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3D Nanoscale Analysis of Protein-Mineral Nanoparticle Interfaces Using Atom Probe Tomography for Understanding Amelogenesis

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The interaction of organics with mineral surfaces plays a significant role in biomineralization. One particular instance highlighting the significance of organic-inorganic interfaces is during enamel formation (or amelogenesis), where at the onset of mineralization it is hypothesized that the protein amelogenin adsorbs onto amorphous calcium phosphate (ACP) nanoparticles, promoting their aggregation and affecting their transformation to hydroxyapatite (HAP). The interaction of amelogenin with calcium phosphate phases at different stages in turn is largely responsible for the unique properties in enamel. However, there remains a lack of understanding of the nanoscale mechanisms involved in the transformation of calcium phosphate minerals in the presence of amelogenin due to the difficulty in effectively tracking the protein distribution on mineral phases. This necessitates developing a sub-nanometer scale, spatially resolved understanding of both metastable and stable phases of calcium phosphate minerals, the boundaries between these phases, and the interactions of these phases with amelogenin from early through to the final stages of HAP mineral growth.

In this study the location of amelogenin relative to the ACP precursor nanoparticles was directly visualized using three-dimensional atom probe tomography (3D APT) to determine mechanisms influencing particle aggregation and transformation to HAP. A systematic and analytical approach was developed for analyzing complex organic-inorganic interfaces with APT. First, a simple protein-mineral interface, i.e. amelogenin adsorbed onto the surfaces of HAP microparticles, was analyzed by APT to develop a protocol for analyzing the spatial distribution of protein at complex interfaces (Figure 1a). Mass spectra analysis combined with 3D atomic maps reveal that the amelogenin protein is effectively identified using CO-species (i.e. CO^{1+} and CO_2^{1+} species at 28Da and 44Da, respectively) and that it adsorbs onto the HAP surface, as expected. In turn, this protocol was applied to probe the protein distribution in amelogenin-mineralized HAP nanoparticles (Figure 1b). A previously developed specimen preparation technique was also utilized to successfully capture the HAP nanoparticles within APT tips.[1] In contrast to the simple protein-mineral interface highlighted by the microparticles, amelogenin was found to be heterogeneously and nonrandomly distributed across aggregated nanoparticles. Furthermore, the protein was occluded and/or trapped within the phosphate phase, thus providing insight into the potential mineralization mechanisms during early stages of mineralization. This study provides information on the treatment of these systems for APT analysis and development of systematic analytical approaches needed to effectively probe complex organic-inorganic interfaces which can be applied to a broad range of systems.

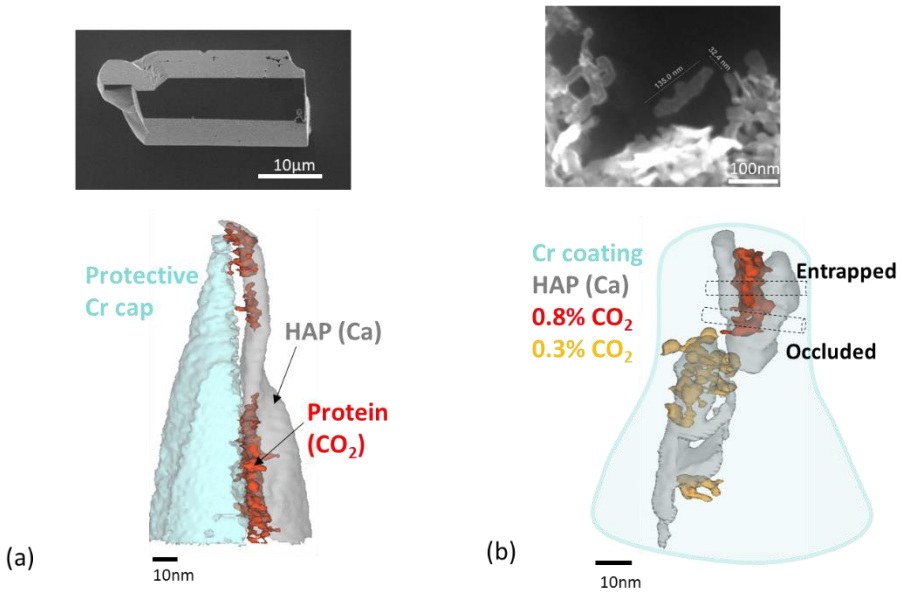

Figure 1. Representative electron micrographs and 3D APT chemical reconstructions for (a) amelogenin adsorbed on an HAP microparticle and (b) amelogenin-mineralized HAP nanoparticles.

References

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