Available online at www.sciencedirect.com
ScienceDirect
Journal of Inorganic Biochemistry 102 (2008) 311–317
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

TEM study of the morphology of Mn^{2+}-doped calcium hydroxyapatite and β-tricalcium phosphate

I. Mayer [a,*], F.J.G. Cuisinier [b], S. Gdalya [a], I. Popov [c]

[a] Department of Inorganic and Analytical Chemistry, Hebrew University, Jerusalem 91904, Israel
[b] INSERM U 424, Centre de Recherches Odontologique 1 Place de l'Hospital, F-6700 Strasbourg, France
[c] Unit Nanoscopic Characterization, Hebrew University, Jerusalem 91904, Israel

Received 25 April 2007; received in revised form 4 July 2007; accepted 13 September 2007
Available online 29 September 2007

Abstract

Mn-doped carbonated hydroxyapatites (HA) were prepared by precipitation method. Ca-deficient HA samples were obtained by this method with the characteristic hexagonal apatite structure. Scanning transmission electron microscopy (STEM) of two HA samples with two different Mn content has shown that their morphology depends on their Mn content. In case of relatively low (0.73%) Mn content (HAMn1), platelet crystals about micron size and needle-like crystals up to 100 nm were observed, while with 1.23% Mn (HAMn2) crystals were smaller, needle-like and with sizes up to 400 nm only. Mn-doped TCP samples were prepared by two methods. In one case it was obtained by direct solid-state reaction with the characteristic rhombohedral structure of β-TCP and with composition of $Ca_{2.7}Mn_{0.3}(PO_4)_2$. TEM pictures of crystals of this sample were tens of micron and submicron size with visible faces. Crystals of β-TCP obtained by high temperature partial transformation of sample HAMn2 to β-TCP were found by TEM to be smaller, micron sized, drop-like shaped, sensitive to beam radiation. These results indicate that the morphology of Mn doped β-tricalcium phosphate samples depends on the method of their preparation. Morphological properties of HA and TCP are discussed and it is suggested that the smaller and less perfect HA crystals with the higher Mn-content as well as the less perfect TCP crystals obtained by transformation of HA to TCP might be of more biocompatible character.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Hydroxyapatite; Tricalcium phosphate; Manganese; TEM; Morphology

1. Introduction

Synthetic calcium hydroxyapatite (HA), because of its analogy to the mineral components of bones and teeth, is one of the most important implantable materials due to its biocompatibility, bioactivity and osteoconductivity. HA is widely used in dentistry and orthopedics, to repair bone defects and as coating material for metallic implants [1]. In order to make synthetic HA comparable to that of natural bone tissues, cationic or anionic substituents have been added to them. Considering the anionic substituents CO_3^{2-} is found in natural apatites in substantial amounts, ~4% in teeth and ~6% in bones. The position in which the carbonate group enters in the crystalline structure of the apatite is variable, with higher content B type (CO_3^{2-} substituting in PO_4^{3-} sites) or A-type (CO_3^{2-} substituting in OH^- sites) in young bone. Trace amounts of F^- and Cl^- can be incorporated in HA [2] and enhance its thermal stability [3]. Trace amounts of Na^+, Zn^{2+}, Fe^{3+}, Cu^{2+}, Pb^{2+}, Sr^{2+} and Si are found as well in bones and teeth [4]. Two of these ions Cu^{2+} and Pb^{2+} are toxic, cumulative in animals and humans. Doping HA with Mg^{2+} improves activity of biological cells [5] and co-substitution with Na^+ and Mg^{2+} ions influences its thermal stability [6].

The importance of morphology, size and crystallinity of implanted HA materials was shown in a study in which HA was implanted into a male rat tibia [7]. The morphology and size of the implanted HA affected the intrusion of bone

* Corresponding author. Tel.: +972 2 6585214; fax: +972 2 6585319.
E-mail address: isaacm@vms.huji.ac.il (I. Mayer).

0162-0134/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2007.09.004

marrow derived cells among HA material, and this cell intrusion was important for regulating the newly formed bone mass. High crystalline sintered HA was stable and remained in its initial shape for long periods. However, low crystalline HA was absorbed and disappeared during bone remodeling.

ß-Tricalcium phosphate (β-TCP) is as well biocompatible and was found in many cases advantageous compared to HA [8,9] and was also studied in HA/TCP composite materials [10,11]. The influence of dopants, like Mg^{2+}, Zn^{2+} and Si on the physical, mechanical, and biological properties of β-TCP, used with implants, was investigated [12–14]. HA and β-TCP differ in their crystal structure and can be identified by their powder X-ray diffraction patterns. HA crystallizes in a hexagonal and β-TCP in a rhombohedral structure [15,16].

In previous studies [17,18] Mn^{2+}-containing HA and TCP were studied. The motivation for the addition of Mn^{2+} ions to HA was due to the fact that divalent Mn^{2+} has been linked to the activation of integrins [19], a family of receptors which mediate cellular interactions with extracellular matrix and cell surface ligands. In the presence of Mn^{2+} ions the ligand affinity of integrin increases and cell adhesion is promoted. In further works the beneficial effect of Mn-doped HA thin films was demonstrated [20,21]. When human osteoblasts were cultured on the surfaces of such thin films deposited on etched Ti substrates, biological tests have shown that the Mn-doped HA coatings favour osteoblasts proliferation, activation of their metabolism and differentiation.

EPR spectroscopy has proved that manganese is divalent in the sintered samples and seems to partly occupy the Ca^{2+} sites in the β-TCP structure.

The present work was aimed to determine morphological properties of Mn-containing HA and TCP samples by TEM. HA samples with different Mn content (0.73 and 1.23%) and β-TCP samples prepared in two ways: (a) via the heat treatment of Mn containing HA and (b) by a direct high temperature solid-state reaction of the mixtures of $CaCO_3$, $(NH_4)_2HPO_4$ and $Mn(NO_3)_2$ were selected for this study.

2. Experimental

2.1. Synthesis of Mn-containing HA samples

Samples of HA with Mn (0.1–4.0%) and with carbonate (1.0–3.0%) were prepared by a precipitation method [22]. A phosphate solution [3.7 g $(NH_4)_2HPO_4$ in 200 ml triple distilled water (TDW)] was added dropwise to a calcium solution [9.47 g $Ca(NO_3)_2 \cdot 4H_2O$ in 200 ml TDW]. Carbonate was added from a 1 M $NaHCO_3$ stock solution and Mn from a 0.025 M $MnCl_2$ solution. For the preparation of HA samples with Mn^{2+} the pH of 5.8–6.0 was chosen, because it was found that at this pH Mn^{2+} is not oxidized to a higher valence state. The pH was maintained constant during precipitation by adding NaOH solution using a Mettler pH-stat automatic titrator. The precipitation was carried out during 2 h under controlled temperature (85 °C), and following this the temperature was raised to boiling point and the system was refluxed for 2 h. The sample was then washed with TDW and dried overnight in air at 120 °C.

2.2. Synthesis of Mn containing β-TCP

2.2.1. Synthesis of β-TCP by heating precipitated HA samples

Precipitated HA samples (as described above) were heated in a porcelain crucible during 5 h up to 800 °C in an electric furnace. HA at this temperature transforms partially or almost completely to β-TCP.

2.2.2. Synthesis of β-TCP by high temperature solid-state reaction

$Ca_{2.7}Mn_{0.3}(PO_4)_2$ was prepared from a stoichiometric mixture of $CaCO_3$, $(NH_4)_2HPO_4$ and $Mn(NO_3)_2$ according to the chemical reaction:

$$2.7CaCO_3(s) + 2(NH_4)_2H(PO_4)(s) + 0.3Mn(NO_3)_2(s) \rightarrow Ca_{2.7}Mn_{0.3}(PO_4)_2 + 2.7CO_2(g) + 4NH_3(g) + 3H_2O(g) + 0.3N_2O_5(g)$$

The mixture was thoroughly mixed in an agate mortar, heated in an alumina crucible at 300 °C for 3 h, crushed, mixed and then heated at 1100 °C overnight. The gaseous products of the reaction leave the system after the formation of $Ca_{2.7}Mn_{0.3}(PO_4)_2$.

2.3. Characterization methods

The Ca, P and Mn contents of the samples were determined by ICP-atomic emission spectroscopy with a precision of ±0.1%, ±0.5%, and ±0.3% for Ca, P and Mn, respectively.

Crystal phase was determined by powder X-ray diffraction (XRD) with a Philips Automatic Diffractometer using Cu Kα radiation. The samples were scanned in the 2θ range of 20–55°. The lattice parameters were calculated by a least-square computer program. Maximum deviation of the lattice constants was ±0.003 Å.

Infrared spectroscopy (IR) characterization was made by a Nicolet FTIR spectrometer using samples (~1 mg) pressed into pellets with KBr (~150 mg). The carbonate content of the samples was estimated by IR analysis using the extinction ratio of the carbonate (1420 cm^{-1}) and phosphate (575 cm^{-1}) bands. Carbonate is determined by this method with an accuracy of ±5% [23].

Samples were also examined by scanning transmission electron microscopy (STEM). For this purpose the samples were ground and sonicated in ethanol. The obtained suspension was deposited onto 400 mesh carbon coated copper grid. All the study was performed with Tecnai F20 G^2 (FEI Company) operated at 200 kV and equipped with

Download English Version:

https://daneshyari.com/en/article/1318085

Download Persian Version:

https://daneshyari.com/article/1318085

Daneshyari.com