Stabilization and Assembly of Mineral Clusters Guided by Enamel Proteins.

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Dental enamel, being the most mineralized vertebrate tissue, is extremely well preserved in the paleontological record and thus a favorite subject for studies of vertebrate evolution, archeology and paleoclimate. Enamel is a structurally complex hierarchical nanomaterial. Although, mature enamel is 95% carbonate apaptite it starts as a network of nanocrystaline arrays suspended in a protein matrix, with the mineral phase comprising only 10% of its volume. Despite these differences in composition, the mineral organization in nascient and mature enamel is identical. While the organic matrix is believed to regulate mineral formation, the basic mechanisms of enamel mineralization are poorly understood. Here we present our recent studies of calcium phosphate mineralization *in vitro* in the presence of the major enamel matrix protein, amelogenin. These experiments were carried out on carbon-coated electron microscopy (EM) grids, and studied in conventional and cryo-EM. We found that amelogenin induces formation of parallel arrays of apatitic crystallites that are structurally similar to the basic building blocks of enamel, enamel rods. Importantly, these structures only formed when monomeric, rather than preassembled, amelogenin was introduced to the mineralization solutions. These results suggest that the mineralization occurs not on the preformed organic matrix as in other systems but instead via cooperative interactions between forming crystals and assembling proteins.[*1*] Our cryo-EM studies show that amelogeinin undergoes stepwise assembly via oligomers that then organize into higher order structures. Our results indicate that amelogenin oligomers stabilize calcium phosphate prenucleation clusters and organize them into linear chains. Subsequently, the clusters fuse together to form needle-shaped mineral particles which further organize into parallel arrays.[*2*] We find that the mechanism of enamel formation is very different from the templated mineralization that is observed in other biomineralization systems. Specifically our results indicate that amelogenin oligomers can stabilize prenucleation clusters and organize them into mesostructures prior to their crystallization. This mechanism in which protein assemblies fully control organization of the initial mineral phase enables formation of intricate structures that cannot be obtained via classical crystallization from supersaturated solutions. These studies supported by NIH grants R01DE016376 (to H.C.M.), R01DE016703 (to E.B.) and PA grant SAP 4100031302 (to J.F.C.)

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Fluid inclusions give chemical, physical, biological, and climatic insights into acid saline lake and groundwater systems

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Acid saline lakes and associated shallow groundwaters represent amongst the Earth's most extreme aqueous chemistries. Modern lakes and groundwaters in Western Australia and Chile have pHs as low as 1.5, total dissolved solids as high as 32%, unusually high concentrations of Al, Si, and Fe, and many other atypical chemical characteristics. Permian lake environments in the U.S. midcontinent had even more extreme water compositions.

Fluid inclusions in halite and gypsum from modern and Permian acid saline lakes record specific physical, chemical, and biological conditions of these environments. Primary inclusions in these chemical sediments trap shallow lake water, air bubbles, crystals of other minerals, and microorganisms. Isolated fluid inclusions in early diagenetic phases of halite are remnants of shallow groundwaters and yield their chemical compositions.

A variety of traditional methods, including petrography, freezing/melting microthermometry and laser Raman spectroscopy, and innovative variations, such as homogenization of artificially-nucleated vapor bubbles and UV-vis petrography, produce data detailing environmental conditions. Other traditionally used methods, such as laser ablation ICP-MS, are not effective due to the nature of the inclusions. Making synthetic solutions that match the complexity of natural acid saline inclusions is another challenge. Ongoing efforts include the analyses of modern and Permian air and documentation of microorganisms and organic compounds within these fluid inclusions (Fig. 1).

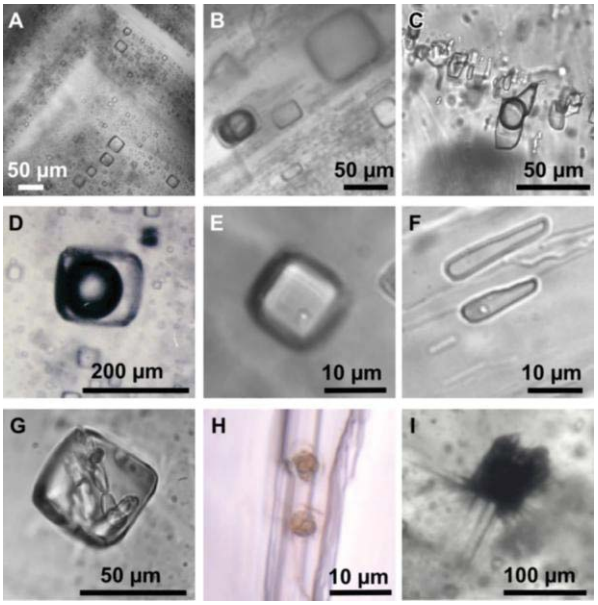

Figure 1: Primary fluid inclusions in acid saline halite and gypsum. A. Fluid inclusions along growth bands in Permian Nippewalla Group halite, Kansas. B. Air in inclusion in modern halite, Western Australia. C. Air in inclusion in modern gypsum, Western Australia. D. Air in inclusion in Permian halite, Nippewalla Group, Kansas. E. Cocci-shaped bacterium/archaeon suspect in Permian halite, Nippewalla Group, Kansas. F. Cocci-shaped bacterium/archaeon suspect in modern gypsum, Western Australia. G. Organic compounds and crystals in fluid inclusion, Permian Nippewalla Group halite, Kansas. H. Algal or pollen suspects in modern gypsum, Western Australia. I. Microbial community in inclusion, Permian Opeche Shale halite, North Dakota.