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RESEARCH ARTICLE

A CRYSTAL CHEMISTRY OF HYDROXYAPATITE : A REVIEW

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Abstract

In chemistry of inorganic crystals, the octacalcium phosphate (OCP) is an apatite based crystals and having a hydrated layers which used in producing of needle or plate-shaped hydroxyapatite (HAP) nanocrystals. Although, the crystals is prepared by a dissolution precipitation reaction. These reaction led to a hexagonal HAP nanocrystals formation under hydrothermal condition from OCP at 180°C for 3 hours with pH of solution adjusted to 5.5 and incorporating dicarboxylate e.g. succinate $(OOC.(CH_2)_2.COO)^{2-}$ 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. Since the crystal system of HAP is hexagonal and its crystalline size in the longitudinal direction of various (a,b,c) axes depending on the thickness of the laminated plate-shaped HAP crystals. Here, their size as perpendicular to the (100) plane which is calculated by introducing of Scherrer's equation, $D100 = K\lambda/(\beta \cos\theta)$. The organically modified OCP which generated to HAP have unique nanostructure with micrometer thickness are characterized by using of SEM, FTIR and X-ray diffraction analysis.

Introduction:-

Indeed, in 'apatite world' 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], but, a bone like nanocrystaline apatite have been formed *via* using self assembled liquid crystals [2]. 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 and dentistry etc[3,4]. Although, fabricated biological hydrogels loaded biphasic calcium phosphate (BCP) nanoparticles have also been reported for bone tissue regeneration[5]. Especially characteristics transformation behaviours of octacalcium phosphate (OCP; $Ca_8(HPO_4)_2.(PO_4)_4.5H_2O$) to HAP have been reported[6], which is different from those of other calcium phosphate compounds under hydrothermal conditions (in *vitro-vivo*)[7-9]. The HAP can be synthesized from various calcium orthophosphates such as α- and β- tricalcium phosphate (TCP; $Ca_3(PO_4)_2$) and OCP as well[10]. For TCP, since HAP is generated by a dissolution precipitation reaction, there is no correlation between 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- and β-TCP particles under hydrothermal conditions[11,12]. Plate-shaped OCP crystals are transformed to laminated thin plate- shaped HAP nanocrystals under hydrothermally and characterized the resultant HAP. The OCP

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crystal is composed of apatite and hydrated layers producing plate-shaped crystals[13,14]. Where, the hydrogen phosphate ion (HPO_4^{2-}) in the hydrated layers can be substituted or incorporated by dicarboxylate such as succinate ions into OCP crystal structure has been reported[15-18]. The molecular structure of succinic acid and its ion is shown in figure 1.

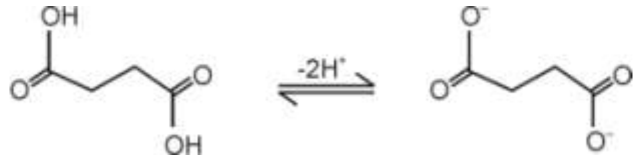

Figure 1:- The molecular structure of succinic acid ($HOOC.(CH_2)_2.COOH$) and succinate ion $(OOC.(CH_2)_2.COO)^{2-}$.

Experimental

In experimental procedure, a dicarboxylic acid such as succinic acid based modified octacalcium phosphate (OCP; $Ca_8(HPO_4)_2.(PO_4)_4.5H_2O$) in incorporated with succinate ion has been synthesized by a previously reported method[19,20]. Here, the our prescribed work is performed well by adapted from the work which described by T. Yokoi et al[21]. The required materials as chemicals and regents have been laboratory based standard is used. In this method, 20 mmol of succinic acid ($HOOC(CH_2)_2COOH$); 99.5%, (Wako Pure Chemical Industries Ltd., Osaka, Japan) 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, Nacalai Tesque Inc., Kyoto, Japan) has been suspended in the dicorboxylic acid solution and 10.0 mmol of phosphoric acid (H_3PO_4; 85% aqu. soln, Wako Pure Chemical Industries Ltd) 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 CONTROL or 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.

The crystalline phases of the different hydroyapatite (CALPHOS or CONTROL and Suc- or SUC 20) sample products have characterized by powder X-ray diffraction (XRD; RINT-2000, Rigaku Co., Tokyo, Japan) using Cu-Kα radiation.

The chemical structures of the given samples have characterized by using of Fourier- transform infrared (FTIR) spectroscopy (Frontier MIR/NIR, Perkin-Elmer Japan Co., Ltd., Kanagawa, Japan) as using the KBr tablet method. The morphologies of the formed samples have been characterized by scanning electron microscopy (SEM; SU-8000, Hitachi, Ltd., Tokyo, Japan) as well.

Results and Discussion:-

Actually, in this paper we have effort and try to mentioned that, about the succinate incorporated OCP, although, following a procedure well reported[19]. The report reveals that the Ca/P molar ratio of OCP with incorporated or complexed succinate (SUC- or 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 3 hours.

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 control or calphos and Suc-20 before and after hydrothermal treatment was white and non of the color may observed visually. Hence, succinate ion decomposition may not occur under the hydrothermal conditions. Here, in figure 2 we have shown the XRD and SEM magnification image of OCP which are as starting materials for transformation reaction.

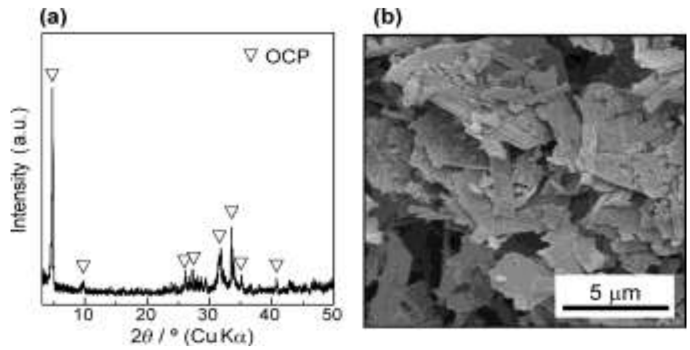

Figure 2:- The XRD pattern (a), and sem image (b) for ocp before hydrothermal treatment.

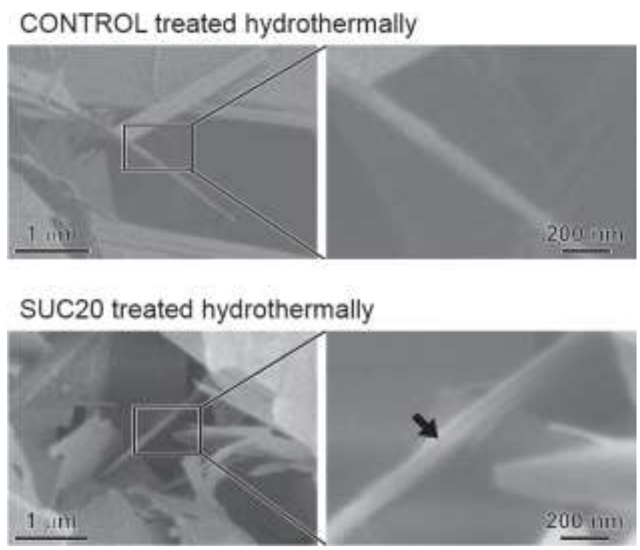

Figure 3:- SEM magnification images of Control or Calphos and suc-20 under hydrothermally treatment (at 180^{0}C for 3 hours), with an arrow which indicate a dark line at center of HAP nanocrystal system.

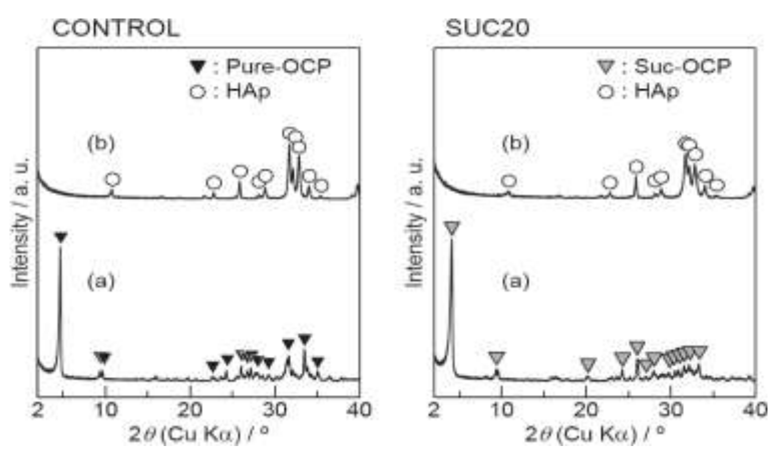

Figure 4:- The XRD patterns of samples control and suc20 under {before (a), and after (b)} hydrothermal treatment at 180 °C for 3 hours.

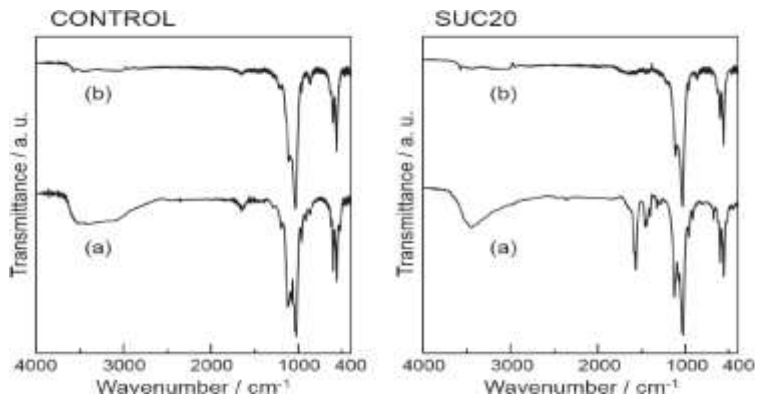

Figure 5:- The FTIR spectra of samples control and suc20 under before (a), and after (b) hydrothermal treatment at 180 °C for 3 hours.

Although, the crystal morphology of the various samples (CALPHOS or CONTROL, Suc-20, Suc-OCP and Pure-OCP) before and after hydrothermal treatment at 180℃ for 3 hours have been well assigned[20,22]. Here, the figure 3 have shown the scanning electron microscopy (SEM) observation of dark line at HAP crystal's center and the crystalline phases of the different products are being characterized by powder X-ray diffraction (XRD) as in figure 4. The figure 5 indicated FTIR spectra with the absorption peak of HPO_4^{2-} located in the hydrated layer is detected at 1193cm^{-1}, [23,24]. 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 and 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 carbonate ions in its crystal lattice, the absorption peaks corresponding to the carbonate ion are not detected in our samples. In respect of crystalline phase the FTIR spectral observation are in line with XRD results.

$5\ Ca_8(HPO_4)_2(PO_4)_4.5H_2O \rightarrow 4\ Ca_{10}(PO_4)_6(OH)_2 + 6\ H_3PO_4 + 17H_2O$ ………….(1)

In transformation reaction of OCP to HAP nanocrystals under hydrothermal condition as shown in equation 1, the pH of used solvent are 5.5 but its value may decrease in treatment conditions because the formation of phosphoric acid (H_3PO_4). In crystal morphology of the samples before and after hydrothermal treatment at 180℃ for 3 hours 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[13,14]. 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 present 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.

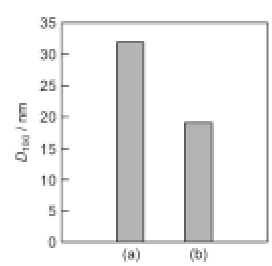

Figure 6:- The D_{100} values of hydroxyapatite (HAP) prepared from, (a) CALPHOS and (b) Suc-20, calculated by using of Scherrer equation.

Since, a survey reveals that the crystallic system for 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 crystals[14,21,25,26]. These crystallite size perpendicular to the (100) plane which are calculated by the using of Scherrer equation (as equation 2) to compare the thickness of the plate-shaped HAP crystal of CALPHOS and Suc-20 after hydrothermal treatment at 180℃ for 3 hours.

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

Here, the $D100$ 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 and θ is the diffraction angle. Figure 6 show that the $D100$ values of samples as HAP synthesized from Suc-20 are smaller than those of HAP prepared from CALPHOS. Thus, calculation of crystallite size support as well as SEM agreement that ,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.

Conclusion:-

In conclusion, we have reported the preparation and characterization of a hydroxyapatite (HAP) nanocrystals *via* hydrogen phosphate (HPO_4^{2-}) ions substitution in hydrated layers of octacalcium phosphate (OCP) by dicarboxylate as succinate ions ($(OOC.CH_2\text{-}CH_2.COO)^{2-}$. These crystals are transformed through hydrothermal precipitation reaction at 180℃ for 3 hours with adjusted pH to 5.5 and incorporated to dicarboxylic (e.g. succinic) acid having Ca/P molar ratio expected to be 1.56±0.02. In transformation of thin plate-shape OCP to laminated hexagonal HAP nanocrystals there are morphology of OCP may retained. During incorporation of organically 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 crystalline size and thickness of generated hexagonally plate-shaped HAP are calculated by introducing of Scherrer equation as $D100 = K\lambda/(\beta \cos\theta)$. The characteristic observation of produced hexagonally HAP nanocrystals have been studied by using of SEM, FTIR and powder XRD technique as well.

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