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LS-4-P-3117 Structural and functional characterization of Odontogenic Ameloblast-associated (ODAM) and Amelotin (AMTN) proteins

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Introduction: The junctional epithelium (JE) is the portion of the gingiva that attaches to the tooth enamel. This specialized structure seals off the periodontal tissue from the aggressive environment of the oral cavity. A loss of its integrity leads to periodontal disease followed by bone and ultimately tooth loss. The adhesion mechanism of the JE to mineralized tooth surfaces is provided by an epithelial structural complex consisting of an atypical basal lamina (BL) and hemidesmosomes. The interaction with a mineralized matrix instead of connective tissue therefore requires special components. Two proteins, odontogenic ameloblast-associated (ODAM) and amelotin (AMTN), encoded by members of the secretory calcium binding phosphoprotein gene cluster, have been identified by our laboratory as novel components of this BL. Proteins from this cluster stabilize Ca and PO4 ions in tissue fluids and regulate their deposition onto extracellular matrices. ODAM and AMTN were shown to co-localize at cell-tooth interfaces suggesting that they might interact (Fig1). **Problematic**: In the literature, little information is available on the physicochemical properties and structure of ODAM and AMTN. They contain some post-translational modifications but they do not possess structural homologues. Our aim is to structurally characterize these proteins and their potential interactions. **Methods**: Proteins were produced using bacterial or eukaryotic systems. They were purified using His-tag affinity and gel-filtration chromatography and then characterized by atomic force microscopy (AFM) and transmission electron microscopy (TEM). For AFM studies, proteins at 0,1mg/ml on 50mM Na_2HPO_4 were incubated 5minutes on HOPG, rinsed three times with distilled water, and air dried. **Results**: With gel-filtration, we have obtained AMTN complexes of 5 monomers and ODAM complexes of ≈20 monomers. AFM images of ODAM and AMTN alone presented globular complexes with average sizes of approximately 32 nm and 36 nm and average heights of ≈2.5 nm and 2.8 nm, respectively. Mixing the proteins resulted in globular complexes with an average size of 84 nm and an average height of 8 nm, suggesting the existence of interactions between them (Fig2). **Conclusion**: Our data show for the first time structural images of ODAM and AMTN. They suggest that AMTN and ODAM are capable of self-assembly and that protein-protein interactions occurs among them. Exploring the molecular arrangement and interactions of proteins in this BL is essential to understand how the JE maintains its unique adhesive relationship with the tooth.

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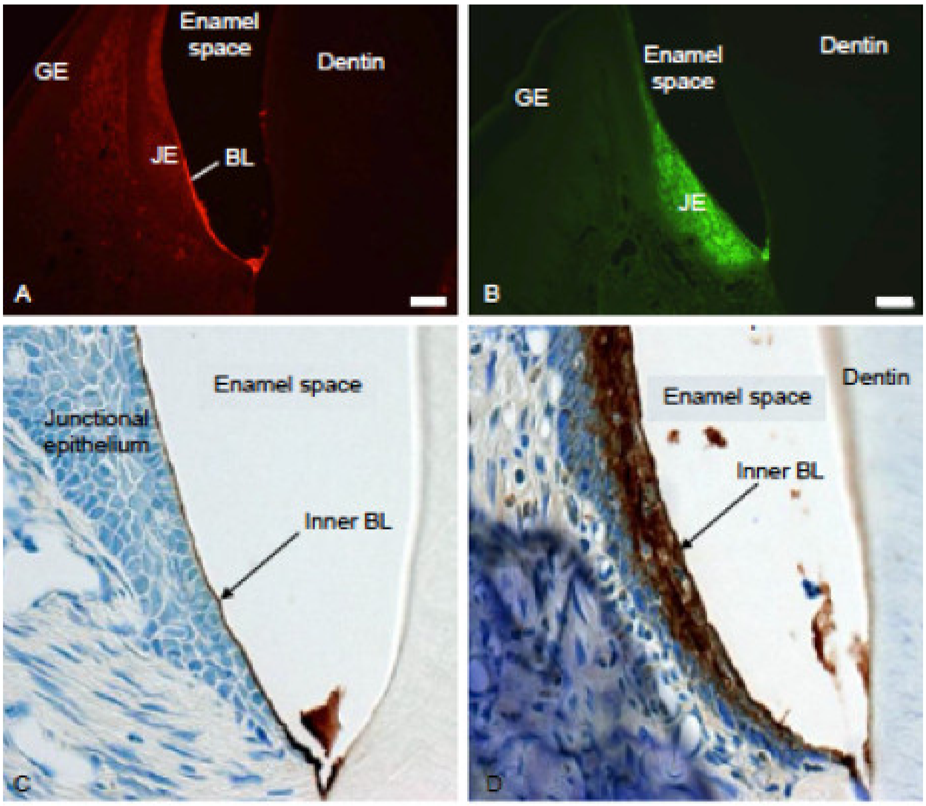

Fig. 1: Immunofluorescence (A, B) and Immunoperoxydase (C, D) preparations for ODAM (B, D) and AMTN (A, C) in the junctional epithelium (JE). ODAM localizes at the ameloblast– enamel interface where there is an atypical basal lamina (BL) and is also present among the cells of the JE. Labeling for AMTN is restricted to the inner BL. GE: Gingival epithelium

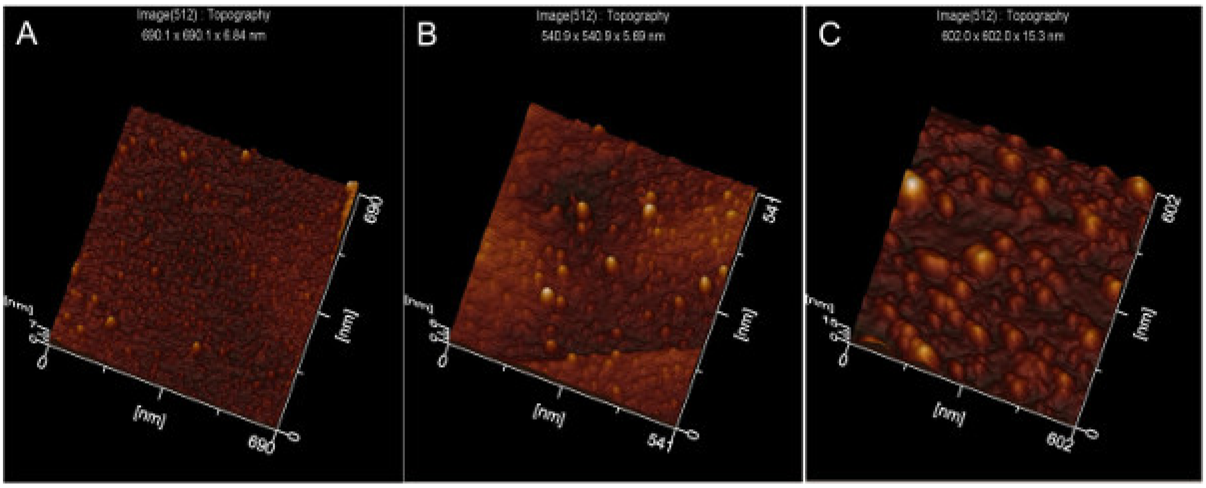

Fig. 2: Highly magnified AFM observation of (A) ODAM and (B) AMTN proteins alone at 0,1mg/ml on HOPG surface. (C) Changes in structure can be observed when ODAM and AMTN (0,1mg/ml) are incubated together.