Direct Observation of Hydroxyapatite Nucleation from Amorphous Phase in a Stoichiometric Calcium/Phosphate Aqueous Solution

Sujin Kim, Hyun-Seung Ryu, Hyunho Shin, Hyun Suk Jung, and Kug Sun Hong*
School of Materials Science and Engineering, Seoul National University, Shillim-dong, Seoul, 151-744, Korea

(Received July 12, 2004; CL-040823)

Nucleation of hydroxyapatite (HAP) nanocrystals from amorphous calcium phosphate (ACP) nanoparticles in a stoichiometric calcium/phosphate aqueous solution was directly observed by dark field transmission electron microscopy. This observation is a direct evidence of internal arrangement process for the ACP–HAP conversion, while it is against the view of ACP dissolution/reprecipitation for HAP formation in a stoichiometric calcium/phosphate aqueous solution.

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is currently being used as a biomedical material because of its excellent biocompatibility and bioactivity attributed to its chemical and structural similarities to bone and tooth minerals. Hydroxyapatite formation in aqueous systems has been intensively investigated for both studies of biological mineralization and of synthesis of biomedical materials. When hydroxyapatite precipitates from highly supersaturated, alkaline or neutral solutions, a metastable amorphous calcium phosphate (ACP) phase forms rapidly in the initial stages of the reaction and transforms to the more stable apatite phase.[1] This amorphous phase has a Ca/P molar ratio of 1.5, and on the basis of an X-ray radial distribution function[2] it is considered to consist of nearly spherical $Ca_9(PO_4)_6$ clusters, or Posner's clusters, which are close-packed to form the larger spherical particles with water molecules in the interstices. Although ACP–HAp conversion has been extensively studied, there remains considerable uncertainty as to the exact mechanisms of the crystallization reaction. Some authors have suggested an ACP dissolution–HA nucleation process[3,4] is responsible for the reaction, while others have explained the crystallization process occurring with an internal rearrangement process.[5–9] With a view to identifying correct crystallization mechanisms, in the present work, crystallization process has been quenched at selected intervals of reaction time for in situ observation of the reaction products. This work presents a direct experimental evidence HAp nucleation, which supports the view of internal rearrangement process.

The induction time and rate of crystallization of ACP into HAp is influenced by numerous parameters such as degree of supersaturation, composition of the solution, ionic strength, pH, temperature, and additives.[2–8,10–14] In the present work, a highly supersaturated solution with a pH adjusted to 11 with a Ca/P molar ratio of 1.67 (the same as the stoichiometric composition of crystalline HAp) was selected. The precipitation was initiated by rapid mixing of $Ca(NO_3)_2 \cdot 4H_2O$ (98.0%, Junsei Chemical Co., Ltd.) and $(NH_4)_2HPO_4$ (99.0%, Junsei Chemical Co., Ltd.) solutions. Solutions were prepared with carbonate-free, deionized water. Prior to mixing, the pH of each solution was adjusted by the addition of NH_4OH to provide the final mixed solution. CO_2 contamination was avoided by bubbling nitrogen gas through the mixed solutions. All experiments were performed at 20 °C. At selected time intervals, aliquots were withdrawn, press-filtered and washed 4 times with carbonate-free, deionized water. The resulting samples were quenched with liquid nitrogen and freeze-dried.

The phases of the dried samples were analyzed using powder X-ray diffraction (XRD). Changes in the morphology of the precipitates were observed via transmission electron microscopy (TEM). The freeze-dried powders were dispersed in ethanol, and then drops of the suspension were placed on carbon-coated grids. The grids were observed with the Philips CM-20 operated at 200 kV. Low magnification (×50000) bright field (BF) as well as dark field (DF) images and selected area diffraction (SAD) patterns were recorded. Fast focusing and short exposure time were used to minimize electron-beam induced damages.

Figure 1 shows the XRD patterns of the samples taken at selected time intervals and the change in the HAp (211) peak intensity as a function of reaction time. The intensity of HAp peaks becomes appreciable since about 7 h and increases rapidly thereafter. After about 16 h of reaction time it does not increase much, indicating that the crystallization is almost completed.

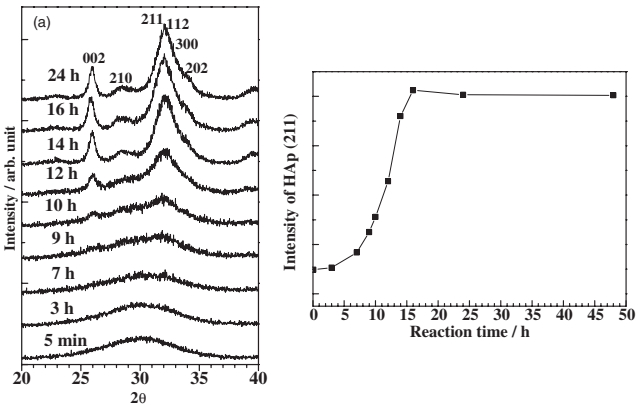

Figure 1. XRD patterns of the samples obtained at selected intervals of reaction time.

The BF images of the precipitates withdrawn after different reaction times through TEM and their corresponding SAD patterns are shown in Figure 2. As the reaction time increases, clusters of spherical individual particles (Figure 2a) change to clusters of rod-like or acicular individual particles (Figure 2c). As confirmed by the XRD result and the SAD, particles with spherical morphology in 5-min sample are amorphous (diffusive rings in SAD, Figure 2a), while the 48-h specimen is crystalline (Figure 2c). Although no apparent rod-like specimens are observed in the BF image of 7-h specimen, tiny spots on the SAD pattern indicate that the particles in 7-h specimen have HAp nanocrystals to be shown up more clearly later.

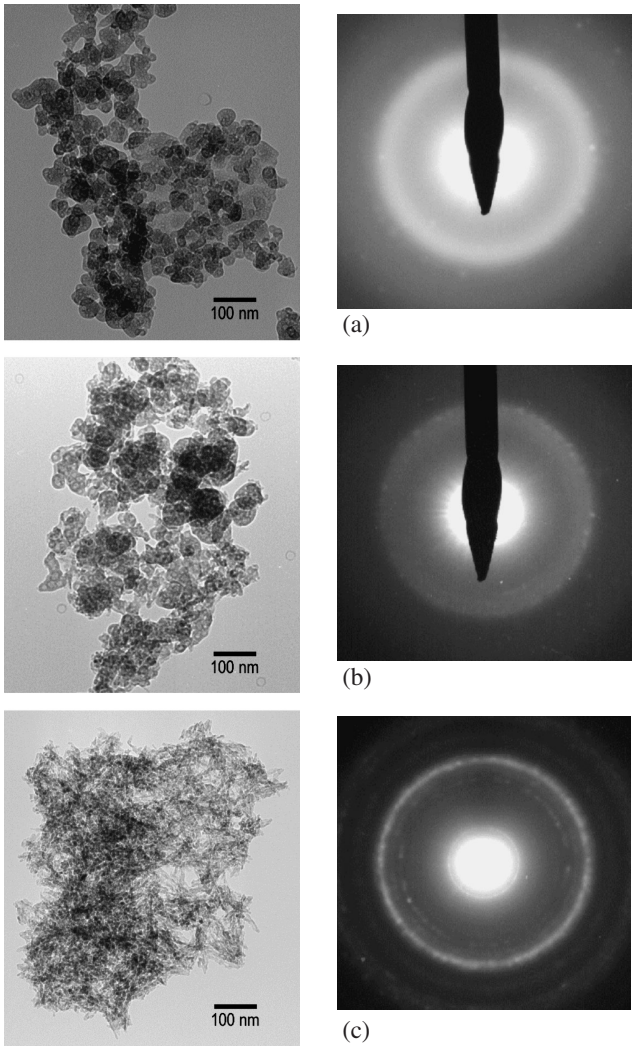

Figure 2. BF images and SAD patterns from the samples taken at (a) 5 min, (b) 7 h, and (c) 48 h.

Having studied the overall change of morphology and crystallinity with reaction time as in the above, more detailed investigation is necessary for the specimens in which crystallization just started for proper identification of the crystallization mechanism. In Figure 1, the intensity of HAp peak in XRD is appreciable from about 7 h though the actual crystallization could start earlier, which may be due to the detection limit of XRD. In this sense, TEM and SAD results from 3-h specimen (Figure 3) would reveal the situation when crystallization has just started, while precipitates taken after longer reaction duration also showed the similar feature at some locations. In the BF image in Figure 3, the morphology of the individual particles is spherical (shape of ACP), not of rod-like shape (HAp crystals). However, the SAD pattern in Figure 3 shows very weak diffraction rings. The DF image also shows many tiny bright spots. The SAD and DF results are the evidence of the presence of tiny crystals. Considering these with the morphology in BF image, nuclei of the HAp crystals are believed to be nucleated directly from ACP and they will develop to rod-like or acicular shapes later. Actually no apparent evidence of dissolution of ACP has been

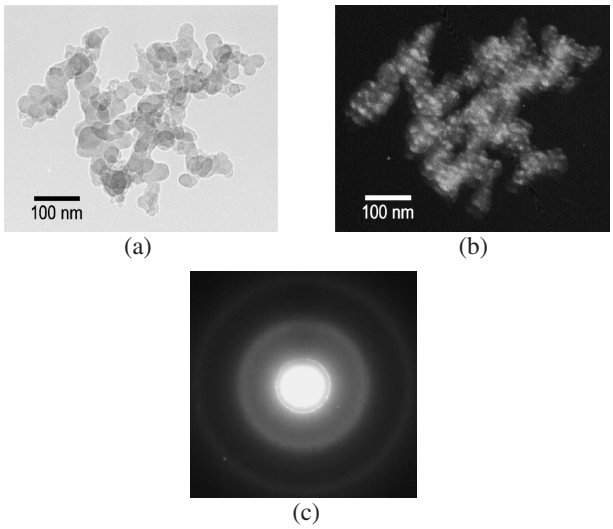

Figure 3. BF image (a), DF image (b), and SAD pattern (c) from particle clusters where crystallization has just started.

available in the literature while some indirect evidences, such as extended X-ray absorption fine structure (EXAFS), have been presented to support the internal rearrangement process. In the present work, no considerable size reduction of ACP spherical particles has been observed with reaction time before the crystallization occurs: no decisive reason for ACP dissolution can be conceived of as well. Thus, our observation serves as a direct evidence of the internal rearrangement process at least in a stoichiometric calcium/phosphate solution.

References

1 E. D. Eanes, I. H. Gillessen, and A. S. Posner, *Nature*, **208**, 365 (1965).
2 A. S. Posner and F. Betts, *Acc. Chem. Res.*, **8**, 273 (1975).
3 A. L. Boskey and A. S. Posner, *J. Phys. Chem.*, **77**, 2313 (1973).
4 S. Lazic, *J. Cryst. Growth*, **147**, 147 (1995).
5 J. E. Harries, D. W. L. Hukins, C. Holt, and S. S. Hasnain, *J. Cryst. Growth*, **84**, 563 (1987).
6 F. Abbona and A. Baronnet, *J. Cryst. Growth*, **165**, 98 (1996).
7 K. Onuma, A. Oyane, T. Kazunori, T. Katsuharu, G. Treboux, N. Kanzaki, and A. Ito, *J. Phys. Chem. B*, **104**, 10563 (2000).
8 K. Onuma and A. Ito, *Chem. Mater.*, **10**, 3346 (1998).
9 M. Tamai, T. Isshiki, K. Nishio, M. Nakamura, A. Nakahira, and H. Endoh, *J. Mater. Res.*, **18**, 2634 (2003).
10 N. C. Blumenthal, A. S. Posner, and J. M. Holmes, *Mater. Res. Bull.*, **7**, 1181 (1972).
11 T. P. Feenstra and P. L. de Bruyn, *J. Phys. Chem.*, **83**, 475 (1979).
12 W. Kibalczyc and K. Bondarczuk, *J. Cryst. Growth*, **71**, 751 (1985).
13 C. Liu, Y. Huang, W. Shen, and J. Cui, *Biomaterials*, **22**, 301 (2001).
14 L. M. Rodriguez-Lorenzo and M. Vallet-Regi, *Chem. Mater.*, **12**, 2460 (2000).
15 A. Rodrigues and A. Lebugle, *Colloids Surf., A*, **145**, 191 (1998).

Published on the web (Advance View) September 11, 2004; DOI 10.1246/cl.2004.1292