Biogenic Magnetite in Higher Organisms and the Current Status of the Hypothesis of Ferrimagnetic Magnetoreception

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1. Abstract

Crystals of biogenic magnetite are now known in five phyla ranging from bacteria through chordates. These ferrimagnetic particles are precipitated in a diverse variety of sizes and shapes, and range in their magnetic properties from super-paramagnetic to single-domain and multi-domain. In pelagic fish, particles of single-domain magnetite are arranged in linear chains in tissue within the dermehnoid bone at the front of the snout, and afferent nerves ramify in close proximity to the tissue. Several tests of the ferrimagnetic hypothesis are discussed.

2. Introduction

It is fitting for this volume to contain in-depth discussions of the current status of the hypothesis of magnetite-based magnetoreception in higher organisms. Magnetite (Fe₃O₄) is at present the only known mineral that has ferromagnetic properties and is precipitated directly by organisms [1], and it is clearly responsible for the magnetotactic behavior of numerous micro-organisms [2,3]. This hypothesis of magnetoreception is at present the only one for which there are concrete examples in nature, and from which direct inferences to higher organisms can be made.

In this chapter, we will review briefly the new information which has been gained during the past five years, largely through the development of new, ultrasensitive analytical techniques and by the use of existing procedures in new ways. In parallel with this, physical constraints on the operation of this system are on magnetite biomineralization and magnetoreception [4].

3. Biogenic Magnetite in Animal Tissue

The most significant discovery with regard to the ferrimagnetic hypothesis of magnetoreception was made by Lowenstam [5] when he recognized that the teeth of polyplacophoran molluscs (the chitons) were mineralized with magnetite. Subsequent work by Lowenstam and his associates [1,6-10] has shown clearly that this mineral is a true biochemical precipitate (Fig. 1).

The discovery of geomagnetic sensitivity in honeybees, homing pigeons, and bacteria in the early 1970s [11-14] coupled with the work of Lowenstam cited earlier led directly to the hypothesis that biogenic magnetite might be responsible [15-17]. An important aspect of this work was the introduction of the moment magnetometers using

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Superconducting Quantum Interference Devices (SQUIDs) which had been developed for the study of rock magnetism; these devices permit the detection of subnanogram quantities of magnetite in tissue samples, and results from these instruments led quickly to the discovery of biogenic magnetite in the bees and birds [15,18].

These initial results quickly led to a flurry of reports of ferromagnetic material in animal tissues (summarized in [4]). Unfortunately, few attempts were made in many of the earlier works to characterize the material precisely, and many authors were not careful in distinguishing true biogenic particles from environmental contaminants. For reasons discussed below, it is our opinion that many of these studies are suggestive but not necessarily relevant to the problem of how higher organisms perceive the magnetic field. Whatever their source, however, the possible presence of ferrimagnetic material in tissues must be considered when evaluating the effects of steady magnetic fields on living systems.

Our discussion of biogenic ferrimagnetism will be divided into three phases based on the magnetic properties of the particles, including, in order, those of superparamagnetic, multi-domain, and single-domain size (Fig. 2).

Superparamagnetism. Superparamagnetic particles of magnetite are those which fall in the lower portion of Fig. 2 (e.g., particles less than about 40 nm in size). Although these particles are uniformly magnetized, the direction of this spontaneous magnetic moment is not stable relative to the crystal axes, and any small magnetic or thermal fluctuation will cause the moment to wander in response. It is therefore impossible to put a magneto-mechanical torque on these particles, and it is consequently highly unlikely that they could be coupled directly with a mechanoreceptor to form the basis of a magnetoreceptor (several alternative possibilities are suggested
Magnetic stability diagram for rectangular parallelepipeds of magnetite (after [50]). Solid lines represent the theoretically and experimentally determined boundaries between multi-domain, single-domain, and superparamagnetic crystal sizes, with the approximate size and shape ranges for observed biogenic magnetites superimposed. The three dotted lines crossing the single-domain and superparamagnetic areas represent contours of equal grain volume and hence constant magnetic moment, \( \mu \). Each curve is scaled such that the ratio \( \mu B/kT \) (\( -\Gamma \)) is respectively 0.1, 1, and 10 at 300 K in the 50 \( \mu T \) geomagnetic field.

Superparamagnetic grains in a worker bee. The relaxation time for a magnetic domain increases exponentially as the temperature is lowered. Therefore, some grains which are superparamagnetic at room temperature will be single-domain when cooled to 77 K. Conversely, any remanent remanence held by these particles at low temperature will disappear as they warm in field-free space. The temperature at which the remanence is lost indicates the grain size responsible. Curve 1 shows the remanence of one bee as it warmed up inside the field-free space within a SQUID magnetometer. About 200 million particles 32-56 nm in diameter are required to account for the remanence lost upon warming. Curves 2 and 3 show subsequent warming cycles without remagnetization. From [17].

Honeybees are at present the only organisms known to possess material of this type (Fig. 3), and at the time of discovery it was thought that these particles might play a role in the bees' magnetic sensitivity [19]. We now feel, however, that a single-domain receptor offers a better explanation for the honeybee compass receptor due to the consistency of the accuracy of the dance with the Langevin function [20]. The function of the superparamagnetic particles thus remains a mystery.

Multi-domains. Multi-domain particles are those which plot in size and shape in the upper-right portion of Fig. 2 (sizes generally
> 0.1 μm). We are on quite firm ground when we suggest that these particles are not important for magnetoreception. Particles of this type will spontaneously form internal domains, each of which has atomic moments aligned in randomly-varying directions (e.g., [21]). The effect of this process is dramatic: the net magnetization per unit volume is negligible in most natural cases, and these particles cannot in any conceivable way serve as a magnetoreceptor. However, the ability of these particles to gain large magnetic moments after exposure to an applied magnetic field has led several authors to suggest that they might have a sensory role (e.g., [22, 23]). In nature, such magnetite particles need to be exposed to fields in excess of 5 mT to acquire a magnetic moment strong enough to overpower gravity and align them in the 50 μT geomagnetic field.

Application of this strong field temporarily realigns the domain structure and aligns their individual moments; the moment acquired by the particles slowly decays with time after the external field is removed. Magnetizing fields of this strength are extremely rare in nature; the most common source is the electrical generation in the neighborhood of a lightning strike. It is highly unlikely, however, that an animal would survive such an encounter long enough to find a use for any newly magnetized particles! It is not yet known whether organisms actually precipitate multi-domain particles of magnetite. Perry et al. [24] found magnetite spheres up to 50 μm in diameter distributed in various tissues. Surface morphologies of these particles are quite distinct from other spherical magnetite particles produced by industrial or cosmic sources. It is not yet clear whether these are single particles or aggregations of smaller crystals as are the chiton teeth. In contrast, magnetite particles reported from the guitarfish sacculus [22] closely resemble inorganic grains picked up from the environment for use in the inner ear by elasmobranchs [25].

Single-domains. These are particles which plot in the central region of Fig. 2, and are characterized by a uniform and stable direction of magnetization throughout the particle volume. The magnetic vector is held fixed relative to the crystallographic axes, and is usually pinned so as to parallel the long axis of the particle (see [21] for a discussion). These particles therefore will rotate like compass needles in an external magnetic field and can produce a measurable torque on neighboring structures. When the particles are aligned in a chain like those in a bacterial magnetosome, the torques will add vectorially to produce a large net moment for the entire structure. The magnetic orientation energies for typical single-domain particles in the geomagnetic field are comparable to or larger than that of the background thermal energy (kT) which tend to disrupt alignment; values of this ratio (μB/kT = Γ) equal to 0.1, 1, and 10 are shown as dotted lines through the single-domain field on Fig. 2.

It is not surprising that all crystals of biogenic magnetite from magnetotactic bacteria measured to date with transmission electron microscopy (TEM) fall within this single-domain field. A mutant bacterium which made particles of any other size would be replaced quickly through natural selection [17]. It follows that single-domain crystals are the most plausible structures for use as magnetoreceptors in higher organisms as well. Rock magnetists have developed a battery of techniques for detecting the presence of single-domain crystals, and it is clear from such studies that they are widespread in animal tissues (see papers in [4]). However, these indirect studies cannot rule out the possibility of contamination, and for some organisms like cetaceans [26] this can be a significant problem.
We feel that the best approach to overcome these problems is to extract the ferromagnetic particles from these tissues [27-29] and then characterize their composition with X-ray and electron diffraction and their crystal morphology with TEM. Single-domain magnetite is too small for viewing with the TEM, and it is quite clear that the higher resolution of the TEM is a necessity (e.g., [30,31]). Several extraction studies of this sort have been done on pelagic fish [27-29,32], and the techniques have improved to the point where recognizable particles of biogenic magnetite crystals can be viewed with the TEM (Figs. 4, 5). It appears that some of these extracted crystals, particularly from pelagic fish (Fig. 5) appear to form chains similar in many respects to those in the magnetotactic bacteria, and studies of the interparticle interaction effects strongly support this interpretation [27,28,33]. These chains of single-domain particles have been interpreted as the remnants of some as-yet unidentified magnetoreceptor [27].

Other studies along similar lines reveal the need to be extremely careful in sample preparation. An earlier study [34] reported the detection of fine-grained magnetic material in two forms of murine tumor (YC-8 lymphoma and Lewis lung carcinoma). Tissue samples first were carefully extracted with glass tools, and the magnetic material was found to be uniformly distributed through the tumor masses. Levels of saturation remanence suggested the presence of between one and ten crystals per cell, and the two tumor types differed by a factor of two in their median coercivities.

Fig. 4.
Grains of single-domain magnetite extracted from the dermethylmold tissue of yellowfin tuna. Note the presence of several small chains of particles, somewhat like those in bacterial magnetosomes. Scale bar = 0.1 μm. TEM micrograph courtesy of S-B.R. Chang. From [27]
Fig. 5.
Oblong grains of single-domain magnetite extracted by the authors from the heads of homing pigeons. Scale bar = 0.05 μm. Samples courtesy of C. Walcott. The magnetite particles are the blunt, opaque objects, while the more translucent, elongate material is some as yet unidentified crystalline material.

(i.e., the particles within each cell line were of nearly the same size, but they differed greatly between the cell types). A subsequent extraction attempt on material apparently removed with nonmetallic surgical instruments yielded magnetic material, but X-ray diffraction revealed a dominant component of metallic iron, presumably from the extraction tools. Experience of this sort has proven the need to exercise extreme care to prevent contamination in all phases of this work, and in addition indicates use of clean-lab facilities wherever possible (e.g., [35, 36]).

Resolution of the ultrastructure of these as yet hypothetical organelles is a major goal of our present research. After three years of experimentation, we have finally developed TEM fixation techniques for the yellowfin tuna which neither introduce ferromagnetic contaminants nor chemically alter ultrafine-grained magnetite. Coercivity spectra from our fixed tissue from the tuna dermestomoid sinus (reported in [27]) match those of the original material, and the remanence levels suggest the presence of about 5 ppb magnetite as noted earlier. At such low concentrations, this search for the magnetoreceptors is still a "needle-in-the-haystack" problem. However, we have identified nervous tissue within the blocks and hope to trace these to sensory structures through serial sections.

Several investigators have tried to use histologic staining techniques for locating biogenic magnetite in tissue samples [37-40], and several have claimed to locate the magnetoreceptors with these
methods. Our work with tuna leads us to view these claims with caution. As noted by Kuterbach et al. [37], magnetometric studies constrain much less than 0.6% of the stainable iron to be in a magnetic phase; in fact, if magnetite were responsible for all the stainable iron claimed both by Baker et al. [38] and by Reason and Nichols [40], the total magnetic moment for their samples would be larger than measured by several orders of magnitude. We have found that these staining procedures also reveal numerous deposits of ferric iron in histologic sections of the tuna dermethylids. However, when we remove all of the non-magnetite iron with a dithionite-citrate-bicarbonate treatment [41] and then stain for the remaining iron, nothing is visible. In retrospect, this is hardly surprising as the chains of magnetite are at the threshold of optical resolution at best.

4. Discussion

It seems clear from the work outlined above that biogenic magnetite of single-domain size suitable for use in magnetoreception is widespread among higher organisms. The next question is whether the existing behavioral data in magnetically sensitive animals is compatible with the ferrimagnetic hypothesis of magnetoreception. For a simple compass response there is certainly no problem, as only a few tens of bacterial-like chains at most would provide an extraordinary sensitivity [15-17], but with the present SQUID magnetometers we would not be capable of finding them. There is, however, an easy method to test for the presence of a ferrimagnetic compass. Kalmijn and Blakemore [42] performed a simple pulse-remagnetization experiment on the magnetotactic bacteria which succeeds in turning a north-seeking organism into a south-seeking one. This switch occurs when the strength of the applied magnetic pulse exceeds the coercivity of the magnetite particles in the magnetosome, and when the pulse is applied antiparallel to its direction of magnetization. Pulses that are too weak or are aligned parallel to the particle moments produce no effect.

Four animals have been reported as having directional (rather than axial) magnetic compass responses in laboratory environments. These are elasmobranch fish ([43], but see the discussion in [44]), salmon fry [45], salamanders [46,47], and bobolinks [40]. We feel that the mechanism underlying these responses is still unknown, even in the elasmobranchs. A pulse-remagnetization experiment along the lines discussed in [42] will test this mechanism. Our only attempt at performing this experiment so far has been on the salmon fry (in collaboration with T. Quinn), but this failed because the fish failed to exhibit the initial compass response reported by Quinn [44].

The apparent sensitivity of honeybees and migratory animals to small anomalies or variations in the background field (on the order of 0.1% and less) is more difficult to test directly against the various transduction hypotheses (see [44] for a discussion of this behavioral evidence). Rosenblum et al. [47] present a rather strong argument against electrical induction as the mechanism, as it would imply the presence of electroreceptors several orders of magnitude more sensitive than those known from elasmobranchs (e.g., [48]). No such conceptual problem has yet been found for the ferrimagnetic hypothesis, however, and several plausible mechanisms for achieving high sensitivities have been proposed [17,49]. All these models are similar in supposing that the organisms use some form of averaging network to integrate small signals from several million magnetite-based sensory organelles. The magnetometry discussed earlier has
consistently revealed the presence of more than enough single-domain magnetite for this purpose.

In summary, ferrimagnetic material of biogenic origin is abundant in animal tissue, and the particles present meet all known physical and biological constraints for use in a magnetoreceptive organelle. Other magnetite particles are also present with apparent non-sensory functions, and together all forms of this material must be evaluated in assessing the influence of steady magnetic fields on living system.

Acknowledgments.

Supported through NSF grants BNS83-00301 and EAR83-51370, an NIH biomedical research support grant, and gifts from the W.M. Keck Foundation and the Weyerhaeuser Corporation. Caltech Division of Geological and Planetary Sciences Contribution # 4319.

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