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Synthesis of octacalcium phosphate with incorporated succinate and suberate ions

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Abstract

Octacalcium phosphate (OCP) has a layered structure composed of apatitic and hydrated layers. HPO_4^{2-} in the hydrated layer can be substituted by dicarboxylate ions. The synthesis of octacalcium phosphate with incorporated dicarboxylate ions (octacalcium phosphate carboxylate, OCPC), was investigated through the effects of the initial concentration of dicarboxylic acid on the formation of OCPC. Succinic acid (Suc) and suberic acid (Sub) were used as dicarboxylic acids for preparing OCPC, and crystalline phases of the products were characterized by powder X-ray diffraction (XRD). When the amount of added dicarboxylic acid was 1–5 times the amount corresponding to the stoichiometric composition of OCPC, incorporation of dicarboxylate ions progressed with increasing amount of added dicarboxylic acids, although OCP without incorporated dicarboxylate ions was also present. When the amounts of added Suc and Sub were larger than 10 times the stoichiometric amount in OCPC, a single OCPC phase was detected in the powder XRD patterns. Amounts of Suc and Sub greater than 10 times the stoichiometric amounts facilitated formation of the OCPCs and inhibited formation of OCP. The incorporation of dicarboxylate ions into OCP competes with incorporation of HPO_4^{2-}. Hence a high concentration of dicarboxylic acids is required for complete incorporation of dicarboxylate ions in OCP.

Keywords: A. Powders: chemical preparation; B. Nanocomposites; E. Biomedical applications; Octacalcium phosphate

1. Introduction

Calcium phosphates are familiar compounds that are applicable to bone reconstruction in biomedical applications [1]. Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) and β-tricalcium phosphate (β-TCP; $Ca_3(PO_4)_2$) are popular crystalline phases for use as ceramic biomaterials for bone substitutes, i.e. artificial bone. Octacalcium phosphate (OCP; $Ca_8(HPO_4)_2(PO_4)_4{\cdot}5H_2O$) is a thermodynamically metastable phase under physiological conditions [2], and is considered to be a precursor of formation of HAp in bone tissue [3]. Dicalcium phosphate anhydrous (DCPA; $CaHPO_4$), dicalcium phosphate dihydrate (DCPD; $CaHPO_4{\cdot}2H_2O$), α-tricalcium phosphate (α-TCP; $Ca_3(PO_4)_2$) and tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$) are available as bioactive pastes for bone fillers [4,5]. Of these calcium phosphates OCP has a particularly characteristic crystal structure with triclinic crystals, and a layered structure composed of apatitic and hydrated layers [6]. The layered structure is constructed by hydrogen phosphate ion (HPO_4^{2-}), which plays the part of a pillar in the hydrated layer. The HPO_4^{2-} ion forms a Ca–HPO_4–Ca bond and links two apatitic layers, and can be substituted by dicarboxylate ions. Monma and Goto reported that dicarboxylate ions incorporated into the OCP structure expand the interplanar spacing of the (100) planes of OCP [7]. Typical dicarboxylic acids that can be incorporated into OCP are aliphatic dicarboxylic acids [8], and OCP with incorporated dicarboxylate ion (OCPC; OCP carboxylate) is regarded as an organic–inorganic hybrid. A previous report indicated that OCP incorporating aspartate ions showed specific adsorption of an aldehyde [9]. Hence OCPCs are expected for use as an absorbent having specific adsorbability. Additionally, OCPCs are good candidate for

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novel materials implying bone substitutes with unique characteristics derived from incorporated organic molecules, in addition to high biological affinity of OCP.

Several methods for synthesis of OCPC have been reported [10–15]. Conventionally, OCPC is synthesized by hydrolysis of α-TCP in the co-presence of a dicarboxylic acid [10–13]. Kamitakahara *et al.* reported that OCPC with high crystallinity can be synthesized from calcium carbonate ($CaCO_3$), phosphoric acid (H_3PO_4) and a dicarboxylic acid [14]. The synthetic procedure allows wider compositional variation than the method using α-TCP as starting material. Although OCP with incorporated succinate ion was obtained without other phases by this process, OCPCs with incorporated adipate and suberate ions were formed with a precipitate of OCP that did not have dicarboxylate in its crystalline structure. In the formation of OCPC, HPO_4^{2-} and dicarboxylate ion compete for incorporation into the hydrated layer of OCP. Hence the concentration of dicarboxylic acid is one of most important factors in the formation of OCPC. However, the effects of the concentrations of dicarboxylic acids on formation of OCPC have not been investigated. In the present study we examined the formation of OCPC in the presence of high relative concentrations of dicarboxylic acids. We used succinic acid (Suc; $HOOC(CH_2)_2COOH$) and suberic acid (Sub; $HOOC(CH_2)_6COOH$), since they have simple chemical structures and are familiar dicarboxylic acids for incorporation into OCP.

2. Experimental procedures

Varying amounts (0, 1, 2, 4, 10, 20 or 50 mmol) of succinic acid (Suc, $HOOC(CH_2)_2COOH$; Wako Pure Chemical Industries Ltd., Osaka, Japan) or suberic acid (Sub, $HOOC(CH_2)_6COOH$; Wako Pure Chemical Industries Ltd.) were dissolved in 200 cm^3 of ultrapure water, and the pH was adjusted to 5.5 by addition of an appropriate amount of ammonia solution (NH_3; 25% aqueous solution, Wako Pure Chemical Industries Ltd.). 16.0 mmol of calcium carbonate ($CaCO_3$ (calcite); Nacalai Tesque Inc., Kyoto, Japan) was suspended in the dicarboxylic acid solution, then 10.0 mmol of phosphoric acid (H_3PO_4; 85% aqueous solution, Nacalai Tesque Inc.) was mixed with the suspension. The suspension was stirred at 60 °C for 6 h. Changes in pH of the suspension were measured using a glass-electrode type pH meter (D-53, Horiba Ltd., Kyoto, Japan). The precipitates were isolated by vacuum filtration then dried at 40 °C overnight. The synthesis procedure is shown in Fig. 1.

Sample powders were placed on a glass sample holder, and the crystalline phases of the precipitates were identified by powder X-ray diffraction (XRD; RINT 2100V, Rigaku Co., Tokyo, Japan) in the range $2^\circ \leq 2\theta \leq 40^\circ$ with 0.02° step size and scan rate 2.0° min^{-1}, using Cu Kα radiation. To analyze changes in crystalline phases during reaction, sample powders were obtained by vacuum filtration of 2 cm^3 aliquots of reaction mixtures. The small amounts of sample powders thus obtained were placed on a non-reflecting silicon substrate, and the crystalline phases of the samples characterized by powder XRD. The morphologies of the precipitates were observed by scanning electron microscopy (SEM; JSM5600, JEOL Ltd., Tokyo, Japan), after application of a thin gold coating. Hereafter, the samples are denoted as Suc*X* and Sub*Y*, where *X* and *Y* indicate the amount (mmol) of added Suc and Sub, respectively. A sample synthesized in the absence of dicarboxylic acid was designated the Control sample.

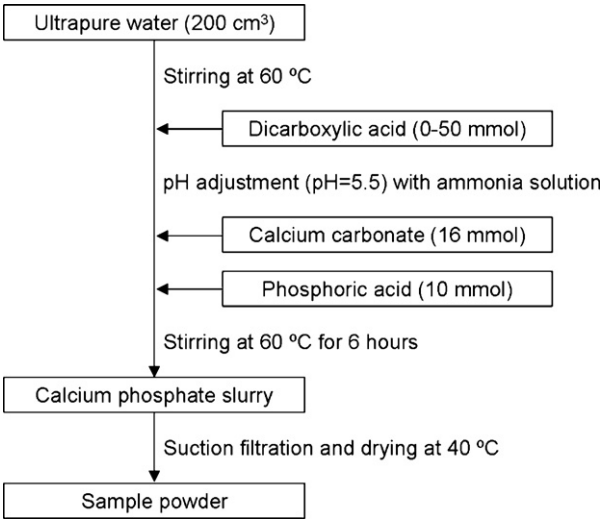

Fig. 1. Procedures for preparing OCP incorporated with succinic acid (Suc) or suberic acid (Sub).

3. Results

Crystalline phases were characterized according to powder diffraction files #74-1301, #11-0293 and #47-1743 for assignment of OCP without dicarboxylate, DCPD and calcite, respectively. Hereafter OCP without dicarboxylate is abbreviated as Pure-OCP. We assigned the powder XRD patterns of OCP with incorporated Suc (Suc-OCP) or Sub (Sub-OCP) based on a previous report [10]. The interplanar spacing of (100) of OCP is expanded by the substitution of HPO_4^{2-} in the hydrated layer by dicarboxylate ions. Hence the 100 reflections of OCPCs are detected at lower angle than that of Pure-OCP. The 100 reflections of Pure-OCP, Suc-OCP and Sub-OCP were detected at $2\theta = 4.7^\circ$, 4.1° and 3.3°, respectively. Fig. 2 shows powder XRD patterns of the samples synthesized in the presence of Suc: diffraction peaks assigned to OCP were detected in all of those samples. Diffraction peaks assigned to calcite were detected in the Control sample and Suc1. The powder XRD patterns in the range $2^\circ \leq 2\theta \leq 6^\circ$ in Fig. 2 show that Pure-OCP was detectable in the Control sample and Suc1. Diffraction peaks assigned to Suc-OCP were detected in Suc2, Suc4, Suc10, Suc20 and Suc50. A small shoulder peak was detected at about $2\theta = 4.7^\circ$ in the powder XRD patterns of Suc2 and Suc4. Fig. 3 shows powder XRD patterns of the samples synthesized in the presence of Sub; diffraction peaks assigned to OCP were detected in all of those samples. Diffraction peaks assigned to calcite were detected in the Control sample, Sub1 and Sub2. According to the powder XRD patterns in the range $2^\circ \leq 2\theta \leq 6^\circ$ in Fig. 3, diffraction peaks assigned to Pure-OCP were detected in the Control sample, Sub1, Sub2 and Sub4. In

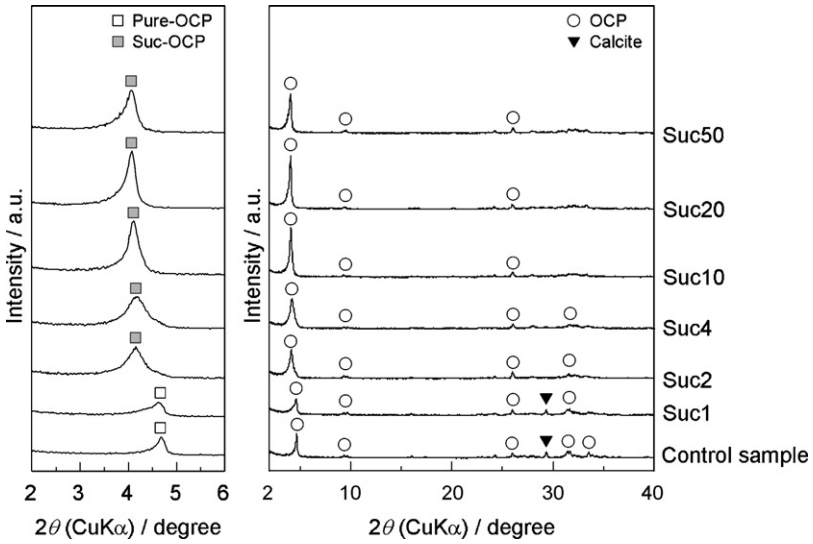

Fig. 2. Powder X-ray diffraction (XRD) patterns of the samples formed in the presence of succinic acid (Suc). The left hand figure shows XRD patterns in the 2θ range 2–6°.

Sub10, a very small peak was detected at about $2\theta = 4.7°$. Diffraction peaks assigned to Sub-OCP were detected in Sub2, Sub4, Sub10, Sub20 and Sub50.

The SEM images in Fig. 4 show that plate-like crystals were formed in all of the samples. The OCP crystals formed in the presence of Suc were larger than those formed in the absence of Suc, and the same trend in crystal size was observed for OCP samples formed in the presence of Sub. There was no apparent dependence of crystal size on the amount of dicarboxylic acid added to the reaction mixture.

Fig. 5 shows the changes in pH during reactions of the samples in the presence of Suc. At the start of the reaction, calcium carbonate was mixed with dicarboxylic acid (Suc) solution with pH = 5.5. The pH of the slurry was increased by addition of calcium carbonate, and the magnitude of the increase in pH decreased with increasing amount of Suc in the reaction mixture. After 2 min phosphoric acid solution was added to the calcite slurry. The pH of the suspension decreased immediately after the addition of phosphoric acid, followed by a gradual increase in pH: the magnitude of these pH changes decreased with increasing amount of Suc.

To clarify the changes in the crystalline phases of precipitates during the synthesis of OCP and OCPC, the Control sample and Suc20 were investigated by powder XRD of the precipitates extracted at various times. The XRD results are shown in Fig. 6, where the reaction time was measured as the time elapsed after addition of calcium carbonate. At 1 min, calcite was detected in the Control sample and Suc20, and no other crystalline phases (such as compounds of calcium and succinic acid) were detected. After 2 min phosphoric acid solution was added to the slurry. At 3 min, calcite and a small amount of dicalcium phosphate dihydrate (DCPD;

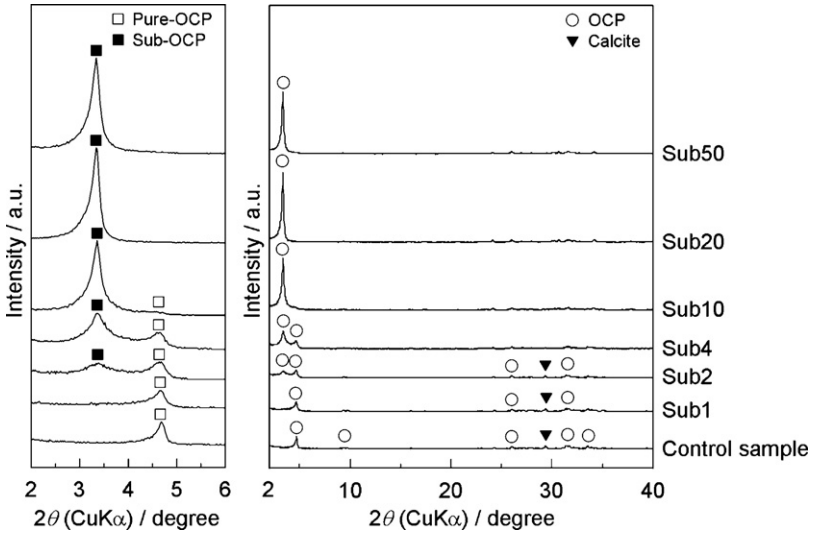

Fig. 3. Powder X-ray diffraction (XRD) patterns of the samples formed in the presence of suberic acid (Sub). The left hand figure shows XRD patterns in the 2θ range 2–6°.

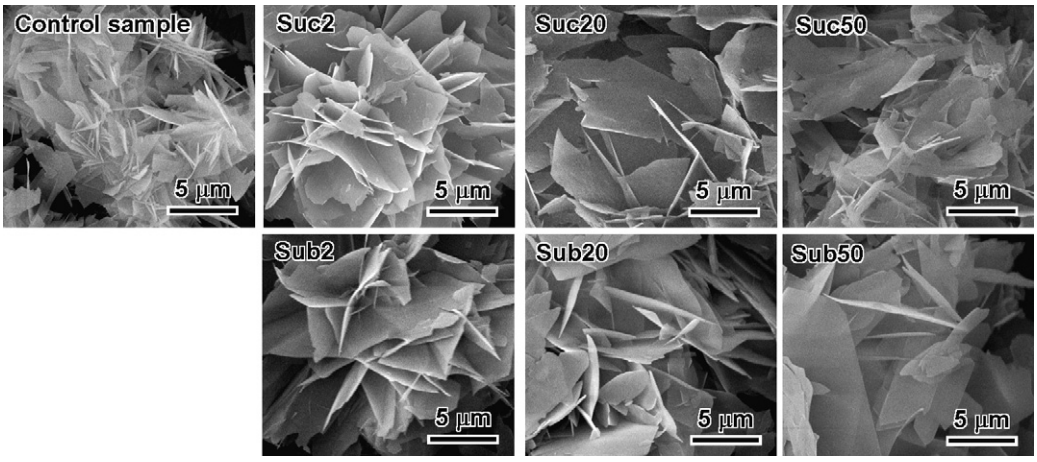

Fig. 4. Scanning electron microscope (SEM) images of the samples formed in the presence and absence of succinic acid (Suc) and suberic acid (Sub).

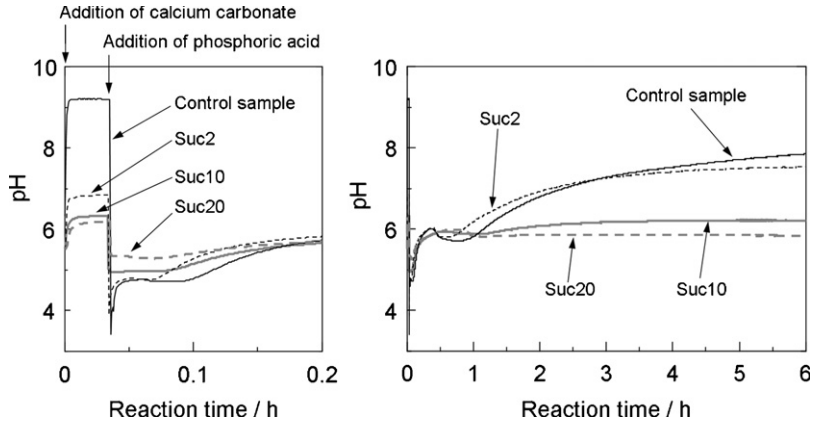

Fig. 5. Reaction time dependence of the pH in the Control sample, Suc2, Suc10 and Suc20. The left hand figure shows the reaction time dependence of the pH in the period 0–0.2 h. The reaction time was measured as the time elapsed after addition of calcium carbonate.

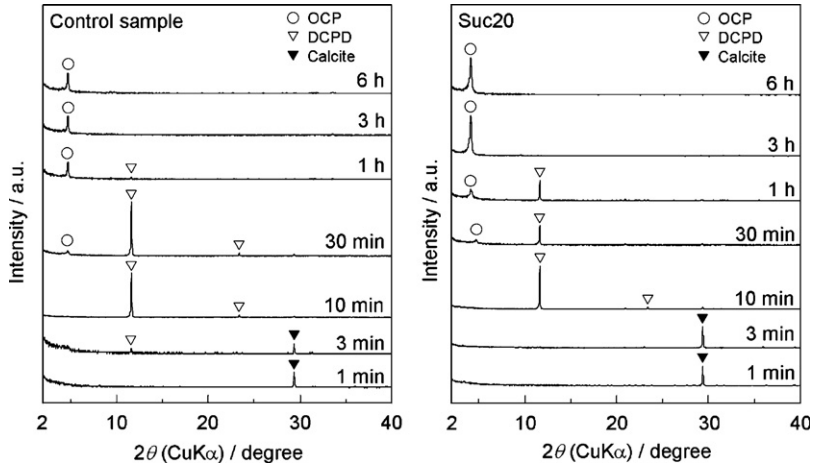

Fig. 6. Reaction time dependence of the crystalline phases in Control sample and Suc20. The reaction time was measured as the time elapsed after addition of calcium carbonate.

$CaHPO_4{\cdot}2H_2O$) were detected in the Control sample, and calcite was detected in Suc20. At 10 min, DCPD was detected in both of those samples, and DCPD and a small amount of Pure-OCP were detected at 30 min in the Control sample and Suc20. In the Control sample the intensity of diffraction peaks assigned to DCPD decreased, while the peak assigned to Pure-OCP increased in intensity after 1 h. In Suc20, a diffraction peak assigned to Suc-OCP, rather than Pure-OCP, was detected

at 1 h. After 3 h and at longer times Pure-OCP and Suc-OCP were detected in the Control sample and Suc20, respectively. Pure-OCP and Suc-OCP were formed following the formation of DCPD. For the Control sample, the presence of calcite was indicated by the powder XRD patterns in Fig. 2, but not by the powder XRD patterns in Fig. 6. This difference may be attributed to amount of calcite in the latter case being smaller than the detection limit of XRD.

4. Discussion

The general composition of OCPC can be represented as $Ca_8(HPO_4)(OOC(CH_2)nCOO)(PO_4)_4{\cdot}mH_2O$ [14]. The molar ratio of (calcium ion):(phosphate ions):(dicarboxylate ions) is thus 8:5:1. We used 16 mmol of $CaCO_3$ and 10 mmol of H_3PO_4, hence 2 mmol of dicarboxylic acid was the stoichiometric amount for OCPC. When the added amounts of Suc and Sub were 1 mmol, diffraction peaks attributable to OCPC were not detected (Figs. 2 and 3). Thus OCPC was not formed under conditions in which the amount of dicarboxylic acid present was smaller than the stoichiometric amount for OCPC. When the added amount of Suc was >2 mmol Suc-OCP was mainly formed. However, a small shoulder peak was detected at about $2\theta = 4.7^\circ$ in Suc2 and Suc4 (Fig. 2). In Sub2 and Sub4, Pure-OCP and Sub-OCP were formed. Sub-OCP was mainly formed with addition of >10 mmol of Sub, although the small shoulder peak was still detected at $2\theta = 4.7^\circ$ in Sub10 (Fig. 3). We attempted semiquantitation of the amounts of Pure-OCP, Suc-OCP and Sub-OCP by using the intensities of the 100 reflection peaks of Pure-OCP and OCPC. The 100 reflections of Pure-OCP, Suc-OCP and Sub-OCP were detected at $2\theta = 4.7^\circ$, 4.1° and 3.3°, respectively. F_{OCPC}, which shows the semiquantitative fraction of Suc-OCP and Sub-OCP, was defined by the equation

$$F_{OCPC} = \frac{I_{OCPC}}{I_{Pure\text{-}OCP} + I_{OCPC}} \quad (1)$$

where $I_{Pure\text{-}OCP}$ and I_{OCPC} are the 100 reflection peak intensities of Pure-OCP and OCPC, respectively. Fig. 7 shows the relationship between F_{OCPC} and the amounts of added dicarboxylic acids. The $F_{Suc\text{-}OCP}$ value increased dramatically with increase from 1 to 4 mmol of the amount of Suc. These results show that substitution of HPO_4^{2-} by Suc progressed substantially with an increase of the amount of Suc from 1 to 4 mmol. The $F_{Sub\text{-}OCP}$ values increased with an increase from 1 to 10 mmol of the amount of Sub. When the amounts of Suc and Sub ranged from 1 to 10 mmol, the $F_{Suc\text{-}OCP}$ values were larger than the $F_{Sub\text{-}OCP}$ values. Since Suc and Sub are incorporated in HPO_4^{2-} sites of the hydrated layer, the molecular sizes of the succinate and suberate ions, compared to that of HPO_4^{2-}, influence the formation of the Suc-OCP and Sub-OCP. The closer size of succinate ion to HPO_4^{2-} than suberate ion may cause the enhanced formation of the Suc-OCP under the same initial concentrations of Suc and Sub in the solution. Thus the more excess amounts of suberate ion were required to achieve the formation of Sub-OCP free from Pure-OCP than those of succinate ion. When the amounts of added Suc and Sub were >20 mmol, the F_{OCPC} values were almost unity. Hence we conclude that the presence of a large excess of Suc or Sub (more than 10 times the stoichiometric amounts) facilitated formation of OCPCs and inhibited formation of Pure-OCP. We consider that incorporation of dicarboxylate ions competes with incorporation of HPO_4^{2-} in OCP. Hence, it was reasonable that incorporation of dicarboxylate ions occurred preferentially with an increasing amount of dicarboxylic acid.

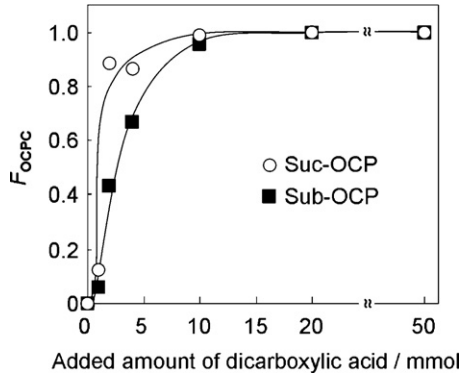

Fig. 7. Relationship between F_{OCPC} and the amounts of dicarboxylic acids. F_{OCPC} is the semiquantitative fraction of OCPC in the samples. The F_{OCPC} values were calculated from the intensities of the 100 reflections of Pure-OCP ($I_{Pure\text{-}OCP}$) and OCPCs (I_{OCPC}).

As noted above the OCP crystals synthesized in the presence of the dicarboxylic acids were larger than those synthesized in the absence of the dicarboxylic acids (Fig. 4). The origin of these findings is decreasing crystal growth rate and inhibition of nucleation of OCP resulting from adsorption of dicarboxylate ions on the crystal surfaces of OCPC, and chelation of calcium ions by dicarboxylate ions. OCP displays mainly the (100) face, which is calcium ion-rich [16]. Consequently, dicarboxylate ions interacted with calcium ion and adsorbed on the (100) face. The crystal growth rate in the [100] direction should decrease by adsorption of dicarboxylate ion. As a result, the crystals tended to expose more (100) face in the presence than in the absence of dicarboxylic acids. The chelation of calcium ions by dicarboxylate ions could affect crystal size. The activity of calcium ions was decreased by chelate formation, hence the degree of supersaturation with respect to OCPC was reduced. The nucleation frequency of OCPC was decreased, and crystal growth of OCPC could occur preferentially. Thus crystal sizes were increased by the addition of dicarboxylic acids.

Changes in pH during the reaction decreased with increasing amount of Suc (Fig. 5), because of the buffering action of Suc. pH buffering by Suc occurs in the pH range 3–7, because pK_{a1} and pK_{a2} of Suc are 4.19 and 5.48, respectively [17]. The acid dissociation constants, K_{a1} and K_{a2}, of a dicarboxylic acid, are defined by

$$K_{a1} = \frac{[H^+][HOOC(CH_2)_nCOO^-]}{[HOOC(CH_2)_nCOOH]} \quad (2)$$

$$K_{a2} = \frac{[H^+][^-OOC(CH_2)_nCOO^-]}{[HOOC(CH_2)_nCOO^-]} \quad (3)$$

pH increase caused by addition of calcite was inhibited by the pH buffering action of Suc, and pH decrease caused by the addition of phosphoric acid was also inhibited (Fig. 5). Through the overall reaction, pH changes decreased with increasing amount of Suc. The values of pK_{a1} and pK_{a2} for Sub are 4.52 and 5.40, respectively [17]. Hence pH changes would decrease with increasing amount of added Sub, because of its buffering action.

OCPC was formed via DCPD under our experimental conditions (Fig. 6), and the formation process of OCPC was almost the same as that reported by Kamitakahara *et al.* [14]. The present study shows that formation of Suc-OCP occurred at 1 h, following the formation of Pure-OCP at 30 min, in Suc20 (Fig. 6). These findings imply that the rate of formation of Pure-OCP was greater than that of Suc-OCP, and the stability of Suc-OCP was higher than that of Pure-OCP under our experimental conditions.

5. Conclusions

We investigated the effects of dicarboxylic acid concentration on formation of octacalcium phosphate carboxylate (OCPC). Incorporation of succinate (Suc) and suberate (Sub) ions into the OCP structure was attempted. When the amount of dicarboxylic acid in the reaction mixture was 1–5 times the amount corresponding to the stoichiometric composition of OCPC, incorporation of dicarboxylate ion progressed with an increasing amount of dicarboxylic acid. When the amounts of Suc and Sub present were larger than 10 times the stoichiometric amounts, OCPC was formed without Pure-OCP formation. Large excess of Suc and Sub (>10 times the stoichiometric amounts) facilitated formation of OCPCs and inhibited formation of Pure-OCP. Since the incorporation of dicarboxylate ions competes with incorporation of HPO_4^{2-} into OCP, incorporation of dicarboxylate ions occurred preferentially with an increasing concentration of dicarboxylic acid in the reaction mixture.

Acknowledgments

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