

REVIEW ARTICLE

Amelogenin: A novel protein with diverse applications in genetic and molecular profiling

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ABSTRACT

Tooth enamel is a unique entity among all mineralized tissues because of the presence of high mineral content. It is non collagenous and does not undergo resorption and remodelling. Its formation occurs through a transient collaborating network of enamel matrix proteins which controls hydroxyapatite crystal growth and orientation. Amelogenins constitute about 90% of the total enamel matrix proteins and play a major role in enamel bio mineralization. Amelogenin isoforms coalesce into nanospheres thus dictating the width and thickness of apatite crystals. The X and Y copies of amelogenins do not undergo homologous recombination, thus preferring it for sex determination in modern forensics. Recently, it was discovered that application of amelogenin to diseased periodontal tissue surfaces enhanced the regeneration of all the periodontal tissues. Additionally, low molecular mass amelogenin polypeptides have also been thought to possess osteogenic potential. Recent data regarding usage of immunohistochemical markers for mesenchymal stem cells suggested that amelogenin has the capacity to induce the recruitment of mesenchymal stem cells directly or indirectly during regeneration of the supporting periodontal tissues. Thus, our current concepts of dental enamel formation should be reviewed thoroughly so that this information could be applied to clinical circumstances where this understanding may be particularly relevant.

Key words: Amelogenesis imperfecta, amelogenin, hydroxyapatite, nanospheres

INTRODUCTION

Tooth enamel is a unique entity among all mineralized tissues because of the presence of high mineral content in it.^[1] It is non collagenous and does not undergo resorption and remodelling. The process of enamel formation occurs extra cellularly through the interaction of the various proteins that are assembled together to form a transient network, which controls the hydroxyapatite crystal growth, its structure and orientation with each other. This protein network is called as enamel organic matrix which is synthesized and secreted by specialized cells known as ameloblasts.^[2]

The enamel matrix proteins are generally classified into three broad categories which have common features among them. They can be enumerated as: first group is amelogenin which is a 20-kDa hydrophobic protein; second one is enamelin a 65-kDa acidic protein which also includes tuftelin and the third non-amelogenin, non-enamelin group, represented by ameloblastin (also known as amelin or sheathlin).^[2] Enamel matrix proteins interact with each other as well as with the hydroxyapatite crystals. These biochemical interactions are important to guide the growth of hydroxyapatite crystals and influence their structure and orientation. Two main enamel proteinases have also been named as an integral part of enamel matrix. They are matrix metalloproteinase-20 (MMP-20)/enamelysin and kallikrein 4 (KLK4), which are expressed in the developing enamel at different times and have different roles to play. They modulate the network of enamel matrix proteins thus affecting their interaction with each other and with the developing hydroxyapatite crystals.^[2]

STRUCTURE

Amelogenin constitute about 90% of the total enamel matrix

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proteins and play a major role in the mineralization and morphological changes in enamel. The human amelogenin gene has been located on X-chromosome at Xp22.1–p22.3 and on Y chromosome at Yp11.2 with 90% of the transcripts expressed from the X-chromosome and the rest from the Y-chromosome.^[3] The X and Y copies of amelogenin gene do not undergo homologous recombination, which makes it the most preferred genetic marker for sex determination in modern forensic science.^[1]

The basic structure of amelogenin comprises of two well-demarcated self-assembly regions: first is the amino-terminal domain-A (hydrophobic and contains amino-acid residues 1-42) and the other region is carboxy-terminal domain-B (hydrophilic and contains amino-acid residues 157-173). Amelogenin has the ability of binding to hydroxy apatite crystals by its domain-B which is hydrophilic in nature. Amelogenin molecules usually form nanospheres due to the binding capacity of both the hydrophilic areas in the amino as well as the carboxy terminal. Central region (C-domain) of amelogenin forms the dense central area of the nanospheres which are surrounded by extended tails of both the terminals.^[4] The function of domain-B is assumed to be critical only in the early stage of formation of enamel as it gets cleaved in a short time as the protein is secreted into the extracellular space [Figure 1].^[2]

ISOFORMS OF AMELOGENIN

Amelogenin has a variety of isoforms present in the enamel matrix which are generated by alternative splicing of primary amelogenin RNA transcript. There are three schools of thought about exhibition of varied isoforms by amelogenin: first is the presence of two distinct sets of genes located in both the sex chromosomes, second is the production of numerous amelogenin mRNAs by alternative splicing and lastly they are proteolytic processed after secretion.^[2]

Majority of the isoforms can coalesce into spherical structures called nanospheres averaging about 20 nm in diameter and containing about 100 amelogenin molecules [Figure 2].^[1]

ROLE OF AMELOGENIN IN ENAMEL FORMATION

Nanospheres have a direct correlation with the spacing of enamel crystallites. Since enamel crystals eventually grow until they contact adjacent crystals, the nanosphere dimension may ultimately dictate the width and thickness of enamel crystals.^[1] Amelogenin nanospheres have been detected *in vivo* as “beaded rows” along the C axis of developing enamel crystallites, suggesting their close interaction with the crystal surface. Hence, it was suggested that the organized assembly of amelogenin nanospheres into collinear arrays is critical at the initial stage of mineral deposition adjacent to the dentino-enamel junction, both when oriented nucleation occurs (most likely as a result of other enamel proteins interacting with

the structured amelogenin framework) and before amelogenin processing and degradation [Figure 3].^[5]

After the orientation of the crystals is achieved, amelogenin protein begins to be processed at its C terminus. This results in the formation of the most abundant amelogenins proteolytic

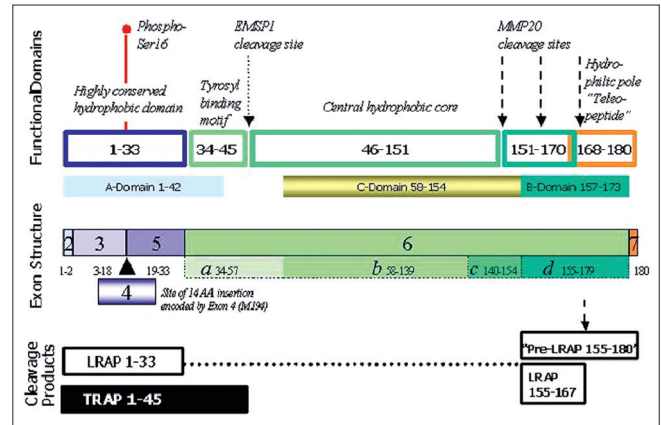


Figure 1: Photomicrograph showing the structure of amelogenin gene

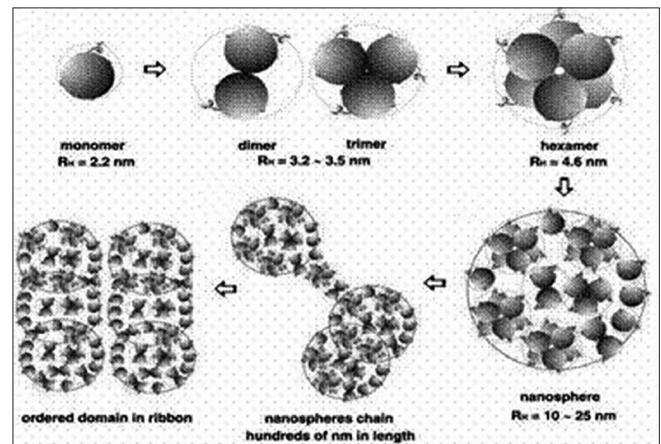


Figure 2: Photomicrograph showing a schematic model of amelogenin self-assembly

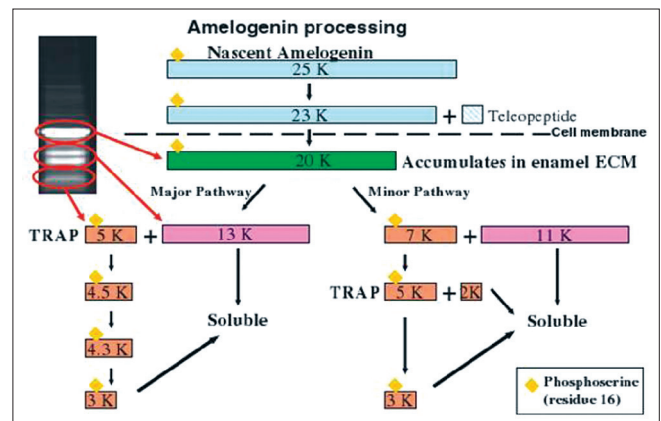


Figure 3: Photomicrograph showing extracellular amelogenin proteolytic processing in which it is processed into smaller peptides by specific proteases

product which has the potential to affect the morphology of calcium phosphate crystals in a very specific manner. Recent *in vitro* studies have demonstrated that amelogenins lacking the hydrophilic C terminus increase the ratios of length/width and thickness/width of octacalcium phosphate crystals by specifically interacting with the side faces.^[5] After their secretion, amelogenins are almost immediately processed in a stepwise and controlled manner and eventually are removed from the extra cellular space during the late maturation stage. Massive degradation of the enamel extracellular matrix components (mostly amelogenins) is concomitant with the rapid growth of enamel crystals, creating a highly organized structure that is almost completely inorganic (>90% mineral). The highly elongated and oriented ribbon like carbonated hydroxyapatite crystals in mature enamel are quite different in size and morphology from those of bone, cartilage and dentin, which have a plate like morphology.^[5]

Amelogenin has two other properties worth mentioning. First, intact amelogenin binds tightly to enamel crystals. This binding is mediated by the C-terminal segment, which is removed by proteinases a short time following amelogenin secretion. As a consequence, amelogenin cleavage products have a low affinity for enamel crystals. Second, amelogenin is rich in the amino acid histidine which is capable of absorbing hydrogen ions and thereby buffering the enamel fluid.^[1]

During enamel development and mineralization, the secreted amelogenins are lost from the tissue along with most of the other enamel matrix proteins, due to sequential degradation by specific proteases. They are replaced by mineral ions, calcium and phosphorus, which eventually results in fully mineralized hard and mature enamel.^[3] *In vitro*, experiments conducted by Bouropoulos and Moradian-Oldak strongly suggest that the 32-kDa amelogenin and amelogenin cooperate to promote nucleation of apatite crystals and propose a possible novel mechanism of mineral nucleation during enamel bio mineralization.^[2]

MUTATIONS IN AMELOGENINS

As mentioned before, amelogenins are present on both the sex chromosomes. Mutations of this gene present on X-chromosome induce amelogenesis imperfecta which is a genetically induced disorder presenting with two different characteristic malformations of enamel namely hypoplastic or hypo-mineralized. The malformed enamel in such cases show the presence of less inorganic and a higher organic content as compared to normal enamel.^[2] Mutations in the carboxy terminal of amelogenins results in a hypoplastic phenotype whereas mutations present in the amino terminal results in a hypo-mineralized phenotype. Most likely reason for the double phenotypic expression observed *in vivo* could be a reduction in the rate of hydrolysis of amelogenin and subsequent mineralization patterns.^[2]

FORENSIC DENTISTRY

All research done upto now points to a major fact that amelogenins has a distinct difference in size and pattern of nucleotide sequence in male and female enamel. This difference between the two enamel phenotypes is a sensitive sex determinant for very minute DNA samples produced from unknown human skeletal/dental remains. Amelogenin gene present on X-chromosome has 106 base pairs in length whereas this gene present on Y-chromosome has 112 base pairs. This information can provide us a strong distinction between male and female amelogenins as well as highlights this fact that females have two identical amelogenin genes present on X-chromosome whereas males have two different genes, present on both the sex chromosomes. This difference in male and female genotypes can be utilized as a indispensable tool having great specificity, sensitivity and financially suitable for modern forensic science.^[6]

Mutations that knockout the human X-chromosomal copy of the amelogenin gene result in genetic enamel malformations known as X-linked amelogenesis imperfecta or AI. In females with this condition, there is a Lyonization effect; that is cohorts of ameloblasts that inactivate the X-chromosome carrying the mutated amelogenin gene produce normal enamel. Clinically, the teeth of affected females show alternating vertical bands of defective and normal enamel. So in this way it can help us in determining the sex of an individual.^[1]

REGENERATION OF TISSUES

For decades, amelogenin was considered to be a protein of epithelial origin which was present only in enamel. More recently, distinct isoforms of amelogenin have also been discovered in places other than enamel like dentin matrix, odontoblasts, in remnants of Hertwig's root sheath and in periodontal ligament (PDL) cells. The expression of ameloblasts has also been found in long bone cells such as osteocytes, osteoblasts, osteoclasts, some bone marrow cells, and articular cartilage, chondrocytes of the articular cartilage and in cell layers of the epiphyseal growth plate. Different functions of amelogenins may be attributed to the following facts: firstly presence of a relatively large number of amelogenin alternatively spliced mRNA translated polypeptides and secondly it is expressed in different tissues (calcifying and soft tissues) and of different embryonic origin.^[7]

A major discovery that points out a new role for enamel matrix proteins to play was the fact that the application of enamel matrix protein extracts to diseased periodontal tissue surfaces enhanced the regeneration of all the periodontal tissues. In addition to that, low molecular mass amelogenin polypeptides have also been thought of as in cell signalling pathways and are considered to possess osteogenic potential.^[7]

Purified recombinant human amelogenin is now being utilized to study the underlying mechanisms associated with

the normal physiologic functions of amelogenin in normal, diseased and regenerating hard and soft tissues.^[3] In the last few years, a mixture of enamel matrix proteins, Emdogain (composed primarily of amelogenin) was acknowledged and till then has been used successfully to promote repair of both hard and soft periodontal tissues. Function of leucine-rich amelogenin peptide (LRAP) as a signaling molecule to induce osteogenic differentiation has been demonstrated in different cellular contexts. Despite the identification of LRAP receptor and attempts to address mechanistic functions for LRAP for its osteo-inductive property, the signaling pathway responsible for the osteogenic effect of LRAP remains largely unclear. The discovery that LRAP activates Wnt/b-catenin signalling pathway to stimulate osteogenesis makes LRAP amenable to novel therapies and interventions to treat Wnt-related bone diseases.^[8]

FUTURE PERSPECTIVE AS TUMOR MARKERS

Normally, amelogenin gene is expressed in dental tissues with distinct temporal and spatial restriction pattern only at the time of odontogenesis. Thus, the study of amelogenin gene and protein expression in odontogenesis and odontogenic neoplasms also pose an important and interesting topic in the field of developmental biology and oral pathology.^[9] Kumamoto *et al.* in 1980 had examined amelogenin immunohistochemically in several benign and malignant odontogenic tumors and is now regarded as a potentially useful polypeptide for identification of odontogenic epithelial components.^[10] At the present time, genes responsible for amelogenesis and dentinogenesis imperfecta, oligodontia and many other disorders affecting the hardness, color, size, shape and number of teeth are being identified. In the future, genetic and molecular testing will be available to identify the specific mutations that can cause inherited disease in a given family. So, it is the duty of dental professionals to perform these tests so as to make a positive diagnosis and to improve treatment decisions.^[11,12]

The expression of amelogenin gene also has been observed in various sites such as dentin matrix, odontoblasts, remnants of Hertwig's root sheath, periodontal ligament cells in long bone cells (osteocytes, osteoblasts, osteoclasts, bone marrow cells), chondrocytes of the articular cartilage, so in future it can also be used as a tumor marker in the neoplasms of these locations.^[7]

DISCUSSION

Amelogenins are the most studied enamel matrix proteins. They account for more than 90% of the matrix proteins in the secretory stage of enamel formation, comprising the major components of the supramolecular transient framework, which is absolutely necessary for normal enamel crystals growth and architecture.^[2] They are hydrophobic molecules that self-assemble *in vitro* and *in vivo* into nanospheric structures, which regulate the oriented and elongated growth, shape and size of the enamel mineral crystal.^[7] Recent knowledge on

amelogenin's primary and quaternary structures has allowed the development of *in vitro* experimental systems for the study of amelogenin-mineral interactions and interpretation of the function of this structural protein during amelogenesis.^[2]

Mutations in the X-chromosomal copy of the amelogenin gene have been associated with the hereditary disease amelogenesis imperfecta, which illustrates the importance of amelogenin in developing enamel.^[3] Amelogenin is expressed from genes on the X and Y chromosomes with about 90 percent of all RNA transcripts coming from the X-chromosome. The X and Y copies of the amelogenin gene do not undergo homologous recombination (the trading of DNA that occurs between equivalent segments on paired chromosomes). Because of this the amelogenin gene is the preferred genetic marker for sex determination in forensics.^[1] Therefore, both the amelogenin genes on corresponding X and Y chromosomes are functionally distinct in the way that they might epitomize the only X/Y gene pair in which X linked inactivation may act on the X chromosome locus itself so that males potentially have more genetic output than females although the transcriptional yield of both X and Y loci are qualitatively and quantitatively different.^[12]

However, in more recent years different isoforms of amelogenin have also been found in the dentin matrix and the odontoblasts, during cementogenesis in remnants of Hertwig's root sheath and in periodontal ligament (PDL) cells. Very recently, amelogenin expression has also been described in long bone cells; osteocytes, osteoblasts and osteoclasts, and some of the bone marrow cells. The relatively large number of amelogenin alternatively spliced mRNA translated polypeptides and the fact that amelogenin is expressed in different tissues (calcifying and soft tissues) and of different embryonic origin, possibly reflect different functions of amelogenin.^[7]

Recent data available regarding usage of immunohistochemical markers for mesenchymal stem cells suggested that amelogenin has the capacity to induce the recruitment of mesenchymal stem cells directly or indirectly during regeneration of the supporting periodontal tissues.^[7]

In the past years, a major contribution of enamel matrix proteins, Emdogain was recognized and was very successful in clinical practice for promoting the repair of both hard and soft periodontal tissue. Emdogain is composed principally of amelogenin including the alternatively spliced amelogenin isoform called leucine-rich amelogenin peptide (LRAP).^[8] In addition, low molecular mass amelogenin polypeptides have since been associated with cell signaling and have been suggested to have osteogenic potential.^[3] Normally, amelogenin gene is expressed in dental tissues with distinct temporal and spatial restriction pattern only at the time of odontogenesis.^[9] Amelogenin, due to its ability to form a supermolecular network can also be utilized as a model to delve into the intricacies of tissue-specific enamel biomineralization.^[13] Thus, the study of amelogenin gene and protein expression

in odontogenesis and odontogenic neoplasms also pose an interesting question in the field of developmental biology.^[9]

CONCLUSION

Enamel formation is a highly complex process and major understanding is yet required to understand the whole sequence of enamel bio mineralization. Until now, all major enamel matrix proteins and proteinases have already been cloned, characterized and their recent involvement in many cases of amelogenesis imperfecta has strengthened their foothold in the process of enamel development. However, there is still an unanswered question in the literature about the processing of enamel matrix proteins and the way they align together to interact with themselves and with the developing enamel crystals. More recently, the important realization that leucine-rich amelogenin peptide activates many signaling pathways to stimulate osteogenesis makes it amenable to novel therapies to treat Wnt-related bone diseases and also may shed light on the mechanism by which amelogenin stimulates periodontal tissue regeneration in clinical dentistry. Recombinant enamel proteins are currently being tested for their effects on crystal growth, but significant breakthroughs are needed before anything like dental enamel is prepared *in vitro*. In the long run, stem cell research combined with the recent advances in our understanding of tooth development may lead to the growth of teeth in culture for use as dental implants. Thus, our current concepts of dental enamel formation should be reviewed thoroughly so that this information could be applied to clinical circumstances where this understanding may be particularly relevant.

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