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Hydrogen Phosphate (HPO_4^{2-}) ions Substitution in Hydrated Layers of OCP by Dicarboxylate ions to Hexagonal HAP Nanocrystals System

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Received 27 June 2020; Accepted 07 July 2020; Published 19 August 2020

Abstract

The octacalcium phosphate (OCP) crystals are apatite bases with hydrated layers in producing of needle or plate-shaped hydroxyapatite (HAP) nanocrystals, which prepared by a dissolution precipitation reaction. The reaction led to a hexagonal HAP nanocrystals formation under hydrothermal condition *via* OCP at 180℃ for 3h with pH of solution adjusted to 5.5 with incorporating dicarboxylate or succinate ions having Ca/P molar ratio is expected to be 1.56±0.02, where the morphology of OCP are retained. During incorporating of succinate ions in OCP crystals, the hydrogen phosphate (HPO_4^{2-}) ions in the hydrated layers of OCP are being substituted by succinate ions. These organically modified OCP which generated to HAP with unique nanostructures have been characterized by using of SEM, FTIR and X-ray diffraction analysis. Since the crystal system of HAP is hexagonally and its crystalline size in the direction of various (a,b,c) axes depending on the thickness of the plate-shaped HAP crystals where their size as perpendicular to the (100) plane is calculated by using of Scherrer equation $D100 = K\lambda/(\beta \cos\theta)$. The formed hexagonal HAP are used in soft and hard tissue engineering system for biomedical application.

Keywords: Hydroxyapatite, octacalcium phosphate, succinate ion .

1. Introduction:

Indeed, the bio-apatite are indispensable for which the general formula is $Ca_5(PO_4)_3X$, where X=F,Cl or OH, since they are key component of bone and teeth. Recently, synthetic apatites that permit bone grafts are now abailable[1].The hydroxyapatite (HAP,$Ca_{10}(PO_4)_6.(OH)_2$ which is the main inorganic components of hard tissue such as bone and teeth and they are used in medicinial application have attracted a great attention including several application such as artificial organs, tissue engineering, medical devices & dentistry etc[2,3]. Although ,fabricated biological hydrogels loaded biphasic calcium phosphate nanoparticles have also been reported for bone tissue regeneration[4]. Especially characteristics transformation behavious of octacalcium phosphate (OCP,$Ca_8(HPO_4)_2.(PO_4)_4.5H_2O$) to HAP have been reported, which is different from those of other calcium phosphate compounds under hydrothermal conditions,(in *vitro* & *vivo*)[5-7]. The HAP can be synthesized from various calcium orthophosphates such as α- & β- tricalcium phosphate (TCP,$Ca_3(PO_4)_2$) and OCP as well. For TCP, since HAP is generated by a dissolution pricipetation reaction, there is no correlation betbeen the crystal shape of the original TCP particle and the shape of the HAP particles generated. Generally,needle shaped HAP crystals are formed from granular α- & β-TCP particles under hydrothermal conditions[8,9].

Herein, a plate-shaped OCP crystals are transformed to laminated thin plate- shaped HAP nanocrystals under hydrothermally and characterized the resultant HAP. The OCP crystal is composed of apatite and hydrated layers producing plate-shaped crystals[10,11]. Where, the hydrogen phosphate ion (HPO_4^{2-}) in the hydrated layres can be substituted or incorporated by dicarboxylate such as succinate ions into OCP crystal structure has been reported[12,13]. The molecular structure of succinic acid/ion is shown in figure -1.

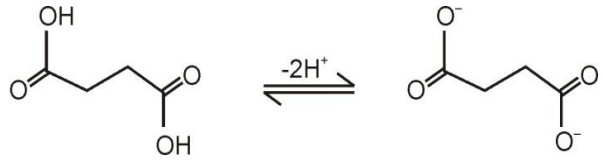

Figure 1. The structure of succinic acid ($HOOC.(CH_2)_2.COOH$) & its succinate ion $(OOC.(CH_2)_2.COO)^{2-}$.

2. Experimental

The experimental procedure for succinic acid based modifided octacalcium phosphate (OCP; $(HPO_4)_2.(PO_4)_4.5H_2O$) with incorporated succinate ion has been synthesized by a previously reported method[14,15], which are adapted from the work described by T.Yokoi et al[16]. The required materials, chemicals/regents have been labotic bases standard, used. In this method, 20 mmol of succinic acid ($HOOC(CH_2)_2COOH$); 99.5% is dissolved in 200 cm^3 of ultra pure water, where the pH of solution is adjusted to 5.5 by adding an appropriate amount of ammonia solution (aqu.NH_3 soln.;25%). The 16.0 mmol of calcium carbonate ($CaCO_3$; calcite) has been suspended in the dicorboxylic acid solution and 10.0 mmol of phosphoric acid (H_3PO_4;85% aqu.soln.) is mixed with the suspension. Then suspension is stirred at 60℃, after about 3h, the pH of the suspension is reduced to 5.0 by using 1.0 mol. dm^{-3}HCl

solution and after 30 minutes, the precipitates has been isolated by vacuum filtration and gently rinsed with ultra pure water and ethanol(C_2H_5OH), followed by drying overnight at 40℃.

The sample which synthesized in solution containing 20 mmol of succinic acid is denoted as Suc-20 as well as OCP those not containing dicorboxylate ion is also synthesized by using 16.0 mmol of $CaCO_3$ and 12.0 mmol of H_3PO_4 which may denoted as CALPHOS . Now, CALPHOS(0.10g) and Suc-20(0.10g) are added to a 28-cm^3 teflon vessel with 10cm^3 of ultra pure water. The samples have encapsulated in an autoclave, and then hydrothermally treated at 180℃ for 3h. These hydrothermal treatment condition under which the phase transformation is completed in a short time may selected because as the reaction time become longer, the morphological differences in the morphology of generated hexagonal HAP due to different starting materials disappear due to aging, where hydrothermally treated sample has collected by vacuum filtration and it dried overnight at 40℃, respectively.

3 Results & Discussion :

Earlier as we reported and mentioned that, the succinate incorporated OCP has been reported herein following a procedure well reported[14]. The report reveals that the Ca/P molar ratio of OCP with incorporated or complexated succinate (Suc-OCP) ion is expected to be 1.56± 0.02. The transformation of Suc-20 have proceeded under hydrothermal condition and Suc-OCP is completely transformed to HAP by hydrothermal treatment at 180℃ for 3h. There is no by- products such as dicalcium phosphate anhydrous are detected by XRD analysis. It is reported that the colour of Suc-OCP changed from white to light brown upon heat treatment at 450℃ in an air due to residual corbon formation. Notable, the colour of both CALPHOS & Suc-20 before and after hydrothermal treatment was white and non of the colour may observed visually. Hence succinate ion decomposition may not occur under the hydrothermal conditions.

The crystal morphology of the samples (CALPHOS, Suc-20, Suc-OCP & Pure-OCP) before and after hydrothermal treatment at 180℃ for 3h have been well assigned[15,17]. Where the crystalline phases of the different products are being characterized by powder X-ray diffraction (XRD), scanning electron microscopy (SEM) and other instrumentation. The absorption peak of HPO_4^{2-} located in the hydrated layer is detected at 1193cm^{-1}. This peak is not absorbed for Suc-20 because HPO_4^{2-} is replaced by the succinate ion. The observation peaks arising from the COO stretching and CH_2 bending modes of the complexated succinate ion are observed at 1565,1460 & 1300 cm^{-1}. After the hydrothermal treatment, the absorption peak corresponding to HAP are detected for both hydrothermally treated CALPHOS and Suc-20. Although, in some cases, hydrothermally synthesized HAP includes corbonate ions in its crystal lattice, the absorption peaks corresponding to the corbonate ion are not detected in our samples. In crystalline phase`s terms the FTIR spectral observation are in line with XRD results

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$$5Ca_8(HPO_4)_{2-n}(OOC(CH_2)_2COO)n(PO_4)_4.mH_2O+8H_2O \longrightarrow$$
$$4Ca_{10}(PO_4)_6(OH)_2+(6-5n)HPO_4^{2-}+5n(OOC(CH_2)_2COO)^{2-}$$
$$+12H^++5mH_2O\ (0 < n \leq 1)\ldots\ldots(1)$$

The transformation from Suc-OCP to hexagonal HAP is proposed to proceed from the reaction which are shown as above in eq. 1. In crystal morphology of the samples before & after hydrothermal treatment at 180℃ for 3h have displayed that, both the as-synthesized and hydrothermally treated CALPHOS sample are composed of plate-shaped crystals several micrometers in size, although the crystalline phase is changed from OCP to hexagonal HAP. Therefore, for pure-OCP, the crystal morphology is almost retained after phase transformation[10,11]. Similarly, to CALPHOS there is no change in the macroscopic morphology for Suc-20. These finding strongly suggested that the phase transformation mechanism for Suc-OCP is similar to that of pure- OCP. On the basis of SEM images report of the different samples we observed that, the HAP crystals, where the thickness of HAP crystals, formed by the hydrothermal treatment of CALPHOS is in range 50 - 150nm similar to those of plate-shaped crystals before hydrothermal treatment. The persent observation have shown the dark line (S- line) are found at the centre of the Suc-20 crystal after hydrothermal treatment, which can attributed to the gap between two thin-plate crystals. In other words, the hexagonal HAP crystal synthesized from OCP with incorporated succinate ion is likely composed of laminated thin plate-shaped crystals and ought to be thinner than the HAP crystal generated from pure-OCP.

Since, the crystal system of HAP is hexagonal, where the crystallite size in the direction of the various axes (a,b,c) dependent on the thickness of the plate-shaped HAP crystal[11,16,18]. The crystallite size perpendicular to the (100) plane which are calculated by the using of Scherrer equation (as eq.-2) to compare the thickness of the plate-shaped HAP crystal of CALPHOS and Suc-20 after hydrothermal treatment at 180℃ for 3h.

$$D100 = K\lambda/(\beta \cos\theta)\ldots\ldots[2]$$

Where, $D100$ is the crystallite size perpendicular to (100) plane, K is Scherrer constant (=0.9), λ is the wavelength of incident X-ray (0.154 nm), β is the full width at half-maximum of the 100 reflection peak for HAP & θ is the diffraction angle.The $D100$ values of samples as HAP prepared from Suc-20 are smaller than those of HAP prepared from CALPHOS. The SEM and crystallite size calculation also supports the presence of dark line (S- line) corresponding to gap between to thin-plate crystals, therefore, the HAP crystal which are obtained from Suc-20 likely have laminated nanostructures. Where, the elimination of succinate ion from interlayer of OCP crystal is necessary for the transformation from OCP with incorporated succinate ion to HAP. The laminated nanostructure is formed probably because the succinate ions inhibit crystal growth in the thickness direction.

4 Conclusion:

In the present articles, we have reported the biological apatite as octacalcium phosphates (OCP) based hexagonal hydroxyapatite (HAP) nanocrystals transformation under hydrothermal precipitation reaction at 180℃ for 3h with adjusted pH to 5.5 with incorporating dicarboxylic acid as succinic acid having Ca/P molar ratio expected to be 1.56±0.02. In transformation of OCP to laminated thin plate-shaped hexagonal HAP nanocrystals, there are morphology of OCP may retained. During incorporation of succinate ions into OCP crystal, the substitution of hydrogen phosphate (HPO_4^{2-}) ions in hydrated layer of OCP are replaced by succinate ions. The characterization of hexagonally HAP nanocrystals have been well studied by using SEM, FTIR and XRD pattern. The crystalline size and thickness of generated hexagonally plate-shaped HAP are calculated by introducing of Scherrer equation as- $D100 = K\lambda/(\beta \cos\theta)$. Where, $D100$ values of the sample are smaller than the thickness of the thin plate crystals as observed by SEM. This is probably due to broadening of the reflection peak derived from sample characteristics and the optical

system of the X-ray diffrectometer. The generated hexagonal HAP nanocrystals from apatite OCP crystals are applicable in soft and hard tissue engineering in biomedical uses.

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