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Synthesis of inorganic and organic crystals mediated by proteins in different biological organisms. A mechanism of biomineralization conserved throughout evolution in all living species

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ABSTRACT

The synthesis of crystals through biomineralization is a process of protection and support preserved in animals, protists, moneras, plants and fungi. The genome of every species has evolved to preserve and/or modify the formation of one or another type of crystal, which may be of the organic or inorganic type. The most common inorganic crystals identified in organisms include calcium carbonate ($CaCO_3$), calcium phosphate (CaP), calcium oxalate (CaOx), magnetite or greigite, and sulfides of cadmium (CdS), mercury (HgS) and lead (PbS). Organic crystals are of the protein or ice type. The formation of both types of crystals requires biomolecules such as proteins. This paper reviews the proteins involved in the synthesis of different crystals in distinct biological systems, in order to understand how each organism has adapted its genome to preserve essential mechanisms such as biomineralization, which has enabled them to survive in a changing environment for millions of years.

1. Introduction

The formation of crystals in biological systems is a process called biomineralization that takes place from bacteria to humans, and is perhaps the mechanism most conserved during evolution that connects inanimate matter with living beings. The earliest record of existence of life was detected in ~3465 million years old microbial fossils in a bedded chert unit of the Early Archean Apex Basalt of northwestern Australia [1], and recently Dodd et al. (2017) describe putative fossilized microorganisms that are at least 3770 and possibly 4280 million years old in ferruginous sedimentary rocks in Quebec, Canada [2]. This fact allows us to consider the possibility that these ancestral microorganisms carried out both intracellular and extracellular mineralization, biologically induced by the precipitation of minerals present in the environment [3], a characteristic that has been preserved in organisms through evolution. Biomineralization allows organisms to generate materials that provide both protection and support. The distribution of biogenic minerals among the five kingdoms shows that they are synthesized by animals, protists, monerans, plants and fungi [4]. Calcite is one of the minerals that has been conserved in organisms for approximately 500 million years. This polymorph of calcium carbonate ($CaCO_3$) is more common than the aragonite form [3]. Examples of biosynthesized $CaCO_3$ crystals include mollusks, spicules of some marine sponges, pearls, shells and nacre; also teeth, bones and otoliths of all vertebrates including humans as well as the eggshells of birds and sea urchins [5–14]. Other calcium crystals, such as calcium phosphate (CaP) and calcium oxalate (CaOx), are found in humans [9] and in plants [15,16]. Interestingly, there are microorganisms such as magnetotactic bacteria that can form magnetite or greigite crystals [17]. In nature, magnetite is a mineral produced at elevated temperatures and pressures in igneous and metamorphic rocks, but it cannot be generated in the biosphere [3]. This fact shows how magnetotactic bacteria were able to adapt their genome to synthesize crystals of magnetite and survive in their environment. In addition to bacteria, yeasts also produce crystals through biomineralization. Some of these yeasts are *Saccharomyces cerevisiae* [18], *Schizosaccharomyces pombe* and *Candida* species such as *C. albicans, C. glabrata, C. krusei* and *C. parapsilosis* [19]. *S. pombe* can form crystals of cadmium sulfide (CdS) [20–22], while *Candida* species form crystals of CdS, HgS or PbS [19]. Among the microorganisms that are able to form non-inorganic crystals as a defense mechanism is the bacterium *Bacillus thurigiensis*, an insect pathogen [23] that has a parasporal body which is a crystalline protein with insecticidal properties [23,24]. Another type of non-inorganic crystals are ice crystals; the bacteria that has been reported as having great ability to form ice crystals are those of the genus *Pseudomonas*, such as *P. syringae, P. fluorescens, P. viridiflava* and *P. Borealis*, as well as strains of *Erwinia herbicola* and *Xanthomonas* [25–27]. However, in order to make possible the formation of any crystal of biogenic origin,

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either organic or inorganic, organisms require the help of proteins, which are one the main biomolecules involved in biomineralization processes [28]. The identification of proteins that participate in the synthesis of crystals is very important because it allows the obtainment of crystals *in vitro* emulating the *in vivo* conditions [29]. The aim of the present article is to review the role of proteins involved in the formation of different types of crystals in a variety of organisms. This is to understand how each organism has been able through evolution to adapt its genome to preserve essential mechanisms such as biomineralization, allowing them to survive for millions of years.

2. The proteins involved in the formation of inorganic crystals are mainly anionic biomolecules

2.1. Calcium crystals

The formation of inorganic crystals mediated by biomolecules in any species is of great interest in many areas such as biochemistry, biomaterials, industry, biosensors and medicine, among others [9,30,31]. Biomolecules that favor nucleation, crystallization, and size of inorganic crystals are mainly proteins, lipids and carbohydrates [13,32,33], with a primary role of proteins [28]. The identification of these proteins has been very important as it has allowed crystals to be obtained *in vitro* emulating the *in vivo* conditions [29]. The most common biomineral whose synthesis is directed by proteins is $CaCO_3$ (Fig. 1) [13,28].

$CaCO_3$ crystals are found in mollusks, spicules of some marine sponges, pearls, shells and their nacre, in avian eggshells, sea urchin, teeth, bones and otoliths [5–14]. These crystals may take the form of aragonite, calcite and vaterite [34–36]. As calcite, they can present/display rhombohedral shape, foliate or chalk structures, while aragonite can be prismatic, granular prismatic and foliated, and finally the vaterite can adopt multiple structures [35–37]. The shell of the pearl oyster *Pinctada fucata* is of special interest because it presents two types of crystalline structures of $CaCO_3$ [12], the outer layer is formed by prismatic calcite, while the inner nacreous layer is aragonite [12,14]. It has been shown that the proteins involved in formation of the outer layer originate from the epithelial cells of the mantle, while those involved in the inner layer come from the pallial region [14]. The first protein that was identified in *P. fucata* was a 60-kDa polyanionic protein called nacrein which, by cDNA analysis, revealed two domains, one of which is similar to carbonic anhydrase [38]. It has been suggested that the function of nacrein is to inhibit the precipitation of $CaCO_3$, so that it is considered as a negative regulator in the calcification of the extrapallial space [39]. In addition to nacrein, other proteins in *P. fucata* that participate in the formation of nacreous and prismatic layers are MSI60 (Gly-Ala rich) and MSI31 (Gly-Glu rich) proteins [40]. It was also found that β-chitin and silk-fibroin determine polymorphism for the synthesis of aragonite and calcite crystals [41]. Other proteins that induce the formation of aragonite crystals are from the family called N16 [42]. Within this family, three open reading frames were identified with a high content of acidic (Gly, Tyr, Asn, and Cys) and aromatic amino acids. The acidic amino acids were found in four domains of the sequences and possibly these are the regions that can serve for adsorption to other components of the aragonite crystals and in this way controlling the formation of the crystals [42]. MSI7 is also a small molecular weight protein (7.3 kDa) identified in *P. fucata* [43], which is like MSI31 [40]. It is believed to accelerate nucleation and the precipitation of $CaCO_3$, determining the texture of the mother-of-pearl. The matrix protein, Prismalin-14, is also an anionic protein identified in the prismatic layer of *P. fucata* [44]. By means of scanning electron microscopy it was found that Prismalin-14 affects the crystallization of $CaCO_3$ *in vitro* and by means of *in situ* hybridization it was found that the Prismalin-14 mRNA is expressed in the inner part of the shell. As a result, Zuzuki et al. (2004) suggest that it is involved in the prismatic shell layer [44]. Another protein that participates in the precipitation of calcium carbonate is N19 (19 kDa), which is a negative regulator of calcification in the pearl oyster [45]. Amorphous calcium carbonate-binding protein (ACCBP) is an extra-pallial fluid protein which is believed to inhibit calcite growth and induce the formation of amorphous calcium carbonate, as well as to recognize the different crystalline phases of $CaCO_3$, a function that would allow ACCBP to inhibit the growth of undesired aragonite crystalline faces and thus to maintain controlled shell formation [46]. Pif97 and Pif80 are acidic matrix proteins that are bound to the crystals of aragonite and regulate the formation of nacre [47]. In order to elucidate the formation of nacreous aragonite, a work was carried out in which the role of the matrix proteins described above was evaluated in the initial stages of nacre formation [14]. It was found that Nacrein, MSI60, N16, N19 and Pif 80 have similar expression patterns during nacre growth, suggesting that these proteins regulate the nucleation of aragonite and growth with the inner shell film [14]. In contrast to these proteins that favor the synthesis of aragonite, PfN44 is an acidic matrix protein that inhibits the crystallization of aragonite, but participates in the formation of the shell of *P. fucata* [48]. PfN44 can bind calcium and magnesium, but has a stronger affinity for magnesium. During crystallization of $CaCO_3$, it regulates the magnesium content of the crystalline carbonate polymorphs and stabilizes magnesium calcite to inhibit the synthesis of aragonite (Fig. 2) [48].

In the synthesis of the nacre of *P. fucata*, as described above, acidic proteins have been identified. However, interestingly, a basic protein, PfN23, has also been identified which was localized specifically in the nacreous aragonite layer [49]. This suggests that this protein participates in the control of the synthesis of aragonite crystals in nacre. The PfN23 sequence showed that it has a putative signal peptide at the N-terminus, a negatively charged region, a hydrophilic region, a disordered region, and a C-terminal basic tail [49].

Other species where protein-mediated $CaCO_3$ crystal synthesis has been reported include otoliths in *Oryzias latipes* [13], in bird eggshells such as *Struthio camelus* (ostrich) [5,6,50], *Dromaius novaehollandie* (Emu) [5], *Gallus gallus* (chickens) [7], *Meleagris gallopavo* (wild turkey) [11], and *Taeniopygia guttata* (Zebra finch) [10], in the mollusk nacre layer [51], and in humans [9].

The Stm-I protein has been identified in the formation of otoliths in *O. latipes* [52,53]. Through biochemical, biophysical and *in silico* analyses, the recombinant Stm-I protein was found to have properties of a coil-like intrinsically disordered protein, which affects the shape, size and number of calcium carbonate crystals [13,52]. Interestingly, the

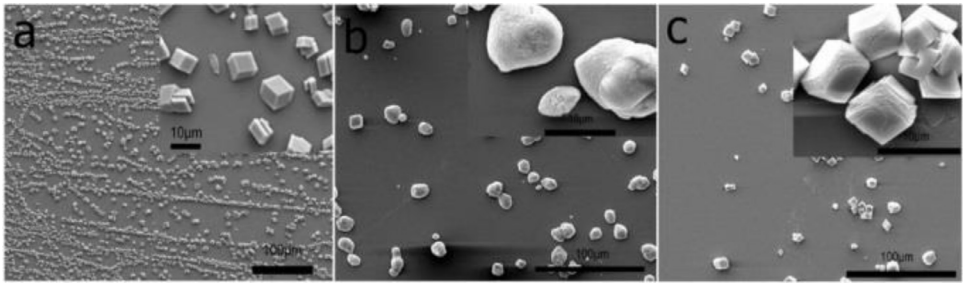

Fig. 1. Influence of proteins in crystals formation. Scanning electron microscope (SEM) images of $CaCO_3$ crystals obtained without the addition of any proteins (a) and in the presence of $\sim 2.5\ \mu g\ mL^{-1}$ ESM-P (b) and ESM-N (c). ESM: EDTA-soluble matrix; P: prismatic layer; N: nacreous layer. Reprinted from C. Liu, L. Xie, R. Zhang, Heterogeneous distribution of dye-labelled biomineralizaiton proteins in calcite crystals, Sci. Rep. 5 (2015) 18,338, with permission from SpringerNature, under a Creative Commons Attribution 4.0 International License.