Biomimetic magnetite mediated by magnetosome proteins vs. ALH84001 meteorite magnetite: Are both comparable?

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Abstract
The suggestion in 1996 that the Martian meteorite ALH84001 could contain proof of possible biologic activity in the past have generated a huge controversy that last until today. One of the most discussed evidence is the presence of magnetite crystals that resemble those produced by a particular group of bacteria, the so called magnetotactic bacteria (MTB). These microorganisms are the only known example of biologically controlled biomineralization among the prokaryotes and exert an exquisite control over the biomineralization process of intracellular magnetite that result in crystals with very unique features that, so far, cannot be replicated by inorganic means. These unique features have been used to recognize the biological origin of natural terrestrial magnetites, but the problem arises when those same biogenecity criteria are applied to extraterrestrial magnetites. Most of the problems are caused by the fact that it is not clear whether or not some of those characteristics can be reproduced inorganically. Magnetosome protein mediated magnetite synthesis seems to be the best approach to obtain magnetosome-like magnetites, and such strategy may help clarify what is the specific biosignature of magnetotactic bacteria.
Key words: ALH84001; Magnetite; MPMS (Magnetosome Protein Mediated Synthesis); Magnetotactic bacteria.

Magnetita biomimética mediada por proteínas del magnetosoma vs. magnetita del meteorito ALH84001: ¿Son ambas comparables?

Resumen
La sugerencia en 1996 de que el meteorito marciano ALH84001 pudiese contener pruebas de posible actividad biológica en el pasado ha generado una gran controversia que aún persiste hoy. Una de las evidencias más discutidas es la presencia de cristales de magnetita que se asemejan a aquellos producidos por un grupo particular de bacterias, las bacterias magnetotácticas (MTB). Estos microorganismos son el único ejemplo de mineralización controlado biológicamente conocido entre procariotas y ejerce un control delicado sobre el proceso de biomineralización de la magnetita intracelular que resulta en la formación de cristales con características únicas que, hasta ahora, no han podido ser replicadas por medios inorgánicos. Estas características únicas se han usado para reconocer el origen biológico de magnetitas terrestres naturales, pero el problema aparece cuando los mismos criterios de biogenicidad se aplican a magnetitas extraterrestres. La mayoría de los problemas se deben a que no está claro si alguna de esas características puede ser reproducida inorgánicamente. La síntesis mediada por proteínas del magnetosoma parece ser la mejor aproximación para obtener magnetitas similares a las de los magneto-

somas, y dicha estrategia podría ayudar a clarificar cual es la biosignatura específica de las bacterias magnetotácticas.
Palabras clave: ALH84001; Magnetita; MPMS (Síntesis mediada por proteínas del magnetosoma); Bacterias

Summary: Introduction. 1. Magnetosome Proteins and MPMS experiments 2. MPMS and ALH84001 magnetite comparison 3. Conclusion. References.

Normalized Reference
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Introduction

Magnetite ($Fe^{2+}Fe^{3+}{}_2O_4$) is a ferrous diferric oxide mineral that has strong ferromagnetic properties at room temperature. Many different processes can originate magnetite either inorganically or biologically, i.e. by chemical means or mediated by microorganisms, either through a biologically induced or controlled process (Jiménez-López et al., 2010).

Inorganically produced magnetite occurs naturally in a wide range of geological environments on Earth. These geological environments include igneous and metamorphic rocks, skarns and low-temperature hydrothermal and sedimentary deposits. In addition, the wide biomedical and technological application that nano-sized magnetite has (Lang et al., 2007; Matsunaga et al., 2007), has driven the development of a huge range of laboratory synthesis techniques. According to Jiménez-López et al. (2010), inorganic magnetite can be produced through two different ways: (1) precipitation as a primary mineral phase or (2) formation as a secondary mineral phase. As a secondary phase, magnetite is formed by the decomposition of another mineral which suffers a specific transformation. Specifically, most of the studies have been performed on magnetite formed by the thermal decomposition of iron carbonates (Golden et al., 2001; Golden et al., 2004; Jiménez-López et al., 2010). As a primary mineral phase, magnetite precipitates from either a homogeneous or a heterogeneous solution. It can be formed at both room and high temperatures, from aqueous solutions and/or from gels and, also in the absence or presence of organic additives. Magnetosome protein mediated synthesis (MPMS) is especially relevant. In this case mineralization takes place in the presence of one or more recombinant magnetosome proteins. MPMS is interesting, on one hand, to understand the biomineralization process of magnetosomes and to infer the role of magnetosome proteins. On the other hand, from the applied field point of view, MPMS might be used to inorganically produced biomimetic (magnetosome-like) magnetic nanoparticles.

Organically produced magnetite can be formed through a process controlled by an organism, or indirectly, where the presence of an organism generate suitable conditions

for magnetite precipitation, although this process is not controlled by the organism itself. Many organisms from different taxonomic groups are known to produce magnetite such as some algae (Torres de Araujo et al., 1986); some mollusca (Nesson and Lowestam, 1985); honeybees (Gould et al., 1978), sea turtles (Perry et al., 1985) or birds (Presti, 1985). However, the most well-known organisms that are able to produce magnetite are some bacteria. Bacterial magnetite can be produced by two different pathways. One of them is the biologically induced mineralization (BIM). In this case, the suitable conditions for magnetite precipitation are created by bacterial metabolic activity and/or bacterial cell walls, membranes and debris. The dissimilatory iron reduced bacteria (DIRB), like *Geobacter metalireducens* or *Shewanella oneidensis* are among those able to carry out that process. These bacteria can reduce the Fe^{3+} anaerobically when they use such a cation as the final electron acceptor in an anaerobic respiration (Bazylinski et al., 2007). As a result, both iron cations, Fe^{2+} and Fe^{3+}, become available in the surroundings of the bacteria, promoting magnetite precipitation. This magnetite is a waste product, since it is useless to these bacteria. In fact, these magnetite crystals pose no difference with those produced by exclusively inorganic pathways under similar physic-chemical conditions. However, one notable exception was reported by Vali et al. (2004), who observed that under low partial pressure of CO_2, *Geobacter metallireducens* is able to produce tabular single domain magnetite crystals.

Conversely, the biologically controlled mineralization (BCM) is the process where an organism tightly controls the mineral production at the genetic level. In the case of bacterial magnetite, a group known as magnetobacteria is able to produce magnetite through BCM. Magnetotactic bacteria (MTB) are a ubiquitous and taxonomically diverse group that have in common the capacity to passively align along the Earth magnetic field lines and actively swim along them. They are able to respond to the magnetic field because MTB produce magnetite crystals (Fe_3O_4) or rarely, greigite (Fe_3S_4), each crystal surrounded by a double lipid membrane, being that the so called magnetosome (Komeili, 2007). The magnetic particles produced by this group are key for their survival. These organisms live in a specific zone of the water column, called oxic-anoxic transition zone (OATZ). MTB are microaerophilic organisms, that is to say, they required a small concentration of oxygen, neither too high nor totally absent. To find that goldilocks zone, MTB align themselves with the lines of the geomagnetic field thanks to their magnetic particles and swim along them until they find the OATZ. This process, so called magnetoaerotaxis, represents a huge ecological advantage, because it allows MTB to reduce the three spatial dimensions to only one, and therefore, they can respond to changes in OATZ depth rapidly (Pérez-González et al., 2010a). Magnetosomes are so needed for MTB that their formation is strictly regulated at the gene level, all related genes concentrated in a region of the MTB chromosome called the magnetosome island (MAI) (Ullrich et al., 2005). Several genes codifying proteins essential for membrane invagination, iron transportation and reduction, maintaining the pH within the magnetosome and directly involved in magnetite nucleation and growth have been reported

by a number of authors (Grünberg et al., 2004; Tanaka et al., 2006; Scheffel et al., 2008; Murat et al., 2010; Komeili, 2012; Raschdorf et al., 2013). Such an exquisite degree of control results in magnetite with unique properties not, to the present, replicated by any chemical means.

The controversy came in 1996, when McKay et al. reported that around 28% of the magnetite included within the carbonate globules in the Martian meteorite ALH84001 presented those unique features only found in terrestrial MTB magnetite (Thomas-Keprta et al., 2000). Once terrestrial contamination was ruled out by stable isotope analyses, some biogenicity criteria were established to try to differentiate the biotic or inorganic origin of natural magnetites (Thomas-Keprta et al., 2000). Those criteria are listed below.

Single-Domain (SD) Size and Restricted Anisotropic Width/Length Ratios

Both size and crystal morphology determine how well magnetite crystals can work as discrete magnets (Thomas-Keprta et al., 2000). Particles smaller than 30nm have a superparamagnetic behavior, with no permanent magnetization. Particles bigger than 120 nm present several magnetic domains with a lower net magnetization. Only particles within the size range from 30 to 120 nm act as single magnetic domain. Bacterial BCM has a very controlled size between this size range required to be a single magnetic domain and, moreover, display restricted width/length ratios to maximize their function (Thomas-Keprta et al., 2000).

Chemical Purity

Magnetite produced by magnetobacteria is generally stoichiometrically pure. The MTB exclude compounds like Ti, Al, Cr or Mn even if they are present in the media (Thomas-Keprta et al., 2000). A number of authors have demonstrated the difficulty for magnetosome magnetite to incorporate foreign cations. This may be related to the fact that the incorporation of impurities into the structure reduce the total magnetization, and so, the total magnetic efficiency of the particle. In this context, Mn incorporation has been detected in magnetosome magnetite (Prozorov et al., 2014), although only as trace levels, while such incorporation is greater and extended to other cations in biologically induced magnetite and inorganically produced ones (Jiménez-López et al., 2010; Amor et al., 2015).

Crystallographic Perfection

Electron microscopy studies reveal that MTB magnetite crystals are essentially free of inner defects, with minor exceptions of {111} twinnings (Devouard et al., 1998). The crystallographic lattice lack of defects contributes to enhance the particle net magnetic moment. As direction [111] is the easy axis in magnetite, that twinning does not necessarily affect the magnetization of the crystals.

Nonetheless, more research is needed about this criterion. For instance, Pérez-González et al. (2010b) observed organic matter incorporation in magnetite induced by *Shewanella oneidensis* and determined that such incorporation can induce alterations in the crystal structure of the magnetite. However, such incorporation has not been observed so far in magnetosome magnetite.

Magnetite Chains

MTB are known to arrange their magnetite crystals into chains. The cell magnetic moment is increased in this way, because it is the sum of the individual moments of each magnetite crystal. This maximizes the cell magnetic moment, allowing the organisms to overcome the Brownian forces. It has been demonstrated in *Magnetovibrio magneticum* (MV-1) that while a single crystal cannot overcome that forces, the alignment of 20 of them can (Frankel and Blakemore, 1980). Although it was thought that magnetite chains tend to collapse when the organism die (Kirschvink, 1982), Kobayashi et al. (2006) observed that magnetosome linearity persists long after cells are disrupted. Nevertheless, this last criterion does not seem very trustful to recognize biotic origin, since inorganic magnetite chains have been reproduced in the laboratory (Liu and Chen, 2008).

Unusual Crystal Morphology

Magnetite crystals shapes in many MTB present particular shapes [e.g. bullet-shaped, cubo-octahedral, elongated-prismatic (Jogler and Schüler, 2009)], that are not common among those produced inorganically. That particular morphology is opposed to the general rule that tends to reduce the surface free energy by making the minerals of the isometric crystal system (like magnetite) to adopt isotropic forms (Ichnose et al., 1992). Biological elongation allows particles have bigger volumes and therefore, bigger magnetic moments without falling in a multi-domain state. Thanks to that, cells can create less magnetosomes to achieve the same orientation energy (Thomas-Kerpta et al., 2000).

This criterion has been very much investigated with the purpose of producing magnetosome-like magnetites by inorganic means, either by changing the physical-chemical conditions of the experiment or by introducing proteins that may affect the nucleation and/or the growth of the magnetite crystals, thus affecting the final morphology and shape of those magnetites. This topic is going to be treated in greater detail below.

Crystallographic Direction of Elongation of Magnetite Crystals

Another characteristic of magnetite crystals in bacterial magnetosomes is the tendency for the crystals to be elongated along the chain length in one of the [111] directions. Although the reasons for that trend are not clear, it is believed that it is another factor

that contributes to increase the magnetic moment (Thomas-Kerpta et al., 2000). Nevertheless, this is not the only possible elongation direction, since elongations in other directions as [100] have also been reported (Körnig et al., 2014).

Other criteria

Other distinct characteristic between magnetosome magnetites and inorganic ones have been proposed by several authors over the years. Statistical distributions of crystal-size (CSD) and shape-factor (SFD) distributions reveals that most CSD curves for MTB magnetite are asymmetric and negatively skewed, while SFD curves are bell-shaped (Arato et al., 2005). Magnetic measurements also have been employed to determine whether or not magnetite samples are potentially biogenic (Weiss et al., 2004a).

Magnetic techniques are mainly based in an effect known as Verwey transition. The Verwey transition is an effect that takes place at a temperature of 122 K, and is characterized by a moderate conductivity above that temperature, and a discontinuous drop in conductance (Verwey, 1939; R. Prozorov et al., 2007). The actual transition temperature is depressed below this value for impure and/or partially oxidized magnetite. Magnetite will demagnetize while cooling through the Verwey transition and then recover part of its remanence upon warming back up to room temperature, with the amount recovered partly depending on the domain state (i.e., crystal size). As a result, the Verwey transition temperature is a sensitive indicator of both composition and crystal size (Weiss et al., 2004a). The presence of a magnetic signature of the Verwey transition is usual in magnetosomes, and can be seen as a sharp change in the magnetic moment, while it is rare in inorganic magnetite formed at room temperature (Prozorov et al., 2007). Also, magnetosomes present different behavior compared to that of inorganic magnetite when analysed by ferromagnetic resonance spectroscopy (FMR), which senses the magnetic anisotropy (Weiss et al., 2004b). This magnetic anisotropy is a product of chain alignment and particle elongation (Kopp et al., 2006). FMR studies reveal differences between biologically controlled and biologically induced and inorganic magnetite. While the first have smaller effective g-factor, which characterized the magnetic moment of a particle, the second have a larger effective g-factor (Weiss et al., 2004b). Measurement of magnetite magnetic properties is a technique that has been already used to determine the presence of putative bacterial magnetite in sediments (Snowball et al., 2002; Paasche et al., 2004).

A close comparison between Martian meteorite ALH84001 magnetite and magnetite produced by *Magnetovibrio blackemori*, strain MV-1, reveals close similarities between both magnetites. According to Thomas-Keprta et al. (2001) both share the features of the six main biogenicity criteria detailed above. Both groups of magnetite crystals have sizes that fall into the single domain ranges. Both also are chemically pure, containing only Fe and O at detectable levels (>150 ppm). MV-1 magnetites have few crystallographic defects that act to attenuate the crystal's ferromagnetic properties, whereas ALH84001 magnetites are defect free. Both populations of magnetite crystals

are truncated hexa-octahedral, elongated along the zone axis [111]. Finally, MV-1 magnetite crystals are aligned in chains within living cells, and the presence of putative magnetite chains has also been reported in ALH84001 (Friedmann et al., 2001). It is important to point out that it is not comply with one of the criteria what makes the natural magnetite a potential biomarker, but, as Thomas-Kerpta et al. (2001) said, natural magnetite could be considered a biomarker if it meets as once all the above mentioned criteria.

Golden et al. (2004) demonstrated that stoichiometric elongated magnetites that resemble those controversial ALH84001 ones could be reproduced inorganically through the thermal decomposition of an iron-rich carbonate. However, Jimenez-Lopez et al. (2012) argued those results since these authors demonstrated that magnetites resulting from the thermal decomposition of an iron-rich phase inherit both the composition and the structure of the precursor. Following their argument, and since ALH84001 magnetites are embedded in a (Ca, Mg, Fe)CO_3 phase, if ALH84001 magnetites were formed by the thermal decomposition of such a phase, they would have trace of Ca and Mg in their structure, but, rather, ALH84001 magnetites are stoichiometrically pure. Moreover, these authors demonstrated that such thermal decomposition was topotactic, as a consequence of CO_2 loss, accompanied by limited atom displacement, and shrinkage along specific [hkl] directions. The consequence of this study is that if ALH84001 magnetites were the result of such a thermal decomposition they should be aligned in chains in which the <441> of the carbonate should be parallel to the [110] of the newly formed magnetite and the [010] of the precursor carbonate should be parallel to the [110] of the magnetite. However, this is not the alignment observed in ALH84001 magnetites. Therefore, the thermal decomposition scenario for the formation of those large, euhedral, chemically-pure, [111]-elongated magnetites found within Ca-, Mg- and Fe-rich carbonates of the Martian meteorite ALH84001 is not plausible and the biological origin cannot be ruled out.

1. Magnetosome proteins and MPMS experiments

MTB magnetite is located in a specific compartment called magnetosome. Magnetosomes are cell membrane invaginations delimiting a functionally enclosed space in where magnetite synthesis is carried out (Komeili, 2007). Since magnetite is key for the bacterial survival, its synthesis inside the magnetosome is tightly controlled in order to guarantee that each magnetite crystal can maximize its own magnetic field, and therefore, enhance its performance in the magnetoaerotaxis. The magnetosome membrane contains several exclusive proteins that are believed to take part in that strictly controlled synthesis. As it has been said in the introduction, the genes that encode those proteins are all grouped in a region of the genome known as MAI (Ullrich et al., 2005).

Magnetosome proteins have four important roles (Nudelman and Zarivach, 2014): a) Protein sorting and magnetosome membrane invagination b) Magnetosome arrangement into a chain structure c) Iron transport and nucleation d) Control of crystal shape and size. Proteins with the functions b, c and d have a special interest in the search for potential biological signatures, because they may confer special features to magnetite crystals that could allow its differentiation from other magnetite crystals.

Two main proteins are known to be responsible for the arrangement in chains of magnetosomes: MamK and MamJ. MamK is a dynamic actin-like protein (Draper et al., 2011) responsible for the magnetosome membrane organization into a chain roughly parallel to the long axis of the cell (Komeili et al., 2006). However, MamK is not only a merely rigid backbone, but also has an active function in positioning and concatenating magnetosome chains (Katzmann et al., 2010). MamJ physically interacts with MamK, with two distinct sequence regions involved in binding to MamK, and that direct interaction allow the formation of the magnetosome chain (Scheffel and Schüler, 2007). Interstingly, mamJ and mamK genes are cotranscribed (Scheffel et al., 2006), strengthen the interpretation that both complement each other as two of the main responsible of the arrangement of magnetosomes into chains.

The proteins responsible for the iron transport and nucleation are MamO, MamE, MamH, MamN, MamM, MamB, MamP, MamT, MamZ, MamX (Nudelman and Zarivach, 2014). Mam E, MamP, MamT, and MamX share a remarkable feature, a CXXCH motif (Quinlan et al., 2011; Siponen et al., 2012; Raschdorf et al., 2013), which is a typical c-type cytochrome motif that acts in the reduction/oxidation of iron. That domain in those proteins seems to be specific of MTB and has been called "magnetochrome", that could be a new, functional, unique class of cytochromes (Siponen et al., 2012; Siponen et al., 2013). This domain is key for the redox control, in which these proteins are involved (Yang et al., 2013; Barber-Zucker et al., 2016; Jones et al., 2015). MamO has been suggested to take part in crystal nucleation (Barber-Zuker et al., 2016). In fact, a single mutation in the genes that encode them is sufficient to abolish magnetite biomineralization (Murat et al., 2010, Yang et al., 2010). MamM, MamB and MamN are transmembrane proteins (Nudelman and Zarivach, 2014) with transport roles. Due to their similarity with of the cation diffusion facilitator family (CDF), MamM and MamB can act primarily as iron transporters (Uebe et al., 2011), whereas MamN may be involved in pH regulation (Lohβe et al., 2014) because it shares homology with Na^+/H^+ antiporters (Komeili, 2012; Nudelman and Zarivach, 2014). MamH and MamZ (MamH-like32) are also believed to be involved in iron transport, because they share high identity to the major facilitator superfamily (MFS) domain (Raschorf et al., 2013). Proteins thought to be responsible for magnetite shape and size control are MamR, MamS, MamC, MamG, MamD, MamF, FtsZ-like, Mms6 and MmsF (Nudelman and Zarivach, 2014). While MamS has a function in the regulation of magnetosome size and morphology (Murat et al., 2010), MamR has been shown to be important for crystal number and size control as well, but is not involved in the control of their morphology.

(Murat et al., 2010). The FtsZ-like protein is a truncated homologous of the FtsZ protein (Ding et al., 2010), but unlike the full length FtsZ, which is still functional in in cell division, FtsZ-like is involved in redox control of magnetite crystallization (Müller et al., 2014) and crystal size and shape (Ding et al., 2010).

MamG, MamF, MamD and MamC are all encoded in the mamGFDC operon. It has been observed that the deletion of the entire mamGFDC operon does not abolish the formation of magnetite crystals, but cells produce crystals that are only 75% of the wild-type size and are less regular than wild-type (Scheffel et al., 2007). These authors also suggested that the MamGFDC proteins have partially redundant functions and control the growth of magnetite crystals in a cumulative manner. Interestingly, MamC, MamF and MamG share some features. All three have transmembrane domains and loops with charged residues facing the magnetosome lumen that are believed to interact with iron cations (Nudelman and Zarivach, 2014; Barber-Zuker et al., 2016). In the same way, they display nearly identical localization patterns (Lang and Schüler, 2008; Valverde-Tercedor et al., 2014).

Mms6 and MmsF are encoded in the mms6 operon (Lohβe et al., 2014) and both are believed to play a role controlling shape, and in the case of MmsF, controlling crystal maturation as well (Tanaka et al., 2011; Murat et al., 2012). An interesting behaviour is that Mms6 and MmsF have been observed to self-assembly in micelles that have high affinity for iron in aqueous solution (Wang et al., 2012; Rawlings et al., 2014), which has also been observed in MamC (Kashyap et al., 2014).

The fact that many different proteins work as a part of a highly coordinated orchestra to build up a chain of magnetosomes and synthetize magnetite in a process that is not fully understood yet is a proof that magnetite crystals are shaped by a genetic code and they play a role that allows MTB survive. Thus, it should not be a surprise that magnetite crystals have a set of particular features with the purpose of enhancing their efficiency. A further and a deep understanding not only of the individual roles of each protein, but also a holistic vision of their interactions could be useful not only for obtaining new nano-sized magnetic biotechnological products, but for checking the strength of magnetite as a biomarker, confirming whether or not the currently established biogenicity criteria are suitable or not to be used with that purpose.

Among these proteins, those controlling the size and morphology of the magnetite crystals may provide a distinct biosignature. Unfortunately, only three of these proteins have been studied so far in in vitro experiments. Although some other proteins and operons have been studied in mutants lacking the specific gene (or genes) of interest (Scheffel et al., 2007; Ding et al., 2010; Murat et al, 2010; Murat et al., 2012; Tanaka et al., 2011; Arakaki et al., 2014), the results are not yet conclusive, because there are many other factors that may influence those results. MamC (Valverde-Tercedor et al., 2015), Mms6 (Ameniya et al., 2007; Prozorov et al., 2007; Arakaki et al., 2010; Rawlings et al., 2016) and MmsF (Rawlings et al., 2014) have been expressed as recombi-

nant proteins, purified and used in biomineralization experiments to study potential biosignatures. Here we briefly present the magnetite precipitation experiments done so far with those recombinant proteins and we describe the obtained nanoparticles.

Mms6

Mms6 has been used to produce nanoparticles of magnetite from aqueous and gel solutions, both in co-precipitation experiments (in which salts of $Fe^{2+}$and Fe^{3+} are added to the aqueous solution) and partial oxidation experiments (in which only a salt of Fe^{2+} is added to the aqueous solution and then partially oxidated). Uniform magnetite nanocrystals of about 30nm with a protein concentration of 5.6 μg/mL have been obtained through co-precipitation experiments (Prozorov et al., 2007; Galloway et al., 2011; Wang et al., 2012). Compared to those formed in inorganic protein-free experiments, protein-bearing particles were bigger and with a narrower size distribution. Sizes of magnetite crystals produced by partial oxidation were slightly smaller (average size of about 20 nm) than those produced by co-precipitation (Amemiya et al., 2007; Arakaki et al., 2010).

Both magnetite crystals obtained through co-precipitation and through partial oxidation from aqueous and gel solutions have a morphology described as cuboidal or cubo-octahedral (Amemiya et al., 2007; Arakaki et al., 2010; Galloway et al., 2011; Rawlings et al., 2016). Furthermore, magnetite (1 1 1) and (1 0 0) crystal faces were also formed, resembling the truncated crystals produced naturally by *Magnetospirillum magneticum* AMB-1 (Ameniya et al., 2007; Arakaki et al., 2010).

Magnetization data obtained from particles formed in the presence of Mms6 show a higher magnetic moment per particle, with much larger remanence lasting well above the blocking temperature, consistent with the presence of magnetite with a well-defined crystalline structure (Prozorov et al., 2007). Additionally, Wang et al., (2012) have reported a blocking temperature (K) of 35 (Mms6- magnetite formed through co-precipitation).

MamC

Magnetite particles produced in presence of MamC in a concentration of 10 μg/mL presented a size range between 20-80 nm, with most of the crystals falling in the range between 30-40nm (Valverde-Tercedor et al., 2015).

The morphology of these particles was reported to be a rhombic, rectangular, or square two-dimensional, with one or two corners frequently missing. These crystals were much better faceted than those produced inorganically as a control (Valverde-Tercedor et al., 2015).

Magnetization mesurements of particles synthetized with MamC showed that particles grown at 10 μg/mL of MamC had a high blocking temperature and a slow magnetization increase, thus having a large magnetic moment per particle, even larger than that measured from Mms6-bearing in vitro magnetite (Valverde-Tercedor et al., 2015).

MmsF

In vitro magnetite synthesis experiments conducted by Rawlings et al. (2014) following the co-precipitation method in the presence of MmsF (10 μg/mL) yielded magnetite particles with an average size of 56nm. These particles produced in the presence of MmsF were larger and showed defined crystal faces, as a difference to the particles produced in the absence of the protein. This result is similar to those obtained both with MamC and Mms6, suggesting similar roles in the control of morphology (Rawlings et al., 2014). The magnetite produced in MmsF-bearing experiments had a high saturation magnetization (129 emu/g), which indicates that high-quality magnetite was produced (Rawlings et al., 2014).

2. MPMS AND ALH84001 magnetite comparison

The question remaining is whether or not MPMS and ALH84001 magnetite crystals are both comparable. Both magnetite crystals do have similarities between them. However, inorganic magnetite produced by thermal decomposition also has features that mimic those traditionally considered as biogenic. Differences and similarities are summarized in Table 1. As it can be seen in Table 1, both magnetite produced through thermal decomposition as well as a subpopulation of around 27% of magnetite crystals from ALH84001 have features that match with all the biogenicity criteria. In contrast, MPMS magnetites, in which the presence of MTB magnetosome protein gives the crystals some specific features, do not meet all of them. Should be remarked, however, that the features of magnetite produced by thermal decomposition correspond to different sets of magnetites obtained through different experiments (Golden et al., 2001; Golden et al., 2004; Jiménez -López et al., 2012; Vuong et al., 2015), whereas the subpopulation of ALH84001 magnetite meet all of them at once.

A closer look also reveals subtle but quantifiable differences among the three sets of magnetite crystals. Thermal decomposition magnetite has a wider size distribution (Jiménez-López et al., 2012), with mean values usually situated just below the SD limit (Golden et al., 2004). Morphologies are varied: from hexagonal, rectangular (Jimenez-Lopez et al., 2012) and euhedral non-elongated (e.g. octahedral, cubic, platy) to irregular shapes (e.g., subhedral and anhedral crystals) and individual whiskers (Golden et al., 2004), being the 66% of the crystals obtained by Golden et al., (2004) elongated on the [111] axis. In addition, it has been demonstrated (Golden et al., 2001; Golden et al., 2006) that defect-free chemically pure magnetite (assuming that magnetite crystals are chemically pure if they have Mg levels comparable or lower than Mg detected by Thomas-Keprta et al., 2000 in ALH84001 magnetite) can be obtained through a thermal-decomposition process as well. Lastly, Vuong et al., (2015) have reported high quality magnetite with a high saturation magnetization for particles produced by thermal decomposition. Although along those experiments crystals that meet the biogenicity criteria have been produced, Jiménez-López et al., (2012) observed that magnetite originated by thermal decomposition retained a chemical and structural inheritance

from their carbonate precursor that has not been observed in ALH84001, backing the proposition of Thomas-Keprta et al., (2009), that the majority of magnetite crystals found in ALH84001 are allochthonous.

Table 1. Comparison between inorganic, MPMS and ALH 84001 magnetite using the features considered as biogenicity criteria. [a]Jiménez-López et al., 2012; [b]Golden et al., 2001; [c]Golden et al., 2004; [d]Golden et al., 2006; [d]Thomas-Kerpta et al., 2000; [e]Bird et al., 2015; [f]Valverde-Tercedor et al., 2015; [g]Amemiya et al., 2007, [h]T. Prozorov et al., 2007; [i]Rawlings et al., 2014; [j]Friedman et al., 2001, [k]Vuong et al., 2015; [l]Weiss et al., 2004b.

Criteria	Inorganic magnetite (Produced by thermal decomposition)	MPMS magnetite	ALH84001 magnetite
Size	Sizes between 5 to 40 nm[a]	Depending of the method, size varies between 20 to 30 nm[f,g,h,i]	Around 27% of crystals with a mean size of 39 x 27 nm.[d]
Crystallographic Perfection	Defect-free magnetite crystals[b]	Crystals better faceted than those produced inorganically.[f,g,h,i]	Truncated hexa-octahedral magnetites are defect free[d]
Chemical Purity	Chemically pure magnetite[d]	Depends of media composition. Co could be incorporated if added to the media[e]	Many magnetite grains observed to be stoichiometrically pure.[d]
Magnetite Chains	Well developed chains of magnetite crystals [a]	Mechanisms for crystal arrangement absent, no chains formed.[f,g,h,i]	Magnetite chains present[j]
Morphology	Hexagonal, to rectangular, rhombic and irregular[a]	Particles tend to be cuboidal to cubo-octahedrical or rhombic, rectangular[f,g,h,i]	Around 27% of crystals with elongated prismatic morphology.[d]
Crystallographic Direction of Elongation	Some magnetite crystals elongated on the [111] axis[c]	No elongation[f,g,h,i]	Around 27% anisotropic, elongated along the [111] growth direction.[d]
Magnetization	Large moment per particle[k]	Larger moment per particle[i]	Larger moment per perticle[l]

Regarding the sets of magnetite crystals synthetised in different MPMS experiments with different proteins, SD size particles with a better trend to crystallographic perfection have been consistently obtained (Amemiya et al., 2007; Prozorov et al., 2007; Rawlings et al., 2014; Valverde-Tercedor et al., 2015). These characteristics not only resemble those present in the ALH84001 magnetite, but also the variability range is lesser than in the thermal decomposition magnetite. Additionally, even though magnetite particles in the three groups have a large moment per particle as magnetization studies reveal, the quality of MPMS magnetite (129 emu/g) (Rawlings et al., 2014) is even higher than those produced by thermal decomposition (78 emu/g) (Vuong et al., 2015). A Verwey transition of 122 K for the ALH84001 magnetite (Weiss et al., 2004b) indicates that the quality of those particles is also higher than the produced by thermal decomposition. Therefore the presence of a MTB recombinant protein gives the magnetite crystals homogeneous features that make them differentiable from those crystals produced solely by inorganic pathways. Thus, MPMS demonstrate that at least some features, like size and crystallographic perfection are strongly conditioned by the presence of a magnetosome protein, and the fact that those features are homogenously present in around the 27% of ALH84001 magnetite cannot be ignored.

3. Conclusions

MTB are known on Earth for producing magnetite crystals with several exclusive features that allow us to differentiate them from other magnetite crystals produced through exclusively inorganic pathways. That features tend to maximize the efficiency of magnetite crystals, which play an important role for the survival of MTB. Moreover, that particular features are the result of the interaction of several proteins, that work in a synergic process to build up a high-quality magnetite crystals whose physical and chemical properties challenge even inorganically produced synthetic particles. That tightly controlled process is driven, in the last instance, by a genetic code in which the protocol of that synthesis is written, and that genetic code has been shaped by evolution through natural selection. As evolution can be seen as one of the most singular processes exclusively associated with life, the particular features of MTB magnetite crystals offer great potential to be used as biomarkers. For that reason, it is extremely necessary to establish non-ambiguous reliable criteria that allow their use to infer the putative presence of life, both on Earth and beyond. In addition, it is also necessary to remark that biogenicity criteria should be applied as a whole and not individually separated to the same set of crystals, because it is only the comply with all of that criteria what would make possible the differentiation between organic and inorganic magnetite sets.

In this context, MPMS offers a valuable technique to evaluate the strength of some biogenicity criteria, and even to find new ones. Now, it is clear that some characteristics like single-domain size or crystallographic perfection are strongly influenced by magnetosome proteins. The problem persists, however, since other inorganic techniques, as

thermal decomposition are able to produce crystals sharing some of those features. More research is needed in order to fix the exclusive features of biogenic magnetite crystals. In this sense future MPMS experiments could have great potential, helping to identify the specific mechanisms that lay behind bacterial magnetite synthesis.

About magnetite crystals within ALH84001, they probably depict a complex history. Thermal decomposition, that has been claimed for many authors as a possible mechanism to explain the occurrence of magnetite crystals in ALH84001 (Golden et al., 2001; Barber & Scott, 2002; Brearly, 2003; Golden et al., 2004), has been discarded by Thomas-Keprta et al., (2009) and Jiménez-López et al., (2012) who have proven that magnetite crystals in ALH84001 are allochthonous, meaning that those crystals were not originated within the carbonate globules that fills the meteorite fractures in which magnetite is embedded. Instead, those crystals might have been washed away from different external sources maybe by the action of water that is assumed to be present in huge quantities in early Mars (Carter et al., 2015) and deposited within the meteorite fractures. If that scenario was true, the problem of magnetite crystals origin would be complicated, because those crystals could have been originated through very different processes, which would make more difficult to determine if one of those could have included the action of any kind of lifeform.

Regarding this last possibility, it is necessary to specify whether a hypothetical magnetobacteria would has been able to thrive given the conditions that were present on ancient Mars. As aforementioned, water was widespread on early Mars surface (Carter et al., 2015), which is an essential requisite for the presence of life as we know it. The second requisite is the presence of both a source of carbon and a source of energy to allow the bacterial growth and reproduction. Putative organic compounds have been detected on Mars (Leshin et al., 2013; Freissinet et al., 2015). In the same way, palaeoenvironments that could support life based on chemiolithotrophy have been identified on Mars as well (Grotzinger et al., 2014). In third place, magnetobacteria are microaerophilic organisms, so they require low concentration of oxygen. The Martian atmosphere has a low concentration of oxygen (Krasnopolsky, 2011) that would allow magnetobacteria to thrive even without the need of magnetotaxis. However, a remanent magnetic field has been detected (Acuña et al., 1999), indicating that a consistent magnetic field was present on Mars in its early history, thus allowing the magnetotaxis to occur. In conclusion, conditions on early Mars were not as hostile as they are today, and could have been able to support some sort of bacterial life.

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