Formation of octacalcium phosphate with incorporated succinic acid through gel-mediated processing

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Calcium phosphate crystals were formed by diffusion of calcium ions into polyacrylamide (PAAm) hydrogels containing phosphate ions and succinic acid. Octacalcium phosphate (OCP) and OCP with incorporated succinate (Suc-OCP) were formed in the PAAm hydrogel. Suc-OCP was formed in the presence of excess amounts of succinic acid. The precipitates of OCP and Suc-OCP had a layered structure, composed of alternating layers of fine or irregular-shaped particles, and large spherical crystals that were the focus of this work. All of the spherical crystals were composed of many flake-like crystals. When the succinic acid concentration was $\leq 0.375 \text{ mol} \cdot \text{dm}^{-3}$ a network-like structure was observed on the spherulites, and for succinic acid concentrations $\geq 0.75 \text{ mol} \cdot \text{dm}^{-3}$ a petal-like structure was observed. These morphological changes were probably caused by adsorption of succinate ions on the (100) plane of OCP.

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1. Introduction

Various minerals are used by organisms for exoskeleton, endoskeleton, cutting/grinding, and ion storage;¹⁾ these mineral phases are referred to as biominerals. We can assume that formation of biominerals is by precipitation of inorganic compounds in a hydrogel medium. For example, crystalline hydroxyapatite (HAp: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) in our bones is formed in collagen hydrogel media.¹⁾

Crystal formation of biominerals in hydrogel media is known as *in situ* fabrication of an organic–inorganic composite, where inorganic compounds are precipitated in an organic gel matrix, and is one of the crystal growth methods for poorly soluble inorganic compounds such as calcium phosphate^{2)–10)} and calcium carbonate.^{11)–13)} HAp–poly(acrylic acid) hydrogel,¹⁰⁾ HAp–fibrin hydrogel,⁶⁾ and HAp–gelatin hydrogel⁴⁾ have been prepared as bone repairing materials by gel-mediated processing, which sometimes gives unique morphology of the crystals formed. The crystal morphology produced by gel-mediated processing is mainly determined by the degree of supersaturation, the rate-determining step of crystal growth, and interactions between gel molecules and crystals or ions.^{14),15)} Under high supersaturation conditions, the morphology of crystals is far from the equilibrium form, while it becomes the near equilibrium form under conditions of low supersaturation. The rate-determining step of crystal growth is associated with the degree of supersaturation. The rate-determining step under high and low supersaturation conditions is diffusion of the ions in a gel, and ion incorporation from the solution into the crystals, respectively.

The concentration of the molecular networks in a gel regulates the diffusion rates of the ions in the gel, and gel concentration is one of the parameters that determines whether the rate-determining step is kinetics limited or diffusion limited.¹⁶⁾ Adsorption of gel molecules on the crystal surface and chelation of ions by gel molecules also affects crystal morphology.

In the calcium phosphate system, both crystal morphology and crystalline phases strongly depend on the degree of supersaturation. We previously investigated the effects of supersaturation on the crystalline phases and morphologies of calcium phosphates precipitated in polyacrylamide (PAAm) hydrogel, by controlling calcium and phosphate ion concentrations.⁹⁾ The crystalline phases of the calcium phosphate were governed mainly by phosphate ion concentration. Under conditions of high concentration of phosphate ion, granular HAp crystals formed near the surface of the gels. When the concentration of phosphate ion was low, spherical octacalcium phosphate (OCP: $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) crystals were formed in the gels. OCP is potentially useful as a bone substitute.

OCP crystals have a layered structure composed of stacked apatitic and hydrated layers.¹⁷⁾ Hydrogen phosphate ion (HPO_4^{2-}) in the hydrated layer can be substituted by dicarboxylate ions ($^-\text{OOC}(\text{CH}_2)_n\text{COO}^-$) and their derivatives. Monma et al. reported that dicarboxylate ions incorporated into the OCP structure expanded the interplanar spacing of the (100) planes of OCP.¹⁸⁾ Various methods for preparation of OCP with incorporated dicarboxylate ions (OCPC: OCP carboxylate) have been reported,^{19)–22)} but synthesis of OCPC by gel-mediated processing has not been reported. In addition, incorporation of dicarboxylate ions into OCP crystals might change the morphology of the crystals. Consequently, it is worthwhile to investigate the formation conditions of OCPC in gel media and the effects of

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Fig. 1. Schematic representation of the gel-mediated processing method used in this study.

dicarboxylic acid on crystal phase and morphology, from the viewpoint of crystal growth in gel media and the chemistry of calcium phosphate. In the present study, we investigated formation of OCPC in PAAm hydrogel, using our previously reported method.⁹⁾ We used succinic acid ($\text{HOOC}(\text{CH}_2)_2\text{COOH}$) as the dicarboxylic acid, because succinic acid is the most familiar dicarboxylic acid for incorporation into OCP.

2. Experimental procedures

2.1 Preparation of PAAm hydrogels containing phosphate ions and succinate ions

Acrylamide monomer solutions containing phosphate and succinate ions were prepared as follows. 0.15 mol of acrylamide (Aldrich, Missouri, USA), 1.5×10^{-3} mol of *N,N'*-methylene-bisacrylamide (Wako Pure Chemical Industries Ltd., Osaka, Japan), 3.0×10^{-3} mol of *N,N,N',N'*-tetramethylene diamine (Wako Pure Chemical Industries Ltd.), 2.0×10^{-2} mol of diammonium hydrogen phosphate (Wako Pure Chemical Industries Ltd.) and various amounts of succinic acid (Suc: $\text{HOOC}(\text{CH}_2)_2\text{COOH}$, Wako Pure Chemical Industries Ltd.) were mixed in about 70 cm³ of ultrapure water. The amounts of Suc added were 0, 4.0×10^{-3} , 3.75×10^{-2} , 7.5×10^{-2} , and 1.5×10^{-1} mol which is equivalent to the amount of acrylamide. When the amount of succinic acid was 4.0×10^{-3} mol, the molar ratio succinic acid:phosphoric acid was 1:5. That value of the ratio corresponds to the stoichiometric composition of OCPC.²²⁾ The pH of the monomer solution was adjusted to 7.9 with aqueous ammonia (Wako Pure Chemical Industries Ltd.) or nitric acid (Wako Pure Chemical Industries Ltd.), and finally the volume of solution was adjusted to 100 cm³ in a volumetric flask. A 20 cm³ aliquot of the monomer solution was transferred by pipette to a glass vessel (capacity 50 cm³, 5 cm in diameter). 4.5×10^{-4} mol of ammonium peroxosulfate (Wako Pure Chemical Industries Ltd.) was mixed with the monomer solution and gelation allowed to take place at 60°C for 24 h. The pH of the gel was measured using a glass electrode-type pH meter (InLab® Solid, Mettler Toledo, Zürich, Switzerland).

2.2 Formation of calcium phosphate

5.0×10^{-2} mol of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; Wako Pure Chemical Industries Ltd.) and 1.0×10^{-2} mol of tris(hydroxymethyl)aminomethane (Nacalai Tesque Inc., Kyoto, Japan) were dissolved in about 60 cm³ of ultrapure water. The pH of the solution was adjusted to 7.0 by addition of $1.0 \text{ mol} \cdot \text{dm}^{-3}$ HCl solution, and the volume of the solution was adjusted to 100 cm³ in a volumetric flask. A 20 cm³ aliquot of the $\text{Ca}(\text{NO}_3)_2$ solution was transferred by pipette onto the surface of the gel. The experimental system is shown in Fig. 1. The glass vessel was covered with a poly(vinylidene chloride) film and a polyethylene lid, and maintained at 40°C. After 1 day, the $\text{Ca}(\text{NO}_3)_2$ solution on the gel was removed and the gel was extracted from the glass vessel. The pH of the solution was measured with a glass

electrode-type pH meter (D-51, Horiba Ltd., Kyoto, Japan). The gel was cut in a vertical direction and the cross-section observed by naked eye and stereoscopic microscope (SZ-CTV, Olympus Co., Ltd., Tokyo, Japan).

2.3 Characterization of crystalline phases of samples by powder X-ray diffraction

The PAAm hydrogel/precipitate composites were ground into paste to characterize the crystalline phase. The paste was placed on a glass sample holder, and the crystalline phases identified by powder X-ray diffraction (XRD; RINT PC2100, Rigaku Co., Tokyo, Japan) using $\text{Cu K}\alpha$ radiation. The crystalline phases of the precipitates were also examined as dried powder samples. The sliced samples were soaked in 30 cm³ of ultrapure water for 1 h to remove unreacted chemicals. This operation was repeated three times, then the rinsed samples were dried at 40°C for 1 day, crushed to powders, and examined by XRD.

2.4 Scanning electron microscopic observation of samples

Rinsed samples (see section 2.3) were dried at 40°C for at least 1 day, coated with a thin Au film, and examined by scanning electron microscopy (SEM; JSM5600, JEOL Ltd., Tokyo, Japan).

Hereafter, the following notations of the prepared samples are used. The notation 0.375Suc means that the concentration of Suc was $0.375 \text{ mol} \cdot \text{dm}^{-3}$ in as-prepared PAAm hydrogel. A wet sample after calcification is denoted by *x*Suc(Wet) and a dried sample by *y*Suc(Dry), where *x* and *y* indicate Suc concentrations.

3. Results

The pH values of as-synthesized gels 0Suc, 0.04Suc, 0.375Suc, 0.75Suc and 1.5Suc were 7.5, 7.6, 7.5, 7.6 and 7.6, respectively, thus the pH was essentially independent of Suc concentration. The pH values, after calcification, of the $\text{Ca}(\text{NO}_3)_2$ solutions that had been in contact with the gels are summarized in Table 1. The highest pH was 6.4 in 1.5Suc, and the lowest was 4.3 in 0Suc and 0.04Suc. The initial pH of the $\text{Ca}(\text{NO}_3)_2$ solution was 7.0 and those of the gels were about 7.6. Thus the pHs of the sample solutions were decreased by the calcification reaction, and with decreasing Suc concentration in the gel.

Figure 2 shows the cross-sections of calcified samples. Before calcification, as-synthesized gels were transparent, regardless of their Suc concentration, hence precipitates were contained in the

Table 1. pH values of the samples after calcification

Sample	pH
0Suc	4.3
0.04Suc	4.3
0.375Suc	5.4
0.75Suc	5.8
1.5Suc	6.4

Fig. 2. Cross-sectional images of the crystals formed with varying succinic acid concentrations.

Fig. 3. XRD patterns of wet-state samples calcified under varying succinic acid concentrations. The examined samples were prepared by grinding the wet-state gels with precipitates. The left figure indicates XRD patterns for the 2θ range $2-7^\circ$.

Fig. 4. XRD patterns of dry-state samples. These samples were calcified under varying succinic acid concentrations. The examined samples were prepared by grinding the dried gels with precipitates after removing unreacted chemicals by soaking in ultrapure water. The left figure indicates XRD patterns for the 2θ range $2-7^\circ$.

white parts of the gels. Periodic precipitation was observed in all of the gels: the number of precipitation bands decreased with increasing Suc concentration.

Figures 3 and 4 show XRD patterns of samples in wet state and dry state, respectively. We assigned the diffraction peaks using the powder diffraction file (PDF) database: the card number of OCP is #74-1301. In all of the wet state samples (Fig. 3) diffraction peaks assignable to OCP were detected. There were obvious peak shifts in the samples containing $\geq 0.375 \text{ mol} \cdot \text{dm}^{-3}$ of Suc (see the left XRD patterns in Fig. 3). Specifically, the

diffraction peak assigned to the 100 reflection of OCP was detected at about $2\theta = 4.7^\circ$ in 0Suc(Wet) and 0.04Suc(Wet), but at about 4.1° in 0.375Suc(Wet), 0.75Suc(Wet), and 1.5Suc(Wet). The interplanar spacings of the (100) planes, d_{100} , of OCP calculated using the Bragg equation are summarized in **Table 2**. d_{100} of OCP was substantially increased with increasing Suc concentration from 0.04 to $0.375 \text{ mol} \cdot \text{dm}^{-3}$. The intensity of the diffraction peak assigned to the 100 reflection of OCP increased with increasing Suc concentration from 0 to $0.375 \text{ mol} \cdot \text{dm}^{-3}$, and was almost invariant with further increase of the Suc concen-

Table 2. The 2θ values of the diffraction peak assigned to the 100 reflection of OCP in wet samples, and the d_{100} values of OCP calculated from these 2θ values

Sample	$2\theta/^\circ$	d_{100}/nm
0Suc(Wet)	4.66	1.89
0.04Suc(Wet)	4.66	1.89
0.375Suc(Wet)	4.06	2.17
0.75Suc(Wet)	4.12	2.14
1.5Suc(Wet)	4.02	2.20

Fig. 5. Stereoscopic microscope image of the cross-section of the calcified sample initially containing $0.75 \text{ mol} \cdot \text{dm}^{-3}$ of succinic acid in the wet state (left), and SEM image of the cross-section of the sample in the dry state (right).

tration. For the 1.5Suc(Wet) sample, unknown peaks were detected in addition to OCP reflections.

In the XRD patterns of the dry state samples (Fig. 4) only diffraction peaks assigned to OCP were detected. The diffraction peak assigned to the 100 reflection of OCP was detected at about 4.7° (2θ) for 0Suc(Dry) and 0.04Suc(Dry), and at about 4.1° for 0.375Suc(Dry), 0.75Suc(Dry) and 1.5Suc(Dry) (see the left XRD patterns in Fig. 4). The positions of the 100 reflection peak were thus almost the same as for the corresponding wet state samples. However, while unknown peaks were detected in the 1.5Suc(Wet) sample, they were not detected in the 1.5Suc(Dry) sample.

Figure 5 shows stereoscopic microscope and SEM images of the cross-section of 0.75Suc as a representative sample. The four-layer structures, with the appearance of geological strata, were observed in both images, and the other samples had similar structure. We denoted the layers (a)–(d) beginning at the top (see the left photograph in Fig. 5). The surface of layer (a) was the interface between $\text{Ca}(\text{NO}_3)_2$ solution and PAAm hydrogel. Layers (a) and (c) were opaque; the thickness of these layers was about 2.0 mm and 1.3 mm, respectively. In layers (b) and (d), precipitated spherical particles were observed in the gel matrix. Layers (a) to (d) in the right SEM images of Fig. 5 correspond to the magnified parts of layers (a) to (d) in the left stereoscopic microscope images. Many fine particles were present in layer (a), and we observed spherical crystals in layer (b). In layer (c) the spherical crystals were not present; irregular-shaped precipitates were observed in this layer. The spherical crystals reappeared in layer (d) and were larger than those formed in layer (b). No crystals were observed below layer (d). Similar morphological trends were observed for 0Suc, 0.04Suc, 0.375Suc and 0.75Suc. The 1.5Suc sample had a similar layered structure with distributions of the particles, but the spherical particles were observed not only in the layers (b) and (d) but also in layer (c). SEM images of the cross-sections of 0.75Suc at high magnifi-

Fig. 6. SEM images of the crystals precipitated in each layer of the calcified sample initially containing $0.75 \text{ mol} \cdot \text{dm}^{-3}$ of succinic acid.

cations are shown in Fig. 6. The images show the morphology of crystals formed in layers (a), (b), (c), and (d) shown in Fig. 5. Layer (a) in Fig. 6 shows the crystals formed in layer (a) of 0.75Suc. The crystals embedded in PAAm gel were spherical and submicrometer to $1 \mu\text{m}$ in diameter. Layer (b) in Fig. 6 shows the typical spherical crystals, $30\text{--}40 \mu\text{m}$ in diameter, which were precipitated in this layer, with petal-like structure on the spherulites. Layer (c) in Fig. 6 shows the irregular-shaped precipitates embedded in this layer of PAAm gel. Layer (d) in Fig. 6 shows typical crystals with petal-like structures on the spherulites formed in this region of the gel. The whole surface of the crystals was lightly covered with PAAm gel. In the other samples, crystals that had almost the same morphology as those observed in 0.75Suc were observed in each layer.

We were interested in the morphology of the crystals formed in layer (d) in Fig. 5. Figure 7 shows SEM images of typical crystals formed in layer (d) of 0Suc, 0.04Suc, 0.375Suc, 0.75Suc and 1.5Suc. In 0Suc and 0.04Suc spherical crystals $35\text{--}50 \mu\text{m}$ in diameter were observed, and network-like structures were found on the surfaces of the spherulites. Although the whole surfaces of the crystals were lightly covered with PAAm gel, we were able to observe the crystal morphology because we previously conducted SEM observation on samples that had been dried or heat-treated to remove PAAm hydrogel. In both cases, the observed crystal morphology was almost the same.⁹⁾ In layer (d) of 0.375Suc, spherical crystals $60\text{--}80 \mu\text{m}$ in diameter and slightly covered with PAAm gel were observed. The network-like structure was also observed on the surface of these crystals. In 0.75Suc, the spherical crystals had petal-like rather than network-like structure, and the diameters of the spherical crystals were $50\text{--}80 \mu\text{m}$. In 1.5Suc, spherical crystals $40\text{--}60 \mu\text{m}$ in diameter were observed. These crystals also had petal-like structure on the spherulites. The structure of the spherulites changed from network-like to petal-like with increasing Suc concentration. The crystals observed in all samples were aggregates of flake-like crystals which became smaller and thinner with increasing Suc concentration. The number of flake-like crystals on each spherulite increased with increasing Suc concentration.

4. Discussion

According to the data in Table 1, the pHs of the experimental systems were decreased by the calcification reaction. The precipitated crystalline phase was OCP (see Figs. 3 and 4).

Fig. 7. SEM images of the crystals precipitated in layer (d) of the samples calcified under varying succinic acid concentrations.

When PO_4^{3-} and/or HPO_4^{2-} was consumed by formation of OCP ($\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$), the following reaction occurred, and hence the pH of the reaction system decreased.

The decrease in pH of the samples containing low concentrations of Suc was larger than that of the samples containing the higher concentrations of Suc. This phenomenon was caused by increase of buffer capacity with increasing Suc concentration. The dissociation constants of Suc are $\text{p}K_{a1} = 4.19$ and $\text{p}K_{a2} = 5.48$.²³⁾ K_{a1} and K_{a2} are defined as follows:

$$K_{a1} = \frac{[\text{HOOC}(\text{CH}_2)_2\text{COO}^-][\text{H}^+]}{[\text{HOOC}(\text{CH}_2)_2\text{COOH}]} \quad (3)$$

$$K_{a2} = \frac{[^-\text{OOC}(\text{CH}_2)_2\text{COO}^-][\text{H}^+]}{[\text{HOOC}(\text{CH}_2)_2\text{COO}^-]} \quad (5)$$

The initial pH of the gels was about 7.6. Calculating from dissociation constant, under these conditions more than 99% of Suc was present as $^-\text{OOC}(\text{CH}_2)_2\text{COO}^-$. The hydrogen ions generated by formation of OCP induced the reverse reactions of Eqs. (2) and (4) thus Suc inhibited the pH decrease in the reaction systems. Consequently, when OCP formed in the presence of a high concentration of Suc, the decrease in pH was slight. On the other hand, when OCP was formed in a medium with low concentration of Suc, the decrease in pH was large because the succinic acid/succinate ion buffer capacity was reduced.

As shown in Fig. 2, periodic precipitation was observed. This phenomenon is known as Liesegang rings. The origin of Liesegang rings is considered to be the fluctuation of ion concentrations produced by diffusion of ions in a gel matrix and precipitate formation.²⁴⁾ According to Fig. 2, the number of precipitation bands decreased with increasing succinic acid concentration, implying that pH buffering by Suc decreased the number of Liesegang rings.

The diffraction peak assigned to the 100 reflection of OCP was shifted depending on Suc concentration (Fig. 3). Monma et al. reported that d_{100} of pure-OCP and succinate-incorporating

OCP (Suc-OCP) are 1.87 and 2.15 nm, respectively.¹⁸⁾ In the present work d_{100} of OCP was found to be 1.89 nm for 0Suc(Wet) and 0.04Suc(Wet), and in the range 2.14 to 2.20 nm for 0.375Suc(Wet), 0.75Suc(Wet) and 1.5Suc(Wet). These values were almost the same as the reported values of d_{100} for pure-OCP and Suc-OCP. It follows that pure-OCP formed in the 0Suc and 0.04Suc hydrogels, while Suc-OCP formed in 0.375Suc, 0.75Suc and 1.5Suc gels. The chemical equation for formation of Suc-OCP is

According to Eq. (6), when Suc concentration is high Suc-OCP is easily formed, and in practice $\geq 0.375 \text{ mol} \cdot \text{dm}^{-3}$ of Suc was sufficient for formation of Suc-OCP. The molar ratio of succinic acid to phosphoric acid in Suc-OCP is 1/5 (see Eq. (6)). The 0.04Suc hydrogel initially contained $0.04 \text{ mol} \cdot \text{dm}^{-3}$ of Suc and $0.2 \text{ mol} \cdot \text{dm}^{-3}$ of phosphoric acid, thus the initial succinic acid:phosphoric acid molar ratio was 1:5. However, pure-OCP rather than Suc-OCP formed in this condition. In conventional wet processing, it is important to adjust the molar ratios of calcium ion, phosphate ion, and dicarboxylic acid in the reaction system to the stoichiometric composition, for formation of OCP.²²⁾ It is interesting that in contrast to conventional wet processing, an excess of Suc was needed for formation of Suc-OCP in the gel-mediated process.

The diffraction peak assigned to the 100 reflection of OCP was shifted in the samples containing $\geq 0.375 \text{ mol} \cdot \text{dm}^{-3}$ of Suc (Fig. 4). When the Suc concentration was $\leq 0.04 \text{ mol} \cdot \text{dm}^{-3}$, the peak shift was not detected. Pure-OCP formed in 0Suc(Dry) and 0.04Suc(Dry), while Suc-OCP formed in 0.375Suc(Dry), 0.75Suc(Dry) and 1.5Suc(Dry). The samples examined were dried after soaking to remove unreacted chemicals that remained in the gel, but Suc remained in the OCP crystals even after the soaking operation. In 1.5Suc(Wet), unknown peaks in addition to those Suc-OCP were detected (see Fig. 3), however they were not detected in 1.5Suc(Dry) (see Fig. 4). Hence, the unknown peaks probably originated from a succinate.

The alternating layers of fine or irregular-shaped particles and large spherical particles were observed in the cross-section of the samples. The cross-section of 0.75Suc as a typical sample is shown in Fig. 5. The alternating layers were derived from the

fluctuation of ion concentrations. Layers (a) and (c) (see the right photograph in Fig. 5) were composed of a number of fine crystals and irregular-shaped crystals, respectively (see Layer (a) and Layer (c) in Fig. 6). These layers formed under high supersaturation conditions in which the nucleation rate was high, hence a number of fine crystals tended to form under those conditions. In addition, we previously reported that irregular-shaped OCP crystals formed under high supersaturation conditions.³⁾ Layers (b) and (d) were composed of large spherical crystals (see Layer (b) and Layer (d) in Fig. 6). These layers were formed under low supersaturation conditions, in which crystal growth preferentially occurred rather than nucleation, due to the low nucleation rate, hence large crystals formed under those conditions. Spherical crystals, which were similar to the crystals observed in layers (b) or (d), were found in layer (c) of 1.5Suc. This finding indicated that a high concentration of Suc decreased the degree of supersaturation, and hence spherical crystals were formed in layer (c) of 1.5Suc. The likely reason for this finding was decrease of calcium ion activity by formation of a calcium-succinate complex.

The crystal morphology in layer (d) changed from a network-like structure to a petal-like structure with increasing Suc concentration (see Fig. 7). To discuss the morphological change, it is necessary to consider the effects of the main parameters on crystal morphology, namely degree of supersaturation, incorporation of Suc into OCP crystals, and adsorption of Suc on crystal surfaces. Crystal morphology generally changes depending on the degree of supersaturation. The morphology of the crystals formed at high supersaturation is far from the equilibrium morphology, while the morphology of crystals formed at low supersaturation is almost the equilibrium morphology.¹⁴⁾ In our previous study the morphology of OCP changed dramatically from ribbon-like to spherical shape with increasing degree of supersaturation.³⁾ The morphological change caused by the difference of degree of supersaturation was large. In the present study, however, the contribution of the degree of supersaturation to morphological change was not as large, because all of the crystals shown in Fig. 7 had spherical shape and we were able to assume that these crystals formed under almost the same supersaturation conditions. In 0.375Suc, 0.75Suc and 1.5Suc, Suc-OCP was formed, while, pure-OCP formed in 0Suc and 0.04Suc. Incorporation of Suc into OCP did not result in morphological change. According to Table 2, the d_{100} values of Suc-OCP formed in 0.375Suc, 0.75Suc, and 1.5Suc were almost the same. The data from Table 2 indicate that the substitution rates of HPO_4^{2-} by $^-\text{OOC}(\text{CH}_2)_2\text{COO}^-$ were almost the same in each sample. However, the morphology of crystals formed in 0.375Suc, 0.75Suc, and 1.5Suc gradually changed with increasing Suc concentration, hence incorporation of Suc into OCP was not the main cause of the morphological change of the OCP crystals. The dominant factor in the morphological change was Suc adsorption on the crystal surfaces. A previous report showed that OCP crystals formed in silica hydrogel grew along their c -axis and exposed the (100) plane.⁷⁾ In addition, the intensities of diffraction peaks assigned to the 100 reflection of OCP in all samples were particularly strong (see Figs. 3 and 4). Thus the crystals shown in Fig. 7 also grew in the c -axis direction, exposing mainly the (100) plane, and the observed morphological change was increase of the (100) plane of OCP or Suc-OCP. The (100) plane of OCP is a calcium ion-rich plane,²⁵⁾ and OCP crystals formed in the presence of succinic acid were smaller than those formed in conditions without succinic acid.²⁰⁾ Consequently, succinate ions interacted with calcium ion on the (100) plane

and adsorbed on this plane. As a result, the crystals tended to expose more (100) plane with increasing Suc concentration. In addition, the adsorbed succinate ions inhibited crystal growth in the a -axis direction, and hence the thickness of the flake-like crystals in spherulites decreased with increasing Suc concentration.

5. Conclusions

Formation of calcium phosphate crystals in PAAm hydrogel containing phosphate ions and Suc, by diffusion of calcium ions into the hydrogel, was examined. When the Suc concentration was $\leq 0.04 \text{ mol}\cdot\text{dm}^{-3}$ pure-OCP was formed, while for Suc concentrations in the range 0.375 to $1.5 \text{ mol}\cdot\text{dm}^{-3}$, Suc-OCP was formed. Precipitates of pure-OCP or Suc-OCP formed a layered structure of Liesegang rings. The layered structure was composed of alternating layers of fine or irregular-shaped crystals and large spherical crystals. The spherical crystals were aggregates of flake-like crystals, and their structure changed with Suc concentration. When the Suc concentration was $\leq 0.375 \text{ mol}\cdot\text{dm}^{-3}$ a network-like structure was observed on the spherulites, whereas a petal-like structure was found for Suc concentrations in the range 0.75– $1.5 \text{ mol}\cdot\text{dm}^{-3}$. The main cause of this morphological change was probably adsorption of succinate ions on the (100) plane of OCP.

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