GEOMETRICAL ARRANGEMENT OF MAGNETOSOMES IN MAGNETOTACTIC BACTERIA

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Introduction

In 1975, Blakemore[1] discovered the freshwater magnetotactic bacterium *Aquaspirillum magnetotacticum* which navigates along the magnetic-field direction. Electron microscopic work showed that the magnetotactic bacteria contain magnetosomes which are intracytoplasmic membrane-bound particles of magnetite, Fe_3O_4. The magnetosomes are within the single-domain size range ($\sim$500 Å) of Fe_3O_4. The magnetosomes within cells are often arranged in one or more chains with the chain axis more or less parallel to the axis of motility of the cell. A detailed study of the magnetic properties of magnetotactic bacteria can be found in the paper by Moskowitz.[2]

In this paper, we address the question of the arrangement of magnetosomes, with the intention of shedding light on the mechanism and sequence of the formation of magnetosomes. In a single chain of magnetosomes (Figure 1), all the magnetic moments are parallel to each other along the chain direction. The chain of magnetosomes thus has a permanent magnetic dipole moment equal to the moment of a magnetosome times the number of particles in the chain. The light scattering and birefringence experiments by Rosenblatt et al.[3] confirmed that this was the case, and the resultant average magnetic dipole moment per cell was consistent with estimates based on the number of particles per cell from electron micrographs.

However, there have been many observations that multiple chains exist and the magnetic dipole moment configuration in this case is less clear. The goal of this study was to determine the possible magnetic moment configuration of multiple chains of magnetosomes. We adopted the chain of spheres model of Jacobs and Bean[4] and calculated the magnetic energies of various magnetic moment configurations for each arrangement of magnetosomes. The lowest energy configuration was obtained for each arrangement. It was found that for the square-lattice arrangement of magnetosomes, the two chains would have opposite magnetic moments in order to stabilize this arrangement. However, for the close-packed arrangement of magnetosomes, the corresponding stable configuration would be that the magnetic moments of the two chains have the same orientation. For the purpose of navigating along a magnetic field, the bacteria would prefer the close-packed arrangement to maximize the net magnetic moment. This is indeed the case. Transmission electron microscopy (TEM) micrographs showed mostly close-packed arrangements for double chains (Figure 2). Furthermore, by comparing the energy gain of adding a particle at the end to adding one on the side of a chain, we found that it is always energetically favored to add a particle at the end of a chain. Therefore, from the energy minimization point of view, multiple chains are formed by combining existing single chains rather than by adding new particles one by one on the side of a single chain.

Chain of Spheres Model

The chain of spheres model of Jacobs and Bean[4] can be used to describe the arrangement of magnetosomes. The interaction between two magnetic dipoles is

$$E = \frac{\mu_i \mu_j}{r_{ij}^3}[\cos(\theta_i - \theta_j) - 3\cos\theta_i \cos\theta_j] \ , \qquad (1)$$

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where θ_i is the angle between the magnetic moment of particle i and the line connecting the centers of the two particles, and μ is the magnetic moment of a particle. For a chain of N particles, there are many possible magnetic moment configurations. The simplest configuration is parallel rotation, in which all the particles have moments pointing in the same direction with an angle θ with respect to the chain axis. In this case, the magnetic energy for a chain of N particles is

$$E_N = \frac{\mu^2}{a^3}[1 - 3\cos^2\theta] \sum_{j=1}^{N} \frac{N-j}{j^3}, \tag{2}$$

where a is the diameter of a sphere. Since all magnetic energy has a unit of μ^2/a^3, we will omit that notation for convenience from here on in this paper. At the limit of $N \to \infty$ and $\theta = 0^0$, we obtained the energy per particle $E_0 = -2.404$. This energy E_0 is used as a criterion for determining whether a particular configuration of magnetic moment in multiple chains is stable or not. If the energy per particle of a particular configuration is larger than E_0, then this configuration is considered not stable and the multiple chains will eventually separate.

Double Chains

The simplest multiple chain is a double chain. Two possible particle arrangements are considered: square-lattice and close-packed. An arrangement is defined as the pattern of the centers of all the particles. For simplicity, we considered the situation $N \to \infty$ first and then addressed the effect of finite N later.

For each arrangement, we minimize the interaction energy with respect to the angle θ and find the lowest energy configuration for each arrangement. We considered the parallel rotation configuration first. In the square-lattice arrangement, the magnetic moments in the two chains are anti-parallel, and the magnetic energy per particle due to dipole interaction is

$$E_{ps} = (1 - 3\cos^2\theta)\sum_{j=1}^{\infty} \frac{1}{j^3} + \frac{1}{2}(-1 + 3\sin^2\theta)$$

$$+ \sum_{j=1}^{\infty}\{-1 + \frac{3}{1+j^2}(\sin^2\theta + j^2\cos^2\theta)\}\frac{1}{(1+j^2)^{3/2}}. \tag{3}$$

Minimizing equation (3) with respect to θ, we obtained $\theta = 0^0$ and the energy $E_{ps} = -2.477$. Although the anti-parallel configuration is a stable configuration ($E_{ps} < E_0$), there is no net magnetic moment. The expression for a parallel configuration is similar to equation (3) except the signs change starting at the second term on the right-hand side. The parallel configuration has a net magnetic moment, but the energy, $E = -2.331$, is higher than E_0 meaning there is a repulsion between the two chains and the configuration is not stable. Therefore, the square-lattice arrangement can be stable but has no net magnetic moment.

In contrast, in the close-packed arrangement, the anti-parallel configuration has a magnetic energy per particle

$$E_{pc} = (1 - 3\cos^2\theta)\sum_{j=1}^{\infty} \frac{1}{j^3}$$

$$+ \sum_{j=0}^{\infty}\{-1 + \frac{3}{1+j+j^2}(\frac{3}{4}\sin^2\theta + (\frac{1}{2}+j)^2\cos^2\theta)\}\frac{1}{(1+j+j^2)^{3/2}}. \tag{4}$$

The minimum energy occurs at $\theta = 0^0$ and $E_{pc} = -2.226$. For the parallel configuration, the second term on the right-hand side of equation (4) changes sign, and $E_{pc} = -2.582$. Therefore, the parallel configuration is stable and at the same time has a finite net magnetic moment. The close-packed arrangement would be expected in TEM micrographs of double chains.

The other important magnetic dipole moment configuration is fanning, in which the magnetic moments of successive spheres in the chain fan out by rotating in alternate directions in alternate spheres. For a chain of spheres in the fanning configuration, the magnetic energy per particle is

$$E_f = (\cos 2\theta - 3\cos^2\theta)L_\infty + (1 - 3\cos^2\theta)M_\infty \,, \tag{5}$$

where

$$L_\infty = \sum_{j=1}^{\infty} \frac{1}{(2j-1)^3} \,, M_\infty = \sum_{j=1}^{\infty} \frac{1}{(2j)^3} \,.$$

The lowest energy state for a single chain in the fanning configuration is $\theta = 0^0$ and $E_{f0} = E_0$.

For double chains, in the square-lattice arrangement and anti-parallel fanning configuration, the resultant energy per particle is

$$E_{fs} = E_f + \frac{1}{2}(-1 + 3\sin^2\theta) + \sum_{j=1}^{\infty}\{-1 + \frac{3}{1+4j^2}(\sin^2\theta + 4j^2\cos^2\theta)\}\frac{1}{(1+4j^2)^{3/2}}$$

$$+ \sum_{j=1}^{\infty}\{-\cos 2\theta - \frac{3}{1+(2j-1)^2}(\sin^2\theta - (2j-1)^2\cos^2\theta)\}\frac{1}{(1+(2j-1)^2)^{3/2}} \,. \tag{6}$$

The lowest energy occurs at $\theta = 0^0$ and $E_{fs} = -2.477 = E_{ps}$. In the close-packed arrangement, we consider the parallel configuration only. The magnetic energy per particle in this case is

$$E_{fc} = E_f + \frac{1}{2}\sum_{j=0}^{\infty}\{1 - \frac{3}{1+j+j^2}[\frac{3}{4}\sin^2\theta + (1/2+j)^2\cos^2\theta]\}\frac{1}{(1+j+j^2)^{3/2}}$$

$$+ \frac{1}{2}\sum_{j=0}^{\infty}\{\cos 2\theta + \frac{3}{1+j+j^2}(\frac{3}{4}\sin^2\theta - (1/2+j)^2\cos^2\theta)\}\frac{1}{(1+j+j^2)^{3/2}} \,. \tag{7}$$

The lowest energy occurs at $\theta = 0^0$, and $E_{fc} = -2.582 = E_{pc}$.

Although the fanning configuration does not produce any advantage in terms of energy and the results are the same as that of the parallel rotation configuration, there is a significant difference in the excitations of the energy minimum. The difference in excitations can most easily be seen by comparing the energy for a single chain. For a single chain in the parallel rotation configuration, equation (2) gives $E_p = -2.404 + 3.606\theta^2$ for small θ. Whereas in the fanning configuration, equation (5) gives $E_f = -2.404 + 1.503\theta^2$ for small θ. Therefore, if there is any excitation to a single chain, it will be in the fanning configuration rather than the parallel rotation configuration. Similar result can be obtained for double chains as well.

Formation of Double Chains

The question of where to add a new particle to an existing chain was also considered. This question was studied in the close-packed arrangement only since the square-lattice

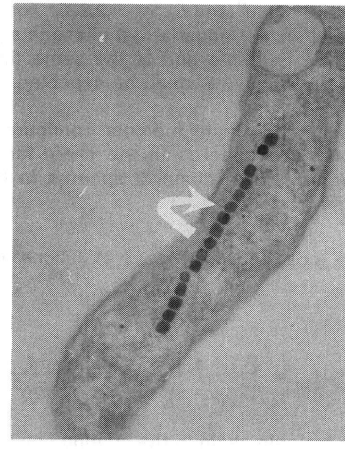

Figure 1. TEM image of an *Aquaspirillum magnetotacticum*, displaying a single chain containing a row of particles.

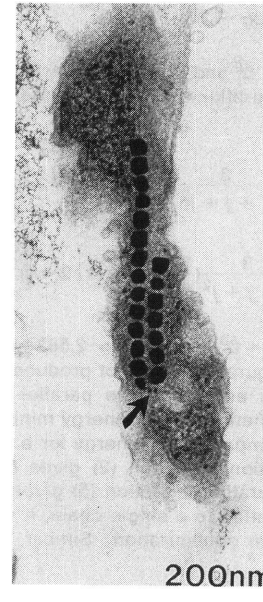

Figure 2. Double-chain formation of magnetosomes in a bacterium.

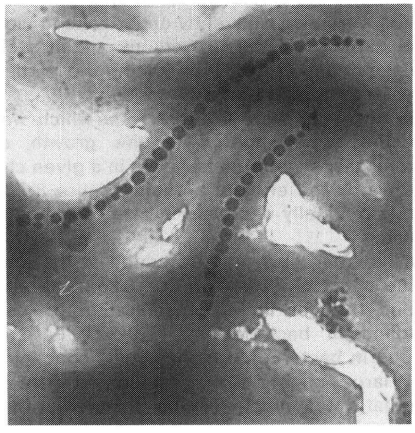

Figure 3. Smaller magnetosomes can be discerned towards the ends of a chain in this image of a bacterium.

arrangement is energetically unfavorable. The potential energy at the end of a chain of N particles is

$$\Delta_e = (1 - 3\cos^2\theta)(\sum_{j=1}^{N-1} \frac{1}{j^3}) \ . \tag{8}$$

On the other hand, the potential energy on the side of a chain Δ_s depends on where the particle is located on the side. If N is even, and the particle sits at the middle on the side,

$$\Delta_s = \sum_{j=0}^{j<N/2} \{2 - \frac{6}{(1+j+j^2)}(\frac{3}{4}\sin^2\theta + (1/2+j)^2\cos^2\theta)\} \frac{1}{(1+j+j^2)^{3/2}} \ . \tag{9}$$

The minimum occurs at $\theta = 0^0$ for both equations (8) and (9). When $N \to \infty$, $\Delta_e = -2.38$ and $\Delta_s = -0.356$, and $\Delta_e < \Delta_s$. In fact, for all N values, $\Delta_e < \Delta_s$. Therefore, from the energy minimization point of view, a new particle is always added to the end of a chain rather than on the side. This result indicates that double chains are formed by combining two existing single chains rather than by adding new particles to the side of a chain. If two chains of magnetosomes have the same number of particles N, there exists a critical N_c such that for $N > N_c$, the two chains will combine, and for $N < N_c$, the two chains will separate. In the close-packed arrangement, using equation (4) and letting the limit of summation be finite, we found $N_c = 5$.

Transmission Electron Microscopy Observations

Analysis of the geometric arrangement of magnetosomes in *Aquaspirillum magnetotacticum* was done by TEM observations of samples that were prepared either by embedding and ultramicrotoming or by separately suspending on carbon films. In Figure 1, a row of magnetosomes, seen in a bacterium, are fairly uniform and form a single straight chain. We found that the particles along a chain are not in a single zone axis orientation but that there is a relative change of crystal orientation in each of the particles in the chain.

So far, no consistent rocking of the crystallographic axis with respect to the chain axis has been found. Double chains were also frequently observed and Figure 2 exhibits an example. It can be noted that the particles in the two chains are in a close-packed arrangement, although, in this particular projection, the lines joining the particle centers appear to have an alternation of 50° and 70° angles between them.

Most of the samples observed were from bacteria which were incubated 4 to 5 days, which corresponds to the upper portion of the growth curves for *Aquaspirillum magnetotacticum*.[5] Not in all cases were the particles in a given chain the same size; particle size varied, especially towards the ends of a chain (Figure 3). As shown in Figure 3, the particles towards the ends gradually decrease in size. This indicates that "new" particles form at the ends of chains.

Other evidence that new particles may be added at the ends of an existing chain is shown in the study of the effect of ferric iron Fe^{+3} concentration on the magnetosomes' production.[5] It was found that for Fe^{+3} concentration of less than 20 μM, very few magnetosomes were found in bacteria. At ~20 μM Fe^{+3} concentration, 10 to 25 magnetosomes were found in bacteria. At higher Fe^{+3} concentrations, ~200 μM, multiple chains and chains with a large number of magnetosomes were observed in bacteria. This result supports the idea that a new magnetosome is added at the end of an existing chain.

Summary and Discussion

It was found that for the square-lattice arrangement of double chains of magnetosomes, the two chains have opposite magnetic moments in order to stabilize this arrangement. However, for the close-packed arrangement of magnetosomes, the stable state is such that the two chains have magnetic moments in the same orientation. For the purpose of navigating along a magnetic field, the bacteria prefer the close-packed arrangement to maximize the net magnetic moment. TEM micrographs show mostly close-packed arrangements for double chains. Furthermore, we considered the question of how the double chains are formed. By comparing the potential energies of a particle sitting at the end and on the side of a chain, we found that a new particle is always energetically favored to be added at the end of a chain. This indicates that double chains are formed by combining two existing chains rather than adding new particles on the side of a chain. For two chains of an equal number of particles N, there exists a critical number N_c such that for $N > N_c$, two chains will combine. Whereas if $N < N_c$, two chains will repel each other. Numerically, we found $N_c = 5$ in the case of a close-packed arrangement.

The present paper has considered only the energetics of various configurations in certain arrangements of magnetosomes so far, but the question of possible thermal fluctuations should be addressed as well. The thermal energy available at room temperature is $kT \sim 4 \times 10^{-14}$ ergs. Whereas the energy scale of magnetic energy is μ^2/a^3 which is $\sim 0.3 \times 10^{-10}$ ergs for $\mu = 6 \times 10^{-14}$ emu and $a = 500$ Å. Therefore the thermal energy is on the order of 1/1000 of the magnetic energy and can be neglected. Even if the separation between magnetosomes were taken into account, the thermal energy is still small compared to the magnetic energy and can be neglected.

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