

MAMMALIAN ENAMELINS: IDENTIFICATION OF CONSERVED REGIONS, EVOLUTION MODE AND MADE USE OF FOR VALIDATION OF MUTATIONS LEADING TO AMELOGENESIS IMPERFECTA

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INTRODUCTION: The uniqueness of dental enamel as a mineralized tissue is reflected in the tissue specificity of its principal matrix constituents, amelogenin (AMEL), enamelin (ENAM) and ameloblastin (AMBN). ENAM is the largest and least abundant non-amelogenin protein in the developing enamel matrix. It represents roughly 1 to 5% of total matrix protein. ENAM is known to possess a SXE motif that is supposed to be a phosphorylation site and a RGD motif that allows protein-cell membrane binding. Intact ENAM (186 kDa) is found only in the surface enamel at the mineralization front near Tomes' process. Among the ENAM cleavage products, the 32 kDa (amino acids 136-241) is the most characterized. It represents up to 1% of total enamel protein in late stage of enamel maturation, and it accumulates in the deeper rod and inter-rod enamel, where it is hypothesized to bind the sides of developing enamel crystals and regulate their shape. The importance of ENAM for proper enamel formation is manifested in the autosomal-dominant form of *amelogenesis imperfecta* (AIH2), displayed by individuals with defective ENAM alleles.[1, 2]

A recent study of AMEL has shown that evolutionary analysis is a useful approach to understand protein evolution, reveal conserved regions, which have certainly important functions, and highlight residues that could lead to a genetic disease if modified. [3, 4]. We have performed an evolutionary analysis of ENAM in mammals (i.e., 250 millions years of evolution) with these objectives in mind.

METHODS: ENAM sequences were obtained in 34 representatives of the main mammalian lineages by blasting complete and raw sequenced genomes in databases. Sequence alignment was done by using Se-Align v2.0 program.

RESULTS & DISCUSSION: The evolutionary analysis reveals that only the SXE motif is

conserved in mammalian ENAM. The RGD motif lacks in some sequences suggesting that it would not be of great importance for the normal protein function. A proline-glutamine rich region, encoded by exon 7 has been identified. This region is homologous to the proline rich region described in AMEL and AMBN sequences. This finding confirms our previous hypothesis of evolutionary relationships of enamel matrix proteins. [4]

The analysis of the 32 kDa ENAM fragment, which is degraded by kallikrein 4 during the late stage of maturation shows that three out of five cleavages sites of this proteinase are conserved, as well as, one asparagine glycosylation site and two serine phosphorylation sites. In addition, two motifs are also well conserved (PYYSEEM and SNE_xGGNP). Such a long lasting conservation indicates an important function for this region. Sequence comparison allows identifying more than 70 amino acids, which were conserved during mammalian evolution. Without doubt, each of them plays an important role for ENAM function, and is a strong "candidate" for AIH2 if it is modified.

CONCLUSION: The evolutionary analysis is an efficient approach for predicting possible function of ENAM. Such a prediction is particularly important as numerous gene sequences are generated with little or no accompanying experimentally determined functional information. In addition our ENAM sequence dataset will be highly useful to highlight residues that could lead to (AIH2) if modified.

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