Enamel synthesis explained

Valerie Vaissier Welborn[a,1]

Tooth enamel, the protective outer layer of the dental crown, is the hardest and most mineralized tissue in the human body. Enamel's unique mechanical properties arise from the hierarchical organization of inorganic and organic matter across length scales. Unlike other biomaterials, such as bone or carapace, the structure of enamel is highly conserved across species, suggesting that it confers significant evolutionary advantages (1). This universality makes enamel an ideal system to study the biomineralization processes that produce materials with properties surpassing those of many synthetic materials. In PNAS, Bai et al. (2) unveil the detailed mechanism by which the organic matter contained in enamel guides the oriented growth of the mineral phase. Further, Bai et al. (2) demonstrate how this mechanism can be replicated to grow enamel in vitro, resulting in a material with a microstructure resembling that of natural enamel.

Enamel Composition

Enamel consists of over 95 wt% (carbonated) apatite, a calcium phosphate mineral that can be found in all mineralized tissues in vertebrates (3). Apatite crystals grow predominantly along their c axis, thereby displaying elongated shapes. In mammalian enamel, these elongated crystals align parallel to one another, effectively forming an enamel rod that can reach tens of micrometers in length. Between these rods, space is filled by apatite crystals whose principal direction progressively deviates from the rod axis, as shown in Fig. 1. The most distant interrod crystal is positioned at a 60° angle with respect to the enamel rods. Finally, each rod is enveloped with a sheath of organic matter, which makes up only 1 to 2 wt% of enamel. The preferred orientation of these apatite crystals yields the unique microstructure that gives enamel its mechanical properties. The work of Bai et al. (2) provides strong evidence that a self-assembled protein scaffold guides the oriented growth of apatite crystals in developing enamel tissues.

The role of organic matter in providing a scaffold for enamel minerals to grow has long been recognized (4, 5). More specifically, enamel forms within an
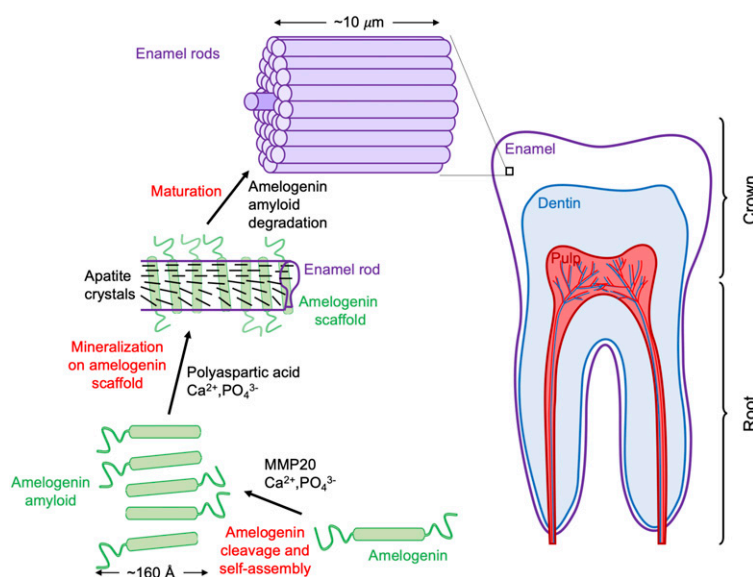
Fig. 1. In PNAS, Bai et al. (2) unveil the molecular mechanisms responsible for the oriented growth of apatite crystals in enamel tissues. The product of the enzyme MMP20 self-assembles into amyloid-like structures that guide the growth of apatite minerals along one direction. The packing of the resulting rods gives enamel its unique microstructure and therefore its mechanical properties.

organic matrix composed of unique proteins secreted by ameloblasts, specialized cells whose function is to develop enamel tissues (6). However, after this development stage, also called the secretory stage, enamel enters a maturation phase where most of the organic matter is degraded. The transition to the maturation phase is marked by the expression of the proteolitic enzyme kallikrein-related peptidase 4 (KLK4), which degrades the existing enamel matrix proteins (7). Additionally, the genes coding for the enamel matrix proteins in ameloblasts are down-regulated, preventing the synthesis of new enamel matrix proteins. With the exception of rodent incisors, the maturation process is the reason why enamel does not have the ability to grow or remodel after being formed. Maturation also removes all evidence of the organic scaffolding that enabled mineral growth, thereby preventing the derivation of a direct relationship

between inorganic and organic matter in enamel (8). Nevertheless, the characterization of developing enamel tissues led to the identification of filamentous protein nanostructures that were believed to be the basis of the organic scaffold (9). By studying mature enamel tissues in KLK4^{-/-} knockout mice, Bai et al. (2) demonstrate that the final orientation of apatite crystals matches the underlying orientation of these filamentous protein nanostructures. Indeed, the organic scaffold remains after maturation in KLK4^{-/-} knockout mice since they lack the enzyme that is responsible for its degradation. By carefully demineralizing mature tissues from these mutant mice, Bai et al. (2) were able to reveal this mature scaffold is a filamentous, amyloid-like protein superstructure.

Oriented Growth of Apatite Minerals

Amelogenin is the most abundant protein in the enamel matrix, followed by enamelin and ameloblastin (3, 10). It is unsurprising then, that the filamentous protein structures in developing enamel tissues are made of amelogenins. To better understand this superstructure, many have studied the self-assembly properties of amelogenin in vitro. Amelogenin is a rather small, hydrophobic protein that is capped by a hydrophilic C terminus with a total of 175 amino acids. This hydrophylic tail on an otherwise hydrophobic protein makes amelogenin self-assemble into nanospheres of about 25 nm in diameter. These nanospheres have often been observed in vitro, leading to the hypothesis that the filamentous structures observed in vivo are chains of amelogenin nanospheres (11). However, over the past decade, Habelitz and coworkers (12) demonstrated that the calcium and phosphate ions present in vivo dramatically influence the self-assembly properties of amelogenin. They showed that under physiological conditions, ion bridges form between amelogenin chains, resulting in amyloid-like protein fibers, consistent with the filamentous structures observed in enamel tissues. Bai et al. (2) also consider the effect of the proteolytic enzyme matrix metalloproteinase-20 (MMP20),

which binds amelogenin, on the resulting protein superstructure. MMP20 cleaves the C terminus off the amelogenin protein (13), producing a shorter peptide that can be replicated from a recombinant protein. Bai et al. (2) show that this MMP20 product also self-assembles into nanofibrils, just like the full-length amelogenin. Overall, Bai et al. (2) provide strong evidence that the MMP20 product self-assembles into amyloid-like fibers in vivo and that it is this superstructure that acts as a scaffold for the oriented growth of apatite minerals. The impact of this finding reaches beyond the field of biomaterials. By linking amyloids to a functional role in the development of enamel, they challenge the assumption that amyloids are only associated with severe pathological conditions.

While the presence of a protein scaffold is essential to guide the orientation of growing minerals in enamel, it is not sufficient to induce growth. Indeed, apatite nucleation, and therefore growth, relies on the availability of calcium and phosphate ions. This role is filled by the other proteins in the enamel matrix, enamelin in particular (14). In studies of knockout mice where the expression of the gene coding for enamelin is suppressed, visibly disorganized enamel is observed. However, unlike amelogenin, it is difficult to obtain recombinant enamelin. Bai et al. (2) use an alternative strategy where they incorporate polyaspartic acid in place of enamelin, in addition to a sustained source of phosphate and calcium ions. This approach was validated by remineralizing the demineralized enamel tissues from KLK4^{-/-} mice, thus obtaining an almost perfect replica of natural enamel. More importantly, Bai et al. (2) were able to synthesize enamel in vitro by mineralizing the recombinant MMP20 product superstructures. This achievement not only serves to ascertain the role of filamentous amelogenin-based structures in guiding the orientation of apatite crystal, but also allows for the synthesis of a material that faithfully reproduces the microstructure of enamel, with broad implications for the field of materials.

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