P33-FINE STRUCTURAL AND IMMUNOHISTOCHEMICAL OBSERVATIONS OF THE COLLAR ENAMEL IN *LEPISOSTEUS* AND *POLYPTERUS*, ACTINOPTERYGIAN FISH

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Introduction

It is commonly accepted that ectodermal enamel covers the tooth crown in reptiles, amphibians and sarcopterygian fish, *Latimeria* and lungfish. A few living representatives of early actinopterygian radiation before the appearance of teleosts, *Lepisosteus* and *Polypterus*, demonstrate collar enamel on the surface of the tooth shaft [1, 2, 3]. In *Lepisosteus* and *Polypterus*, both enameloid and collar enamel are found in the same tooth. Therefore, they are suitable materials to study developmental relationships between enameloid and enamel. In this study, we focus on the morphological features of collar enamel, and on the chemical composition of collar enamel.

Materials and Methods

Collar enamel and dental epithelial cells in *Lepisosteus oculatus* and *Polypterus senegals* were observed by light and transmission electron microscopy, and by light and electron microscopic immunohistochemistry using crude antiserum against porcine 25 kDa amelogenin, and region-specific antibodies against the C-terminus and central region of the porcine 25 kDa amelogenin, respectively [4].

Results

The enamel layer, 600nm-5um thick, containing amorphous fine organic matrix was located between the dentin and inner dental epithelial (IDE) cells in the secretory stage. The layer also continues to the surface of enameloid near the dentin - enameloid junction, suggesting that the enamel covers both dentin and mature enameloid. The layer showed marked immunoreactions to the antiserum against porcine amelogenin in addition to the C-terminal and central region-specific antibodies.

Discussion

The structural features of collar enamel in *Lepisosteus* and *Polypterus* resemble those of the enamel in sarcopterygians and amphibians. The collar enamel in *Lepisosteus* and *Polypterus* shows marked mammalian amelogenin immunoreactivity, as the previous studies reported [2, 5]. It is suggested that amelogenin-like protein is present in the collar enamel. However, the genes related to enamel protein that are present in amphibians, reptiles and mammals, are not found in extant teleost fish. It is proposed that secretory calcium-binding phosphor-protein (*SCPP*) genes are involved in producing enamel-like tissues in teleosts [6]. A recent molecular genetic study reported that the strong expression of the odontogenic, ameloblast associate (*ODAM*) gene, which might be involved in the hypermineralization process at the late stage of enameloid maturation, was detected in the IDE cells of zebrafish [7]. In the present study, it is assumed that the collar enamel in *Lepisosteus* and *Polypterus* is produced as a terminal product of the IDE cells at the late stage of enameloid maturation. The collar enamel is thought to be a precursor of ectodermal enamel in higher vertebrates.

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