Immunohistochemical Localization of SERCA2 in the Ameloblasts of Rat Incisors

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Sarcoplasmic or endoplasmic reticulum Ca²⁺-ATPase (SERCA2) was localized in the ameloblasts of rat incisors by immunohistochemistry. Secretion ameloblasts and maturation ameloblasts, including both ruffle-ended and smooth-ended cells, were labeled with anti-SERCA2 antibodies, with maturation ameloblasts showing stronger binding than secretion ameloblasts. The pattern of labeling was cytoplasmic and diffuse. Papillary layer cells were not labeled with anti-SERCA2 antibodies. These results suggest that the SERCA2 in ameloblasts is intimately involved in mineralization during amelogenesis.

Key words: SERCA2, ameloblasts, rat incisor, immunofluorescence, microscopy

I. Introduction

Ameloblasts are responsible for tooth enamel formation. Secretion ameloblasts produce enamel matrix proteins and secrete them to form the enamel layer. This enamel is lightly mineralized [18, 24]. After the completion of enamel layer formation, the same ameloblasts change both shape and function in order to contribute to enamel maturation, which is an increase of mineralization and a removal of the secreted enamel matrix proteins. These cells are now referred to as maturation ameloblasts [29].

During mineralization in the secretion and maturation zone of the rat incisor, calcium and phosphate ions pass through the enamel organ, which includes ameloblasts, via nearby blood vessels [10, 12, 24, 28], with calcium passing through ameloblasts via intracellular and/or intercellular routes [10, 12]. Calcium-binding proteins such as calbindin, calretinin and calmodulin have been reported to be abundant in ameloblasts, as detected by biochemical analyses and immunocytochemistry [1, 2, 9, 14, 20]. Possible roles for passive buffering of cellular calcium ions or for active transport of calcium ions have been suggested. Furthermore, plasma membrane Ca²⁺-ATPase (PMCA) is present in ameloblasts [3, 4, 6, 7, 19, 27, 30]. Among these calcium homeostasis-related proteins, sarcoplasmic or endoplasmic Ca²⁺-ATPase, SERCA2, has recently been detected in enamel cells by Northern and Western blotting [8]. SERCA2 is presumably localized in the endoplasmic reticulum membrane and functions in calcium buffering of cytosol by the pumping of calcium ions into the endoplasmic reticulum lumen and the storing of calcium ions [5, 15, 22]. Alternatively, SERCA2 may be related to active calcium ion transport via the endoplasmic reticulum, thereby contributing to the increase in mineralization in enamel. Since the exact localization of SERCA2 in enamel cells was previously unknown, we examined SERCA2 protein distribution in enamel by immunohistochemistry. We found that anti-SERCA2 antibodies labeled ameloblasts but did not label papillary layer cells in the maturation zone. Secretory ameloblasts were only weakly labeled.

II. Materials and Methods

Six male Wistar rats (7 weeks, 160–230 g) (Jcl Wistar; Clea Japan, Tokyo, Japan) were used for the immunohistochemical analysis. All animal experiments were performed according to the “Principles of laboratory animal care” (NIH publication No. 85–23, revised 1985) and institutional guidelines for animal care. The animals were perfused under sodium pentobarbital (Nembutal; Abbot, North Chicago, IL) anesthesia with 4% paraformaldehyde in 0.1 M phosphate buffered solution (PB), pH 7.2, at room temperature (RT) for 10 min. They were then immersed in the same fixative at 4°C for 2 hr and the maxillary and mandibular incisors were dissected. After being washed with 0.1 M PB, the teeth were demineralized by immersion in 5% EDTA solution adjusted to pH 7.3 with sodium hydroxide solution, at 4°C for 3–4 weeks. Demineralized incisors were cut transversely into two segments. Both segments were immersed in 25% sucrose in PB overnight, rapidly frozen, and then cut longitudinally (6–8 μm thick) using a cryotome.
Cryosections on glass microscope slides were incubated in 1% bovine serum albumin in phosphate-buffered saline (BSA-PBS) at RT for 30 min. They were then labeled with a monoclonal anti-SERCA2 antibody (clone IID8) (Affinity Bioreagents, Golden, CO, monoclonal anti-SERCA2 antibody IID8 specificity has been previously characterized [13, 21, 25]) diluted 1:50 with 1% BSA-PBS at RT for 60 min, followed by labeling with FITC-conjugated anti-mouse IgG diluted 1:75 at RT for 30 min. Some sections were further labeled with rhodamine-phalloidin (Molecular Probes, Eugene, OR) diluted 1:20 with PBS at RT for 30 min to detect ruffle-ended (RA) and smooth-ended (SA) ameloblasts [16] and were labeled with 1 μg/ml Hoechst 33342 (Molecular probes) for nuclei [17]. Other cryosections were similarly labeled with a monoclonal anti-plasma membrane Ca\textsuperscript{2+} ATPase (PMCA) (clone 5F10) (Affinity Bioreagents), the specificity of which has been previously characterized and reported [3, 4]. Fluorescent images were acquired by an Olympus AX80 fluorescence microscope equipped with a CCD camera (Quantix KAF1401E, Photometrics, Tucson AR) and using MetaMorph software (Universal Imaging, Downingtown, PA).

III. Results and Discussion

Anti-SERCA2 antibodies labeled ameloblast cytoplasm diffusely in the secretion zone (Fig. 1A). Whether the stratum intermedium, stellate reticulum, and outer enamel epithelium were labeled by the anti-SERCA2 antibodies was unclear. To confirm the labeling pattern of anti-SERCA2, anti-plasma membrane Ca\textsuperscript{2+} ATPase (anti-PMCA), which reportedly labels ameloblast plasma membrane [3, 4, 30], was used. Anti-PMCA antibodies labeled secretion ameloblast plasma membrane strongly (Fig. 1B), and thus the labeling pattern of anti-SERCA2 antibodies was totally different from that of the anti-PMCA antibodies. Control sections did not contain any labeled secretion ameloblasts (Fig. 1C).

In the maturation zone, two different ameloblast groups with distinct morphology, namely the ruffle-ended and smooth-ended ameloblasts, are alternatively repeated several times throughout the whole zone [11, 23]. Ruffle-ended and smooth-ended ameloblasts are distinguished by a distal brush border and distal junctional complexes, and smooth distal end and proximal junctional complexes, respectively [11, 16]. They are easily detected by the F-actin pattern produced upon labeling with rhodamine-phalloidin (Rh-Ph) [16]. In the present study, single labeling with anti-SERCA2 showed that ameloblasts, but not papillary layer cells, were positive for SERCA2 (Fig. 2A, B). Furthermore, triple labeling with anti-SERCA2, Rh-Ph and Hoechst 33342 for nuclei revealed that anti-SERCA2 labeled both ruffle-ended ameloblasts with strong Rh-Ph fluorescence at the distal end and smooth-ended ameloblasts with stronger Rh-Ph fluorescence at the proximal end (Fig. 2C, D).

Anti-SERCA2 was localized in the ameloblasts of the rat incisors, with stronger binding observed in the matura-

Fig. 1. Secretion ameloblasts triple labeled with anti-SERCA2 (A), anti-PMCA (B) or 1% BSA-PBS for control (C) (green), rhodamine-phalloidin for F-actin (yellow to red) and Hoechst 33342 for DNA (blue). Anti-SERCA2 labeled the cytoplasm diffusely, whereas anti-PMCA labeled the plasma membrane. In the control section, no ameloblast labeling was observed, whereas proximal and distal terminal webs (arrows in C) labeled by rhodamine-phalloidin and nuclei labeled by Hoechst 33342 were evident. SI, stratum intermedium; A, ameloblast; E, enamel. Bars=50 μm.
face. In the present study, both ruffle-ended and smooth-ended ameloblasts (RA and SA, respectively) were labeled by anti-SERCA2 antibody, suggesting that the calcium-handling activity of SERCA2 does not differ between RA and SA. This is reasonable given that maturation ameloblasts modulate rapidly: one SA band moves to the next SA band through an RA band in 8–10 hours [23]. On the other hand, plasma membrane Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase is enriched in RA but weak in SA [3], and in the part of the RA zone where the ruffled border of the RA is re-creating, calcium uptake in enamel is lacking [26], indicative of differential PMCA activity in maturation ameloblasts. Thus, the significance of a similar level of SERCA2 reactivity in RA and SA remains to be clarified.

Papillary layer cells were not labeled by anti-SERCA2 antibody in the present study. Although the possible involvement of papillary layer cells in calcium ion transport to the enamel surface has been proposed [20], based on the present results, the SERCA2 protein is probably not related to calcium homeostasis in the papillary layer cells. Enamel epithelial cells are known to contain SERCA2b mRNA and protein, as detected by Northern and Western blotting [8]. The present study suggests that SERCA2 is localized in the ameloblasts, but not in the papillary layer cells, and that SERCA2 probably contributes to calcium homeostasis during enamel formation, especially in the maturation stage.

IV. Acknowledgments


V. References


