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Purification, Characterization, and Biosynthesis of Bovine Enamelins

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Summary. Enamelins were extracted from developing bovine enamel with 0.5 M EDTA, 4 M guanidine HCl, and purified by DEAE-Sephacel, Sephacryl S-200, and high-performance gel filtration chromatography. Four distinct enamelins having molecular weights of 70, 45, 30, and 28 K daltons were isolated. Their amino acid compositions were found to be rich in Pro, Glu, Gly, and Asp. Low molecular weight enamelins (45, 30, and 28 K) were more abundant in Pro, Gly, and Phe. Two-dimensional electrophoretic pattern of enamelins revealed several spots that immunoreacted to monoclonal anti-enamelin antibody raised in mice. Enamelins were found to be comprised of heterogeneous proteins as well as amelogenins. Biosynthesis of enamelins was investigated by incubating the bovine ameloblast cell layer, and several radioactive enamelins were identified by the use of two-dimensional electrophoresis. The data in this study suggest that enamelins were synthesized by the ameloblasts.

Key words: Enamel proteins — Enamelins — Biosynthesis — Monoclonal antibody.

During tooth morphogenesis, epithelial cells differentiate into secretory ameloblasts, which synthesize and secrete enamel matrix proteins [1–3]. Immature enamel matrix consists of about 30% proteins by weight, almost all of which is removed from the tissue with increasing mineralization [4–7].

Termine et al. [8] divided enamel proteins into two classes, amelogenins and enamelins, based on their solubilities, molecular weights, and amino acid compositions. Quantitatively, amelogenins occupy most of the organic matrix in developing dental enamel, having lower molecular size than enamelins. The rate of disappearance of amelogenins by degradation to smaller-sized molecules and removal from the matrix during enamel maturation is more rapid than that of enamelins [9–12]. Amelogenins have been studied more extensively than enamelins because of their abundance in the enamel matrix. The complete amino acid sequence [13], cDNA sequence [14] of bovine amelogenins, and cDNA sequence of murine amelogenin [15] were reported.

Enamelins have a high affinity to bind hydroxyapatite crystals with considerable tightness even though they are present in smaller quantities. Their distinctive acidic property along with their higher carbohydrate content distinguishes them from the amelogenins [8, 16–18], and enamelins appear to remain until later stages of enamel maturation than amelogenins. Enamelins significantly retarded crystal growth seeded on the apatite surface from metastable solutions in vitro [19].

Monoclonal anti-amelogenin antibodies have been developed that could distinguish enamelins as discrete molecules different from amelogenins [20–22]. Recently, Rosenbloom et al. [23] reported monoclonal anti-enamelin antibody and they described that amelogenins and enamelins did not cross-react each other with their respective antibodies.

The present study was designed to identify and characterize enamelins of bovine developing enamel. Enamelins were purified by using high resolution techniques. They were then investigated by two-dimensional gel electrophoresis, and mono-
clonal antibodies to them were produced. Furthermore, in order to investigate the biosynthesis of enamelines, bovine ameloblast cell layer was incubated in $^3$H-proline-containing medium and the incorporation of radioactivity into protein fractions was detected by two-dimensional electrophoresis. The result indicated that enamelines were also heterogeneous proteins like amelogenins. In addition to immunological methods, a two-dimensional gel electrophoretic technique was found to be useful in distinguishing the two protein classes because enamelines and amelogenins had different isoelectric points. A biosynthetic study showed that enamelines were synthesized by ameloblasts.

Materials and Methods

Preparation and Extraction

Enamelins and amelogenins were extracted from enamel at the secretory stage having a cheesy consistency from a developing permanent incisor in 1-year-old bovine mandible according to the method of Termine et al. [8]. The preparation and purification procedures are outlined in Figure 1. The enamel sample was first extracted with 4 M guanidine HCl ("G" extract) and then with 4 M guanidine HCl and 0.5 M EDTA ("E" extract). Each extract was concentrated by ultrafiltration (YM-10 filters, Mr. = 10,000 cut off; Amicon Corporation, Lexington, MA), dialyzed against distilled water at 4°C, and then freeze-dried before further purification.

Ion-Exchange Chromatography

In the first purification step, enamel "E" extract dissolved in 7 M urea, 0.05 M Tris buffer pH 7.4 was chromatographed by a DEAE-Sephacel ion-exchange column (2.5 cm x 10 cm, Pharmacia Fine Chemicals, Uppsala, Sweden). A linear salt gradient was applied (0–1 M NaCl, total 60 ml or 0–0.3 M NaCl, total 600 ml) at a flow rate of 30 ml/hour and fractions (10 ml each) were collected and pooled.

Gel Filtration Chromatography

DEAE-bound fractions (0–0.3 M NaCl gradient elution, bound fractions 2–4) were eluted on a Sephacryl S-200 gel filtration column (2.5 cm x 100 cm tandem column, Pharmacia Fine Chemicals) with 4 M guanidine HCl, 0.05 M Tris buffer, pH 7.4 at a flow rate of 16 ml/h. Fractions (5.3 ml each) were collected and reapplied on a Sephacryl S-200 column under the same conditions. Further purification was done by HPLC gel filtration column (Protein Pak 125, 7.8 mm x 30 cm tandem column, at a flow rate 1 ml/minute, Waters Associates, Milford, MA). The eluate was monitored at 230 nm.

Analytical Procedures

Amino acid composition was analyzed on a sample hydrolyzed