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# Rod-shaped Hydroxyapatite Nanoparticle Formation by Maturation with AOT

Jun Oshitani,* Nobuhide Kataoka, Mikio Yoshida, Kuniaki Gotoh, and Koreyoshi Imamura
*Division of Chemistry and Biochemistry, Graduate School of Natural Science and Technology,*
*Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530*

(Received June 28, 2011; CL-110541; E-mail: oshitani@cc.okayama-u.ac.jp)

Amorphous calcium phosphate nanoparticles were synthesized by mixing aqueous solutions of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$. Then the particles were matured with sodium bis(2-ethylhexyl) sulfosuccinate (AOT) at various temperatures. Rod-shaped hydroxyapatite (HAp) nanoparticles were formed at maturation temperature ≥40 °C. These results indicate that addition of AOT not during the particles synthesis but during the maturation at more equal critical temperature is a key to form the rod-shaped HAp nanoparticles using AOT.

Hydroxyapatite (HAp) particles are generally utilized as artificial bone materials[1,2] and chromatomediums.[3,4] In order to enhance the potential of HAp particles for other applications, it is important to synthesize HAp particles which have various morphologies. For instance, the rod-shaped HAp particles have been studied as a composite material for polymers to control the mechanical properties.[5,6]

The surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT) is used to prepare nanosized W/O microemulsions.[7] The W/O microemulsions containing AOT have been used for nanoparticle formation;[8–12] where the nanoparticles are formed as W/O microemulsions and act as templates, when two kinds of W/O microemulsions containing reactants in the water phase are mixed together. Sarda et al.[12] reported that the rod-shaped HAp nanoparticles are formed using the AOT-base W/O microemulsions and focused on an interaction between calcium ions and sulfonate headgroups of AOT at the W/O interface for the origin of the rod-shaped HAp nanoparticle formation. Recently, we reported that rod-shaped HAp nanoparticles are formed by mixing an aqueous solution containing AOT and calcium ion with an aqueous solution containing phosphate ion (not using the W/O microemulsions),[13] which means that the oil phase is not necessarily required for the rod-shaped HAp nanoparticle formation. Then, we had a question; which stage of the particle formation is responsible for the AOT effect? HAp particle formation consists of two stages; the nucleation stage for seed crystal formation and the maturation stage for crystal growth. In this study, we focused on the maturation stage for the AOT effect of the rod-shaped HAp nanoparticle formation.

$Ca(NO_3)_2$ (≥99.5%), $(NH_4)_2HPO_4$ (≥99.0%), 10% aqueous ammonia from Wako Pure Chemicals, and AOT (98%) from Aldrich were used. All reagents were used as received. Pure water of 18.2 MΩ cm was prepared using a Direct-Q System (Nihon Millipore). 0.5 M solutions of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$, 1 M solution of $NH_3$, and 0.02 M solution of AOT were separately prepared with pure water. pH values of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ solutions were adjusted to 10 by adding an appropriate amount of 1 M solution of $NH_3$. 5 mL of $Ca(NO_3)_2$ solution and 3 mL of $(NH_4)_2HPO_4$ solution were mixed, where the Ca/P ratio was 1.67. After the mixture was agitated for 30 min at 20 °C, the mixture was centrifuged for 30 min at 3500 rpm. Then the amorphous particles were obtained (see the XRD pattern shown as "before maturation" in Figure 1). The amorphous particles were dispersed in the AOT solution or pure water and centrifuged for 30 min at 3500 rpm. The dispersion and centrifugation were done totally twice for each. In the case of dispersion with AOT, gel-like material existed on the particles. FT-IR spectrum of the gel and AOT were measured, and these spectra corresponded well. This indicates that the gel consists of AOT. After the second centrifugation, the amorphous particles with and without AOT in glass tubes were stored for maturation at 20–90 °C for 24 h without capping the tubes. Then, the particles matured with AOT were twice washed with acetone to remove AOT. We followed previously described procedures[13] for characterization of the particles after maturation. Briefly, X-ray diffraction (XRD) patterns and images of scanning transmission electron microscopy (STEM) were recorded. Length and width of one hundred randomly selected particles were measured by hand using the images.

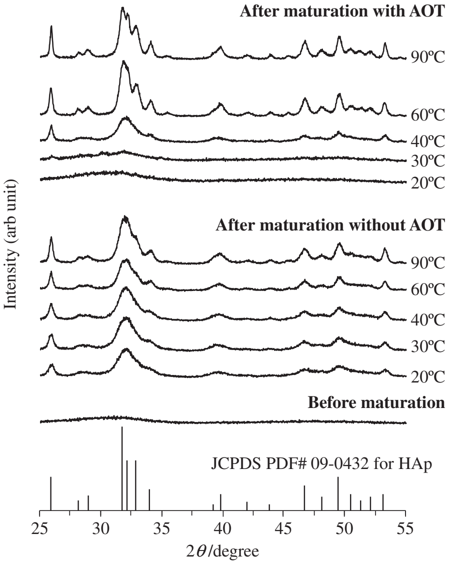

**Figure 1.** XRD patterns of the particles after maturation with and without AOT and before maturation.

Figure 1 shows the XRD patterns of the particles after maturation with and without AOT and before maturation with the JCPDS PDF# 09-0432 for HAp. The broad peak (halo) for the particles before maturation indicates that the particles are amorphous. When the particles are matured without AOT, the pattern shows the HAp pattern regardless of the maturation temperature. In the case of maturation with AOT, the particles matured at 20 °C are amorphous. HAp pattern is visible at 30 °C.

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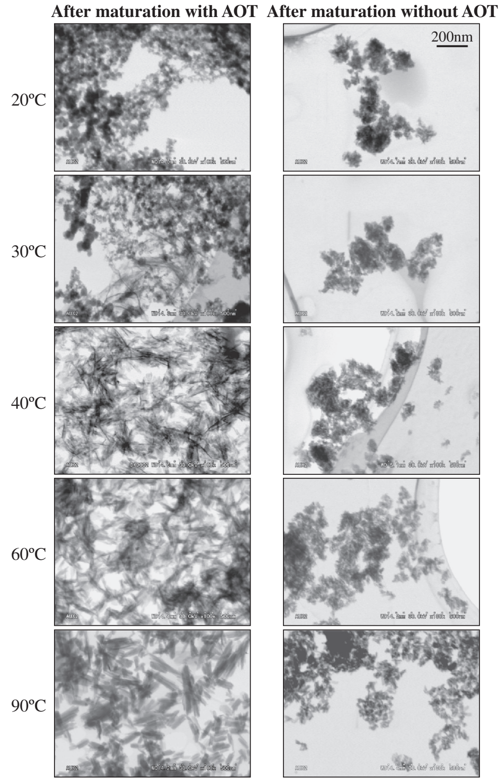

**Figure 2.** STEM images of the particles after maturation with and without AOT.

The particles given at the maturation temperature ≥40 °C are HAp; the peaks become sharper with increasing the maturation temperature because the particle width becomes larger with increasing maturation temperature as shown below. Figures 2 and 3 show the STEM images and the length and width, respectively. The particles matured without AOT are small rod-shaped HAp particles of about 40 nm in length and about 10 nm in width. When the particles are matured with AOT, all of the particles at 20 °C and most of the particles at 30 °C are round amorphous particles. Some of the particles at 30 °C and all of the particles at 40 °C are larger rod-shaped HAp particles. The length is approximately constant at 100 nm regardless of the maturation temperature, while the width increases from 10 to 20 nm with increasing maturation temperature.

Hereafter, the possible mechanism of the rod-shaped HAp nanoparticle formation by maturation with AOT will be discussed. As shown in Figure 2, the small amorphous round particles and the rod-shaped HAp particles exist together in the case of maturation with AOT at 30 °C. This suggests that the small round particles grow to be the rod-shaped particles. The particles were wet at the beginning of the maturation, so dissolution and deposition of the particles might occur during the maturation. In the previous study,[13] as the origin of the rod-shaped HAp nanoparticle formation by mixing an aqueous solution containing AOT and calcium ion with an aqueous solution containing phosphate ion, we focused on the Ca–AOT complex formed by an interaction between a positive charge of a calcium ion and a negative charge of a polar headgroup of AOT.

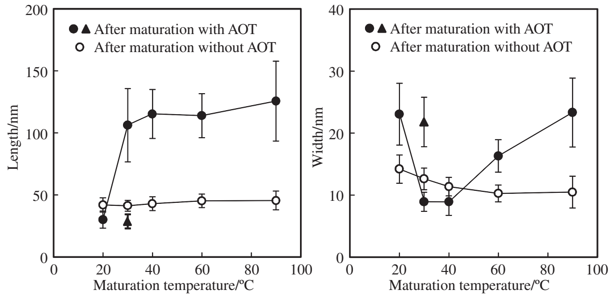

**Figure 3.** Length and width of the particles after maturation with and without AOT as a function of maturation temperature. Black triangle and circle at 30 °C show the mean values of 50 round particles and 50 rod-shaped particles, respectively.

Here we also focused on the Ca–AOT complex. It is known that the surface of the side wall of the rod-shaped HAp particles are rich in calcium ions.[14] Therefore, during the dissolution and deposition, the Ca–AOT complexes are formed and the side wall is surrounded by the Ca–AOT complexes. AOT has two branched carbon chains; hydrophobic interactions act between AOTs. The hydrophobic interactions may inhibit the dissolution and/or the particle growth on the side wall and enhance the particle growth on the surface of the top and bottom wall of the rod-shaped particles.

In summary, the rod-shaped HAp nanoparticles are formed by maturing the amorphous calcium phosphate particles with AOT at the temperature ≥40 °C. This indicates that AOT enhances the HAp particles growth to be the rod-shaped particles during the maturation. The Ca–AOT complex might play a role in the rod-shaped particle formation.

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