Three-dimensional Observation of the Enamel Prisms of *Subhyracodon* Molars

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Abstract

Enamel prisms are a morphological feature and Hunter-Schreger bands are the main microstructure of mammalian tooth enamel. *Subhyracodon* is an extinct rhinocerotoid that inhabited North America in the Oligocene geologic period. The Hunter-Schreger bands of some rhinocerotoids run vertically along the axis of the tooth, and the boundary between bands is distinct in the tangential section. In our observation of *Subhyracodon* tooth enamel, vertical prism decussation along the tooth axis could be observed clearly in the inner three-fourths of the enamel. In this study, the path of the prisms was digitized and reconstructed three-dimensionally. The course of each prism undulated strongly at the border of the bands, and the prisms did not maintain their relative relations but showed positional changes and displacement of adjacent Hunter-Schreger bands.

Keywords :

enamel prism, Hunter-Schreger bands, *Subhyracodon*, 3D reconstruction

Introduction

The microstructure of enamel, such as enamel prisms and Hunter-Schreger bands (HSB), has been known to be of various types among taxa. Kawai (1) reported that the enamel structure of the cheek teeth (molars and premolars) was distinguished among orders of mammals and additional studies divided enamel structure into five types (2, 3). As previously described for rhinocerotoid cheek teeth (3-8), the HSB run vertically along the axis of tooth, whereas horizontal HSB is the most common arrangement in mammalian tooth enamel. In this study, the details of prism decussation in *Subhyracodon*, an extinct rhinocerotoid, have been researched by three-dimensional reconstruction based on serial ground sections for scanning electron microscopy (SEM).

Materials and Methods

Fragments of dry specimens of *Subhyracodon* molar from the Thomas Burke Memorial Washington State Museum in Seattle (USA) (UWBM no. 87034) were examined. Some fragments were embedded in polyethylene resin and sectioned tangentially, vertically, and horizontally. After polishing and slight etching with 1/20 N HCl, the specimens were

sputter-coated with Au-Pd. The specimens were then analyzed under a reflective light microscope and the enamel microstructure was examined by SEM (S2700 ; Hitachi Ltd., Tokyo, Japan). To elucidate the three-dimensional course and profile of the enamel prisms, sections were consecutively ground down in an average of 8- μ m steps tangentially to the dentinoenamel junction to a depth of about 60 μ m for SEM.

The identification of the successive images of prisms was simplified by creating holes as location marks on the enamel surface using an Er: YAG laser (Erwin Advel ; Morita Corporation, Tokyo, Japan) and contact-irradiating with the C400F tip (Morita Corporation, Tokyo, Japan) at 300 mJ/pulse/10 sec. These holes remained in a constant position throughout the series. We used commercially available software on the Windows operating system for this reconstruction. The location marks enabled the register of successive tracings by means of adjusting the function of the Photoshop Element (Adobe Systems Incorporated, San Jose, CA, USA) using 100x magnified SEM images. Separate tracings of each successive outline of 10 adjacent prisms were made on layers of the Photoshop Element using 500x

magnified SEM images. Outlines of corresponding prisms were identified, numbered, traced, and digitized via a graphic tablet (Intuos 3 ; Wacom, Saitama, Japan). Three-dimensional reconstructions were produced with the OZ image rendering software (Rise Corporation, Sendai, Japan). In these reconstructions, each prism could be shown in a different color and could be viewed from any perspective.

Results

In a natural fracture of a *Subhyracodon* molar, horizontal perikymata were seen, and a thin belted structure along the tooth axis was also observed on the surface of the enamel (Fig. 1). On the occlusal surface of the molar, radial HSB from the dentinoenamel junction in the inner two-thirds of the enamel could be observed clearly under a reflective light microscope (Fig. 2). Continuity of HSB was represented in the three-dimensional cutting surfaces of the molar (Fig. 3). In tangential sections, some bands seemed to bifurcate and discontinue on their way to the surface (Fig. 4). In SEM of tangential sections, the arrangement of the prisms belonging to each band seemed to be in the opposite direction as oblique-cut surface. Two or three lines of prisms,

Fig. 1. Perikymata are observed on the surface of the enamel (arrow) and a belted structure along the tooth axis is observed on the surface of the enamel under a reflective light microscope.

Fig. 2. Occlusal surface of the molar. Radial HSB from the dentinoenamel junction could be observed.

Fig. 3. Three-dimensional cutting surfaces of the molar. T, tangential ; H, horizontal ; L, longitudinal.

Fig. 4. Vertical prism bands can be seen under a reflective light microscope. Occlusal direction is toward the top.

Fig. 5. SEM image of a tangential section. Two or three lines of prisms compose the boundary between bands.

Fig. 6. SEM image of a tangential section. Note the shape of the prisms of the boundary between bands.

Fig. 7. SEM image of a horizontal section. The boundary is composed of the longitudinal cutting surfaces of prisms. The enamel surface direction is toward the top.

which appeared as cross-cut surfaces, composed the boundary between bands. The cutting features of boundary prisms were round and more irregular than the features of nonboundary prisms (Figs. 5, 6). In horizontal sections, prism orientation was slightly different between bands and the boundary between bands was composed of longitudinal cutting surfaces of prisms, each of which had different width (Fig. 7). On the longitudinal surface of a natural fracture, changes in prism orientation occurred abruptly at a certain depth with a twisted path (Figs. 8, 9). Directions of the prisms in the same area differed slightly from those of prisms at different depths (Figs. 10, 11).

The three-dimensional visualization of the group of the prisms was a scene viewed from the upper left

Fig. 8. Changes in the direction of prisms. SEM image in the broken longitudinal section.

Fig. 9. Changes in prism direction and distortions. SEM image in the broken longitudinal section.

Fig. 10. Prism arrangement at the boundary area between bands. (SEM image $\times 1000$)

Fig. 11. Prism arrangement in the same area as Figure 10 at a different depth ($40\ \mu\text{m}$). (SEM image $\times 1000$)

Fig. 12. Three-dimensional visualization of the group of the prisms viewed from the upper left oblique. Each prism is shown in a different color with the same letter (A-A' et al.) Arrow : direction of the prism.

oblique and each prism could be shown in a different color for the same letter (A-A' et al.; Fig. 12). This reconstruction showed displacement of position and form in areas of different depths. The prisms were seen to change shape corresponding to their inclines and directions, as indicated by the arrows in Figure 12.

Discussion

Subhyracodon was a cow-size hornless rhinocerotoid that lived during the last Eocene epoch through the Oligocene geologic period in North America (9-11). As previously described for rhinocerotoid cheek teeth (3-8), the HSB of *Subhyracodon* run vertically along the axis of tooth. The arrangement of the prisms belonging to each band seems to be in the

opposite direction as oblique-cut surface and two or three lines of prisms compose the boundary between bands in tangential sections. The different reflectivity of the bands, seen in sections viewed by incident light, are presumed to be due to the differing directions of the prisms in each band. In this specimen, the cutting feature of the boundary prisms was round but getting out shape in place, and more irregular or distortional than the nonboundary prisms. It could be deduced that the prisms in the boundary area became torsional or disordered during amelogenesis.

Osborn (12-15) could follow a group of enamel prisms for a distance of about 100 μm by applying his focusing-through technique to human enamel. From serial semithin sections of tooth germ, Warshawsky et al (16). made a three-dimensional prism profile of rat enamel and Hanaizumi (17) reconstructed the paths of prisms in dog enamel. Radlanski et al (18). presented the arrangement of human enamel prisms by three-dimensional reconstruction based on serial ground sections in 15- μm steps and reported the close similarity with three-dimensional images from optical sections in 2- μm steps by confocal laser scanning microscopy.

The three-dimensional visualization of the group of prisms, obtained from serial SEM sections, showed that the prisms did not simply run straight and parallel, but were moderately bent, twisted, crossed each other, and changed their position. Each prism was distorted and changed shape along the path. This corresponds to the SEM images of the natural surface in the broken longitudinal section (Figs. 8, 9), which indicated changes in the respective directions and vicinity relations of prisms along their path. Some prisms notably represented opposite directions, for example A-A' and B-B' in Figure 11, meaning changing or shifting of the HSB in which they were contained. This indicates, therefore, that there was no rigid division of prisms, regarded as a boundary between HSB, by reason of changes in the directions of prisms contained within the bands. To improve information about the enamel prisms with spatial structure on their path and relative relation-

ship, we need more detailed morphological data and knowledge concerning with ability of ameloblasts to change their tight intercellular attachments for displacement.

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