Gondwana Research 28 (2015) 858–868

Contents lists available at ScienceDirect

Gondwana Research

journal homepage: www.elsevier.com/locate/gr

# Phosphate-dependent morphological evolution of hydroxyapatite and implication for biomineralisation

Shu-Dong Jiang [a], Qi-Zhi Yao [b], Yi-Fei Ma [a], Gen-Tao Zhou [a,*], Sheng-Quan Fu [c]

[a] CAS Key Laboratory of Crust-Mantle Materials and Environments, School of Earth and Space Sciences, University of Science and Technology of China, Hefei 230026, PR China
[b] School of Chemistry and Materials Science, University of Science and Technology of China, Hefei 230026, PR China
[c] Hefei National Laboratory for Physical Sciences at Microscale, University of Science and Technology of China, Hefei 230026, PR China

ARTICLE INFO

*Article history:*
Received 22 October 2013
Received in revised form 13 April 2014
Accepted 13 April 2014
Available online 2 May 2014

Handling Editor: R.D. Nance

*Keywords:*
Hydroxyapatite
Phosphate
Biomineral
Biomimetic mineralisation
Biomineralisation

ABSTRACT

Hydroxyapatite (HAP) with various morphologies was prepared, in the absence of biological or organic molecules, through an ammonia gas diffusion method at room temperature. Contrary to the common consensus that crystal morphology control of biominerals is generally achieved by biological or organic molecules, our results suggest that $PO_4^{3-}$ may also play a crucial role in the special morphogenesis of hydroxyapatite. The morphology, structure and composition of the obtained products were characterised by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), and high-resolution TEM (HRTEM). The FESEM and TEM analyses demonstrate that at a given concentration of $Ca^{2+}$, increasing $PO_4^{3-}$ concentration leads to the formation of hydroxyapatite with various morphologies ranging from porous flower-like spheres, hollow bur-like spheres to solid bur-like spheres. If the $PO_4^{3-}$ concentration remains constant, however, the porous flower-like spheres are always obtained at different concentrations of $Ca^{2+}$. For all concentrations of $PO_4^{3-}$, a series of time-resolved experiments reveal that the initial precipitate is always unstable amorphous calcium phosphate (ACP), and that the generation of the different morphologies originates from the dissolution of amorphous calcium phosphate, followed by the crystallisation and self-assembly of hydroxyapatite. Possible mechanisms are proposed for the formation of HAP with the different shapes and architectures. The dependence of HAP morphology on phosphate concentration suggests that, in biomineralisation, biological genetic and physicochemical factors can cooperatively influence the formation of hydroxyapatite with unusual morphologies and hierarchical structures.



## 1. Introduction

Calcium phosphates are of great significance in a wide range of fields including geology, chemistry, biology, medicine, and material sciences (Dorozhkin, 2007; Bengtsson et al., 2009; Dorozhkin, 2009, 2010). Geologically, the apatite minerals occur as accessory minerals in almost all igneous rocks, metamorphic rocks, veins and other ore deposits; and most commonly as fine-grained and often impure masses as the chief constituent of phosphate rock. The mineral apatite is one of the major reservoirs of phosphorous in the Earth's crust. As such, apatite plays a critical role in a number of geochemical processes. Apatite strongly influences the concentrations of P, Ca and F in surface, ground, and ocean water, and their rare earth element (REE) contents (Köhler et al., 2005; Goddéris et al., 2006; Harouiya et al., 2007). In biological systems, calcium phosphates occur as the principal inorganic constituent of normal (bones, teeth, fish enameloid, and some species of shells) and pathological (dental and urinary calculus and stones, atherosclerotic lesions) calcifications (LeGeros, 1991, 1994; Hesse and Heimbach, 1999; Dorozhkin and Epple, 2002). Structurally, they occur mainly in the form of poorly crystallised nonstoichiometric sodium-, magnesium-, and carbonate-containing hydroxyapatite (often called "biological apatite" or dahllite) (Dorozhkin and Epple, 2002; Dorozhkin, 2010). Amongst these compounds, hydroxyapatite, with the ideal chemistry $Ca_{10}(PO_4)_6(OH)_2$, has also gained considerable attention due to its many functional properties that allow for a wide range of applications such as hard tissue analogues, catalysts, liquid chromatographic columns, and chemical sensors (e.g., Sebti et al., 2002; Vallet-Regi and Gonzalez-Calbet, 2004; Cummings et al., 2009; Liu et al., 2009; Niu et al., 2012).

Living organisms are capable of inducing the crystallisation and deposition of a wide variety of minerals (e.g., Lowenstam, 1981; Skinner and Jahren, 2004; Dorozhkin, 2010), but vertebrates mainly utilise the calcium phosphates in constructing their mineral phases both in normal circumstances in bone, dentin, and tooth enamel and in pathological ectopic mineral deposits. The predominant form of the mineral in all situations is biological apatite. However, the extent of mineralisation in a particular tissue or organ is quite variable, and the mineral crystal size and shape, as well as their packing and organisation may also be variable (Rohanizadeh and Legeros, 2007; George and Veis,

* Corresponding author. Tel./fax: +86 551 63600533.
*E-mail address:* gtzhou@ustc.edu.cn (G.-T. Zhou).

http://dx.doi.org/10.1016/j.gr.2014.04.005
1342-937X/

2008). For example, the basic nanoscale structure of bone consisting of mineralised collagen is usually plate-shaped and exceedingly small (2–4 nm thick and some tens of nanometres long and wide) (e.g., Weiner and Price, 1986; Hu et al., 2010), yet the basic building block of the mature enamel is an enamel rod — a dense array of needle-shaped apatite crystals, roughly 50 nm across and tens of microns long, with their crystalline *c*-axes aligned along the rods. Rohanizadeh and Legeros (2007) reported that *Lingula adamsi* shells consist of apatite crystals of varying size, shape, and orientation in different areas of the shell, and two different types of laminae were identified in *L. adamsi* shells under SEM analysis: compact and stratified laminae. A common consensus is that biological or organic molecules, such as proteins, induce the nucleation of a special polymorph and control the unique morphogenesis of biogenic mineral crystals (e.g., Belcher et al., 1996; Falini et al., 1996; Gower and Tirrell, 1998; Addadi et al., 2006; Politi et al., 2007; Gower, 2008; Mahamid et al., 2008; Tao et al., 2009; Deshpande et al., 2010; Xie and Nancollas, 2010; Yang et al., 2010; Zhou et al., 2010; Gómez-Morales et al., 2011). However, mineral formation in biological tissues always occurs in a fluid phase, which mediates the transport of lattice ions and regular moieties onto crystal surfaces. Some studies involving the various extracellular fluids, which are separated from the mineralising regions of the hard tissues, showed that electrolyte concentrations in a particular tissue or organ have a chemical composition different from that of the other tissues, organs or circulating blood (Howell et al., 1960, 1968; Wuthier, 1969, 1971; Lundgren and Linde, 1987; Larsson et al., 1988; Lundgren et al., 1992; Siqueira et al., 2012). These studies raise the question as to whether physicochemical factors in a particular tissue or organ, such as calcium and phosphate concentrations, ionic strength, solution pH, degree of supersaturation, and even temperature, also contribute to the unique morphogenesis of HAP besides biological or organic molecules.

Here, we report on various hierarchical structures of HAP that were prepared through a simple gas diffusion process in an aqueous system without using any organic templates and/or additives at room temperature. The goal of this study is to examine the influence of phosphate on the development of hydroxyapatite morphology and to reveal the possible contribution of phosphate to hydroxyapatite biomineralisation. The study produced hydroxyapatite particles with various morphologies ranging from porous flower-like spheres, hollow bur-like spheres to solid bur-like spheres at different concentrations of $PO_4^{3-}$. Since no biological or organic molecules were added in our experiments, this excludes biological factors and highlights the influence of physicochemical factors on the fabrication of hierarchical structures of HAP. Contrary to the common consensus that crystal morphology control of biominerals is generally achieved by biological molecules, our results suggest that crystallite size, crystal shape, and packing and organisation of HAP in a particular tissue or organ may also be related to the local phosphate concentration.

## 2. Experimental section

### 2.1. Sample preparation

All starting chemicals are of analytical grade and used without further purification; deionised water was used as solvent. $CaCl_2 \cdot 2H_2O$ was purchased from Silian Chemicals Ltd. Shanghai, China. $(NH_4)_2HPO_4$ and $NH_3 \cdot H_2O$ were from Sinopharm Chemical Reagent Co., Ltd.

The experiments were carried out at room temperature (22 °C). In a typical procedure, 0.1472 g (1 mmol) of $CaCl_2 \cdot 2H_2O$ was dissolved in 20 mL of deionised water with vigorous stirring by a magnetic stirrer to form solution A. Then 0.0792 g (0.6 mmol) of $(NH_4)_2HPO_4$ was dissolved in 20 mL of deionised water to produce solution B. Solution B was introduced into solution A with continuous stirring, and a white suspension was formed. In order to obtain a clear initial mineralization solution, a small amount of 0.1 M HCl was added dropwise into the white suspension while being stirred, and the pH of the initial mineralisation solution was adjusted to 5.0 by addition of 0.1 M HCl or diluted ammonia solution. 25 mL of the homogeneous solution was transferred into a 25-mL beaker with a piece of glass (1.8 cm × 1.8 cm) on the bottom for collecting the precipitate. Each beaker was covered with Parafilm with six punched needle holes and placed in a closed desiccator. 20 mL of diluted ammonia solution was put on the bottom of the desiccator as the source of $NH_3$. A feature of this method is the ability to control the gas diffusion rate ($NH_3$) by simply changing the concentration of the ammonia solution, which regulates the degree of supersaturation in the mineralisation system through the variation of pH. In this manner, confinement of the nucleation and growth of HAP can be achieved, mimicking to a large extent the deposition of HAP in vivo. A similar $CO_2$ gas diffusion method has been extensively applied to the studies on biomimetic mineralisation of $CaCO_3$ minerals (e.g., Albeck et al., 1996; Falini et al., 1996; Pokroy et al., 2006; Zhou et al., 2010). After different mineralisation durations, the beaker was removed from the desiccator, and the piece of glass with the mineralised crystals was separated from the solution, washed with deionised water several times, and allowed to dry at room temperature to obtain the final mineralised product. The dried precipitate was kept in a desiccator for further analysis and characterisation. For other samples, similar procedures were deployed except that the experimental parameters were varied. The detailed experimental parameters and mineralised products are listed in Table 1.

### 2.2. Mineral detection and characterisation

Several analytical techniques were used to characterise the synthesised products. The powder X-ray diffraction (XRD) patterns of the synthesised samples were recorded with a Japan MapAHF X-ray diffractometer equipped with graphite-monochromatised Cu Kα irradiation ($\lambda = 0.154056$ nm), employing a scanning rate of $0.02°s^{-1}$ in the $2\theta$ range of 3–60°. Infrared (IR) spectrum analyses were made on samples palletised with KBr powders in the range 4000–400 $cm^{-1}$, using an infrared Fourier transform spectrophotometer (Nicolet, ZOSX). X-ray fluorescence (XRF) was performed on an XRF-1800 X-ray fluorescence spectrometer at room temperature. Microstructures of the products were observed by JEOL JSM-2010 field-emission scanning electron microscopy (FESEM). Selected area electron diffraction (SAED) patterns, high-resolution transmission electron microscopy (HRTEM) images, and transmission electron microscopy (TEM) images were obtained on a Hitachi model H-800 transmission electron microscope with an accelerating voltage of 200 kV.

## 3. Results and discussion

### 3.1. Results

The phase composition and structure of the mineralised products were first identified by XRD. Fig. 1 shows the typical XRD patterns of the products mineralised for 24 h at phosphate concentrations of 0.015, 0.025 or 0.045 M (samples 1–3), maintaining calcium concentration at 0.025 M. The XRD results reveal that all of the products obtained at the three different concentrations of phosphate are the hexagonal HAP phase with the space group $P6_3/m$ and lattice parameters a = 9.418 Å, c = 6.884 Å (JCPDS file, No. 09-0432), except that the (002) reflections in our samples are particularly strong and sharp. The stronger (002) reflection is most likely related to the preferential orientation of the HAP along the [001] direction. The broadened diffraction peaks from our products may imply that the mineralised products consist of nanocrystals and/or that slight distortions of the crystal lattice occur in the crystals. Corresponding FT-IR spectra of the products (samples 1–3) are presented in Fig. 2. The broad absorption peak between 2800 and 3800 $cm^{-1}$ can be attributed to the O–H stretch of water and HAP. The characteristic band for $H_2O$ at 1640 $cm^{-1}$ further indicates the presence of water in these mineralized products, which should