Role of proteins in the mineralization of collagen fibrils: implication in pathological processes

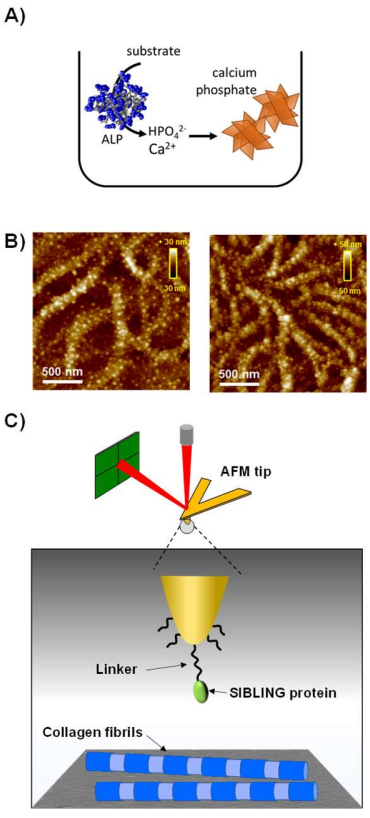
Biomineralization is orchestrated by protein-mineral interactions, where living organisms direct nucleation and growth of inorganic materials. In bone, type I collagen fibrils is suggested to be the place where calcium phosphate (CaP) crystals nucleate and subsequently grow. This process, called intrafibrillar mineralization, is believed to be directed by non-collagenous proteins, present in the extracellular matrix (ECM), even if the role of collagen fibrils during CaP mineralization remains unclear, as it is difficult to achieve in vitro. This hypothesis is mainly made based on observations of late in vitro mineralization events, generally requiring extensive sample preparation procedures prior to imaging or cryogenic preparation.

Meniscus is another connective tissue the ECM of which is mainly composed of type I collagen self-assembled into fibrils. Interestingly, while bone tissue is mineralized, meniscus is not. Moreover, during a pathological remodeling, such as in osteoarthritis, both tissues are damaged. These differences may result from a differential expression of non-collagenous proteins important for tissue mineralization of healthy and pathological bone and meniscus. These proteins mainly include proteins of the small integrin binding N-glycosylated (SIBLING) family.

The main aim of this project is to unravel the mechanism by which SIBLING proteins are involved in the mineralization of collagen fibrils and to provide (i) quantitative insights regarding biomolecular interactions and (ii) continuous real-time monitoring of the spatial distribution of the mineral crystals within collagen fibrils at the nanoscale.

For this purpose, the model system recently developed in the lab (1) will be used and expanded. In this system, one of the two precursors of the CaP minerals is generated in situ by an enzyme, the alkaline phosphatase (ALP). We showed that this release of phosphate allows a control of the mineralization in time and space, in biomimetic conditions (2).

The first step of this study will be dedicated to investigate the role of SIBLING proteins in the mineralization by dynamic light scattering (DLS) (Fig. A). Indeed, we showed that the use of the DLS technique could be powerful tool to follow in real time the mineralization, allowing to identify and isolate key intermediates during the process (3). This study could also be the opportunity to further extend the enzymatic control through the introduction of a second enzyme, thus implementing an enzymatic cascade to generate phosphate.



In a second step, we will take advantage of recent progresses made in the use of atomic force microscopy (AFM) to (i) probe real-time events at biological interfaces, and (ii) record information at the single-molecular level (4). AFM experiments will be conducted on self-assembled collagen fibrils immobilized on a planar surface (Fig. B) and images will be recorded in real-time. AFM will be also used in the spectroscopic mode to probe the interaction between SIBLING proteins and collagen fibrils (Fig. C), to address important issues that remain to be unraveled such as: what kind of force is responsible for the specific binding, if any? What can/cannot be recognized by SIBLING proteins? To this end, we intend to probe events at the single-molecule level using dynamic force spectroscopy between SIBLING proteins and collagen fibrils.
Real-time measurements will be explored to determine the rate of nucleation, while dynamic force spectroscopy will be used to quantify the free energy of binding between individual proteins and collagen fibrils. These data will help to provide a clear view regarding the role of collagen, in combination with SIBLING proteins, in CaP mineralization: a template or a good binder?

Candidate background

The candidate must hold a master degree or equivalent in (bio)chemistry or materials science with solid skills in experimental practice and critical scientific-thinking.

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