SHORT COMMUNICATION

Morphological heterogeneity of matrix vesicles in rat molar tooth germs with relation to predentin maturation

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Abstract

Matrix vesicles (MVs) in thin sections, were classified into four types: electron-opaque MVs, lucid MVs, MVs of medium opacity, and MVs containing needle-shaped inclusions. In freeze-fracture replicas, they were classified into three types by the number of membrane-associated particles (MAPs) on the convex P-faces: MAP-rich MVs, MAP-poor MVs, and MVs with a moderate amount of MAPs. In the course of predentin maturation, electron-opaque MVs and MAP-rich MVs increased in number. It is believed that the increase of these MVs is necessary for the onset of mineralization.

Introduction

It has been already established that matrix vesicles (MVs) initiate mineralization in such mesodermal hard tissues as dentin, cartilage, and bone^{1–4)}. The morphological and cytochemical heterogeneity of MVs is thought to represent their functional varieties^{5–7)}. Many morphological and cytochemical studies on MVs in predentin have suggested that MV membrane play important roles in the initial mineralization of dentin^{4,8–12)}. We examined rat molar predentin by freeze-fracture and thin section electron microscopy to obtain further information on the relation between morphological heterogeneity of MVs and the onset of mineralization.

Materials and Methods

Molar tooth germs of 2- and 4-days old Wistar rats were used. They were fixed in Karnovsky's fixative (4 per cent formaldehyde and 5 per cent glutaraldehyde in 0.1 M sodium cacodylate buffer) at pH 7.3 for 3–4 h at 4°C. After fixation, the tooth germs were rinsed in 0.1 M cacodylate buffer. For thin sectioning, the samples were treated with cacodylate-buffered 1 per cent OsO₄ at pH 7.3 for 1.5 h at 4°C. Then, they were block-stained with 1 per cent uranyl acetate, dehydrated in graded ethanols, and embedded in Epon. The thin sections were stained with both uranyl acetate and lead citrate. For freeze-fracturing, rinsed samples were immersed in 10, 20, 30 and 40 per cent glycerol series buffered with 0.1 M sodium cacodylate buffer for about 80 min in all. Then, they were frozen in Freon 22 cooled with liquid nitrogen. Fracturing and replication were carried out at –105°C and vacuum of 10^{–6} to 10^{–7} torr in a Millitorr FE-600 freeze-etching unit. The thin sections and replicas were observed under a Hitachi HU-12A electron microscope at 75 KV.

Results

Aperiodic collagen fibrils, periodic collagen fibers, cytoplasmic processes of odontoblasts and MVs were observed in predentin before mineralization was initiated. Spherical or oval MVs were scattered among the aperiodic

collagen fibrils and periodic collagen fibers which sometimes existed in close proximity to the odontoblast cytoplasmic processes. The diameters of MVs varied from 40 to 600 nm, though most of them were 70 to 150 nm. MVs increased in number rapidly after their first appearance in the predentin (Fig. 1a, b).

In thin sections, MVs of various appearances were classifiable into four types: highly electron-opaque MVs (type O), electron-lucid MVs (type L), MVs of medium electron opacity (type M), and MVs containing granular or needle-shaped inclusions (type G) (Fig. 2a-d). Table 1 shows the relation between each MV type and size; there are no obvious relations. The ratio of each MV type varied as predentin maturation advanced, as is shown in Fig. 4. The ratio of electron-opaque MVs was greater at the later stage than at the earlier stage, whereas the ratio of electron-lucid MVs decreased.

In freeze-fracture replicas, MVs were observed as hemispherical membranous structures consisting of various sizes and numbers of MAPs on the convex P-face (Fig. 1b). MVs were classifiable into three types according to the MAP numbers on the P-face: MVs with many MAPs (type R), MVs with no or few MAPs (type P) and MVs of an intermediate type (type M) (Fig. 3a-c). Table 2 shows the relation between size and MAP number. The ratio of MAP-rich MVs increased at the later stage, whereas the ratio of MVP-poor MVs decreased (Fig. 5).

Table 1 Relation between type and size of matrix vesicle in thin sections.
Each figure shows the number.
(n=511)

Diameter (nm)	Matrix vesicle type			
	O	M	L	G
0- 99	48	85	17	2
100-199	85	99	91	28
200-299	10	9	24	2
300-	1	4	6	0
Total	144	197	138	32

Table 2 Relation between type and size of matrix vesicle in replicas.
Each figure shows the number.
(n=130)

Diameter (nm)	Matrix vesicle type		
	R	M	P
0- 99	3	3	3
100-199	22	42	16
200-200	14	11	7
300-	4	2	3
Total	43	58	29

Fig. 4 Ratio of each matrix vesicle type in thin sections.

Each angle corresponds to the percentage of each type. "Earlier" represents the stage soon after matrix vesicles appeared in the predentin, and "Later" represents the stage immediately before mineralization began. As for types see explanation in Fig. 2. (n=511).

Fig. 5 Ratio of each matrix vesicle type in replicas.

See explanation in Figs. 3 and 4. (n=130)

Discussion

Our freeze-fracture observations showed various MAP numbers on the predentin MV membranes as described in previous reports¹³⁻¹⁵. Using the freeze-fracture technique, Takagi *et al.*¹⁴ observed MVs in the epiphyseal plate and recognized three different types of MVs, which led them to assume that extensive spreading of MAPs played an important role in mineralization. However, our study did not reveal MVs of the kind to which they referred (compare our Fig. 3 with Fig. 4 in their paper). Nonetheless, in our freeze-fracture study, we found three different MV types in the predentin and that the ratio of MAP-rich MVs increased as initial mineralization approached. This phenomenon may have some relation to the initial mineralization mechanism.

As is well known, in the initial mineralization sites of various tissues, alkaline phosphatase and adenosine triphosphatase activities of MVs increase as mineralization progresses^{5,16,17}. It has been believed that these enzymes promote mineralization in the MVs by hydrolasing the pyrophosphates or transporting the minerals, or by both. It has also been described that the activities of these enzymes increase in conjunction with the approach of initial mineralization¹⁶. Even at this stage, however, MVs showing no such enzymatic activities exist^{12,19,20}. Alkaline phosphatase and adenosine triphosphatase are believed to be associated with plasma membranes. Therefore, our freeze-fracture findings that MVs are morphologically heterogeneous and the ratio of MAP-rich MVs increase as predentin maturation progresses, may correspond to the cytochemical varieties of MVs.

In our observations, the ratio of electron-opaque MVs in sections and MAP-rich MVs in replicas increased as initial mineralization approached. Though it is unclear whether this phenomenon is due to "maturation" of the MVs or to increased generation of such MVs, it is probable that the increase of these MVs is necessary for initial mineralization in the predentin.

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Explanation of figures

Fig. 1(a, b) Survey electron micrograph of a thin section (a), and a freeze-fracture replica (b). Matrix vesicles (arrow head) vary in size, electron-opacity and number of membrane-associated particles.

BM, Basement membrane; OD, Odontoblastic processes; IE, Inner enamel epithelium.

(a), $\times 88,000$. (b), $\times 44,000$.

Fig. 2(a-d) Each type of matrix vesicles in thin sections.

(a), An electron-opaque matrix vesicle (type O). $\times 79,000$.

(b), A matrix vesicle of medium electron-opacity. (type M). $\times 44,000$.

(c), An electron-lucid matrix vesicle (type L). $\times 143,000$.

(d), A matrix vesicle with needle-shaped inclusion (type G). $\times 130,000$

Fig. 3(a-c) Each type of matrix vesicles in replicas.

(a), A matrix vesicle consisting of many membrane associated particles (type R). $\times 87,000$.

(b), A matrix vesicle with moderate amount of particles (type M). $\times 60,000$.

(c), A matrix vesicle with few particles (type P). $\times 77,000$.