Trends in Biochemical Sciences

CellPress

Review

Ferroxidase-Mediated Iron Oxide Biomineralization: Novel Pathways to Multifunctional Nanoparticles

Kornelius Zeth,[1,3,4,5,*] Egbert Hoiczyk,[2] and Mitsuhiro Okuda[3,4]

Iron oxide biomineralization occurs in all living organisms and typically involves protein compartments ranging from 5 to 100 nm in size. The smallest iron-oxo particles are formed inside dodecameric Dps protein cages, while the structurally related ferritin compartments consist of twice as many identical protein subunits. The largest known compartments are encapsulins, icosahedra made of up to 180 protein subunits that harbor additional ferritin-like proteins in their interior. The formation of iron-oxo particles in all these compartments requires a series of steps including recruitment of iron, translocation, oxidation, nucleation, and storage, that are mediated by ferroxidase centers. Thus, compartmentalized iron oxide biomineralization yields uniform nanoparticles strictly determined by the sizes of the compartments, allowing customization for highly diverse nanotechnological applications.

Bacterial Ferroxidase-Mediated Biomineralization

In nature, biomineralization is a widespread phenomenon that leads to the formation of stable and well-ordered carbonate, calcium phosphate, silicate, and iron oxide minerals (Box 1) [1–5]. To achieve biomineralization, cells must precisely control several factors, including the concentration of ions, nucleation processes, the pH, and the redox potential; a task that is usually accomplished through compartmentalization. Compartmentalization offers both highly controlled environments and allows cells to influence the composition, morphology, and size of the developing minerals.

Inside cells, compartmentalized ferroxidases form highly uniform, nanometer-sized iron oxide particles [5–9]. Various such ferroxidase compartments exist in bacteria, ranging from the small Dps cage to mid-size (bacterio)-ferritins to the large virus capsid-like encapsulins (Figure 1) [10–12]. The formation of iron-oxo particles inside these compartments appears to fulfill two functions: first, the rapid uptake, oxidation, and storage of free iron effectively protects the cells from the Fenton reaction, a process that results in the formation of highly toxic hydroxyl radicals (OH) that can damage cellular components such as DNA, lipids, or proteins [13–16]. Second, the formation of iron-oxo particles enables cells to precisely control the storage and mobilization of iron to maintain homeostasis of this highly important and chemically difficult to manage micronutrient [17,18]. Some bacteria [19] and algae [20] possess a further type of iron-oxo-forming compartment. These structures, termed magnetosomes, contain 50–100 nm diameter magnetite (Fe_3O_4) or greigite (Fe_3S_4) cores, formed inside protein–lipid envelopes through poorly understood processes (Figure 1A) [5–9]. Because of their high iron content and unusual magnetic properties, magnetosomes are extensively studied for nanotechnological

Trends

Iron pathways inside the compartmentalized protein cages Dps and ferritin have been extensively studied, and the structural basis for iron translocation and oxidation has been unraveled.

Iron-oxo clusters have so far only been identified in type II Dps enzymes, indicating a probable structural basis for the onset of biomineralization at the atomic level.

Dps, ferritin, and encapsulin provide compartments for the formation of nanoparticles on the length scale of 5–30 nm. This range of diameters allows various applications of defined nanoparticles in bionanotechnology.

The structural features of ferritin have been farthest exploited to produce nanoparticles of variable chemical composition for semiconductor devices and to functionalize molecules for vaccine design and drug delivery.

[1]Universidad del Pais Vasco (UPV)/Euskal Herriko Unibertsitatea (EHU), Department of Biochemistry and Molecular Biology, 48940 Leioa, Spain
[2]University of Sheffield, Department of Molecular Biology and Biotechnology, Sheffield S10 2TN, UK
[3]IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain
[4]Cooperative Research Center (CIC) nanoGUNE Consolider, E-20018, Donostia-San Sebastian, Basque Country, Spain
[5]University of Roskilde, Department of Nature, Systems and Models, Roskilde, 4000, Denmark

*Correspondence: kzeth@ruc.dk (K. Zeth).

 http://dx.doi.org/10.1016/j.tibs.2015.11.011

Box 1. Principles of Biomineralization and Cage-Shaped Proteins (CSPs)

Biomineralization is a process by which living organisms form and maintain highly ordered inorganic mineral structures. This process can occur inside or outside of cells and yields minerals ranging in size from nanometers to centimeters. There are two classifications for mineralization processes at biological interfaces: biologically induced mineralization and biologically controlled mineralization [72]. In prokaryotes, the process of biologically induced mineralization occurs extracellularly and leads to the formation of minerals as a result of metabolite secretion. The minerals formed are induced but not controlled by biomolecules or cells, respectively. By contrast, biologically induced mineralization or biomineralization is often matrix-controlled and allows organisms to create elaborate structures such as bones, teeth, seashells, or exoskeletons. The highly diverse architectures of these structures indicate that their formation follows complex genetically regulated programs resulting in composite materials that help to stabilize and protect the organism from mechanical stress. Because the resulting structures are often mechanically and functionally superior to man-made materials, there is great interest in understanding and mimicking the processes used by organisms or cells to initiate, maintain, and control mineralization [1,69,71,73].

Three conditions have been identified as necessary for biomineralization to occur: (i) the matrix properties must facilitate mineralization, (ii) ions must be supersaturated, and (iii) for access to the matrix for nucleation to be induced, the volume for biomineralization must be limited. Ferritin is certainly the best-studied iron metabolism protein and represents one of the simplest systems to study biomineralization both *in vivo* and *in vitro* [74]. The matrix properties here are provided by the cage architecture (ferroxidase centers, nucleation groove, etc.), the access for ions is provided through translocation channels, which restrict other molecules from reaching the growing mineral, and the protein shell strictly limits the size of the mineral. A similar but more complex process underlies the formation of magnetosomes in bacteria and algae [21,75]. While the nucleation events leading to mineralization are dependent on protein surfaces, the membrane and its integral transport systems strictly limit access to the vesicle interior. Finally, the volume and shape of magnetosomes are further regulated by a complex interplay of membranes and proteins resulting in morphologies ranging from cubic to rectangular, bullet-, tooth-, or even arrow-shaped. Far more complex processes control extracellular biomineralization processes such as the formation of bones [69,73]. In this case a composite amalgam of calcium phosphate, collagen, and water leads to the formation of mineralized tissues whose stiffness and elasticity properties are tightly adapted to local requirements. Thus, combining biomineralization and protein scaffolds can generate high-performance composite materials that are extremely strong, stiff, and low in weight; characteristics that are highly desirable for technically manufactured materials.

applications (Figure 1A) [9,21–23]. Although magnetosomes result from iron-oxo biomineralization, they will not be discussed here because of their distinct phospholipid bilayer shell, their different iron-oxo mineral content, and their specialized function as magnetic sensors; the interested reader is referred to recent reviews [7–9]. In this review we focus on recent advances in our understanding of bacterial ferroxidase-mediated iron-oxo biomineralization and its potential for nanotechnological applications. In particular, our increased understanding of the atomic processes that control iron-oxo formation within these compartments (e.g., mineral growth, protein–atom/mineral interface interactions) hold the promise to design nanoparticles for a wide range of applications. We describe the atomic structures of the ferroxidase-containing compartments, summarize recent results that point towards a converging mechanistic picture of the atomic processes involved in iron-oxo formation, and discuss current and future nanobiotechnological applications.

Principles of Iron Biomineralization in Dps Enzymes

The smallest protein complexes containing ferroxidase centers (FOCs) are the dodecameric Dps (DNA protection during starvation) proteins of approximately 18 kDa which form 9 nm diameter cages with a storage capacity of about 500 iron (III) atoms (Figures 1C, 2A). Despite being initially linked to starvation, the archetypical functions of all Dps enzymes are the detoxification and storage of dispensable free iron (II) [13]. In addition, a subgroup of these enzymes protects the cell's DNA during the stationary phase via a Dps-driven condensation reaction [12,14]. Structurally, each of the 12 Dps monomers are formed by a four-helix bundle, also termed a ferritin-like fold (Figure 2B,C) [24]. Each functional dimer contains two FOCs located at the 1350 $Å^2$ protein interface with two of the four helices, α1 and α4 facing the cavity interior, while α2 and α3 and the connecting helix point outwards (Figure 2C). Three conserved residues form each FOC: an aspartate and glutamate from one monomer, and a histidine from the symmetry-related monomer (Figure 2A,C).