Synthesis of Sulfate-ion-substituted Hydroxyapatite from Amorphous Calcium Phosphate

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Abstract
The composition of Hydroxyapatite (HAp) is expressed by the formula Ca_{10}((PO_4)_6(OH)_2). Many reports have been published on the synthesis of HAp in which Ca ions are substituted by various cations (e.g., Sr, Ba). On the other hand, studies on the synthesis of sulfate-ion-substituted hydroxyapatite (SAp) have rarely been conducted. The present study investigated the conditions for the synthesis of SAp from amorphous calcium phosphate (ACP, Ca_{3}(PO_4)_2•nH_2O) as the starting material, which can readily incorporate various ions into its structure. Sodium sulfate (Na_2SO_4) was added to ACP, and then, the mixture was hydrothermally processed at 220°C for 3 h. SAp obtained under these conditions had a Ca-deficient-type HAp structure. The SO_4/PO_4 molar ratio in SAp increased with increasing amounts of added Na_2SO_4, reaching a maximum value of 0.5, meaning that 1/3 of the PO_4^{2–} ions contained in HAp were substituted by SO_4^{2–} ions.

Keywords: Substituted apatite; Sulfate; Amorphous calcium phosphate; Composition control; Hydrothermal process

Introduction
Apatite is a general term to indicate hexagonal minerals that are represented by the chemical formula M_{10}(ZO_4)_{6}X_2. Apatite of different properties can be synthesized by replacing M, Z, and X with various elements. Among others, the apatite in which M, Z, and X are Ca, P, and OH, respectively, is called Hydroxyapatite (HAp, Ca_{10}(PO_4)_{6}(OH)_2). HAp has good biocompatibility and protein adsorption properties and is an important compound used to produce biomaterials. Moreover, HAp has high ion-exchange capacity, and research concerning the synthesis and functionalization of HAp in which various cations are substituted by virtue of its ion-exchange capacity is underway [1]. Particularly, there are many reports on the synthesis of HAp where Ca is substituted with various cations (e.g., Sr, Ba, Pb, Cd) [2-4]. However, studies on the synthesis of HAp in which PO_4 is substituted with SiO_4 and CO_3 have been reported only occasionally [5-7], while the synthesis of sulfate-ion-substituted hydroxyapatite (SAp), in which SO_4 has been introduced, has been rarely studied.

We conducted a series of studies on the synthesis and properties of amorphous calcium phosphate (ACP, Ca_{3}(PO_4)_2•nH_2O) [8-12]. ACP is a precursor that appears in the initial stage of the formation of various calcium phosphate compounds and is easily crystallized into HAp. If HAp is crystallized in the presence of various cations, various substituted HAp species incorporating different ions can be obtained. Therefore, we investigated the conditions for the synthesis of SAp from ACP as the starting material that are effective for controlling the composition of HAp.

Materials and Methods
Experimental method
ACP was obtained by the hydrolysis of Ca(H_2PO_4)_2•H_2O. The synthesis of SAp was as follows. Sodium sulfate was added to ACP or HAp so that the weight ratio of Na_2SO_4/(ACP or HAp) was 0–6. The mixture was hydrothermally processed at 220°C for 3 h, and then, SAp was obtained after filtering, washing, and drying the processed sample. The same procedure was carried out with stoichiometric HAp as the starting material, for comparison.

Results and Discussion
Synthesis conditions for sulfate-ion-substituted hydroxyapatite
The effect of the amount of added Na_2SO_4 on the SO_4/PO_4 molar ratio in the products is shown in Figure 1. In the cases where HAp was used as the starting material, almost no substitution of SO_4^{2–} ions was obtained. However, when SAp was synthesized from ACP, SO_4 substitution increased with increasing amounts of added Na_2SO_4, reaching a maximum value of 0.5. This result indicates that the weight ratio of Na_2SO_4/(ACP or HAp) affects the SO_4/PO_4 molar ratio in the obtained SAp.

Figure 1: Effect of added amount of sodium sulfate on SO_4/PO_4 molar ratio in sulfate-ion-substituted hydroxyapatite. Starting material: (a) amorphous calcium phosphate, and (b) hydroxyapatite.

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could be observed in the products, not even when the amount of added Na$_2$SO$_4$ was increased. However, in the cases where ACP was used as the starting material, the SO$_4$/PO$_4$ molar ratio in the products tended to increase with the amount of added Na$_2$SO$_4$. The maximum SO$_4$/PO$_4$ molar ratio was about 0.5, implying that about 1/3 of the PO$_4^{3–}$ ions in the structure of HAp were substituted with SO$_4^{2–}$ ions. The chemical formula of the product can be represented by Ca$_9$(PO$_4$)$_4$(SO$_4$)$_2$(OH)$_2$.

The X-ray diffraction patterns of the obtained products are shown in Figure 2. None of the patterns coincided with the Joint Committee on Powder Diffraction Standards (JCPDS) data for HAp (pdf#09-432), and there was no evidence for the existence of sulfate species such as gypsum. Moreover, since the crystallinity of HAp decreased with increasing SO$_4$/PO$_4$ molar ratio, it could be suggested that SO$_4^{2–}$ ions were incorporated in the structure of HAp.

**Structure of sulfate-ion-substituted hydroxyapatite**

The relation between SO$_4$ content and lattice constant, used to study the substitution of SO$_4^{2–}$ ions, is shown in Figure 3. If it is simply assumed that PO$_4^{3–}$ ions (ionic radius: 0.16 nm) and SO$_4^{2–}$ ions (ionic radius: 0.230 nm) in HAp were exchanged, there should have been a change in the lattice constant due to the difference in the ionic radii. However, the results of this experiment showed no change in the lattice constant, not even when the SO$_4$/PO$_4$ molar ratio was increased. Therefore, this result suggested that PO$_4^{3–}$ and SO$_4^{2–}$ ions were not exchanged. The lattice strain calculated by Hall’s equation [13] is shown in Figure 4. The calculated lattice strain of SAp indicated that the lattice strain proportionally increased with increasing SO$_4$/PO$_4$ molar ratio. These results suggested that ions that have the same radii were substituted in the crystal lattice, although no changes in the lattice constant were detected.

The IR absorption spectra of SAp are shown in Figure 5. The HAp obtained by crystallization of ACP in deionized water (SO$_4$/PO$_4$ molar ratio: 0) showed a small, sharp absorption peak at around 3600 cm$^{-1}$ attributable to OH– ions, in addition to a strong absorption peak attributable to PO$_4^{3–}$ ions around 1000 cm$^{-1}$. Moreover, a small absorption peak at around 880 cm$^{-1}$ attributable to HPO$_4^{2–}$ ions was observed. Thus, the obtained HAp was identified as having a calcium-deficient-type HAp structure because the ACP starting material had a Ca/P atomic ratio of approximately 1.5. Furthermore, similar to HAp, an absorption peak attributable to PO$_4^{3–}$ ions was also observed in the obtained SAp, in addition to a slightly weak absorption peak at around 3600 cm$^{-1}$ attributable to OH– ions; hence, it can be inferred that SAp has an apatite structure. Moreover, the absorption at 1180 cm$^{-1}$ attributable to SO$_4^{2–}$ ions, which was absent from the spectrum of HAp, suggested that SO$_4^{2–}$ ions were present in the structure. However, the absorption attributable to HPO$_4^{2–}$ ions, which was detected in HAp, was not observed in SAp. Therefore, we considered that HPO$_4^{2–}$ ions may play an important role in the substitution of SO$_4^{2–}$ ions within the HAp structure.

Based on the results mentioned above, the reaction for the formation of SAp using ACP as the starting material is as follows:

$$3\text{Ca}_3(\text{PO}_4)_2\cdot n\text{H}_2\text{O} \text{[ACP]} \rightarrow \text{Ca}_9(\text{HPO}_4)_2(\text{PO}_4)_4(\text{OH})_2 \text{[Ca-deficient-type HAp]} \quad (1)$$

$$\text{Ca}_9(\text{HPO}_4)_2(\text{PO}_4)_4(\text{OH})_2 + 2\text{Na}_2\text{SO}_4 \rightarrow \text{Ca}_9(\text{PO}_4)_4(\text{SO}_4)_2(\text{OH})_2 + 2\text{Na}_2\text{HPO}_4 \quad (2)$$

**Figure 2:** X-ray diffraction patterns of sulfate-ion-substituted hydroxyapatite in comparison to SO$_4$/PO$_4$ molar ratio. SO$_4$/PO$_4$ molar ratios: (a) 0, (b) 0.28, (c) 0.39, and (d) 0.52. ●: Hydroxyapatite.

**Figure 3:** Relation between SO$_4$/PO$_4$ molar ratio and lattice constant in sulfate-ion-substituted hydroxyapatite.

**Figure 4:** Effect of lattice strain on SO$_4$/PO$_4$ molar ratio in sulfate-ion-substituted hydroxyapatite.

**Figure 5:** FT-IR spectra of sulfate-ion-substituted hydroxyapatite. SO$_4$/PO$_4$ molar ratios: (a) 0 [hydroxyapatite], and (b) 0.52 [sulfate-ion-substituted hydroxyapatite].
When using ACP, for which the Ca/P molar ratio is low, as the starting material, the first step of the reaction involves the formation of the Ca-deficient-type HAp precursor (equation 1). As the Ca-deficient-type HAp incorporates HPO$_4^{2-}$ in its structure, HPO$_4^{2-}$ and SO$_4^{2-}$ ions are exchanged, and SAp is formed (equation 2).

**Conclusion**

SAp could be synthesized using ACP, which is a precursor of HAp, as the starting material. Moreover, the amount of SO$_4^{2-}$ ions incorporated in SAp could be controlled by changing the amount of added Na$_2$SO$_4$, and the maximum SO$_4$/PO$_4$ molar ratio was 0.5. The chemical formula of the obtained SAp corresponded to Ca$_9$(PO$_4$)$_4$(SO$_4$)$_2$(OH)$_2$. Moreover, SAp was considered to be produced by the substitution of HPO$_4^{2-}$ ions with SO$_4^{2-}$ ions, which have similar radii and valences.

**References**