



Synthesis and ultrastructure of plate-like apatite single crystals as a model for tooth enamel

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ABSTRACT

Hydroxyapatite (HAp) is an inorganic constituent compound of human bones and teeth, with superior biocompatibility and bioactivity characteristics. Its crystal structure is hexagonal, characterized by $a(b)$ - and c -planes. In vertebrate long bones, HAp crystals have a c -axis orientation, while in tooth enamel, they have an $a(b)$ -axis orientation. Many methods can be used to synthesize c -axis oriented HAp single crystals; however, to the best of our knowledge, there have been no reports on a synthesis method for $a(b)$ -axis oriented HAp single crystals. In this study, we successfully synthesized plate-like HAp crystals at the air–liquid interface of a starting solution via an enzyme reaction of urea with urease. Crystal phase analysis and ultrastructure observations were carried out, and the results indicated that the particles were single crystals, with almost the same $a(b)$ -axis orientation as tooth enamel. It is hoped that by utilizing their unique surface charge and atomic arrangement, the resulting particles can be used as a high-performance biomaterial, capable of adsorbing bio-related substances and a model for tooth enamel.

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1. Introduction

Hydroxyapatite (HAp), with a stoichiometric composition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the main mineral constituent of natural bones and teeth [1–3]. HAp is an osteoconductive material, and can be used as a biomaterial for artificial bone, cement and teeth roots [2,4–8]. In addition, HAp is widely used as an adsorbent for bio-related substances such as proteins, because it does not cause the degeneration of proteins during the adsorption process [9,10].

HAp has a hexagonal crystal structure with a $\text{P6}_3/\text{m}$ space group, and its unit cell parameters are $a(b) = 0.943$ nm and $c = 0.688$ nm [11,12]. In vertebrate long bones and tooth enamel (Fig. 1a), HAp crystals have preferred c -axis and $a(b)$ -axis orientations, respectively [13–18]. The corresponding c - and $a(b)$ -planes are different in terms of composition and atomic arrangement. For example, the $a(b)$ -planes are rich in calcium ions and are positively charged whereas the c -planes are rich in phosphate or hydroxide ions and are negatively charged. Consequently, these planes exhibit different properties with regard to resorbability, biocompatibility, and sorbability [2,9,10].

Designing crystals with specific orientations and morphology is an effective method for improving the functional properties of materials. Theoretically, as shown in Fig. 1b, if an anisotropic crystal growth rate can be achieved, HAp can be synthesized with either a c -axis

orientation and a fiber-like morphology, or an $a(b)$ -axis orientation and a plate-like morphology [19]. Single-crystal HAp fibers with preferred c -axis orientations can be produced using homogeneous precipitation [19–21] and hydrothermal methods [22,23]. Moreover, by utilizing the particle morphology, resorbability and surface charge, HAp fibers have been developed for use as high-performance biomaterials, such as scaffolds for three-dimensional cell cultures [24,25], implantable bone grafts [26], and carriers for drug delivery systems [27].

Surprisingly, to the best of our knowledge, a synthesis method for single-crystal HAp particles with a preferred $a(b)$ -axis orientation has not yet been reported. The reason may be that the characteristics of HAp crystals make growth along the c -axis easy and therefore preferred. In an earlier study, we synthesized plate-like HAp particles using a homogeneous precipitation process. However, the resulting particles exhibited only weak $a(b)$ -axis orientations and were discovered to be polycrystalline [28].

Interfaces have been widely utilized to synthesize hexagonal plate-like crystals [29–31]. For example, Shrestha et al. prepared hexagonal fullerene crystals using a liquid–liquid interfacial precipitation method involving an interface between isopropyl alcohol and a saturated solution of fullerene in a mixture of benzene and carbon tetrachloride [29]. Chen et al. reported that a PbO nanoplate can be produced at the air–liquid interface at relative lower temperature via interfacial hydrolysis of Pb^{2+} ions [30]. In addition, Wang et al. synthesized larger regular silver plate particles at the liquid–solid interface by combining Langmuir monolayer technique and galvanic electroless deposition technique [31]. In the present study, using a newly developed

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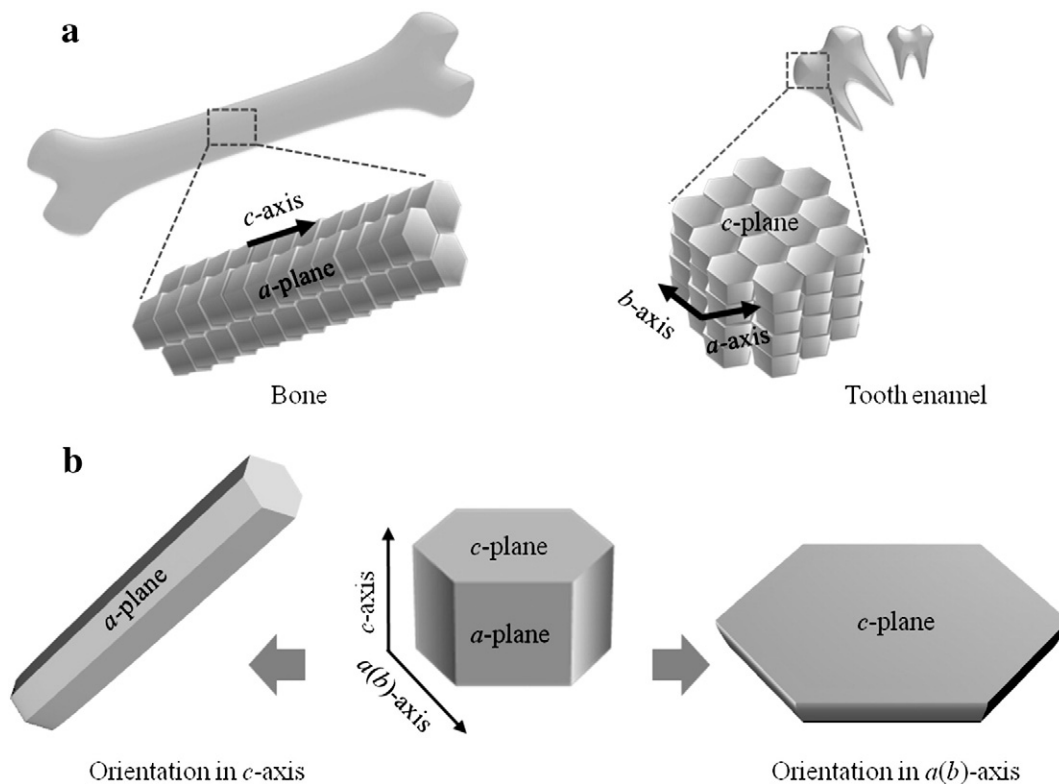


Fig. 1. (a) Orientation of HAp crystals in long bone and tooth enamel, and (b) model of HAp crystal growth.

process, we successfully synthesized plate-like HAp single crystals with a high $a(b)$ -axis orientation at the air–liquid interface of the starting solution.

2. Materials and methods

Plate-shaped HAp with a preferred $a(b)$ -axis orientation was formed at the air–liquid interface of the starting solution via an enzyme reaction of urea with urease. Starting solutions with a Ca/P ratio of 1.67 were prepared by mixing $5.0 \text{ mmol} \cdot \text{dm}^{-3}$ calcium carbonate (CaCO_3 ; Wako Pure Chemical Industries, Ltd., Japan), $3.0 \text{ mmol} \cdot \text{dm}^{-3}$ phosphoric acid (H_3PO_4 ; Wako Pure Chemical Industries, Ltd., Japan), and $1.0 \text{ mol} \cdot \text{dm}^{-3}$ urea ($(\text{NH}_2)_2\text{CO}$, Wako Pure Chemical Industries, Ltd., Japan). An aqueous nitric acid solution (HNO_3 , Wako Pure Chemical Industries, Ltd., Japan) was added to adjust the pH to 3.0. The starting solution (120 cm^3) was dispensed in a glass petri dish ($\phi 116 \text{ mm}$) immediately after adding a 2.734 cm^3 aqueous 0.1 mass% urease solution (activity: $127 \text{ unit} \cdot \text{mg}^{-1}$; from Jack Bean, Wako Pure Chemical Industries, Ltd., Japan), and heated in an incubator at $50 \text{ }^\circ\text{C}$ for 96 h.

The pH value of the solution increased due to hydrolysis of the urea, and as a result, two types of products were obtained, a film-like product formed at the air–liquid interface of the solution, and a second product formed within the solution. Since the degree of orientation of the latter product was low, it was discarded as a by-product. Finally, in order to remove the residual OCP that was present in small amounts in the interfacial product, heat treatment was carried out at $600 \text{ }^\circ\text{C}$ for 2 h in air using an electric furnace (KBF314N1, Koyo Thermo Systems Co., Ltd., Japan).

The crystalline phases of the resulting particles were identified by powder X-ray diffraction (XRD; MiniFlex, Rigaku Co., Japan) using Cu-K α radiation at 30 kV and 15 mA. XRD data was collected under the following conditions: 2θ range of $3\text{--}60^\circ$, scan rate of 2° per minute and sampling width of 0.02° .

The thermal stability of the interfacial product was examined using differential thermal analysis and thermogravimetry (DTA–TG; Thermo Plus TG8120, Rigaku Co., Japan). The DTA–TG measurement was carried out from room temperature to $1200 \text{ }^\circ\text{C}$ at heating rate of $10 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$ under air flow rate of $100 \text{ cm}^3 \cdot \text{min}^{-1}$, using Al_2O_3 powder as reference.

Calcium and phosphorus contents of the interfacial product and the resulting particles were determined by inductively coupled plasma atomic emission spectrometry (ICP–AES; SPS7800, SII NanoTechnology Inc., Japan), and Ca/P molar ratios were calculated.

The particle morphology was characterized using scanning electron microscopy (SEM; JSM6390LA, JEOL Ltd., Japan) at 10 kV. Before observation, all SEM samples were coated with platinum Pt in a vacuum. The ultrastructure of the particles was examined by high-resolution transmission electron microscopy (HR–TEM; JEM–2100 F, JEOL Ltd., Japan) at 200 kV. HR–TEM samples were prepared by dispersing the particles in ethanol and then collecting them on carbon-coated copper grids.

Furthermore, the zeta potential of the particles was measured using a zeta-potential analyzer (ELSZ-2; Otsuka Electronics Co., Ltd., Japan). A specimen suspension was prepared by dispersing the particles in a $10 \text{ mmol} \cdot \text{dm}^{-3}$ NaCl aqueous solution, and the measurements were carried out at $25 \text{ }^\circ\text{C}$ in the range of pH 6.0 to 9.0 ($0.1 \text{ mol} \cdot \text{dm}^{-3}$ NaOH and $0.1 \text{ mol} \cdot \text{dm}^{-3}$ HCl were used to adjust the pH).

3. Results and discussion

When the starting solution was heated at $50 \text{ }^\circ\text{C}$ in an incubator, a film-like calcium phosphate product was formed at the air–liquid interface; the product floated to the top of the liquid phase. The time dependence of the growth process was investigated.

The crystalline phases and particle morphologies following different reaction times were examined using XRD and SEM, and the results are shown in Fig. 2. Approximately 14 h after heating, a thin-film-like

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