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Biochemical, molecular and genetic organisation of bacterial magnetosome for innovating nanotechnology based applications

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ABSTRACT

The great discovery of *Magnetotactic bacteria* is a pleasant surprise for researches engaged in improving the technology related to the material science, as it expresses a magnetic property due to the presence of naturally occurring nano-crystal, attached to cytoplasm membrane. This review focuses on current information of MTB on - biochemical, molecular and genetic organisation within the cells, suggesting the biomineralisation of nano-crystal -magnetosome. Application aspects of magnetosome and perspectives for future research are the additional decided literature of the review.

KEYWORDS

Magnetotactic bacteria;
Magnetosome structure;
Biochemistry;
Genetics;
Functionalization and applications.

INTRODUCTION

Magnetotactic bacteria (MTB) was analyse from mud sample of water bodies, do not have taxonomical significant and represent heterogeneous group of *fastidious prokaryotes* with cellular morphologies including coccid, rod-shaped, vibrioid, spirilloid[1]. The introduction of MTB was the serendipitous discovery by R.P Blakmore as a graduate student, at the University of massachusetts at Amherst. MTB become popular among the researchers due to the due to their magnetic property depends on intracellular structure called Magnetosome[2] by which MTB orient and migrate along magnetic field lines, this behaviour refer to as magnetotaxis[3]. Magnetosome are the nano-crystal consist of magnetic iron mineral particles within the membrane vesicles[1]. The particular size of magnetosome range from 35-120nm, resulting permanent dipole moment by properly organized chains of magnetosome[4]. Bacterial magnetosome have been relevant for synthesis advanced biomaterials, magnetic tapes, magnetic targeting of pharmaceuticals and printing inks[5]. most of the applications of magnetosome depends on increasing productivity of magnetosome in natural condition by improving MTB strain by genetic engendering for future advantages.

Magnetotactic bacteria (MTB)

Despite the great diversity of microorganisms, MTB have several advantageous features found in oxic-anoxic transition zone of freshwater and marine habitat[6]. Studies of freshwater communities of MTB have been conducted by microscopic, cultural molecular-biological approach. The environmental conditions are supportive for the development of specific MTB population[7] In Maine environment, the MTB found in coastal environment. The outstanding feature of MTB was the presences of magnetosome. They are not easily cul-

ture, only few strains are available as axenic culture. The proteobacteria are the best characterized are α-*Magnetospirillum magnetotactium MS-1*, *Magnetospirillum magneticum AMB-1*, *Magnetospirillum gryphiswaldense MSR-1*[8]. The *Magnetospirillum gryphiswaldense* was designate as modal organism for molecular-biological studies on magnetite biomineralisation and applications aspects in different fields due to the availability of genetic system and complete draft genome sequence[9]. *Magnetospirillum gryphiswaldense* was isolated from river near to Greifswald in 1990[10]. Helical in shape, flagellated and 2-3m long and 0.5-0.8μm in diameter. The strain was characterized by a slightly higher oxygen tolerance in comparison[11].

Magnetotaxis and magnetosome in MTB

As the magnetic dipole moment of the cell is usually large, the interaction with the earth's geomagnetic field overcomes thermal force and tend to randomize the orientation of the cell in waterbody[12], while swimming the cell shows passive alignment along geomagnetic field are known as magnetotaxis. The term magnetotaxis, is in fact a misnomers, describe the behaviour of MTB. The magnetotactic cell swims neither up nor down a magnetic field gradient, cells display two-way or one-way swimming behaviour along local field lines[13]. The membrane – enclosed magnetosome mineral crystals consisting either magnetite (Fe_3O_4) or greigite (Fe_3S_4)[14]. These are magnetic minerals with high chemical purity, narrow size range, species-specific crystal morphologies and exhibit specific arrangement within the membrane of cell[15]. Mackinawite (tetragonal FeS) and cubic FeS are several other sulphur minerals have been identify and though to be precursors o Fe_3S_4[16]. The reducing condition in environment promotes the formation of greigite[17]. high degree of structure perfection have been displaced by magnetosome crystal. Magnetosome have dimensions within the order of magnitude of large biomolecules of viruses and can be manipulated by external magnetic field gradient and fallow Coulomb's law [18]. As the intermediate of molecule and solid state, these crystals have physical and chemical properties, characteristics of neither the atom nor the bulk counteports[19]. These crystals are now become an ideal component for construction of nanostructure materials and devices. Synthetically prepared magnetic nanoparticles had been studied widely and applied in various field of biotechnology, such as magnetic drug targeting, magnetic resonance imaging, diagnostics, immunoassays, magnetic separation and magnetic hyperthermia treatment. By conventional inorganic synthesis, aqueous salt solution of $Fe^{2+}$and Fe $^{3+}$ co-precipitate to synthesis magnetic iron oxide particles[20]. PH adjustment and formation of aggregates during synthesis and particle purification become a major problem of bulk solution, although enormous efforts were spent from last few years to generate uniform size particles and a challenge is being here to face for the synthesis of advanced nano-sized magnetic materials with innovative properties to tailored and functionalized according to application. the above maintained problems has been alternative route the biomineralisation process have been suggested for the biosynthesises of magnetic nanoparticles.

Structure and biomineralisation of bacterial magnetosome

The morphologies of magnetite crystals in magnetosome are derived from isometric form {111}, {110}, {100}[21]. Anisotropic crystal growth support the elongated and prismatic structure, which could explained by chemical gradient and ion influx. The specific interaction with biomineralisation-mediating protease favoured or inhibits the growth of particular crystal lattice planes selectively[22]. The magnetite biomineralisation is cover up by the specific take-up of iron as Fe(III) or Fe(II) by the cells from the medium, Fe(III) iron reduced to Fe(II) during uptake. in the cytoplasm and transported to magnetosome vesicle[23], then the iron is re-oxidized to prepare highly reactive Fe(III) oxide-ferrihydrite and later react with dissolved Fe^{2+} to form magnetite by vio-solution[20]. The mutant with less ability of magnetosome was more sensitive to elevated concentration of iron[24]. The magnetite particles quality per cell depends on growth condition. Along with availability of micromolar amounts of iron, the microaerobic conditions are also required for the formation of magnetite. The particles of magnetite are oriented with magnetic easy axis {111} within the cell along the chain direction and one or more parallel straight chains are present to the long axis of cell[3]. In this organisation, magnetosome chains of magnetotactic bacteria confers an average magnetic dipole moment of $5\times10^{-6}Am^2$ to the cell[25]. The passive organisation of cell in the earth magnetic field is due to torque exerted by geomagnetic field on

Review

cellular dipole[12]. Thus, the size and shape of magnetosome, length and orientation of magnetosome chain developed in MTB to generate a magnetic dipole and permanent efficient migration along magnetic field lines.

Biochemistry of magnetosome membrane (MM)

The process of magnetite minimization enables regulation by biochemical pathways. MM provides spatial constrains for shaping crystals morphology. pH and redox potential, together are essential for magnetic biomineralisation. The growth of magnetite crystals is regulated by uptake mechanism and depends on controlled flux of ions over MM to provide supersaturating iron concentration in the vesicle. Hence, MM is a control panel to perform specific function in transport and accumulation of iron, crystallization and redox and pH control[26]. The specification in functioning is known by the analyses of *Magnetospirillum gryphiswaldense*. Almost all the strains of MTB indicates similar structure and mechanism for the formation of magnetosome. Due to the presence of an interparticle connection mediated by MM components, strong tendency to form chain have been indicated. Numbers of common fatty acid are identified from MM extract of modal MTB[27].

Phosphatidylethanolamin and phosphatidylglycerol are polar liquid, abundant compared to ornithinamidlipid and unidentified aminolipid in outer and cytoplasmic MM[28]. Magnetosome is associated with highly specific and complex protein subset with various quantities. Only 0.1% of total cellular proteins is MM-bounded polypeptides[29]. The proteomic techniques and biochemical analysis lead to identify almost all major MM proteins (MMPs)[27]. Approximately 20 major polypeptides have been identified in magnetosome subproteome, limited of them occurring in posttranslational modification of resulting gene and numerous minor constituents were found bounded to isolated magnetosome[30]. The proteins bounded tightly to the magnetosome crystals are embedded within membrane and shows resistance towards proteases and detergent (e.g-MamC, MamF) and loosely attached proteins get dissolved in mild detergent selectively[27]. MM almost consist of proteins with covalently bounded C-type heme and glycoproteins are not detected.

On the bases of analysing sequence, number of proteins families can be associated to MMPs and seem shared by all MTB. MamA is abundant protein of MM with 4-5 copies TPR (tetratricopeptide repeat) motifs, TPR have been identified in growing number of proteins with various function and are known to mediate protein-protein interaction[31]. Therefore MamA act as a receptor in MM interacting with cytoplasmic proteins[32]. MamB and MamM are members of CDF (cation diffusion facilitator) family of metal transporter, comprises the protein respond to efflux pumps of toxic divalentcatians and heavy metal ions, postulated to comprise putative iron transporters[33] and necessary to direct uptake of iron within magnetosome. MamE and MamO are similar to HtrA-like serine protease[34]. HtrA like proteins share conserved trypsin-like protease domain and one or two PDZ domain and act as a molicula chaperones[35]. The MM – associated proteins MamC, MamD, MamG and MamF have known homologues in MTB and represent MTB specific protein families. The repeated motif –MamD, Mms6 and MamG share the conspicuous hydrophobic sequence motifs with rich repeated leucine and glycine residues, indicates the common noticeable features to these particles. The proteins interacts with minerals consist of polyelectrolyte group in cluster[36].

Genetic organization and manipulation of magnetite biomineralisation

Almost, all the MMPs are encoded in a single genomic region known as "Magnetosome Island". the gene encode for magnetosome formation are located in three different operon, placed within 35kb genome of MTB. As can be inferred the available genome data of different MTB, the gene order and amino acid sequences of Mam protein are conserved in other MTB[37]. The numerous genes encoding mobile DNA elements are the characteristic of the region within flanking clusters. The mobil DNA elements phase-associated integrates accounts about 22% of coding sequence and participate in extreme genetic instability of this region under conditions of stationary growth[38]. In short, the entire features are strongly reminiscent of genomic islands in other bacteria. They often encode "accessory" gene function[39]. Thus, the large genome "Magnetosome Island" organised essential gene function for magnetite synthesis and may later distributed by gene transfer later.

Functionalizations and application of bacterial magnetosome

The encapsulated magnetosome crystal or magnetite crystal provides a natural coating within MM to ensure the superior dispersibility of particles and shows excellency to target, modify and functionalise the particles. Beside *in vivo* "tailoring", the genetic engineering altered magnetite crystal and biochemical composition of MM. The design of magnetosome is highly promoting with functionalized surface. The design can be achieved by generations of chimeric protein, specifically displayed on the surface of isolated magnetosome. Magnetosome proteins can be used for the construction of functional genetic fusions[34].

Bacterial magnetosomes become an interesting field in broad range of disciplines in Science and technology. magnetosomes are replacing the synthetic magnetic nanoparticles. In Nanotechnology, magnetosomes are considered suitable as components of nano-motors, nano-generators, nano-pumps and other nanometer-scale devices[40]. Magnetosome have been suggested biomedical applications for magnetic drug targeting, magnetic resonance imaging (MRI), magnetic fluid hyperthermia, magnetofection and procedures for separation of biological entities such as cell, nucleic acid, proteins[41]. The hypethermal treatment of tumors has been effectively successful due to magnetic drug targeting[42]. Some techniques could be used to carry anti-inflammatory, anti-bacterial or blood clotting drug to localized the desires regions. Other than this, the bacterial magnetosome are applicable in the field of biotechnology, for the extraction DNA and mRNA from different cells, blood and tissue[43]. The immunoassay based on magnetosome developed to detect antigene, environmental pollution, hormones and toxic substance[44]. Most of the biotechnological applications are depend on interaction of magnetosome with surface modification to interact with certain target molecule. Numerious *in vitro* application of magnetosomes have been suggested, such as magnetic separation, labelling procedures and biomolecule immobilisation. For instance, the bacterial magnetosomes with dendrimer-modification shows 6 fold higher efficiency of DNA recovery rather than artificial magnetic paticle[45]. The mRNA isolation is facilitated by oligo modified magnetosome[43]. Recently, automation of DNA extraction procedure based on dendrimer-modified particles, peptide and enzymes on magnetic particles; allow selection to reuse immobilized enzymes from a reaction mixture[46]. Bacterial magnetosome particles have been successfully harnessed immobilization of enzyme[47].

The antibodies conjugated to magnetosomes, used for automated immunoassays to detect hormones, toxic substance and environmental pollution[48]. The specific separation of target cell from human blood have been successfully done by antibody-modified magnetosomes[49]. Streptavidin-modified magnetosomes, used for automated discrimination of single nucleotide polymorphism[50]. Magnetic force microscope involve for highly sensitive and quantification[51]. Recently, it has been shown that magnetic nano-tubes can be assembled by incorporation of bacterial magnetosome into peptide nanotubes[52].

Perspectives

The application of bacterial magnetosomes has not been commercially exploded because the mass cultivation of MTB is a big problem as it is too expensive for most practical purpose. The limited practicability of ideas is the lack of details of biochemical and genetic principles with the bacterial cell responsible for magnetite biomineralisation. The mechanism for nucleation and growth of particles is still a critical issue to know. The iron transport mechanism through membrane is under investigation. The efforts regarding magnetotactic bacteria should directed to the genetic and molecular biological analysis of magnetosome formation. It has been analysed that the construction of gene fusion to couple bioactive substances have performed by magnetosome-specific proteins[53]. Once the proper genes for biomineralisation pathway would detected, the gene might be expressed to cultivable host organism, thereby potentially remove the difficulties related to MTB cultivation. A single Gene of *Magnetospirillum* has already been expressed in *E.Coli* but, due to the lack of intracellular compartments limits the heterologous expression of genes related to bacterial magnetosome biomineralisation. The dramatic diversity of MTB should increases the study on related species to know the biomineralisation process within cell. Finally the problem related to the expression of gene could be undertaken by cloning of gene to the host individually. Further detail studies are required to innovate and modify the application aspects of bacterial magnetosome.

Review

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