Influence of Ethylamine on the Crystal Growth of Hydroxyapatite Crystals

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The ability of ethylamine to influence hydroxyapatite (HAp) crystal growth has been studied. HAp crystals were obtained from the mixture of HAp precursor slurry and various amount of ethylamine under hydrothermal condition. The products synthesized with 5 wt% or more ethylamine were plate-like crystals whose grown face was *a*-plane of HAp. Several analyses suggested that the role of ethylamine was the inhibition of crystal growth along a particular axis.

Biomineralization occurring in nature has been attracting attentions, which would provide a novel idea of material design leading to the new functional materials development and frontier of materials science.[1] In biological systems, organic materials have important roles in nucleation, crystal growth and crystal orientation of the mineral phase.[1–3] The organic materials may give the nucleation site, raise ion concentration, etc, and inorganic crystals formed are found to show the specific size, shape and organization. These phenomena are seen in many biological systems. Human bone is a biological produced composite, composed of hydroxyapatite (HAp) and collagen fibrils. The HAp crystals formed are plate-like in shape. Based on the above understanding, we focused on the influence of the organic materials on the HAp crystal growth which shows significantly different characteristics in term of its morphology, with special attention being paid to the role of functional groups. We successfully obtained plate-like HAp crystals with hydroxy group of alcohol.[4] Many proteins are known to be involved with hydroxyapatite formation in human body. Amino group is one of the essential radicals of protein. In this study, the morphological change of HAp crystals was studied in the presence of ethylamine containing amino group.

The HAp precursor slurry was prepared as follows; 0.06 mol $CaCO_3$ and 0.09 mol $CaHPO_4 \cdot 2H_2O$ were agitated in a pot mill using 500 g ZrO_2 balls and 200 mL H_2O for 24 h at 50 rpm to yield the HAp precursor slurry. Various amounts of ethylamine were added to the 200 mL HAp precursor slurry. This mixture was treated in an autoclave with stirring under hydrothermal conditions at 180 °C for 5 h to crystallize the HAp. The products were separated from the obtained slurries by filtration. Phases of the products were analyzed by powder X-ray diffraction (XRD, MAC science, MXP[3]) which employed a graphite monochromated Cu Kα radiation, operation at 20 kV and 40 mA. In order to determine the crystal plane grown extensively by XRD, the products dispersed in ethanol were dropped onto a glass plate and dried to create an oriented layer. Infrared spectra (IR, JASCO, FT/IR-8000S) were measured in KBr pellets. The morphology of the products was observed by transmission electron microscopy (TEM, JEOL, JEM-4000FX) at 300 kV. Copper sample-holders covered with micro grids were used for TEM observation. The Ca and P concentrations of the products and the solutions separated from the slurries were measured by inductively coupled plasma emission spectroscopy (ICP, Seiko Denshi Kogyo, SPS7000A). The Ca/P molar ratios of the products were calculated from the Ca and P concentrations.

The peaks of XRD pattern of the precursor slurry were broad, which indicated the HAp precursor to be incompletely crystallized. However, those of the products treated hydrothermally with various amount of ethylamine were very sharp and the *d* values of these peaks of those products were in good agreement with the JCPDS value of HAp (Figure 1(a)). This indicated that the products were well crystallized HAp independently of the amount of ethylamine added.

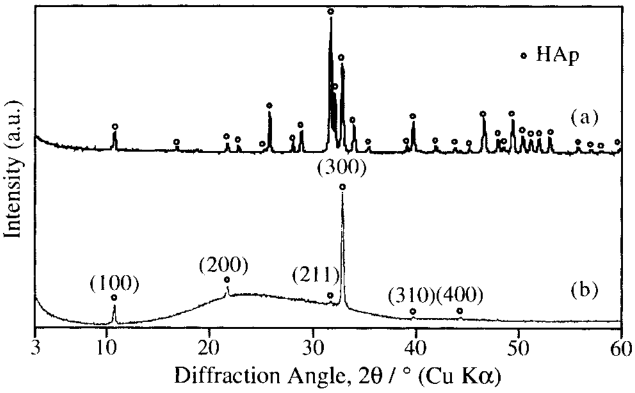

Figure 1. Powder X-ray diffraction patterns of the products synthesized hydrothermally with 15 wt% ethylamine. XRD samples were prepared (a) by packing with the powder products into glass holder and (b) by dropping dispersed powder products onto glass plate to form preferential orientation.

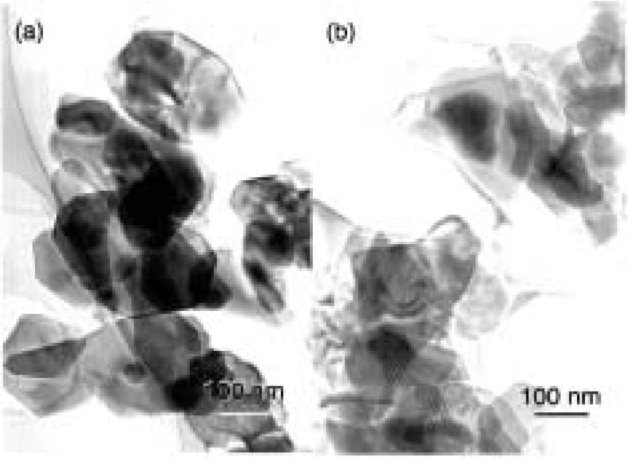

Figure 2. TEM micrographs of the products synthesized hydrothermally (a) without ethylamine and (b) with 15 wt% ethylamine.

TEM images of the products (Figure 2) exhibited that ethylamine addition caused a change in the morphology. For the products synthesized without ethylamine, there were only granular-like images, about 200 nm in size in every field. On the other hand, most of the images of the products synthesized with 5 wt% or above ethylamine were plate-like about 50–300 nm in size, and there were also some dark contrast rod-like images, about 10–50 nm in width and 50–300 nm in length along the edge of micro grids. These dark contrast rod-like images would be considered as the vertical images of the plate-like products. These observation would indicate that the products obtained without ethylamine addition were granular-like crystals, about 200 nm in size, while those prepared with 5 wt% or above ethylamine were plate-like crystals, about 50–300 nm in diameter and 10–50 nm in thickness.

In order to determine the morphology of the products either plate-like or not, the products dispersed in ethanol were dropped onto a glass plate and dried, and this cycle was repeated over than 30 times. If the products were plate-like, the samples formed on a glass plate would make an oriented layer. The XRD result of the sample prepared in this way shows in Figure 1(b). The peaks corresponding to (h00) plane are apparently larger than the other peaks of HAp. These results indicate that the products synthesized with ethylamine were plate-like and the crystal plane grown extensively was defined as the *a*-plane.

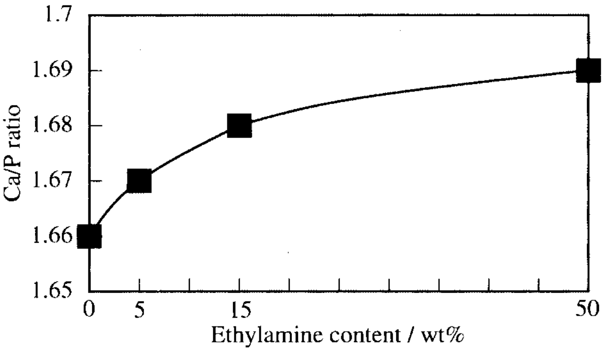

Figure 3. Ca/P ratios of the products synthesized with varying ethylamine content.

Figure 3 shows that addition of ethylamine resulted in an increase in Ca/P ratio of the products. Since HAp is not capable of containing Ca in additional site, this increase in Ca/P ratio would indicate the lack of PO_4. The reason of this lack of PO_4 would be explained by the results of infrared spectra. The infrared spectra of the products synthesized hydrothermally exhibited four bands at 876, 1420, 1456 and 1550 cm^{-1} corresponding to carbonate (Figure 4). In further detail about the bands in the range between 1300 and 1650 cm^{-1}, the bands at 1420 and 1550 cm^{-1} were the band of the carbonates replacing at PO_4 site and OH site in HAp lattice, respectively, and the band at 1456 cm^{-1} was the complex band of the carbonates replacing both of OH and PO_4 sites.[5,6] The measured spectra showed that increasing of ethylamine content caused an increase in the intensity of the carbonate bands replacing at PO_4 site relative to those replacing at OH site. This increase in the carbonate bands at PO_4 site would suggest that ethylamine having amino group interact with PO_4^{3-} ions in the solution and retarded a supply of PO_4^{3-} to HAp nuclei, thus, carbonate ions would be able to occupied PO_4 site easily.

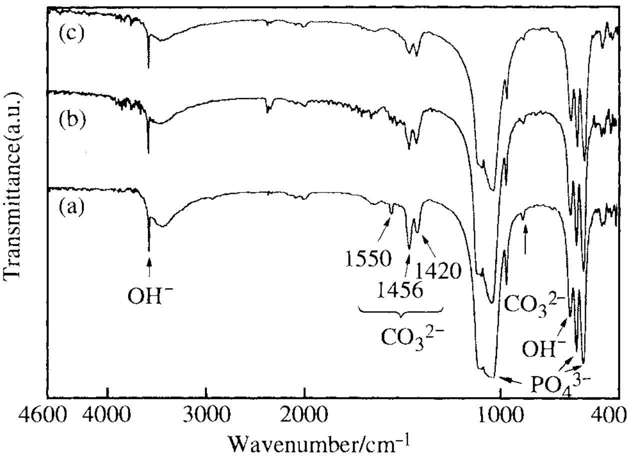

Figure 4. IR spectra of the products synthesized with various amounts of ethylamine: (a) 0 wt%, (b) 5 wt%, and (c) 50 wt%.

This assumption is supported by the change of P concentrations in solutions. The P concentration rose from 0.5 to 20.0 mmol dm^{-3} in solution before and after ethylamine is added into the precursor slurry, even though the Ca concentration decreased from 3.2 to 0.1 mmol dm^{-3}. This rise in the P concentration alone shows that ethylamine induced the dissolution and suppressed the reprecipitation of PO_4^{3-} ions. The role of ethylamine in the anisotropic growth of HAp crystals may be interpreted in term of inhibition of a supply of PO_4^{3-} ions by electrostatic interaction between PO_4^{3-} ions and amino group of ethylamine.

The results clearly showed that the crystal growth of HAp is controlled by organic molecules having amino group, ethylamine. The products synthesized from HAp precursor with 5 wt% or more ethylamine under hydrothermal treatment were plate-like crystals whose grown face was *a*-plane of HAp. FT-IR analysis of the plate-like HAp crystals shows that the carbonate ions replaced at PO_4 site than OH site of HAp. The role of ethylamine would be an inhibitor of HAp crystal growth along a particular axis by interaction with PO_4^{3-} ions in solution.

References

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