SHORT COMMUNICATION

High resolution electron microscopy of apatite crystals with octacalcium phosphate inclusion

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Abstract : Long and thin platy apatite crystals with apatite-octacalcium phosphate (OCP) intergrowth were obtained in a model system of enamel formation in the presence of 1ppm F^- . A high resolution electron microscopic study indicated that each apatite crystal embedded an OCP lamella one to several unit cells thick in the central part of an apatite matrix. The OCP lamella and its (100) plane were parallel to the (100) plane of apatite. The result indicates that the thin plate of OCP crystal acted as a template for the subsequent epitaxial overgrowth of apatite. The cross sections of some apatite crystals exhibited a central line which is similar to a so-called central dark line observed in the tooth enamel and dentine.

Introduction

Although there are many studies on the mechanism of the lengthwise and oriented growth of tooth enamel, the problem is still inconclusive. Roles of fibrous organic matrix¹⁻⁶⁾, enamel proteins^{7,8)}, and secretion flow⁹⁾ are proposed to have some relation to the orientation and the lengthwise growth of enamel crystals, but these hypotheses do not exclude other possibilities.

We have studied the mechanism of the lengthwise and oriented growth of enamel crystals using a model system¹⁰⁻¹⁴⁾ on the bases of a hypothesis that 1) one directional diffusion of Ca^{2+} through the layer of ameloblasts promotes the lengthwise and oriented growth and 2) ribbon-like enamel crystals grow initially as octacalcium phosphate (OCP)^{15,16)}. In our model system, where a cation selective

membrane was used to control Ca^{2+} diffusion, lengthwise and oriented growth of OCP took place on the membrane. Long and thin plate-like OCP crystals grew, on the whole, perpendicular to the membrane and parallel to each other. Previously, we reported that CO_3^{2-} , Mg^{2+} , and F^- affected the crystal growth of OCP and the inhibitory activity of these ions depended on the Ca and PO_4 concentrations and pH of the solution^{13,14)}. Among these ions, F^- is of particular importance, because a small amount of F^- suffices to reduce the caries susceptibility of tooth enamel¹⁷⁻¹⁹⁾. Moreover, it has been reported that F^- induces and accelerates the hydrolysis of OCP^{20,21)}, promotes apatite formation^{22,23)} and improves the crystallinity of apatite²⁴⁾.

The present study was designed to investigate the effect of F^- on the crystal growth of OCP in the membrane system. As a result,

Fig. 1 A high resolution electron micrograph of OCP crystal grown at pH 6.5 and 37 °C in the membrane system without the addition of F^- . The (100) planes of OCP ($d=18.67 \text{ \AA}$) are imaged.

thin and long platy apatite crystals which embedded an OCP lamella in the center were obtained in the presence of 1ppm F^- . This paper reports the effects of F^- on the morphology and the structure of the crystals, which were observed under a high resolution electron microscope.

Materials and Methods

Crystal growth experiments were carried out at 37 °C and at pH 6.5 for 3 days in the membrane system, as has been used in the previous studies^{13,14}. A cation selective membrane (CMV, Asahi Glass Co.) was attached on a small glass bottle which contains 50 ml of 30 mM $Ca(CH_3COO)_2 \cdot H_2O$. The small bottle was used as a Ca^{2+} diffusion source. The small bottle was put in a 7.2 mM of $NH_4H_2PO_4 + (NH_4)_2HPO_4$ solution with or without 1ppm F^- . All the solutions were adjusted to 37 °C and pH 6.5 before the reaction. After the reaction, the membrane was removed from the solution, washed with distilled water for a few seconds and then air dried.

The products were analyzed using a scanning electron microscope (JEOL, T-200, 25 kV), a micro-beam X-ray diffraction camera (Rigaku, Rotaflex UB-200, 50 kV, 150 mA, Cuka α) and a high resolution electron micro-

scope (Akashi, EM 002, 200 kV). For the high resolution electron microscopic observation, the products were embedded in an epoxy resin (EPOK 812, Oken Co.) by the usual procedures. Ultrathin sections perpendicular or parallel to the membrane were cut using an ultramicrotome (LKB, Ultratome III) with a diamond knife. To prevent dissolution of the crystallites, sections were floated on the surface of water which had been saturated with apatite. More than 100 crystals were observed.

Results and Discussion

OCP crystals grew without the addition of F^- , while apatite crystals grew with the existence of 1 ppm F^- . The analysis of the SEM and the X-ray diffraction studies indicated that both OCP and apatite are thin and long platy crystals, which elongated along the c-axis direction, and these crystals had an orientation. The c-axes of OCP or apatite crystals were, on the whole, perpendicular to the membrane and parallel to each other. The crystal size of apatite was smaller than that of OCP.

Figure 1 shows a lattice image of the (100) planes of OCP ($d=18.67 \text{ \AA}$) obtained under F^- free conditions. The (100) fringes of OCP continue up to the edges of the crystal.

Fig. 2 A transmission electron micrograph of apatite crystal grown at pH 6.5 and 37 °C in the membrane system with the addition of 1 ppm F^- . Each crystal exhibits a central line (s) (arrows) along the long axis of the crystal.

Fig. 3 A high resolution electron micrograph of apatite crystal with an OCP lamella in the center of an apatite matrix. The (100) planes of apatite ($d=8.17 \text{ \AA}$) and the (100) planes of OCP ($d=18.67 \text{ \AA}$) are imaged. These planes are parallel to each other.

Fig. 4 (a) A high resolution electron micrograph of a cross-sectioned apatite crystal which contains a central plane and (b) the electron diffraction pattern obtained from the selected area which contains other smaller crystallites. Both the lattice image and the diffraction pattern exhibit hexagonal symmetry, which is characteristic of apatite.

Fig. 5 A high resolution electron micrograph of a longitudinally-sectioned apatite crystal with a central plane. The (100) planes of apatite are imaged and they are parallel to the central plane.

Whereas, apatite crystal which grew in the presence of 1 ppm F^- embedded an OCP lamella in the center of an apatite matrix. The cross sections of these apatite crystals exhibited central lines (arrows in Fig. 2). Figure 3 shows a lattice image of apatite crystal which embedded an OCP lamella with three unit cells thick in the center of an apatite matrix. The (100) fringes of apatite ($d=8.17 \text{ \AA}$) are parallel to the (100) fringes of OCP. According to more than 100 observations, the thickness of the OCP lamella in the a -axis direction was one to several unit cells. In these apatite crystals, the (100) planes of OCP were parallel to the (100) planes of apatite and the OCP lamella located at the center of the crystal. The combined analysis of the X-ray diffraction and the TEM studies indicated that apatite grew on the (100) plane of the thin plate of OCP crystal epitaxially; $(100)_{\text{apatite}} \parallel (100)_{\text{OCP}}$, $C_{\text{apatite}} \parallel C_{\text{OCP}}$ and $b_{\text{apatite}} \parallel b_{\text{OCP}}$.

Some apatite crystals contained a central plane, instead of the distinct OCP lamella. Figure 4a and 5 respectively show a cross-sectioned and a longitudinally-sectioned apatite crystal with a central plane. The (100) planes of apatite are imaged. The central plane is parallel to the (100) plane of apatite. The width of the plane was about 8-12 $\text{\AA}$. Figure 4b is the diffraction pattern of the selected area containing the crystal and a few other crystals. Both Fig. 4a and 4b exhibited the hexagonal symmetry, which is characteristic of apatite.

The present study demonstrates that thin and long platy apatite crystals which embedded a central OCP lamella or a central plane were formed in the membrane system in the presence of F^- . Since the OCP region was too small to act as a coherent diffraction domain, apatite crystals with the OCP lamella did not show any reflection of OCP in their X-ray diffraction photographs. Ca-deficient carbonate containing apatite with a central plane has been synthesized by Nelson *et al.*¹⁶⁾. Their high resolution electron microscopic analysis revealed that the central plane was a two-dimensional OCP inclusion²⁵⁾ and, therefore, they provided a new model of intergrowth of apatite on OCP template^{16,26)}. The formation of apatite with an

OCP lamella can be explained in the same way: A plate-like OCP grew to a certain size (=the critical hydrolysis radius²⁶⁾) before OCP hydrolysis occurred. Apatite crystal grew subsequently on the (100) plane of the thin plate-like OCP crystal, embedding OCP in the center of the apatite crystal. Since F^- was added into the solution where OCP was stable and it is a well known fact that F^- promotes the hydrolysis of OCP^{20,21)}, F^- must have caused formation of the apatite-OCP lamellar mixed crystals by decreasing the critical OCP size before the apatite formation started.

In the present study, many apatite crystals contained a distinct OCP lamella and, moreover, there was a variety in the thickness of the OCP lamella. This suggests that the critical size of OCP crystal is sensitive to the solution condition. The thickness of the OCP lamella will probably decrease when the instability of OCP increases. Further analysis on this point is now on going.

Crystallites of tooth enamel^{1,3,6,27)}, dentine²⁸⁾ and bone²⁹⁾ often exhibit the so-called central dark line when they were observed under a transmission electron microscope. The occurrence of the central line is of particular interest, since it would give important information about the initial stage of crystallization. The possibility that the presence of a precursor phase, such as OCP¹⁵⁾, contributes to the formation of the central line has been pointed out^{16,27)}. Although the existence of OCP has not been confirmed in the precipitates of *in vivo* mineralization, apatite which exhibits a central line was formed under two different experimental conditions, when OCP acted as a template for apatite overgrowth. These results refer to the synthetic ones, but stress a role of a transitory existed OCP in the process of enamel formation.

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