BIREFRINGENCE DETERMINATION OF MAGNETIC MOMENTS OF MAGNETOTACTIC BACTERIA

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ABSTRACT A birefringence technique is used to determine the average magnetic moments $\langle \mu \rangle$ of magnetotactic bacteria in culture. Differences in $\langle \mu \rangle$ are noted between live and dead bacteria, as well as between normal density and high density samples of live bacteria.

INTRODUCTION

Magnetotactic bacteria (1) contain magnetosomes consisting of enveloped Fe_3O_4 particles (2). In the fresh water magnetotactic spirillum *Aquaspirillum magnetotacticum*, the particles are cuboidal, 400–500 Å on a side, and are arranged in a chain fixed in the cell along the axis of motility (3) (Fig. 1). The particles are single magnetic domains and, because interparticle interactions orient the moments parallel along the chain direction, the total magnetic moment per bacterium is equal to the sum of the individual particle moments (4). The average number of particles per bacterium, and hence the average magnetic moment, depends on the conditions of growth; this includes the age of the culture, the number density of bacteria, and the concentration of nutrients and dissolved oxygen (5). We have previously used a light-scattering technique to determine the average length and magnetic moment of bacteria (6). Here we report a magnetically induced birefringence technique for determining the average magnetic moment per cell in cultures of *A. magnetotacticum*. This method is both sensitive and rapid, and the results are unaffected by the presence of nonmagnetic bacteria, dust, or other impurities. Measurements of the average moment per cell of a culture of *A. magnetotacticum* yield results that differ significantly depending on whether the bacteria are living and motile or dead and immotile. This suggests that motility of a bacterium has an additional randomizing effect on its orientation in the field.

METHODS

We assume each bacterium possesses an orientational anisotropy Δp in optical polarizability, with one of the principal polarization components parallel to its long axis, hence parallel to its magnetic moment. An applied magnetic field induces a net birefringence in the sample by orienting the bacteria more or less parallel to the field. The average magnetic dipole moment $\langle \mu \rangle$ per bacterium in the sample can be obtained from a measurement of the field dependence of the birefringence. With the optic axis of the sample parallel to the applied magnetic field, the sample birefringence for noninteracting bacteria is (7)

$$\Delta n = \frac{N \Delta p V}{n_w} \langle P_2(\cos\phi) \rangle, \tag{1}$$

where N is the number density of bacteria, V is the bacterial volume, n_w is the refractive index of the background medium, and P_2 ($\cos\phi$) is the second Legendre polynomial: $P_2(\cos\phi) \equiv (3/2 \cos^2\phi - 1/2)$. ϕ is defined as the angle between the long axis of the bacteria and the optic axis, and the brackets indicate an ensemble average over all bacteria being sampled. For bacteria with magnetic moment μ in a field H at temperature T, the angular distribution function with respect to the field direction is

$$f(\phi, \alpha) = \frac{\alpha \exp[\alpha \cos\phi] \sin\phi}{2 \sinh \alpha} \tag{2}$$

where $\alpha = \mu H / k_B T$ and k_B is Boltzmann's constant. Hence

$$\begin{aligned} \langle P_2(\cos\phi) \rangle &= \int P_2(\cos\phi) f(\phi, \alpha) d\phi \\ &= 1 - \frac{3 \coth\alpha}{\alpha} + \frac{3}{\alpha^2}. \end{aligned} \tag{3}$$

Thus,

$$\Delta n = \Delta n_o \left(1 - \frac{3 \coth\alpha}{\alpha} + \frac{3}{\alpha^2} \right), \tag{4}$$

where $\Delta n_o = N \Delta p V / n_w$.

For $\mu H \ll k_B T$, $\langle P_2(\cos\phi) \rangle \simeq \alpha^2/15$. On the other hand, for $\mu H \gg k_B T$, $\langle P_2(\cos\phi) \rangle \rightarrow 1$. Thus, in the limit of a large α, Δn approaches its saturation value $\Delta n_o = N \Delta p V / n_w$. A measurement of Δn vs. H therefore yields both the average magnetic moment $\langle \mu \rangle$ and the saturated birefringence Δn_o.

EXPERIMENTAL

Measurements were performed with the setup shown in Fig. 2. Light from a HeNe laser (Spectra-Physics Inc., Mountain View, CA Model 124) was attenuated to ~0.3 mW before passing through a pair of crossed

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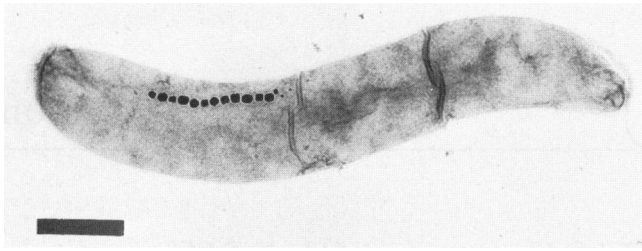

FIGURE 1 Electron micrograph of a typical *A. magnetotacticum*. The black dots are magnetite particles. The number of particles varies with the culture conditions.

Glan-Thomson polarizers (Karl Lambrecht Corp., Chicago, IL), giving an extinction ratio of better than 4×10^{-7}. A set of three orthogonal Helmholtz coil pairs was used to cancel the ambient magnetic field to <0.01 Oe at the sample position, which was located between the two polarizers. A fourth Helmholtz coil pair produced a magnetic field $0 \leq H \leq 40$ Oe perpendicular to the light beam and oriented at 45° with respect to the polarization of the first polarizer. The sample was contained in a quartz cuvette of path length $d = 1$ cm. For a birefringent sample, the light emerging from the sample is elliptically polarized; the phase difference δ_s between the fast and slow components is $\delta_s = k\Delta n d$, where k is the wave-vector of light. Because of the ellipticity produced in the sample, a small amount of light passes through the analyzer and strikes the detector. The intensity I at the detector is related to δ_s by

$$I = I_o \sin^2 (\delta_s/2). \quad (5)$$

I_o is the maximum intensity, obtained when $\delta_s = \pi$ and the light emerging from the sample is linearly polarized but rotated parallel to the analyzer. Due to the finite extinction ratio of the polarizers, the resolution limit for δ_s is $\sim 10^{-3}$; for $d = 1$ cm and $k = 10^5$ cm^{-1} ($\lambda = 6328$ Å), this translates into a resolution limit for Δn of 10^{-8}.

Improved resolution was obtained by placing a Pockel's cell (Lasermetrics Model LMA-4), which produces a voltage-dependent phase shift δ_p, between the sample and analyzer. When the cell's optic axis is oriented orthogonal to both the beam direction and magnetic field, the total phase shift $\delta_{tot} = \delta_s - \delta_p$. Substituting δ_{tot} for δ_s in Eq. 5 and approximating Eq. 5 for small values of δ_{tot}, one obtains

$$I = 1/4\, I_o (\delta_s - \delta_p)^2. \quad (6)$$

If one now modulates δ_p by applying a voltage $V = V_o + V' \sin \nu t$ to the Pockel's cell, a phase shift $\delta_p = \delta_{po} + \delta'_p \sin \nu t$ results, where δ_{po} is the DC shift proportional to V_o, and δ'_p is the amplitude of the AC shift proportional to V'. The signal at the detector is then

$$I = 1/4\, I_o [(\delta_s - \delta_{po})^2 - 2(\delta_s - \delta_{po})\delta'_p \sin \nu t + \delta'^2_p \sin^2 \nu t]. \quad (7)$$

The first term in the brackets is DC and the last term is the sum of a DC component and an AC component at frequency 2ν. Thus the amplitude of the intensity component varying at frequency ν is

$$I_\nu = -1/2\, I_o(\delta_s - \delta_{po})\delta'_p. \quad (8)$$

Because the desired phase shift δ_s is linearly related to I_ν, it can generally be determined with higher resolution than from Eq. 5, where the relation is quadratic.

In the experiment, a lock-in amplifier (Princeton Applied Research Corp., Princeton, NJ, model 5101) was used to measure I_ν. The modulation frequency ν and modulation voltage V' were set at 2,000 Hz and 67 V, respectively. To calibrate the apparatus V_o was first set at 121 V with H = 0 ($\delta_s = 0$); because $\delta_p = (8.3 \times 10^{-4}) \times V$ for our cell, this resulted in $\delta_{po} = 0.1$, producing a measured calibration intensity I^o_ν. V_o was subsequently set equal to zero and I_ν was measured as a function of H. Thus, from Eq. 8

$$\delta_s(H) = \frac{0.1\, I_\nu(H)}{I^o_\nu}. \quad (9)$$

With this procedure, δ_s could be resolved to better than 10^{-4}, giving resolution in Δn to better than 10^{-9}. In typical experiments however, vibrations and thermal drift limited resolution of δ_s to $\sim 1.5 \times 10^{-4}$.

Δn vs. H was measured four times on each of two samples S1 and S2 taken from a single culture (Table 1). S1 was measured with the normal bacterial density present in the culture ($10^7 - 10^8$/cm^3); S2 was centrifuged prior to measurement, increasing the density an order of magnitude. The first two measurements (La and Lb) on each sample were made with the bacteria live. A few drops of formalin were then added to the cuvette, killing the bacteria without changing their shape. The third and fourth measurements (Da and Db) on each sample were subsequently made on dead bacteria.

Measurements of Δn were made for a series of fields between 0.39 and 26 Oe. A two parameter least squares fit to Eq. 4 was performed for each run. A typical run is shown in Fig. 3; values of $<\mu>$ and Δn_o obtained for each run are presented in Table I.

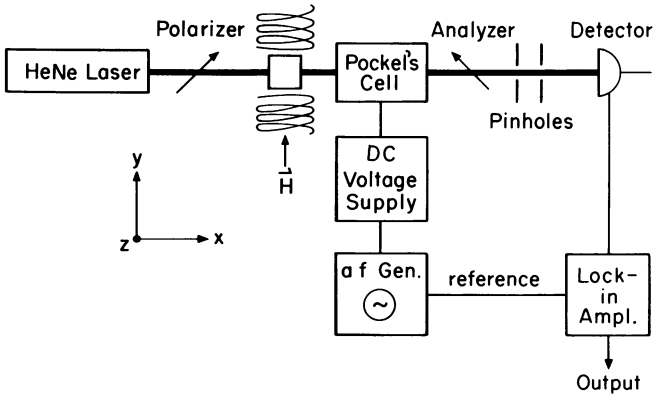

FIGURE 2 Schematic representation of apparatus seen from above. The field H is applied along the y axis and the fast (optic) axis of the Pockel's cell is along z. The polarizer is oriented along $2^{-1/2}(y + z)$ and the analyzer along $2^{-1/2}(y - z)$. The pinholes prevent spurious light from entering the detector.

DISCUSSION

The fits of Eq. 4 to the data are satisfactory, and the reliability of the method is evidenced by the agreement of

TABLE I

Sample	$\Delta n_0 (10^{-8})$	$\mu (10^{-13}$ emu)	Δn rms deviation (10^{-8})
1La	4.29	1.23	0.04
1Lb	4.46	1.19	0.06
1Da	3.89	1.37	0.05
1Db	3.91	1.29	0.06
2La	40.6	0.97	0.4
2Lb	37.5	0.97	0.4
2Da	31.3	1.11	0.7
2Db	31.2	1.34	0.7

Δn_0, μ, and rms deviation in Δn of least-squares fit to Eq. 4 for eight series of measurements. Sample 1 is at normal concentration and Sample 2 is dense. L and D represent live and dead bacteria respectively. The indices a and b refer to the particular run. Because the saturation magnetization of magnetite is 480 emu/cm^3, a typical moment of 1.25×10^{-13} emu corresponds to 2.6×10^{-16} cm^3 of magnetite or per bacterium, or four particles of dimension 400 Å.

the parameters obtained from the duplicate runs for each sample. The errors indicated in Table I and error bar (*b*) in Fig. 3 are root-mean-squared (rms) deviations in Δn from the least-squares fit to Eq. 4. Additional, and potentially larger errors arise from thermal and vibrational drifts in the system, particularly in the Pockel's cell. These drifts occur over a long time period and could result in a systematic error in Δn_0 and $\langle \mu \rangle$ for a particular series of measurements for a given sample. In our experiments, possible drift in the Pockel's cell was checked by determining I_ν° at the end of a series as well as at the beginning. If the results differed by >3% of I_ν(max), the series was discarded. Error bar (*a*) in Fig. 3 reflects drift during the particular run.

Examination of Table I shows that Δn_0 is higher for the concentrated samples, as expected from Eq. 4. Moreover, dilution resulting from the addition of formalin results in an expected lower Δn_0 for both *D* samples relative to the L samples. It appears, however, that $\langle \mu \rangle$ is systematically lower for the L samples than the D samples, where we estimate the error in $\langle \mu \rangle$ to be ±4%. Because microscopic examination shows that formalin killed cells have the same morphology as live cells, we can assume that this difference does not result from structural differences in the chain of particles. Rather, we assume that the motile activities of the live bacteria tend to disorient them relative to the applied field. This system must rigorously be treated in terms of nonequilibrium statistical mechanics; however, the effect of motility on orientation can be approximated by using an effective temperature T_{eff} in Eq. 3, where T_{eff} is 10–20% greater than the thermodynamic temperature T. The temperature T of the medium is virtually unchanged by the motility, either locally or in the sample as a whole.

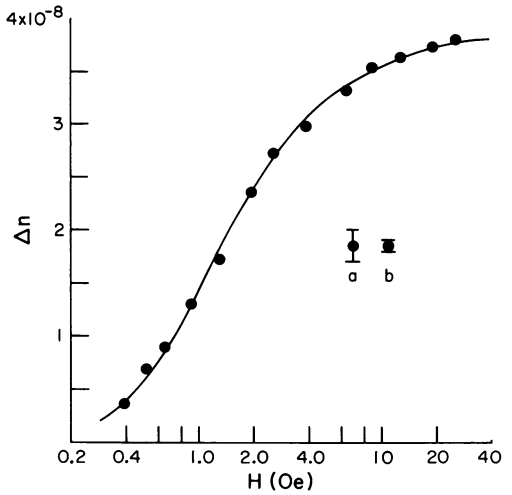

FIGURE 3 Typical data taken from sample 1Da (see Table 1). Curve is a least-squares fit to Eq. 4. Error bar (*a*) arises from a drift in the Pockel's cell and is discussed in the text. Error bar (*b*) is the rms deviation in Δn obtained from the fit to Eq. 4 and is given in Table I.

Another feature of the data is that the live bacteria in sample 2L possess a smaller effective magnetic moment than do those in sample 1L, whereas the moments of their dead counterparts (samples 1D and 2D) are roughly equivalent. Because the concentration in sample 2L is considerably higher than sample 1L, we might expect that the difference in average moment is due to interaction effects between bacteria. Because the moments of dead bacteria are independent of concentration, magnetic interactions can be ruled out. Instead we propose a hydrodynamic effect: the swimming of the bacteria may affect the motions and orientations of nearby bacteria in some way having the effect of further increasing the effective temperature. At lower density these interactions are weaker and the average magnetic moment is higher. This conjecture is presently under further investigation.

CONCLUSION

Measurements of magnetically-induced birefringence provide a rapid and reliable means of determining average magnetic moments in samples of magnetotactic bacteria. The results obtained are comparable to light-scattering determinations and estimates made from electron micrographs (6). The advantage of the birefringence method is that it is insensitive to the presence of nonmagnetic organisms, dust and other suspended particles that would contribute to light scattering.

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REFERENCES

1. Blakemore, R. P. 1975. Magnetotactic bacteria. *Science* (*Wash. D.C.*). 190:377–379.
2. Frankel, R. B., R. P. Blakemore, and R. S. Wolfe. 1979. Magnetite in freshwater magnetotactic bacteria. *Science* (*Wash. D.C.*). 203:1355–1356.
3. Balkwill, D. L., D. Maratea, and R. P. Blakemore, 1980, Ultrastructure of a magnetotactic spirillum. *J. Bacteriol.* 141:1399–1408.
4. Frankel, R. B., and R. P. Blakemore, 1980, Navigational Compass in Magnetic Bacteria. *J. Magn. Mag. Mat.* 15–18:1562–1564.
5. Blakemore, R. P., D. Maratea, and R. S. Wolfe. 1979. Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. *J. Bacteriol.* 140:720–729.
6. Rosenblatt, C., F. F. Torres de Araujo, and R. B. Frankel. 1982. Light scattering determination of magnetic moments of magnetotactic bacteria. *J. Appl. Phys.* 53:2727.
7. Scholten, P.C. 1975. Magnetic measurements on particles in suspension. *IEEE Trans. Magn.* 11:1400–1402.