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Importance of nucleation in transformation of octacalcium phosphate to hydroxyapatite

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ABSTRACT

Octacalcium phosphate (OCP) is regarded as an *in vivo* precursor of hydroxyapatite (HA). It is important to understand the mechanism of transformation of OCP to HA in order to reveal the mechanism of mineralization and help in the development of artificial bone-repairing materials. Herein, we have examined the behavior of OCP in a simulated body fluid (SBF) and pure water. The OCP particles immersed in the SBF at 37 °C did not transform to HA even after 720 h of immersion, though the particles showed crystal growth. In distilled water at 60 °C, the OCP particles transformed to HA but the unreactive period was observed. Although the immersed solution became supersaturated with HA within 12 h of immersion, the OCP was not transformed in the first 36 h of immersion. These results indicate that the nucleation of HA is the rate-determining step in the transformation of OCP to HA.

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is the main inorganic constituent of human bones and teeth. Considering the treatment of diseases and injuries in bone, it is important to understand the mechanism of calcification. Additionally, nowadays calcium phosphates are widely used to repair the bone defects [1–3] and it is also important to understand the behavior of calcium phosphates. Among the calcium phosphates, octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$, OCP) is paid attention in the present study because it is regarded as an *in vivo* precursor of HA [4,5] and has become an important candidate for use as a biomaterial for bone repair [6–9]. OCP was found to be converted to HA and support bone regeneration *in vivo* [6,10]. Therefore, elucidation of the mechanism by which OCP is converted to HA would provide extremely useful information for the design and development of materials for bone repair. The transformation of OCP to HA can be also utilized for the material process [11]. By using this transformation, HA nanorods [12] and plate-like HA particles [13] can be synthesized.

There are several papers about the transformation of OCP to HA [4,14–24]. OCP has a layered structure composed of an apatitic layer, with a structure similar to that of HA, in addition to a hydrated layer. The atomic arrangements of the apatitic layers of OCP and HA are extremely alike [14]. It is suggested that the HA crystals can grow epitaxially on the OCP crystals because of this structural similarity [4,15,16]. LeGeros et al. suggested that the transformation of OCP to HA occurs by the dissolution of OCP and the subsequent precipitation of HA [17]. Iijima et al. suggested that the transformation of OCP to HA proceeds *via in situ* reorganization of lattice ions and/or apatitic clusters [18]. Recently, transmission electron microscope (TEM) observation of *in situ* transformation by electron beam of TEM [19] and nuclear magnetic resonance (NMR) measurement of the transformation [20] were also reported. However, they mainly focused on the crystal growth and discussed the relationship between the OCP structures and the formed HA, and did not focus on the initial transformation reaction, *i.e.* nucleation of HA. We previously examined the transformation of OCP in distilled water and revealed the existence of the unreactive period in the first stage [21]. However, the initial transformation reaction is still unclear.

Moreover, the behavior of OCP under physiological conditions is still unclear. Ban et al. [22] and Yokoi et al. [25] reported that OCP did not transform to HA in a simulated body fluid (SBF), while several researchers [10,23,24] reported the *in vitro* transformation OCP into HA under physiological conditions. These different behaviors may also be explained by revealing the transformation behaviors from the view point of the nucleation.

In the present study, we reveal the importance of the nucleation in the transformation of OCP to HA by immersing OCP in SBF and in pure water. The changes in both the samples and the solutions during the immersion were examined in detail, and the transformation mechanism was discussed from the view point of nucleation.

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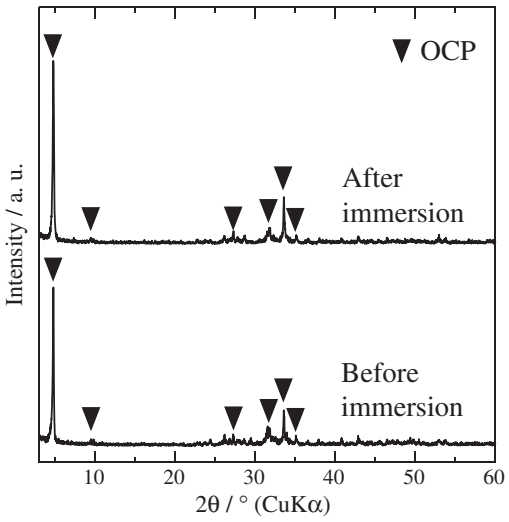

Fig. 1. XRD patterns of sample before and after immersion in SBF at 37 °C for 720 h.

2. Materials and methods

The OCP powder was synthesized from calcium carbonate and phosphoric acid according to the previously reported method [26,27]. A 20 mmol of phosphoric acid (85%, Wako Pure Chemical Industries, Ltd., Japan) was added to 200 cm^3 of distilled water with stirring. The resultant homogeneous solution was heated to 60 °C, and then 26.6 mmol of calcium carbonate (Wako Pure Chemical Industries, Ltd., Japan) was added to the solution with stirring. The resulting suspension was stirred at 60 °C for 6 h. The product was collected using suction filtration, washed with distilled water, and dried at 37 °C for 24 h.

The obtained OCP was immersed in an SBF prepared according to reports by Kokubo et al. [28,29], with ion concentrations similar to those of the human blood plasma. The SBF had a pH value of 7.40, and ion concentrations as follows (in mmol·dm^{-3}): $Na^+ = 142.0$; $K^+ = 5.0$; $Ca^{2+} = 2.5$; $Mg^{2+} = 1.5$; $Cl^- = 147.8$; $HCO_3^- = 4.2$; $HPO_4^{2-} = 1.0$; and $SO_4^{2-} = 0.5$. A 15 mg sample of the OCP powder was placed in a polypropylene tube with 1.5 cm^3 of the SBF. The tubes were kept in an incubator at 37 °C for 720 h. The SBF was replaced every day in order to simulate the fluid circulation within the body. From the preliminary experiment, the decrease in calcium and phosphorus concentrations in the SBF was observed when OCP was immersed in the SBF. As the changes in calcium and phosphorus concentrations in the SBF should affect the reaction of OCP due to the changes in degree of supersaturation, the renewal of the SBF was conducted during the SBF immersion test. After the immersion, the powders and solutions were separated by centrifugation. The powders were collected and washed with distilled water, and then dried at 37 °C for 24 h.

In the next experiment, a 0.10 g sample of fresh OCP powder was placed in a polypropylene tube with 10 cm^3 of distilled water. The tubes were then kept in an incubator at 60 °C for 240 h without exchanging the solution. As the phase change from OCP to HA was not observed in distilled water at 37 °C within 240 h in the preliminary experiment, the temperature was increased to 60 °C to accelerate the transformation reaction. After the immersion, the powders and solutions were separated by centrifugation. The powders were collected and washed with distilled water, and then dried at 37 °C for 24 h. The supernatant solutions were moved to fresh tubes for the subsequent analysis.

The samples were characterized using X-ray diffractometry (XRD; RINT-2200VL, Rigaku, Japan) with CuKα radiation. Diffraction lines derived from OCP and/or HA were detected in all samples, with no other lines evident. The OCP content in the immersed powders was quantitatively determined using an internal standard method. As the standard, Si powder (NIST, USA) was mixed with the immersed samples, and the OCP content was quantified using a calibration curve prepared by plotting the ratio of OCP and Si integrated intensities against the OCP content in the known mixtures of OCP, HA, and Si. The 100 diffraction line ($2\theta = 4.7°$) of OCP and the 111 diffraction line ($2\theta = 28.4°$) of Si were used for the preparation of the calibration curve. The morphology of the immersed samples was observed using scanning electron microscopy (SEM; SU8000, Hitachi, Japan) and transmission electron microscopy (TEM; HF-2000, Hitachi, Japan). The particle sizes of the samples before and after immersion in the SBF for 720 h were measured from the obtained SEM images. The pH value and calcium and phosphorus concentrations of the solutions after the immersion were examined using a standard pH electrode and inductively coupled plasma atomic emission spectroscopy (ICP-AES; ICAP6000, Thermo Fisher Scientific, USA), respectively. Using the values for the pH and ion concentrations, the degree of supersaturation of the solution was calculated according to a previously reported method [18,30,31]. The degrees of supersaturation of OCP (S(OCP)) and HA (S(HA)) were calculated as shown in Eqs. (1) and (2), respectively.

$$S(\mathrm{OCP}) = \sqrt[8]{\frac{a^4\left(ca^{2+}\right)a\left(H^+\right)a^3\left(PO_4^{3-}\right)}{K_{sp}(\mathrm{OCP})}} \tag{1}$$

$$S(\mathrm{HA}) = \sqrt[9]{\frac{a^5\left(ca^{2+}\right)a^3\left(PO_4^{3-}\right)a\left(OH^-\right)}{K_{sp}(\mathrm{HA})}} \tag{2}$$

The activity coefficient was obtained using the Debye–Hückel equation, and the activities ($a(Ca^{2+})$, $a(PO_4^{3-})$, $a(H^+)$ and $a(OH^-)$) were

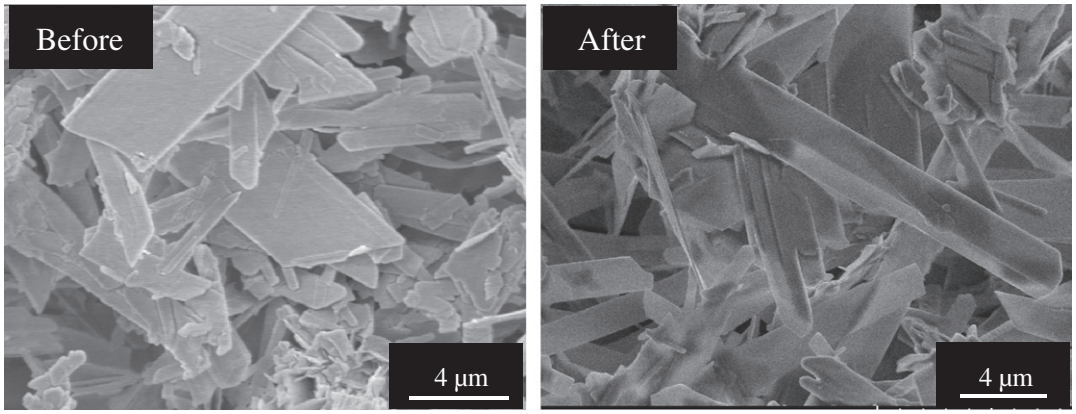

Fig. 2. SEM images of sample before and after immersion in SBF at 37 °C for 720 h.