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Editorial

Transient precursor strategy in mineral formation of bone

Abstract

The strategy in biomineralization of initially depositing a less ordered mineral and then transforming it into a more crystalline mature phase is probably widespread among invertebrates. The report in this issue by N.J. Crane, V. Popescu, M.D. Morris, P. Steenhuis, M.A. Ignelzi, *Raman spectroscopic evidence for octacalcium phosphate and other mineral species deposited during intramembraneous mineralization*. Bone (In press), using micro-Raman spectroscopy to study early mineral deposits in mice calvaria, provides strong evidence that the transient precursor strategy also occurs in vertebrates.

Keywords: Biomineralization; Calcification; Transient bone mineral; Octacalcium phosphate; Raman spectroscopy

Precipitation in vitro often involves the initial formation of a disordered more soluble phase that subsequently transforms into a less soluble and usually more ordered form (Ostwald-Lussac Law of Stages). If however a nucleation substrate is present, the mature phase may be induced to form directly. As most biological mineralization processes appear to control the nucleation step, often resulting in the formation of oriented crystals, it has been tacitly assumed that the nucleation process is responsible for the formation of the first mineral phase. This may not be the case. Studies of various invertebrate mineralization processes show that biology may well be utilizing both approaches to control mineral formation: the transient precursor strategy as well as the nucleation strategy. The study by Crane et al. [1] in this volume provides strong evidence that this may also be the case in vertebrates.

The first unequivocal evidence for the presence of a transient precursor mineral phase in biomineralization was presented by Lowenstam and colleagues in their studies of the formation of magnetite in the tooth of the chiton, a mollusk that lives in the rocky intertidal zone ([2]; reviewed in [3]). They showed that the first mineral formed is a poorly ordered hydrated iron oxide phase called ferrihydrite, which after a few days transforms into crystalline magnetite [2]. It was subsequently shown that the other mineral phase in the chiton tooth, carbonated apatite (the same mineral present in bone and teeth), also forms via a precursor phase, in this case amorphous calcium phosphate (ACP) [4].

In 1989, Lowenstam and Weiner [3] listed all the biological mineralization processes they were aware of that involved a precursor mineral phase. Among the 8 processes listed (Table 3.2) is the transformation of octacalcium phosphate (OCP) into carbonated apatite in bones and teeth, the one reported now by Crane et al. [1]. A more detailed discussion of this phenomenon by Lowenstam and Weiner elsewhere in the book (p.164) relates to the large body of evidence amassed by the pioneer of this subfield, Walter Brown, in support of an OCP precursor phase [5]. As convincing as he was, he did not have the "smoking gun" evidence now presented by Crane et al. [1]. The "hard" but indirect evidence available at the time was an in vitro study by Nelson et al. [6], who showed that the core of synthetic carbonated apatite crystals contains an OCP-like phase. As this so-called central dark line also exists in biological carbonate apatites, it is reasonable to expect that they too form via an OCP precursor phase. More observations of this nature have since been reported. For example, Tohda et al. [7] reported the presence of OCP in first-formed enamel crystals. X-ray diffraction studies of the bones of human fetuses point to the presence of a calcium deficient hydroxyapatite phase [8]. Fascinating high resolution TEM images of the very early formed mineral deposits in vertebrate tissues also provide evidence for the presence of relatively disordered phases [9]. Studies of this type, however, involve a lot of sample manipulation and usually dehydration, leaving open the question of the nature of the early formed mineral phase.

Detection of a transient mineral phase is very difficult. Unless the biological process naturally separates the stages of mineral formation (as is the case in the chiton teeth), it is a real challenge to analyze the first-formed mineral deposits in a tissue without altering them by for example, dehydration or irradiation, and before they transform into a more mature phase. In order to partially alleviate this problem, we studied mineralization processes in various invertebrates that rapidly

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produce relatively large amounts of mineral more or less synchronously, and used detection techniques that can be readily applied and preferably do not involve dehydration. The most useful in this regard is micro-Raman spectroscopy; the technique used by Crane et al. [1]. The laser beam can be focused through a microscope to an area of a few microns diameter, and the sample can be wet. Furthermore, being a spectroscopic technique, Raman is very sensitive to variations in the atomic order of the phase being analyzed, including disordered phases. Amorphous calcium carbonate has been found to be the first-formed transient mineral phase in both forming larval and adult echinoderm skeletons [10,11], larval mollusk shells [12], crustacean cuticles [13] and is inferred to be present in corals [14]. The mature mineral formed in these phyla is either calcite or aragonite. With this broad taxonomic distribution, it is reasonable to assume that the transient precursor phase strategy is widespread.

Crane et al. [1] adopted a similar strategy. They used micro-Raman spectroscopy to monitor mineral formation at the suture boundaries of mice calvaria, and by adding FGF2 to the medium, they induced rapid and somewhat synchronized mineralization. The spectra obtained show the presence of OCP. This I regard as "smoking gun" evidence that OCP is a precursor phase of carbonated apatite. In fact, there is also an indication that amorphous calcium phosphate (ACP) may form before the OCP.

The possibility that the transient mineral strategy is used by vertebrates has been investigated since the 1960s. Termine and Posner [15] proposed that ACP may be a precursor phase in bone formation. In 1972, Fuhredi-Milhoffer et al. [16] showed that in vitro the first-formed phase is ACP. This subsequently transforms into OCP and finally into carbonate apatite. Glimcher [17] reviewed the state of affairs in 1984 and concluded that whereas there is no evidence that ACP is a mature phase in bone, the possibility that it is a precursor phase in bone formation has not been excluded. Is the report by Crane et al. [1] the end of the story? Probably not. It certainly represents a milestone achievement in what really is a very tricky subject. Furthermore, the results need to be confirmed independently and in other vertebrate mineralization processes.

In the better understood transient calcium carbonate forming systems (reviewed in [18,19]) there is good reason to expect that the transient mineral forming strategy is coupled to the nucleation strategy; namely that the first-formed phase is subsequently induced to crystallize from a nucleating substrate. The first-formed amorphous calcium carbonate phases do not diffract X-rays, but have been shown to have short range order that in two cases resemble the structure of the mature phase [20,21]. The biological system somehow imposes this order. It also initially prevents the mineral from crystallizing in an uncontrolled manner, and subsequently triggers its transformation into the mature phase. Clearly, there must be a complex mechanism in place to perform all these tasks, including apparently dedicated proteins [22] often pre-positioned in a 3-dimensional framework. One possible advantage of using a disordered phase initially is that its shape can be molded "at will"; something that is difficult to do with the de novo growth of crystals. Another advantage is that the amorphous mineral phase is in essence a highly concentrated solution, and upon crystallization, a lot less water needs to be removed from the mineralization site [19]. If indeed ACP is the initial phase in bone formation, it is perhaps at this stage that the mineral can be introduced into the very small spaces (gaps) within the collagen fibril.

Transient precursor mineral formation is clearly a fundamental issue to be understood in bone and tooth mineralization. Walter Brown and colleagues [5] recognized this and Crane et al. [1] have moved the field forward significantly. Much still needs to be learned about the macromolecules that may well be orchestrating the process, and whether or not the precursor phases are structurally tailored as they are in invertebrates. With this information in hand, the issue of whether or not some pathologies can be ascribed to the malfunctioning of this process, can also be addressed.

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