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Atomic scale modeling of iron-doped biphasic calcium phosphate bioceramics

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ABSTRACT

Biphasic calcium phosphates (BCPs) are bioceramics composed of hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) and beta-Tricalcium Phosphate (β-TCP, $Ca_3(PO_4)_2$). Because their chemical and mineral composition closely resembles that of the mineral component of bone, they are potentially interesting candidates for bone repair surgery, and doping can advantageously be used to improve their biological behavior. However, it is important to describe the doping mechanism of BCP thoroughly in order to be able to master its synthesis and then to fully appraise the benefit of the doping process. In the present paper we describe the ferric doping mechanism: the crystallographic description of our samples, sintered at between 500 °C and 1100 °C, was provided by Rietveld analyses on X-ray powder diffraction, and the results were confirmed using X-ray absorption spectroscopy and ^{57}Fe Mössbauer spectrometry. The mechanism is temperature-dependent, like the previously reported zinc doping mechanism. Doping was performed on the HAp phase, at high temperature only, by an insertion mechanism. The Fe^{3+} interstitial site is located in the HAp hexagonal channel, shifted from its centre to form a triangular three-fold coordination. At lower temperatures, the Fe^{3+} are located at the centre of the channel, forming linear two-fold coordinated O-Fe-O entities. The knowledge of the doping mechanism is a prerequisite for a correct synthesis of the targeted bioceramic with the adapted (Ca + Fe)/P ratio, and so to be able to correctly predict its potential iron release or magnetic properties.

Statement of Significance

Biphasic calcium phosphates (BCPs) are bioceramics composed of hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) and beta-Tricalium Phosphate (β-TCP, $Ca_3(PO_4)_2$). Because their chemical and mineral composition closely resembles that of the mineral component of bone, they are potentially interesting candidates for bone repair surgery. Doping can advantageously be used to improve their biological behaviors and/or magnetic properties; however, it is important to describe the doping mechanism of BCP thoroughly in order to fully appraise the benefit of the doping process.

The present paper scrutinizes in detail the incorporation of ferric cation in order to correctly interpret the behavior of the iron-doped bioceramic in biological fluid. The temperature dependent mechanism has been fully described for the first time. And it clearly appears that temperature can be used to design the doping according to desired medical application: blood compatibility, remineralization, bactericidal or magnetic response.

1. Introduction

The mineral mass of bone is dominated by nanocrystalline non-stoichiometric hydroxyapatite (HAp, chemical formula $Ca_{10}(PO_4)_6(OH)_2$, Ca/P ratio of 1.67) [1–4]. Non-stoichiometry is mainly assumed by few weight percent of carbonate substitution

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and also calcium deficiency, nevertheless many trace elements participate to the non-stoichiometry. Tricalcium phosphate (β-TCP, chemical composition $Ca_3(PO_4)_2$, Ca/P ratio of 1.5) has a Ca/P ratio close to that of HAp and presents higher solubility under biological conditions [5,6]. HAp and BCP (biphasic calcium phosphates composed of a mixture of HAp and β-TCP) have been investigated for biomedical applications in reconstructive surgery (hard tissue replacement implants and bone prosthesis coating) due to their excellent bioactivities, biocompatibility and osteoconductivity [5–10]. In addition, the doping effect can advantageously be used, among other levers, to improve the biomedical properties of HAp-based ceramics [11]. Nanocrystalline bone mineral contains numerous essential trace elements [4,9]. The role of many of these ionic species in hard tissues is not fully understood, because of the difficulties encountered in monitoring and quantifying their proportions, which vary according to dietary alteration and to physiological and to pathological causes. However, it is commonly accepted that these various ions play a major role in the biochemistry of bones, enamel and dentine by a substitution process [12]. Our previous results on Zn-doped BCP samples highlighted that a fine description of the incorporation mechanism of the doping element remains a significant factor in correctly interpreting biological behavior, namely due to the different solubility of the two HAp and β-TCP phases [13–15]. The incorporation of the doping element in one or the other phases will deeply modify its potential release in biological fluid. The HAp structure is known to accept various ionic substitutions, as has been demonstrated for carbonate [16,17], silicate [18–21], borate [22,23] and alkaline earth cations Mg^{2+} [24–26] and Sr^{2+} [27,28]. Nevertheless our recent results on the Zn-doping mechanism demonstrated that substitution is not the only mechanism to be considered: insertion into an interstitial site [13], as also described for bevolite (the Sr equivalent with composition $Sr_{10}(PO_4)_6(OH)_2$) [29], has been established. This doping mechanism is temperature-dependent [14]. Zn-doping elements can be located in drastically different local environments. The transfer from the six-fold coordinated calcium site substitution in β-TCP at moderate temperature to the two-fold coordinated insertion in HAp at higher temperature is a significant phenomenon.

Following our Zn-doping insertion mechanism studies we undertook a systematic study of BCP doping by the 3d-metal cation series from manganese to zinc. The present paper is devoted to the specific case of ferric cations. Iron is an essential trace element in bones and teeth, is a micronutrient essential for various biological processes and is an important component of several metalloproteins. Iron represents approximately 35 and 45 mg/kg of body weight in adult women and men, respectively. In the intestinal lumen, iron exists in the form of ferrous and ferric salts, although most dietary inorganic iron is in the form of ferric salts [30]. Recent studies have shown that the presence of Fe^{3+} affects the crystallinity and solubility of HAp [31–34], while small amounts of iron were found to have a positive impact on the biomedical properties of HAp [35–37]. The blood compatibility, and more generally the biocompatibility and non-cytotoxicity, of Fe^{3+}-doped HAp has recently been demonstrated, with improved bactericidal and mineralizing properties compared to undoped HAp [38–40]. Biomagnetic calcium phosphate ceramics, incorporating magnetic ions and exhibiting ferromagnetic properties, play an important role in medicine. Doped magnetic HAp could be useful for biological applications such as magnetic resonance imaging (MRI), cell separation, drug delivery and heat mediation for the hyperthermia treatment of cancers [39,41].

Despite the recently-described insertion mechanism for Zn^{2+} [13–15] and despite the cationic size difference between Fe^{3+} (0.64 Å, CN6) and Ca^{2+} (1.00 Å, CN 6) [42], a substitution mechanism at calcium crystallographic sites is commonly considered in the literature as for all cations. In the present study, a detailed structural description of Fe-doped HAp is investigated to clarify the situation. Series of BCP samples (HAp being the main phase) are synthesized using the sol-gel method with different iron doping levels and with thermal treatments between 500 °C and 1100 °C. In addition to a long-range order investigation performed using Rietveld refinement on X-ray powder diffraction patterns, the local order is finely described thanks to X-ray absorption spectroscopy and ^{57}Fe Mössbauer spectrometry.

2. Materials and methods

2.1. Sol-gel elaboration of Fe-substituted BCP samples

The sol-gel method previously proposed by the authors was used to synthesize one undoped and four Fe-doped series of BCP samples [14]. Briefly, to produce 2 g of undoped BCP powder, 4.7 g of $Ca(NO_3)_2{\cdot}4H_2O$ (Aldrich) and 0.84 g of P_2O_5 (Avocado Research chemicals) were dissolved in ethanol (anhydrous, >99.5%, Aldrich) under stirring and refluxed at 85 °C for 24 h. The solution was maintained at 55 °C for 24 h to obtain a consistent gel, and then further heated to 80 °C for 10 h to obtain a white powder. Finally, the powder was sintered for 15 h. Heat treatments were performed at 500 °C, 600 °C, 700 °C, 800 °C, 900 °C, 1000 °C and 1100 °C (series of seven samples with the same chemical composition). To prepare the Fe-doped samples, the required amount of $Fe(NO_3)_3{\cdot}9H_2O$ (Sigma-Aldrich) was added to the solution, simultaneously with $Ca(NO_3)_2{\cdot}4H_2O$ (Sigma-Aldrich). Nominal compositions were calculated assuming the insertion of Fe^{3+} cations at the interstitial crystallographic site of hydroxyapatite; i.e. constant Ca/P = 1.67. In the following, samples are labeled '*x*Fe-*T*' with *x* = 00, 15, 25, 50 and 75 for samples with respectively the targeted nominal $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}Fe_{0.15}(PO_4)_6(OH)_{1.55}O_{0.45}$, $Ca_{10}Fe_{0.25}(PO_4)_6(OH)_{1.25}O_{0.75}$, $Ca_{10}Fe_{0.50}(PO_4)_6(OH)_{0.50}O_{1.50}$ and $Ca_{9.875}Fe_{0.75}(PO_4)_6O_2$ compositions. Deprotonation of hydroxyl anions was first assumed to counterbalance the excess interstitial Fe^{3+} positive charges (i.e. three H^+ protons substitution by one Fe^{3+}), followed by calcium deficiency for the higher iron amount in the 75Fe-*T* series. A total of 35 samples, distributed in five series according to chemical compositions, were synthesized and analyzed. Elemental analyses of the samples using ICP-AES confirmed that iron added in the solutions were well incorporated in the precipitates.

Sample color were sintering temperature-dependent. Powders obtained after the sol-gel process were white. Heat treatment at 500 °C produced light grey samples, which became orange when the temperature increases and attained a rust color at 1000 °C (the greater the iron content, the more pronounced the coloration is). Finally, heat treatment at 1100 °C resulted in light purple powders.

2.2. X-ray powder diffraction (XRPD) and Rietveld analyses

XRPD patterns were recorded on a X'Pert Pro Philips diffractometer, with θ-θ geometry, equipped with a solid X-Celerator detector and using Cu Kα radiation (λ = 1.54184 Å). XRPD patterns were recorded at room temperature in the interval $3° < 2\theta < 120°$, with a step size of $\Delta 2\theta = 0.0167°$ and a counting time of 200 s for each data value. A total counting time of about 200 min was used for each sample (some raw data are showing in Fig. SI1a from supplementary information). A XRPD pattern was collected from pure NIST standard LaB_6 using the same experimental conditions in order to extract the instrumental resolution function to improve peak profile fitting during Rietveld refinements.