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Fine structural and cytochemical mapping of enamel organ during the enameloid formation stages in gars, *Lepisosteus oculatus*, Actinopterygii

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Summary During cap enameloid formation in gars (*Lepisosteus oculatus*), the dental epithelial cells that constitute the enamel organ were observed by means of transmission electron microscopy and enzyme cytochemistry to detect the hydrolytic enzyme activities, alkaline phosphatase (ALPase), acid phosphatase (ACPase), calcium-dependent adenosine triphosphatase (Ca-ATPase) and potassium-dependent *p*-nitrophenylphosphatase (K-NPPase) (sodium, potassium-activated adenoshine triphosphatase (Na-K-ATPase)). The enameloid formation process in gars was divided into three stages: matrix formation, mineralisation and maturation. The enamel organ consisted of the outer dental epithelial (ODE) cells, stellate reticulum (SR), stratum intermedium (SI) and the inner dental epithelial (IDE) cells during the whole of the cap enameloid formation stages. During the matrix formation stage, many cisternae of rough endoplasmic reticulum and widely distributed Golgi apparatus, in which the procollagen granules containing cross-striations were often found, were remarkable elements in the IDE cells. During the stage of mineralisation, the IDE cells were tall columnar, and infoldings of distal plasma membrane of the IDE cells became marked. The most developed Golgi apparatus was visible at this stage, and large secretory granules containing fine granular or tubular materials were found in the distal cytoplasm that was close to the infoldings of the distal end. Many lysosomes that were ACPase positive were seen near the Golgi apparatus and in the distal cytoplasm of the IDE cells. ACPase positive granules often contained the cross-striation structure resembling procollagen, suggesting that the procollagen is degenerated in the IDE cells. During the maturation stage, the distal infoldings became unclear, and there were no large granules containing tubular materials, but many ACPase positive lysosomes were still present in the IDE cells. Non-specific ALPase was detected at

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the plasma membrane of the IDE cells at the mineralisation and maturation stages. K-NPPase was markedly detected at the plasma membrane of the IDE cells at the maturation stage. These results demonstrate that the IDE cells might be mainly involved in the removal of degenerated organic matrix from enameloid during the later formation stages. Strong Ca-ATPase activity was observed at the entire plasma membrane of the stratum intermedium cells, and there was slightly weak activity at the plasma membrane of the IDE cells during the mineralisation and maturation stages, implying that these cells are related to the active Ca transport to the maturing enameloid. It is likely that although the structure of the enamel organ is different, the function, especially at the mineralisation and maturation stages, is similar to other actinopterygians having well-mineralized cap enameloid.

Introduction

Gars, *Lepisosteus*, are extant ray-finned bony fishes, and have both cap enameloid and collar enamel in their conical teeth at the jaw.[1–3] The cap enameloid is a mixture product formed by odontoblasts and dental epithelial cells, while collar enamel is thought to be formed solely by the dental epithelial cells.[4,5] Recently, stratum intermedium (SI) cells were found in the enamel organ during odontogenesis in gars.[6] These features make gars an important species when we consider the evolution of dental hard tissues, especially the relationship between enameloid and enamel, and the evolution of the cells concerning odontogenesis in vertebrates, because it is commonly agreed that ectodermal enamel covering the tooth crown is usually remarkable in amphibians, reptiles and mammals, and SI cells appear in mammalian tooth germs.

During the odontogenesis in gars, cap enameloid is firstly formed, and then collar enamel and the bulk of dentin are produced toward the jaw bone, so that it is important to examine the functions of the dental epithelial cells during the cap enameloid formation and to compare them with the cases of other bony fishes, prior to the observation of the amelogenesis in the collar area. The formation of enameloid in fishes is also quite an interesting subject for the biomineralisation study. Several aspects of odontogenesis in gars were reported,[1–3,7,8] and the morphological features of the dental epithelial cells and their calcium-dependent adenosine triphosphatase (Ca-ATPase) activity during the enameloid mineralisation stage were preliminarily noted by Sasagawa and Ishiyama.[6] However, there is no integrated knowledge concerning the fine structure of tooth germs throughout the enameloid formation and there are no cytochemical data except for the Ca-ATPase mentioned above to with which study the functions of the cells forming enameloid. The aim of this study was to investigate the fine structure and histocytochemical characteristics of the dental epithelial cells constituting the enamel organ during cap enameloid formation stages, and to reveal their morphological and functional features in each stage.

Materials and methods

Five gars (gar-pikes), *Lepososteus oculatus* (total length 11—55 cm), Actinopterygii were used in this study.

After anesthetisation in MS222 (SIGMA, St. Louis, MO) or cold water with ice, and decapitation, tooth-bearing jaws were removed. For light microscopy, the jaws were placed in Bouin's fixative for 12 h. The specimens were embedded in paraffin wax after dehydration, and serial sections were stained with hematoxylin—eosin (H—E). For transmission electron microscopy, the specimens containing tooth germs were placed in Karnovsky's fixative (0.1 M cacodylate buffer with 1.7% NaCl, pH 7.4) for 6—10 h at 4 °C. Selected specimens were demineralized with 4.13% solution of EDTA-2Na (pH 7.4) after the initial fixation. Then, the specimens were fixed in a 1% solution of osmium tetroxide buffered with cacodylate or mixed with a 1.5% solution of potassium ferricyanide. After dehydration, these specimens were embedded in Araldite-Epon 812 or Epon 812 alone. Semithin sections were cut, stained with toluidine blue and examined under a light microscope. Ultrathin sections were cut with a diamond knife, and then stained with uranyle acetate and lead citrate. The sections were examined using a transmission electron microscope (JEOL JEM-1200EX or JEM-1010).

For enzyme cytochemistry, the portions containing tooth germs were placed in 4% paraformaldehyde-0.2% glutaraldehyde fixative (0.1 M cacodylate buffer with 1.7% NaCl or 0.05 M HEPES buffer, pH 7.4) for 3 h at 4 °C, and then demineralized in a 2.5—4.13% solution of EDTA-2Na for 9—12 days at 4 °C. The specimens were embedded in OCT compound (MILES, Elkhart, USA) after immersion in 2 M of sucrose solution for 2—3 h, and were immediately