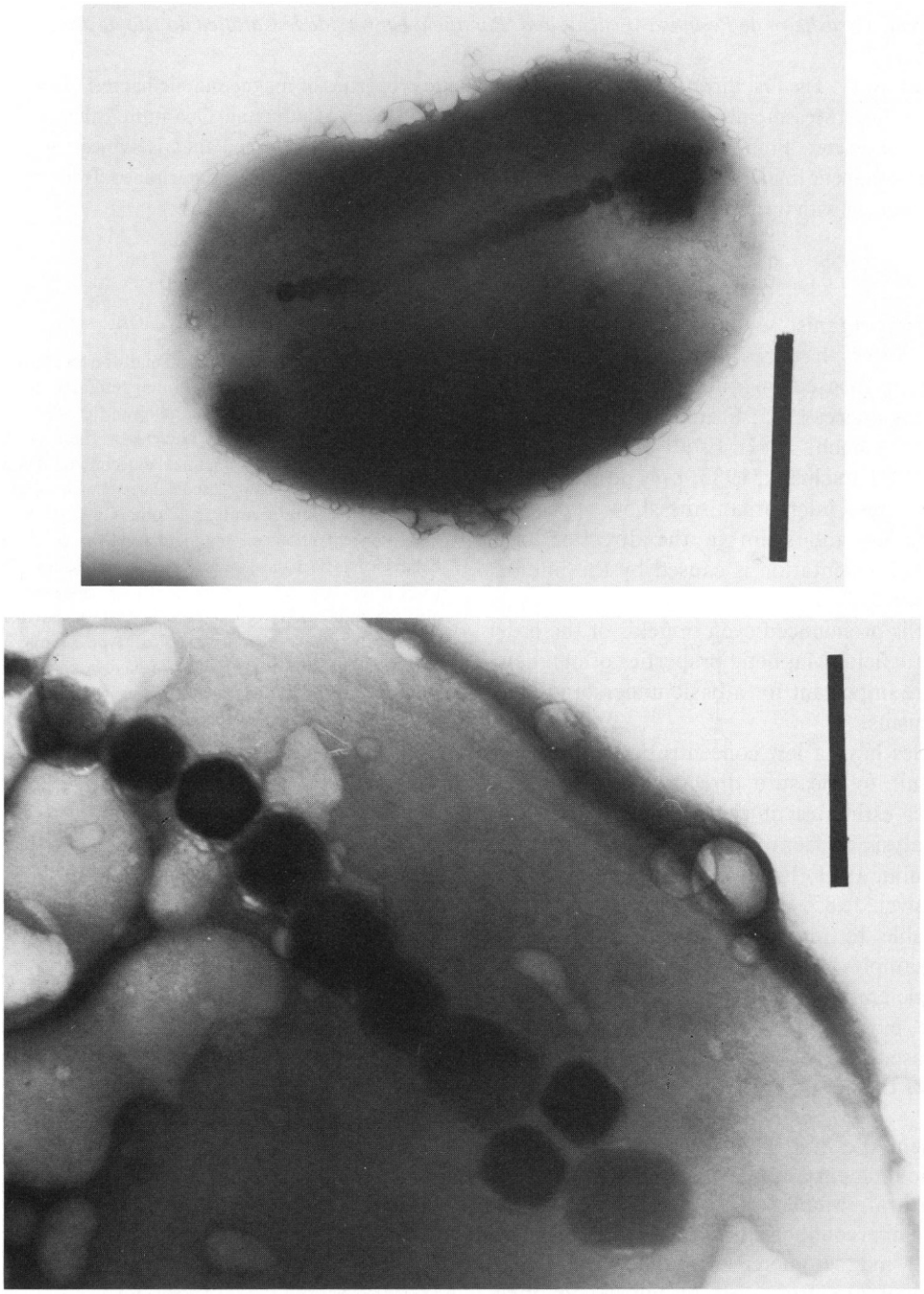

FIGURE 2 (*a*)Electron micrograph of magnetotactic bacteria (transmission E.M.). Bar represents 1 μm. (*b*) Crystal chain detail. Bar represents 2,500 Å.

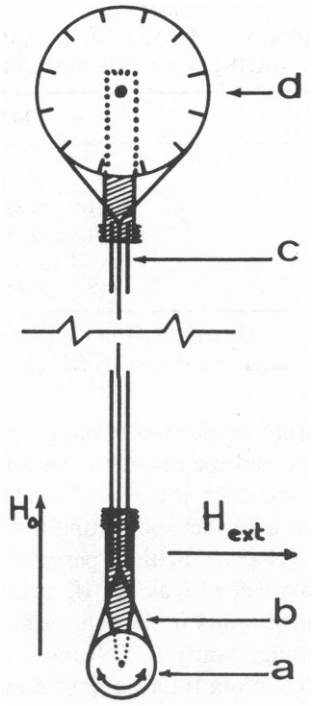

FIGURE 3 Sample holder and accessories, (*a*) spherical sample holder, (*b*) cotton wire, (*c*) inox tube, and (*d*) external pulley. H_0 is the steady field of the magnetometer and H_{ext} is the magnetic field for sample orientation.

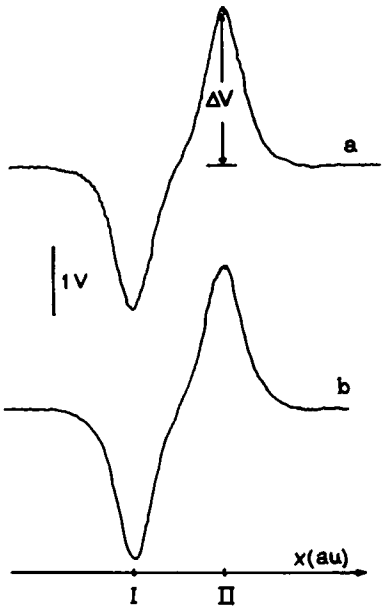

FIGURE 4 SQUID signal at 4.2 K of bacterial suspension frozen to nitrogen liquid temperature. Horizontal axis is related to the sample position in arbitrary unit. I and II are the positions of the two flux transformer coils. Steady field $H_0 = 0$. Sample oriented under a field of (*a*) 6 kG, (*b*) 2 kG.

remanent magnetizations. In a steady field the signals show two contributions: that of the bacteria and that of the sample holder with glutaraldehyde solution. We have found that the glutaraldehyde solution is diamagnetic while the sample holder is slightly paramagnetic, both being isotropic.

The magnetization of the samples, M (in electromagnetic units) has been obtained from the following expression:

$$M = \frac{\phi_0 \Delta V}{20 \times 10^{-3} \cdot f \cdot 4\,\mu A_{eff}}, \qquad (1)$$

where $\phi_0 = 2.07 \times 10^{-7}$ G cm^2 is the magnetic flux quantum, ΔV is the half peak-to-peak signal intensity (see Fig. 4) in volts obtained on the XY recorder and corrected for the background contribution, $f = 1.39 \times 10^{-2}$ is magnetic flux transfer factor characteristic of the instrument used, and $A_{eff} = 0.64$ cm^2 is the effective area of the samples. The sample holder and glutaraldehyde contribution to the susceptibility measurements is $1.8 \pm 0.6 \times 10^{-6}$ emu.

Because the contribution of the cell can be neglected, the bacteria in suspension behave, at room temperature, to a good approximation as a paramagnetic liquid. We assumed that the orientations of the magnetic moments of the sample are not changed during the freezing process and that they have the same average orientation as in the liquid sample. The average orientation of the bacteria in an external field, H_{ext}, is directly related to the Langevin function for paramagnetism, and hence we write

$$M = M(H_{ext}) = M_0 \langle \cos\theta \rangle, \qquad (2a)$$

where $M(H_{ext})$ and M_0 are, respectively, the magnetizations of the sample oriented in a field H_{ext} and of the fully oriented one; $<\cos\theta> = \mathcal{L}(m H_{ext}/kT)$ and $\mathcal{L}(x) = \coth x - 1/x$ is the Langevin function, m is the magnetic moment of a cell, and $kT = 4.1 \times 10^{-14}$ ergs is the thermal energy at 300 K. Eq. 2a assumes, implicitly, that the fluid is composed of noninteracting permanent magnetic dipoles and $<\cos\theta>$ is their average orientation.

We need the magnetization M_0 of the completely oriented sample and its concentration to determine the magnetic moment, m, of a bacterium. Hence we have measured the remanent magnetization ($H_0 = 0$G), of the sample oriented in magnetic fields of 2 kG and 6 kG; we obtained, within experimental error, the same value, $2.0 \pm 0.1 \times 10^{-4}$ emu. We can conclude that for orientation fields of 2 kG and higher the magnetization M of the sample is saturated, i.e., its value is M_0 and the $<\cos\theta>$ is equal to 1 in Eq. 2a. M_0 is related to the magnetic moment, m, of a bacterium by

$$M_0 = c \cdot V \cdot m, \qquad (2b)$$

where c ($= 3.1 \pm 0.7 \times 10^8$ cells/cm^3) is the sample concentration and V ($= 0.38$ cm^3) is the sample volume. We obtained an average magnetic moment of $1.8 \pm 0.4 \times 10^{-12}$ emu for a bacterium.

In anisotropy experiments the sample holder was held at

a position, relative to the transformer coils, that gave the maximum signal. The sample was then rotated by intervals of ~0.48 rad. Typical dependences of the signal on the sample orientation are shown in Fig. 5. These curves were fitted to the expression

$$M = M'' \cos \beta, \tag{3}$$

where β measures the orientation of the sample relative to the direction of the orientation field, H_{ext}.

Table I shows the measured values of M'' for a few fields H_{ext}, with $H_0 = 20$ G. Eq. 3 is associated with a magnetized ferro- or ferri-magnetic behavior, as confirmed by the existence of the remanent magnetization of $H_0 = 0$. We observed that the signal preserves its ferrimagnetic periodicity even when the sample is frozen in a low magnetic field.

The magnetic moment can also be obtained from electron microscopy. The electron microscopy of samples used in the present work shows, even after the freezing procedures, a great number of whole bacteria with a chain of geometrically regular high-density regions (Fig. 1). These regions are analogous to those found in other magnetotactic microorganisms (Frankel and Blakemore, 1980; Towe and Moench, 1981; Esquivel et al., 1983). We can estimate the magnetic moment of a bacterium if we assume that these regions are composed of 80% magnetite (Fe_3O_4) (Towe and Moench, 1981; Blakemore, 1982). The shape of the crystal of the magnetotactic bacteria is presented on the electron micrograph in Fig. 1. The hexagonal shape is the only one observed. The total magnetic moment of a bacterium is thus equal to the product of the volume of each region, the number of regions, and the magnetization per unit volume of magnetite (480 emu/cm^3). The average total magnetic moment obtained by this procedure is $1.3 \pm 0.4 \times 10^{-12}$ emu.

CONCLUSIONS

Here we have presented magnetic properties of samples of magnetotactic bacteria collected directly from the natural habitat. Although there exist data on magnetic moments of such samples from electron microscopy (E.M.) measurements and motion analysis, such data are estimates based on certain hypothesis; SQUID measurements give a direct

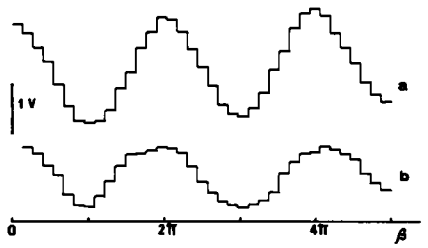

FIGURE 5 Anisotropy curve of the SQUID signal with $H_0 = 20$ G for a sample oriented under a magnetic field of (*a*) 300 G and (*b*) 5 G. The β-angle lies between the directions of magnetization and the external magnetic field.

TABLE I
MAGNETIZATION FOR A SAMPLE OF MAGNETOTACTIC BACTERIA

H_{ext}(G)	M''
	emu
2	$(1.5 \pm 0.2) \times 10^{-5}$
5	$(4.8 \pm 0.9) \times 10^{-5}$
25	$(5.2 \pm 0.4) \times 10^{-5}$
300	$(9.7 \pm 0.2) \times 10^{-5}$

Sample magnetization, M'' (see Eq. 3 in the text), dependence on the external field, H_{ext}, measured with a steady field $H_0 = 20$ G.

result. E.M. estimates depend strongly on assumptions of the crystal shape and the magnetic concentration. Motion analyses are based on a theoretical model that considers the bacterium as a perfect sphere and it does not take into account any flagellar or medium perturbations. The error in the values obtained with a SQUID susceptometer is due mainly to the uncertainty in the concentration counting. In our results this uncertainty is 20% and it can be improved by doing more counting than we have done. The magnetization uncertainty is limited to 5% using a SQUID magnetometer. There is good agreement of the SQUID magnetometer results with the E.M. estimates and this shows that the E.M. estimates provide a good method for obtaining information about magnetic moments of these bacteria. Our measurements show that an average magnetic moment for these natural bacteria is $1.8 \pm 0.4 \times 10^{-12}$ emu.

Because previous studies of cultured magnetotactic bacteria have shown that the conditions of growth alter significantly the magnetic properties of these organisms, such studies cannot provide knowledge about the adaptability and the importance of magnetic orientation effects in nature. Up to now most of the measurements of the average magnetic moment of magnetotactic bacteria grown in culture media were performed using light scattering (Rosenblatt et al., 1982*a*), birefringence (Rosenblatt et al., 1982*b*) and electron microscopy (Frankel and Blakemore, 1980; Frankel, 1984) the results depending on the condition of growth and sample concentration. On the other hand, there were indirect determinations of the magnetic moment of natural samples made by the analysis of motion and by E.M. (Kalmijn, 1981; Esquivel et al., 1983; Lins de Barros and Esquivel, 1983; Esquivel and Lins de Barros, 1986). We have presented here a direct method of studying magnetic properties without all these restrictions.

We conclude that, at 4.2 K, a frozen oriented bacterial suspension with concentration of ~10^8 cells/cm^3 behaves like a ferro- or ferri-magnetic material. If there were a relevant paramagnetic contribution to the magnetization, the anisotropy curve should have a component with the characterisitc period of π, superimposed on the 2π ferrimagnetic curve measured. At room temperature this sus-

pension behaves like a superparamagnetic fluid since each crystal is a single-domain crystal of magnetite and the chain of particles has a permanent dipole moment.

The measured average magnetic moment gives $mB_0/kT \sim 9$ (magnetic to thermal energy ratio) for $B_0 \sim 0.2$ G (local geomagnetic field). This ratio is sufficient for efficient magnetic orientation in the Earth's field (Lins de Barros and Esquivel, 1983; Esquivel and Lins de Barros, 1986). Magnetic concentration together with the use of the SQUID provides a reliable method for determinating the average magnetic moment of small samples of magnetotactic microorganisms collected directly from the environment. This is important for studying adaptability and orientation mechanisms in the natural habitat and for understanding the role of magnetotaxis in the preservation of these organisms.

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