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The ACC strategy in biomineralization: the case of earthworm's amorphous spherulites

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The occurrence of amorphous calcium carbonate (ACC), an hydrated and highly soluble form of solid $CaCO_3$, seems to be a common feature in all carbonate forming organisms such as mollusks, corals, echinoderms and crustaceans. The ubiquity of ACC in these Ca-carbonate biomineralizing systems, as a precursor of further crystalline phases, has recently open the interesting question about if the formation of an amorphous phase is a necessary step in the calcification process of all organisms and consequently, whether it would be possible to define the "amorphous precursor estategy" as a general mechanism in biomineralization. Neverthelees, although ACC appears to be widespread in these organisms very little is known about its particular role in the biomineralization scheme of the different phyla.

The formation of $CaCO_3$ spherulites in the calciferous glands of earthworms is a particular case of calcareous biomineralization involving the presence of ACC as a transient precursor phase [2]. Interestingly, the formation of crystalline carbonates via ACC in these organisms is not connected with skeleton building so it must play another functional role. In addition, the transient transformation stages can be followed by in situ spectrometric techniques and therefore, earthworms provide and adequate model to analyse the mutual interactions between ACC-solvent-and crystalline phases.

In this study, we have analysed the morphological and structural transformations from the initial ACC spherulites until the formation of the crystalline phases: vaterite (and/or aragonite) and finally calcite, is accomplished. The characterization of ACC was done by performing in situ FT-IR, together with and HREM and Debye scherrer -XRD.

The structural results were interpreted in the light of the histological study of the gland. The geometry of the secretory epithelium of the calciferous gland, as evidenced by TEM [2], shows the presence of irregulary shaped cells with their apical surface consisting of dendritic indentations and club-shaped expansions extending to the inter-lamella spaces. The cell basal area is extremely folded and contains abundant mitochondria and membranous infoldings. This morphology could provide the explanatory mechanism by which the calcium present in the blood enters into the gland. Furthermore, the presence of spherulites in the interlamellar space and wrapped by an organic matrix suggest that the calcification process is under organic control.

Further information of the intermediate transformation stages in the ACC spherultiths was provided by the FE-SEM analysis. That shown the collapse of the internal structure of spherulits into radially distributed regions associated with the organic matrix. As ACC is a highly soluble form of $CaCO_3$ that includes a water molecule in the structure, its transformation to one of the crystalline polymorphs, would necessarily involve the release of H_2O and the Ca^{+2} ion. As a result of this, a gradual decrease in volume at the inner part of the spherulites is observed.

Taken these findings together, we conclude that the transformation of the initially stabilised ACC occurs via a 'source-sink mechanism'. Accordingly, the uptake of Ca by the cells (sink) causes a decrease in the [Ca^{+2}] of the extracellular fluid. This, in turn, will induce the dissolution of the spherulites (source), with the subsequent release of H_2O and Ca^{+2} so that the Ca activity in the extracellular fluid is maintained. As result of this process,

while the concentrations of ACC become unsaturated, both vaterite and calcite are still supersaturated and crystallization can be then initiated via an heterogeneous nucleation mechanism at the spherulite surfaces. This mechanism can be used by the orgnism as an efficient way to control the concentration of Ca+2 in the extracellular fluid within its physiological range.

References

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