Hydrothermal synthesis of one-dimensional hydroxyapatite particles using calcium sodium nitrilotriacetate as a calcium reservoir

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One-dimensional hydroxyapatite (1D-HAP) particles were synthesized via a hydrothermal process using calcium sodium nitrilotriacetate (CaNa–NTA) as a Ca2+ reservoir. CaNa–NTA dissolved slowly at 150°C to enable Ca2+ to be supplied to the aqueous phase to react with phosphate and hydroxide ions. The average length and width of the 1D-HAP particles were 6.0 μm and 60 nm, respectively; the former depended on the amount of CaNa–NTA loaded but the latter did not. We investigated the formation of 1D-HAP particles by powder X-ray diffraction measurement and electron microscopy observation.

Key-words : Hydroxyapatite, Calcium hydroxyphosphate, Needle shape, Solid reservoir, Dissolution/re-deposition process

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Hydroxyapatite [Ca10(PO4)6(OH)2, HAP] is a biomineral that makes up the bones and teeth of vertebrates. Because of biocompatibility, synthetic HAP can find applications such as production of artificial bones and dental implants.1–3) HAP is also applicable to chromatographic materials that purify biomolecules such as IgG.3,4) At the same time, HAP has been used in various non-biological applications, such as support for Au cluster catalysts.5) Calcium halophosphate, in which F and/or Cl substitutes for OH, is known as a host for phosphors.6) Much like various other inorganic materials,8–11) one-dimensional (1D) HAP particles have attracted attention recently and have been synthesized mainly via hydrothermal processes.12–15) For example, amorphous calcium phosphate (ACP) gel,12) β-Ca3(PO4)2,13) and CaH2(PO4)·5H2O14) have been employed to preserve Ca2+ and PO43−. Adenosine 5′-triphosphate has been used as a phosphate source for reaction with Ca2+ in the aqueous phase.15) In this study, we propose calcium sodium nitrilotriacetate (CaNa–NTA)16,17) as a novel Ca2+ reservoir for hydrothermal synthesis of 1D-HAP particles.

CaNa–NTA was precipitated by adding 20 mL trisodium nitrilotriacetate (Na3–NTA) aqueous solution (0.5 mol/L) to 100 mL Ca(NO3)2 aqueous solution (0.1 mol/L) under magnetic stirring at 25°C. The precipitation of CaNa–NTA was complete at 30 min. The precipitate was washed with distilled water using a centrifuge and then freeze-dried to obtain CaNa–NTA powder (~85% yield by gravimetry). The molar ratio of Ca:Na:O was evaluated as 13.5:10.1:76.4 by energy dispersive X-ray spectrometry (EDX, EDAX Genesis XM2T attached to a Hitachi H-7650 transmission electron microscope), and the composition of the obtained CaNa–NTA powder was estimated as N(CH2COO)3–Ca1.1Na0.8 as a result.

To synthesize 1D-HAP particles, 0.25 g (1 mmol) of the CaNa–NTA powder was dispersed in 10 mL distilled water with the assistance of ultrasonication. In a water bath at 25°C, 10 mL Na2HPO4 aqueous solution (0.4 mol/L) was added to the CaNa–NTA dispersion under magnetic stirring. The dispersion was then transferred to a Teflon-lined autoclave and placed in an air oven at 150°C for 45 h. The pH changed slightly from 8.95 to 8.78 (measured at room temperature) as the reaction proceeded to completion. The obtained precipitate was centrifuged 3 times and redispersed in distilled water. All the chemicals were purchased from Wako Pure Chemicals (Osaka, Japan) and used as received.

Figure 1 shows field-emission electron microscopy (FE-SEM) and transmission electron microscopy (TEM) images of the as-obtained HAP particles (FE-SEM JEOL SEM-6700F operated at 15 kV, Pt-coated; TEM Hitachi H-7650 operated at 100 kV). As shown in Fig. 1(a), the particles had a one-dimensional shape. The average length and width were estimated as 6.0 μm and 60 nm. The average length and width were estimated as 6.0 μm and 60 nm.
nm, respectively (aspect ratio 100). The specific surface area was estimated as 21.2 m²/g by Brunauer-Emmett-Teller (BET) analysis (MicrotracBEL BELSORP-max). Fig. 1(b) shows the lattice images stacked in the length direction. The separation between the adjacent lattice images was 3.4 Å, which corresponded to {002} of the hexagonal HAP. Thus, the 1D-HAP particles were grown in the c-axis direction, like those synthesized by procedures in the literature. The Ca/P ratio was estimated as 1.64 (by TEM-EDX), which was somewhat lower than the stoichiometric ratio (1.67).

Figure 2 shows the evolution over time of the X-ray diffraction (XRD) pattern of the precipitate during the hydrothermal reaction (Bruker D8 Advance, Cu Kα radiation, λ = 1.5418 Å). As the reaction proceeded, the intensities of the XRD peaks for HAP increased while those of the CaNa–NTA peaks decreased. The corresponding TEM images in Fig. 3 also indicate the growth of 1D-HAP particles with a decrease in the size and population of CaNa–NTA particles. TEM-EDX mapping images in Fig. 4 show that Na and P are localized at the triangular and 1D silhouettes, respectively, while Ca is detected in both regions. Therefore, the triangular and 1D silhouetted particles correspond to CaNa–NTA and HAP, respectively. Thus, it is suggested that HAP particles grow by consuming Ca²⁺ ions that are supplied from CaNa–NTA, probably through a dissolution/re-deposition process via the aqueous phase.

The size of the 1D-HAP particles, on the other hand, depended on the content of CaNa–NTA loaded in the reaction vessel, as shown in Fig. 5. The mean length was roughly proportional to the CaNa–NTA content, i.e., 1.0, 2.9, and 6.0 μm for 0.25, 0.5, and 1 mmol of CaNa–NTA, respectively. However, the corresponding mean widths were similar: 60, 57, and 63 nm. Note that the reaction with 2 mmol of CaNa–NTA was still incomplete at 45 h. As the yields were closely similar (~90%), the mean volume of the particles seemed to be proportional to the amount of CaNa–NTA, suggesting that the number of particles formed was practically independent of the amount of (or the total surface area of) CaNa–NTA. We therefore suggest that the nucleation of 1D-HAP occurred in the aqueous phase and was controlled by the concentration of Ca²⁺ in equilibrium with CaNa–NTA, and was thus independent of the CaNa–NTA content in the system.

Although HAP particles can be synthesized hydrothermally using a soluble calcium salt such as Ca(NO₃)₂, they tend to be much shorter than those in the CaNa–NTA system. This difference is due to the presence of NTA, but it should also be clarified whether the CaNa–NTA precipitate or NTA⁻⁻ dissolved in the
aqueous phase actually contributed to the formation of 1D-HAP particles.

We examined the aforesaid possibility by synthesizing HAP particles using Ca(NO$_3$)$_2$ without and with the addition of Na$_3$-NTA aqueous solution. 10 mL Na$_2$HPO$_4$ (0.4 mol/L) was added to 10 mL Ca(NO$_3$)$_2$ (0.1 mol/L) under magnetic stirring at 25°C to obtain a white gel-like ACP precipitate instantaneously.$^{20}$ 2 mL of distilled water or Na$_3$-NTA (0.5 mol/L) was then added to the ACP dispersion. The ACP dispersions reacted hydrothermally at 150°C for 20 h.

Figure 6 shows TEM images of the HAP particles synthesized with Ca(NO$_3$)$_2$ in the absence and presence of Na$_3$-NTA, where the pH values after the reaction were 7.42 and 8.67, respectively. Similar particles were obtained under both sets of conditions, although the particle lengths were slightly different, probably due to the difference in pH. Even when synthesized using Na$_3$-NTA, moreover, the particles were much smaller than those synthesized using CaNa-NTA, as shown in Fig. 1. Thus, it was confirmed that CaNa-NTA contributed to the formation of 1D-HAP particles, while the released NTA$^{2-}$ had no effect as a growth modifier.

The rod-shaped particles in Fig. 6 suggest that the intrinsic growth of HAP is anisotropic, which means the growth rate constants of the a- and c-planes are different ($k_a$ and $k_c$, respectively). In addition, the anisotropic growth could be enhanced when the reactants surrounding the a-plane flow out and are used for preferential growth of the c-plane.$^{21,22}$ That is, as suggested by the concentration profile in the diffusion layer around a growing particle,$^{23}$ the concentration gradient between the a- and c-plane regions promotes diffusion of the reactants, if $k_b$ is sufficiently small to allow reaction-limited growth whereas $k_c$ is sufficiently large to allow diffusion-limited growth.

At the same time, a sufficient supply of reactants for each particle is also required for progress of the anisotropic growth, as shown in Fig. 5. Since the amount of reactants distributed to each particle is inversely proportional to the number of particles, $n$, the nucleation process plays an important role in the synthesis of 1D-HAP particles. Neucleation theory suggests that $n$ is a function of the molar supply rate of reactants, $Q_a$, and the volume increase rate of the nucleus, $v$, as

$$n = Q_aV_m/v \quad (1)$$

where $V_m$ is the molar volume of the particles.$^{24}$ Thus, a small $Q_a$ and/or $v$ is preferred.

The experimental results suggest that $Q_a/v$ in the CaNa-NTA system (Fig. 1) is much smaller than in the ACP system (Fig. 6), where the dissolution rate of CaNa-NTA (or ACP) and the growth rate of HAP particles are regarded as $Q_a$ and $v$, respectively. The CaNa-NTA system required a longer reaction time than the ACP system, suggesting slower $Q_a$ in the former, since $Q_a$ is concordant with the overall reaction rate. In addition, the smaller $Q_a/v$ ratio leads to a lower supersaturation ratio, $S$.$^{25}$ Since $S$ is a determinant factor of $v$ in general, it seems difficult to assume a larger $v$ in the CaNa-NTA system. It is more likely that the smaller $Q_a$ is the dominant factor contributing to the smaller $v$ that resulted in the 1D-HAP particle formation.

We conclude, therefore, that the CaNa-NTA precipitate is applicable as a Ca$^{2+}$ reservoir for the hydrothermal synthesis of 1D-HAP particles through regulation of nucleation by its slow dissolution rate. It is expected that the resulting 1D-HAP particles, which have a narrow width with a high aspect ratio, offer high efficiency in adsorbing proteins, supporting catalyst nanoparticles, etc., due to the large specific surface area of the a-plane.

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