Exploring energetics of confined nucleation for bioapatite formation

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Although bioapatite formation in fibrillar collagen is a key process for biomineralization of bones and teeth, there has been little exploration of its nucleation pathways and their energy barriers under nanoscale confinement, which significantly influences the physico-chemical properties of mineral phases. Recently, we observed that the CaP nucleation pathway in confined collagen gap regions (e.g., ~2 nm high and ~40 nm long) is clearly distinct from the nucleation occurring in unconfined extrafibrillar spaces. Our findings suggest two different nucleation energy barriers, with and without nano-confinement. However, due to the complexity of collagen's fibrillar structure and the lack of *in situ* analytical tools, evaluation of nucleation energy barriers for these two mineralization behaviors is challenging. In this study, we separately evaluated the energy barriers to nucleation in confined gap regions and unconfined extrafibrillar spaces using *in situ* synchrotron-based small-angle X-ray scattering. *N*ucleation rates were measured in the simulated body fluids at different supersaturations (i.e., $ln(IAP/K_{sp})$ ranged 0.59-1.13, with respect to amorphous calcium phosphate, where *IAP* is the ion activity produect and K_{sp} is the solubility product). Without any nucleation inhibitors, nucleation dominantly occurs in the extrafibrillar space. However, with polyaspartic acid as a nucleation inhibitor in the extrafibrillar space, most nucleation occurred in the confined collagen gap regions, leading to nucleation of 2-dimensional bioapatite whose size is close to the gap dimension in size. Consequently, the active surface area for nucleation is limited only to the sidewalls of nuclei, which decreases the surface energy and thus lowers the total nucleation energy barrier. Based on these observations, we developed a new modified classical nucleation theory to better reflect the geometry of the confined space of collagen gap regions. Our findings highlight the importance of energetics on nucleation of bioapatite in the nanoscale confined spaces and proved a new framework to explain the energy landscape during biomineralization.