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SOLUTION-MEDIATED TRANSFORMATION OF OCTACALCIUM PHOSPHATE (OCP) TO APATITE

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Abstract

OCP crystals were hydrolyzed in solutions containing Ca^{2+}, Mg^{2+}, HPO_4^{2-}, CO_3^{2-}, F^-, citrate or P_2O_7 ions. Products of hydrolysis were analyzed using scanning (SEM) and transmission (TEM) electron microscopy, infrared spectroscopy and x-ray diffraction.

Results demonstrated that the OCP to Apatite (AP) transformation is influenced by: (1) types of ions in solution: inhibited by Mg^{2+}, citrate or $P_2O_7^{4-}$; facilitated by F^-, CO_3^{2-}, HPO_4^{2-} or Ca^{2+} ions; (2) ionic concentrations; (3) solution pH; (4) OCP crystal size. SEM showed needle-like micro-crystals on the surfaces and ends of OCP macrocrystals. TEM showed side-to-side and end-to-end arrangements and presence of central defects in the apatite crystals. IR spectra showed the incorporation of CO_3, or HPO_4, the HPO_4 incorporation being least from F-containing solutions. These results suggest that OCP to AP transformation occurred by the process of dissolution of OCP and subsequent precipitaton of Ca-deficient apatites, incorporating CO_3^{2-}, HPO_4^{2-} or F^- present in solution.

These results indicate that the observed stabilty of OCP in pathological calcifications may be due to the presence of Mg^{2+}, citrate and/or $P_2O_7^{4-}$ and/or low levels of CO_3^{2-}, F^-, Ca^{2+}, HPO_4^{2-} ions in the biological fluids.

Key Words: octacalcium phosphate (OCP), apatite, magnesium, carbonate, fluoride, scanning electron microscopy, transmission electron microscopy, infrared spectroscopy, x-ray diffraction.

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Introduction

Octacalcium phosphate, $Ca_8H_2(PO_4)_6.5H_2O$, OCP, and pure calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, HA, are very similar in crystallographic structures [4-6, 43]. OCP is known to transform to apatite in vitro [4, 8, 9, 16, 32, 44] and is an intermediate phase in the transformation of amorphous calcium phosphate, ACP, to apatite [13, 38, 48]. Based on the structural similarities of OCP and HA, the ability of OCP to transform to apatites and on the reported similarities in the crystal morphologies of synthetic OCP crystals with those of bone and enamel apatite crystals, OCP is believed to be a necessary precursor in the formation of biological apatites (the mineral or inorganic phases of enamel, dentin, bone and other pathological calcifications) [4-7, 10, 15, 36, 42, 44, 47, 49]. In vivo, OCP is frequently observed as a major crystalline component of human (but not of animal) dental calculi [20, 23, 36, 46], and on rare occasions, observed with urinary stones [36] and other soft-tissue calcifications [20, 35]. However, except for isolated reports [40], OCP has not been observed associated with normal calcifications, e.g., enamel, dentin, bone [25]. Thus, it appears that in vivo, OCP is meta-stable in pathological calcifications (principally human dental calculi) and unstable or transitory in normal calcifications.

The purpose of this study was to investigate the conditions which influence the OCP- to-apatite transformation from solutions in vitro and the mechanism for such transformation in order to gain insights into its transformaton in vivo. Factors investigated included: types of ions present in the hydrolyzing solution (calcium, magnesium, phosphate, carbonate, fluoride, citrate or pyrophosphate); solution pH and concentration; hydrolysis period. The extent of hydrolysis and products of hydrolysis were characterized using scanning (SEM) and transmission (TEM) electron microscopy, infrared (IR) absorption spectroscopy and x-ray diffraction. Preliminary results have been published [32] and presented [33,35] but not published.

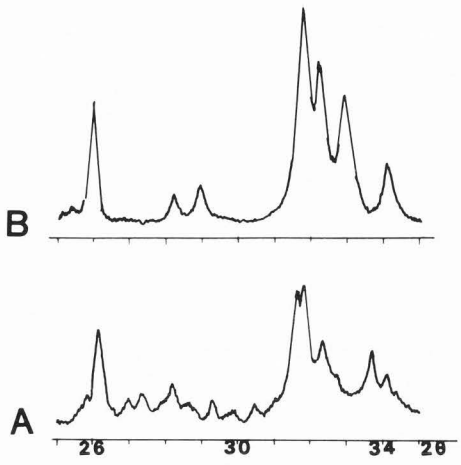

Fig. 1: X-ray diffraction patterns of OCP before (A) and after (B) hydrolysis in solutions containing F (0.001M/L), resulting in the formation of (F,OH)-apatite indicated by a-axis dimensions 0.9417 nm compared to 0.9443 nm for apatites obtained by hydrolysis in F-free solutions.

Materials and Methods

Preparation of OCP. OCP was prepared by the precipitation method previously described [32, 34]. Briefly, the method consisted of a slow drop by drop addition of calcium solution (250 ml, 0.04M $Ca(Ac)_2$) to slowly stirred phosphate solution (100ml 0.1M NaH_2PO_4 + 650 ml distilled H_2O, pH adjusted to 5) maintained at 60°C in a water bath; or by precipitation at 80°C, with the pH of the phosphate solution adjusted to pH 4 using 0.1M HAc.

Table 1. IR ABSORPTION BANDS OF OCP AND APATITES*

IR bands, OCP	IR bands, Apatites*	Assignments **
3800-3000 cm^{-1}	3700-3000 cm	H-O-H, H_2O of crystallization (OCP) adsorbed H_2O (AP)
	3580	O-H, (OH) group
1615	1615	H-O-H, H_2O of crystallization (OCP) adsorbed H_2O (AP)
	1454, 1414	C-O of CO_3 groups in CO_3-AP
1280, 1200		P-OH bending modes, HPO_4 groups
1108, 1194	1119, 1098 (F-AP) 1114 (CO_3-AP)	P-O and P-OH, HPO_4 and PO_4 groups
1078, 1060(sh) 1040, 1024	1080(sh), 1030	P-O in HPO_4 and PO_4 groups
964	965 (F-AP) 961 (CO_3-AP)	P-O or PO_4 group
910, 869	865 (AP)	P-OH stretching mode of HPO_4 groups
	873 (CO_3-AP)	C-O
	630	O-H of OH group
628, 600, 561	620, 600, 564 (F-AP) 600, 563 (CO_3-AP)	P-O of PO_4 groups
525		HO-PO_3 bending mode in HPO_4
470, 450	471	P-O of PO_4 groups
344	368 (F-AP) 365,sh (CO_3-AP)	P-O of PO_4 groups

*apatites obtained by hydrolysis of OCP in solutions containing Ca^{2+}, HPO_4^{2-}, F^-, or CO_3^{2-}.
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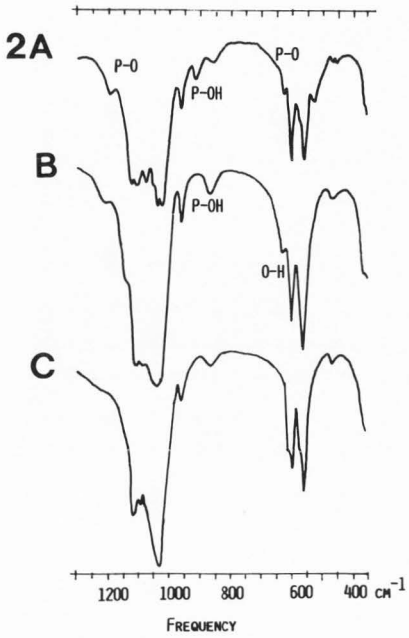

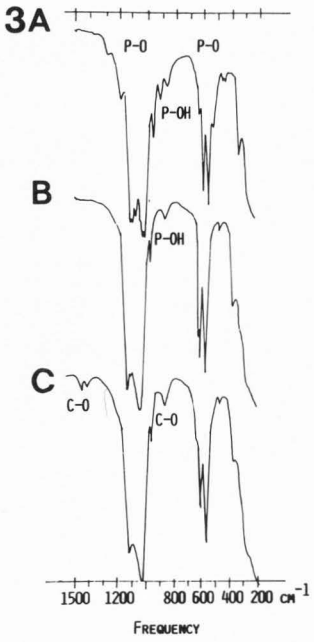

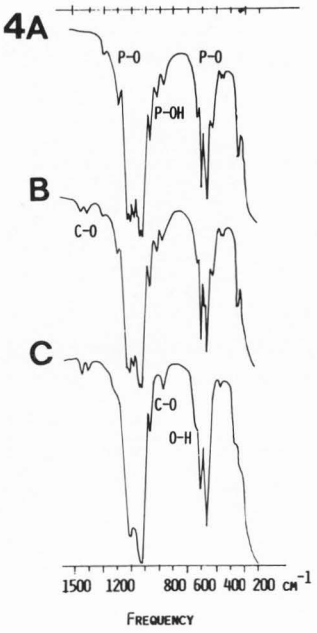

Fig. 2: IR spectra of OCP before (A) and after hydrolysis in solutions containing Ca^{2+}, 0.02M/L HPO_4^{2-} (B) and F^- (C). Both apatites incorporated HPO_4^{2-} as indicated by absorption bands at 865 cm^{-1} in (B) and (C) which are different from the HPO_4^{2-} bands in OCP at 869 and 910 cm^{-1} (A). HPO_4 present is less in F-containing AP (C) than in F-free AP (B).

Fig. 3: IR spectra of OCP before (A) and after hydrolysis in solutions containing F^- (B) and CO_3^{2-} (C) ions. F-incorporation in AP is indicated by lower a-axis dimension and greater resolution of PO_4^{3-} absorption bands: 119, 1090 1030 and at 620, 600 and 564 cm^{-1} (B). CO_3-incorporation is indicated by C-O absorption bands at 1454, 1414 and 873 cm^{-1} and less resolution of the PO_4 absorption bands (C).

Fig. 4: IR spectra of OCP before (A) and after hydrolysis in solutions containing 0.01M/L CO_3^{2-} (B) and 0.02M/L (C), 60°C, 72h. (B) shows incomplete hydrolysis, by the mixed spectra of OCP and CO_3-containing AP. (C) shows complete hydrolysis, spectrum only of CO_3-apatite [26,27].

Hydrolysis of OCP. Crystals characterized as OCP by x-ray diffraction, IR, light microscopy and SEM and having a Ca/P ratio of 1.34 [32,34] were suspended in slowly stirred or unstirred solutions containing different ions (Ca^{2+}, 0.01 to 0.05M/L; HPO_4^{2-}, 0.01M/L; CO_3^{2-}, 0.01 to 0.05M/L; F^-, 0.001 to 0.003M/L; citrate, (0.005M/L; or pyrophosphate, 0.001M/L) at 60°C for periods of 12, 14, 48, 72, 144 hours. The initial pH of the hydrolyzing solutions were 5, 7.5 or 9. The solid/solution ratios were 5mg OCP/5ml solution and 20mg OCP/2ml solution. In some cases, the OCP crystals were ground before hydrolysis. All hydrolysis experiments were made at least in triplicate.

Analyses. SEM analyses were made on coated specimens using a Hitachi 5450 instrument operating at 15kV. High resolution TEM were made on ultra-thin sections of embedded materials without decalcification prior to embedding and observed with JEOL TEMSCAN 200 CX operating at 200 kV. The IR absorption spectra were recorded on a Perkin-Elmer 983S double grating spectrometer using normal slit width and normal scanning speed over the frequency range, 4000 to 200 cm^{-1} frequency range, using KBr pellets (0.7 mg sample/300mg KBr). Assignments of the IR absorption bands were based on previous studies [1, 2, 14, 17, 19, 22-34]. X-ray diffraction analyses of powdered materials were made on a Philips APD 3520, using copper target, x-rays generated at 40kV, 20 mA; receiving slit, 1°, scanning speed, 1/8° 2θ per min. The x-ray diffraction patterns were identified according to earlier studies [22-34].

Results

The following factors were observed to influence the transformation of OCP to apatite in solutions:

(1) solution pH: OCP was indefinitely stable in acidic solutions (pH 5), except when the solutions contained F^- ions (0.001M/L), transformation to (F,OH)-apatite (Figs. 1B,2C) as deduced from its a-axis dimension, 0.9427 nm, compared to 0.9378nm for fully substituted F-apatite and 0.9443 nm for F-free apatite;

(2) temperature of hydrolysis: extent of hydrolysis was greater at higher temperatures (60°C vs 37°C);

(3) period of hydrolysis: the longer the duration of hydrolysis, the more complete the transformation of OCP to apatite;

(4) crystal size: under identical experimental conditions, powdered OCP was hydrolyzed to a greater extent than unpowdered OCP

Fig. 5: SEM of OCP crystals after hydrolysis in solution containing 0.01M/L CO_3^{2-}, for 12h at 60°C., showing very few microcrystals on the surfaces compared with Fig. 6. (bar = 0.5 μm)

crystals;

(5) ionic concentration: the higher the concentration, the greater the extent of transformation, as shown in the case of Ca-containing solutions and CO_3-containing solutions (Fig. 4);

(6) type of ions present in the hydrolyzing solution: (a) OCP was stable in solutions containing Mg^{2+} (0.02M/L), even in the simultaneous presence of Ca^{2+} (0.02M/L), Mg/Ca =0.2, but hydrolyzes to apatite in the presence of Ca^{2+} and absence of Mg^{2+} ions (Fig. 6C); was stable in solutions containing citrate ions (0.005M/L), or in solutions containing pyrophosphate (0.001M/L). Products of hydrolysis from solutions containing Mg^{2+} or citrate or pyrophosphate ions gave IR absorption spectra identical to those given by OCP before hydrolysis; (b) OCP in solutions containing Ca^{2+} (Figs. 2B, 3, 4C); HPO_4^{2-}, F^- (Figs.1B, 2C, 3B); or CO_3^{2-} (Figs.3C, 4B, 4C), OCP to apatite transformation was observed. The extent of transformation was greater from F-containing solutions than from CO_3-containing solutions (Fig.5 vs Fig.6), in spite of the much lower concentration of F^- (0.001M/L) compared to CO_3^{2-} (0.01M/L).

OCP in dry storage remains indefinitely stable unlike some types of ACP which transforms to poorly crystallized apatite after about one month in dry storage, or unlike brushite or DCPD, $CaHPO_4.2H_2O$, which partially transforms to monetite, $CaHPO_4$, upon dry storage. Another serendipitous observation was that OCP grown in silica gel systems [28] was stable even after 17 years in the gel.

IR absorption analyses showed that apatite obtained by OCP hydrolyses were not stoichiometric apatite (HA), i.e., $Ca_{10}(PO_4)_6(OH)_2$, with Ca/P of 1.67, but are Ca-deficient apatites (AP) as

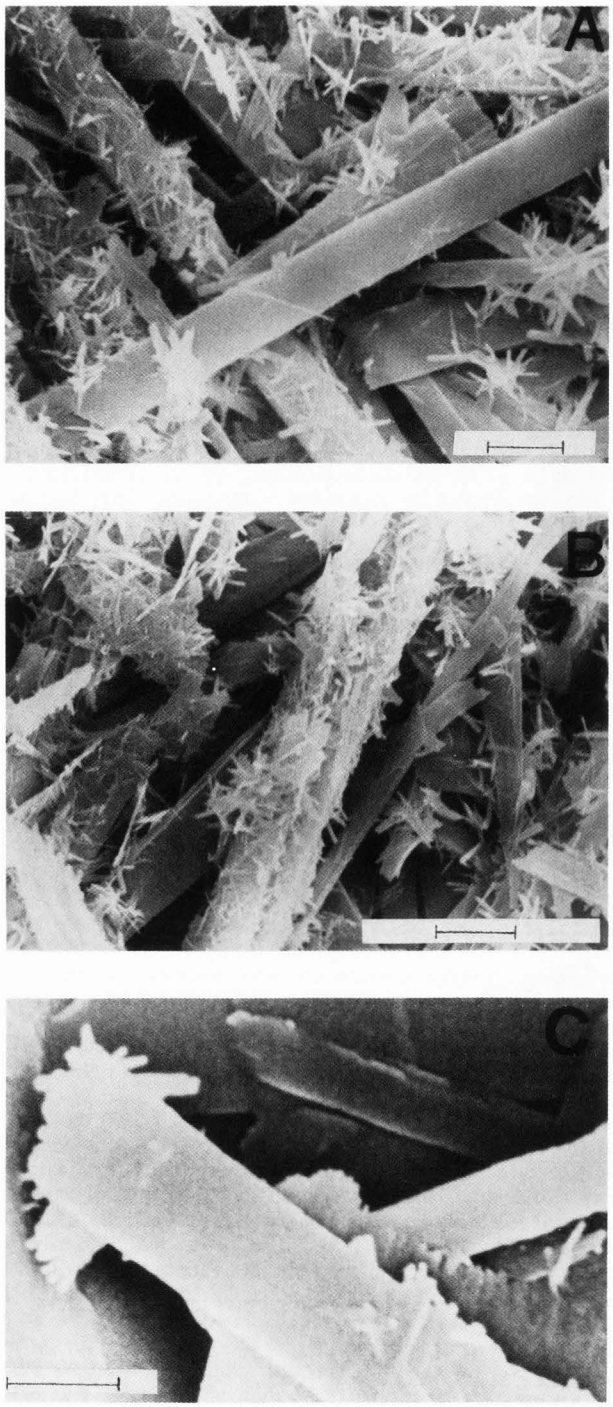

Fig. 6: SEM of OCP crystals after hydrolysis in solution containing F^- ions (0.001M/L), for 12h, at 60°C., showing abundance of microcrystals on the surfaces of the OCP macrocrystals (A,B). After 8h, microcrystals as finger-like projections at the ends of the OCP crystals were observed (C). These microcrystals were identified as apatite by electron diffraction (Fig. 8). Average crystal widths: OCP, 1μm; microcrystals, 40 to 70 nm. (Bars = 2μm for A and B, and 1μm for C).

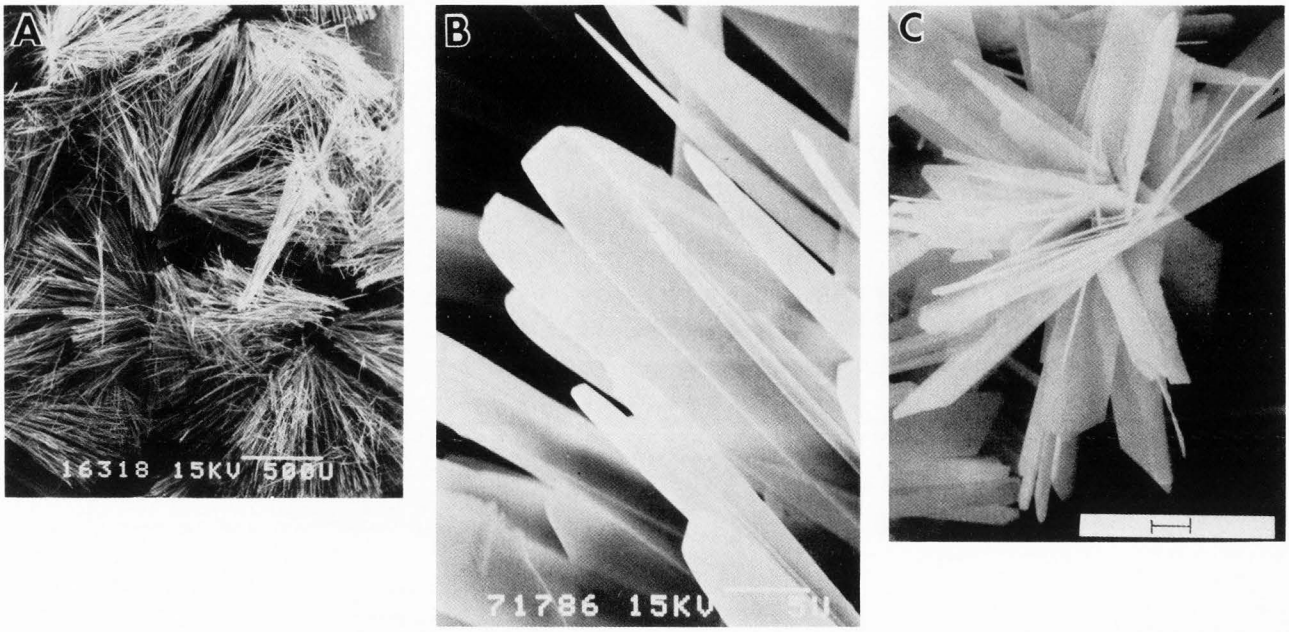

Fig. 7: SEM of OCP crystals used in this study showed long blades of OCP originating from a common center (A, B). Note the smooth surfaces and smooth ends of the individual OCP blades before hydrolysis. While most preparation of OCP consisted of long crystals (A,B), clusters of shorter blads (C) had been infrequently observed in some preparations [32]. (Bars = 500μm for A, 0.5μm for B, and 5μm for C).

indicated by its non-stoichiometric Ca/P (1.59 to 1.61) except if obtained in the presence of carbonate or fluoride [26], and as indicated by the incorporated HPO_4^{2-} (Figs. 2,3); the amount of HPO_4 incorporated being least in apatites obtained when OCP was hydrolyzed in F-containing solutions (Fig. 2C compared to 2B). The absorption bands attributed to the presence of HPO_4^{2-} groups occur at different frequencies in the spectra of OCP compared to those in apatite, and in some cases, bands are present in the spectra of OCP but not in those of apatite (Table 1). For example, one of the absorption bands due to the HPO_4^{2-} groups has its maximum at 869 cm^{-1} in OCP but at 864 cm^{-1} in AP (Figs. 2,3,4). The other bands associated with the P-OH stretching mode of HPO_4 groups observed at 913 and 527 cm^{-1} in the spectra of OCP (Figs. 2A, 3A, 4A, 4B) were absent in the spectra of AP (Figs. 2C, 3B, 3C, 4C). The absorption bands due to the PO_4 group v_4 bending mode (triply degenerate) observed at 628, 600, and 561 cm^{-1} in OCP were observed at 618, 600 and 564 cm^{-1} in (F,OH)-apatites (Figs. 2A, 2C, 3A, 3B; Table 1). Apatites obtained when OCP crystals were hydrolyzed in CO_3-containing solutions were CO_3-substituted, the CO_3^{2-} groups principally substituting for some of the PO_4 groups (Figs. 3C,4B,4C), confirming earlier observations [9,32]. The absorption bands due to the CO_3^{2-} groups incorporated in the apatite were observed at 1454 and 1414 cm^{-1} in partially hydrolyzed OCP (Fig. 4B) and also in completely hydrolyzed OCP (Fig. 4C), in the latter case, the additional band at 873 cm^{-1} also due to CO_3 absorption band, may overlap the HPO_4 band usually observed at 864 cm^{-1} in the spectra of CO_3-free apatites (Figs. 2,3,4). The criteria used for deciding that the OCP was completely hydrolyzed was the absence of any OCP lines in the x-ray diffraction (e.g., Fig. 1) and absence of any absorption bands attributed to OCP [14, 32, 34, Table 1] in the IR spectra (Figs. 2B, 2C, 3B, 3C, 4C).

SEM investigations of partially transformed OCP showed the presence of needle-like micro-crystals on the surfaces (Figs.6A, 6B) or at the ends of the macro-crystals appearing as finger-like projections (Fig. 6C). The abundance of these micro-crystals were greater in OCP partially hydrolyzed in F-containing solutions (Fig. 6) compared to those hydrolyzed in CO_3-containing solutions (Fig. 5). SEM of OCP crystals before hydrolysis showed smooth surfaces (Fig. 7A) and smooth terminals (Fig. 7B). OCP crystals used for these studies were predominantly those with long blades (Fig. 7). In some preparations, OCP with shorter blades (e.g.Fig. 7C) similar to those reported earlier [34, 44, 45] have been infrequently observed with the more preponderant clusters of long blades [30, 32] shown in Figs 7A, 7B. Regardless of whether the OCP crystals grow as long or short blades, they grow as spherulitic aggregates, growing from a common center. The cause for the difference in morphology of individual OCP crystals may be related to the method of preparation: directly, by precipitation [30, 32], or by homogeneous crystallization [43,44] or growth in gel systems [28, 30]; and, indirectly, by the hydrolysis of DCPD [4, 9, 47], or by seeded growth [45].

SEM showed that the microcrystals resulting from the hydrolysis of OCP were always needle-like or acicular. These microcrystals were identified by electron diffraction as apatite. TEM analyses

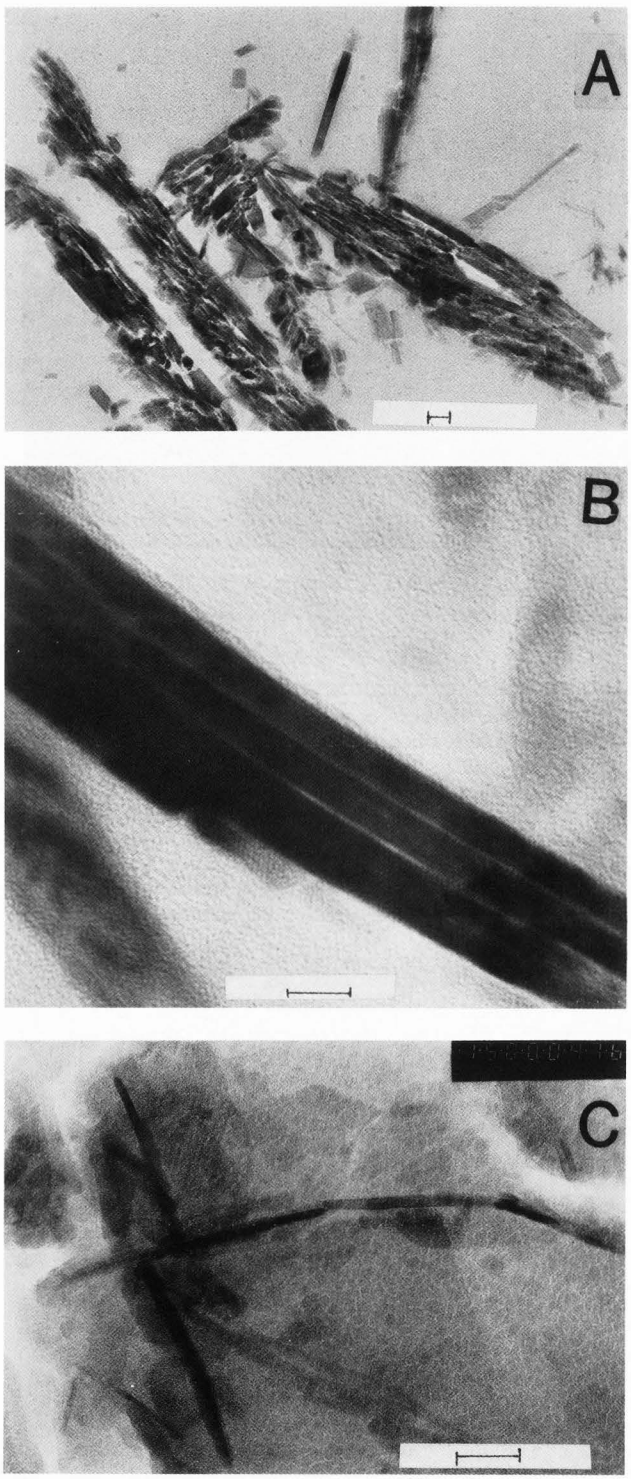

Fig. 8: TEM of apatite crystals obtained by the hydrolysis of OCP, showing side to side (A,B) and end to end (A, C) arrangements. The width of the crystals are about 30 to 50 nm. (bar = 100 nm (A); 40 nm (B); 200 nm (C)).

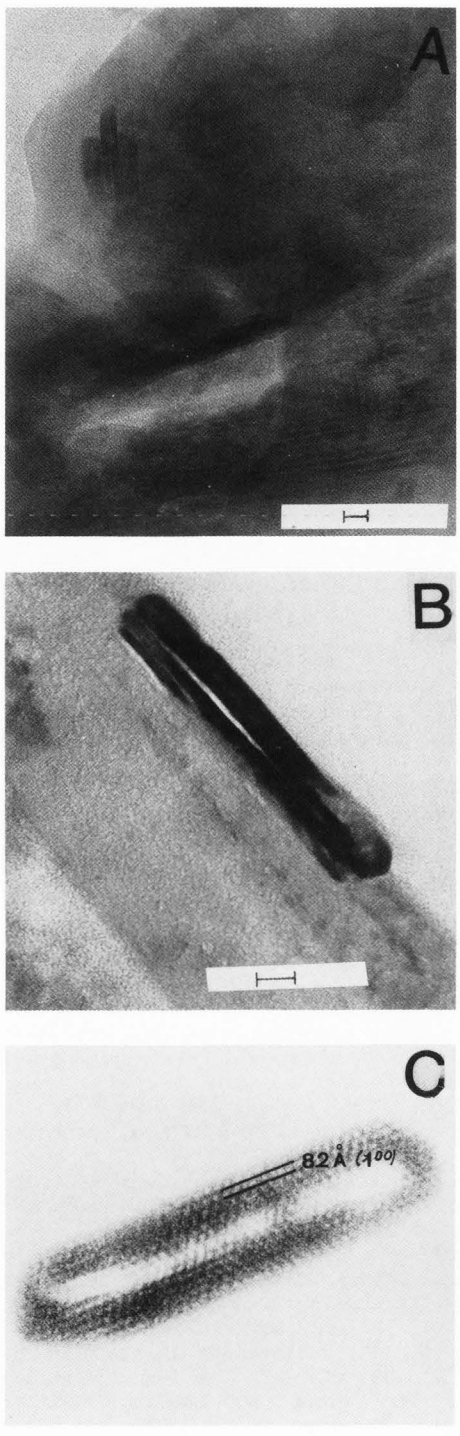

Fig. 9: TEM of apatite crystals obtained by OCP hydrolysis showing central defects and apparent twinning. In (C), the 8.2A spacing represents the (100) plane of the apatite. (bar = 10 nm (A, B), (C), lattice plane = 0.82 nm)).

showed that the apatite crystals resulting from the hydrolysis of OCP were arranged side-to-side like book pages (Figs.8A, 8B) and end to end (Figs. 8A, 8C). Central dark lines (Figs. 9A, 9B) and central defects (Fig. 9C) were observed in these apatites similar to the central features observed in TEM of biological apatites of enamel, dentin and bone [3, 11, 12, 18, 21, 37, 41, 42,

49]. TEM measurements of the apatite crystals showed them to be smaller (30 to 50 nm) than the microcrystals shown by SEM (40 to 70nm) on the surfaces or ends of the OCP macrocrystals which have an average length of about 1μm (Fig.6).

Discussion and Conclusions

This study described the *in vitro* solution-mediated transformations of OCP to apatites *in vitro* when OCP crystals were hydrolyzed in solutions containing CO_3^{2-}, F^-, Ca^{2+}, or HPO_4^{2-} ions (Figs. 1-6); the stability of OCP in acidic solutions, unless the acidic solutions contained F^- (Fig. 2C); and the stability of OCP in solutions containing Mg^{2+}, citrate or pyrophosphate ions. The inhibitory effect of citrate and magnesium on the transformaton of OCP to apatite observed in this study confirmed those reported earlier [4, 6, 30, 38]. The inhibitory effect of Mg^{2+} on the OCP-to-AP transformation was stronger than the promoting effect of Ca^{2+}, when Mg^{2+} and Ca^{2+} ions were simultaneously present. Earlier studies demonstrated the transformation of OCP to CO_3-apatites from solutions containing CO_3^{2-} ions [9, 30] and in the presence of $CaCO_3$ [16]; the latter describing the hydrolysis product as CO_3-substituted OCP or OCPC [16]. Previous preliminary studies have also demonstrated the transformation of OCP to (F,OH)-apatite in F-containing solutions [30].

Brown et al [4] concluded that OCP can form apatite by either of two processes: (i) dissolution of OC followed by direct precipitation of HA, and/or (ii) hydrolysis of OCP *in situ*. In the present study, the transformation of OCP to apatite is believed to be principally by the dissolution of OCP, since the calcium and/or phosphate (PO_4^{3-} and HPO_4^{2-}) ions of the apatite crystals obtained can only be provided by the dissolving OCP crystals. The apatite obtained by the hydrolysis of OCP in this study is *not* stoichiometric apatite (Ca/P = 1.67) referred to as HA, but Ca-deficient apatites, referred to as 'AP', based on the following observations: (a) presence of HPO_4^{2-} absorption bands in the IR spectra of the apatites obtained by the hydrolysis of OCP (Figs. 2B, 2C, 3B); (b) larger a-axis dimensions of the apatite compared to HA (e.g., 0.9443 nm for CO_3-free or F-free AP, compared to 0.9422 nm for HA; (c) Ca/P ratio lower than the stoichiometric value of 1.67 apatite; (d) presence of numerous defects in individual apatite crystals as observed in TEM (Figs. 9A-C) which should not be present in perfect HA crystals. Thus, in this study, the OCP-to-AP transformation appeared to follow the reaction:

$$Ca_8(HPO_4)_2(PO_4)_4.5H_2O \text{ [OCP]}$$

$$\downarrow$$

$$Ca^{2+} + HPO_4^{2-} + PO_4^{3-} + OH^-$$

$$\uparrow$$

$$(Z) = CO_3^{2-},\ HPO_4^{2-},\ \text{or } F^-$$

$$\downarrow$$

$$(Ca_{10-x}Na_x)(HPO_4,CO_3)_x(PO_4)_{6-x}(OH,F)_2$$

[Ca-deficient apatite, AP]

The CO_3-apatites obtained by the hydrolysis of OCP in CO_3-containing solutions at 37 and 60°C gave similar IR spectral characteristics as those obtained by the hydrolysis of dicalcium phosphate dihydrate, DCPD, or brushite, $CaHPO_4.2H_2O$ at similar conditions of temperature and solution composition [29] and by the hydrolysis of monetite, $CaHPO_4$, in similar solutions but at 95-100°C [22, 27]. While it can be argued that DCPD-to-AP transformation can proceed by way of OCP [4,9], e.g., DCPD to OCP to AP, the same cannot be said for the monetite or DCP transformation to apatite, since OCP does not form at 95 to 100°C.

The HPO_4^{2-} incorporated in the apatites formed in solutions containing Ca^{2+} or F^- or phosphate (PO_4^{3-} + HPO_4^{2-}) ions can come from the solution and from the dissolving OCP crystals. The incorporation of F^- in the apatite appear to minimize HPO_4^{2-} incorporation thus allowing for the formation of apatite with higher Ca/P molar ratio [24].

The side-to-side and the end-to-end arrangement of the apatite crystals (Fig.9) may be 'represent OCP crystals that have hydrolyzed by a layer-by-layer mechanism which introduces fissures between the layers' [Brown, private communication]. This type of mechanism would be expected for an *in situ* hydrolysis of OCP [6]. The presence of central defects in the apatites obtained by the hydrolysis of OCP (Fig. 9C) has been observed in biological and synthetic apatites [3, 10, 11, 12, 18, 21, 41, 49].

The apparent stability of OCP in human dental calculi [23, 30, 34, 46] and other pathological calcifications [20, 35, 36] may be due to any or a combination of factors which inhibit transformation of OCP to apatite, namely: low pH, presence of critical levels of Mg^{2+}, pyrophosphate or citrate ions and absence or very low levels of CO_3^{2-}, PO_4^{3-}, Ca^{2+}. Transformation of OCP to apatite in biological systems can proceed either by *in situ* hydrolysis or by dissolution of OCP and precipitation of apatite incorporating ions present in the micro-environment or both, as also previously proposed by Brown et al [4-7]. However, formation of apatites *in vivo* directly or by the hydrolysis of calcium phosphate phases other than OCP (e.g, DCPD) cannot be completely ruled out until further experimental evidence becomes available.

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Discussion with Reviewers

W.E.Brown: The cited structural, chemical and morphological evidence for the role of OCP in the formation of biominerals are in themselves powerful indicators, but there are many other types of evidence which support the OCP-precursor-hydrolysis mechanism [ref.4-7]. But in applying *in vitro* results to the *in vivo* situation, a most important factor is the presence of crystal growth poisons in serum which impede the direct formation of HA from its ions. In this respect, it is unfortunate that none of the electron photomicrographs showed crystals that had been treated with solutions containing Mg^{2+}, citrate or $P_2O_7^{4-}$. These components may have prevented the direct formation of AP, leaving the OCP-*in situ* hydrolysis mechanism as the only effective process for forming AP. A comment on this matter along with the photomicrographs requested above could be helpful to the reader.

Authors: We appreciated the many good suggestions in your review. We (LeGeros and Daculsi) will be following up on one of your suggestions and will be investigating ultrastructurally OCP crystals hydrolyzed in the presence of the ions you mentioned. Our previous studies demonstrated that under conditions which allowed the formation of OCP, the presence of citrate ions caused the formation of poorly crystallized apatite and the presence of pyrophosphate ions caused the formation of amorphous calcium phosphate,ACP [30]; under conditions which allowed the formation of AP (calcium-deficient apatites), the presence of citrate caused a reduction in its crystallinity and the presence of pyrophosphate ions promoted the formation of ACP even at preparation temperature of 95-100C which does not allow the formation of OCP [22,24 and LeGeros etal, 1975, Coll Internat CNRS No. 230: Physico-chimie et Cristallographie des Apatites d'interet Biologique, pp. 105-115]. We agree that in serum, the presence of magnesium, citrate and pyrophosphate ions could interfere with the formation of apatite, either by preventing the hydrolysis of OCP to apatite, preventing the direct formation of apatite, or preventing the formation of OCP in the first place.

W.E.Brown: One of the interesting features of the microcystals is that they do not seem to have epitaxial orientations relative to the larger OCP crystals (Fig.6) even though this might be expected. They look as though they formed in a stirred suspension and attached themselves randomly to the larger crystals. Do the authors agree?

Authors: The hydrolysis experiments were made in unstirred suspensions. While SEM (Fig. 6) did not seem to demonstrate epitaxial orientation between the apatite microcrystals and the OCP macrocrystals, TEM (Fig. 8) demonstrated epitaxial orientation of apatite crystals with each other and apparently with the OCP, i.e., the apatite microcrystals appeared to form in the same space once occupied by the OCP macrocrystal.

G. Hirai: The apparent stability of OCP in human dental calculi may be due to the presence of a critical level of Mg^{2+}. If the ionic concentration of Mg^{2+} is over the critical level, the whitlockite, which is also one of the important constituents of dental calculi, will be produced. Do you have any idea on this problem?

Authors: Mg-substituted whitlockite, b-TCMP, similar to those found in human dental calculi can be obtained *in vitro* either directly by precipitation, or indirectly by the hydrolysis of DCPD (brushite) at 37 or 60°C) or of DCP (monetite) at 95-100C when the Mg/Ca molar ratio in the solution exceeds 0.1 [12, 22, 24, 34; LeGeros (1984),in Tooth Enamel IV, R.W.Fearnhead, S. Suga (eds), Elsevier, pp.32-36; LeGeros etal (1989), Proc. Magnesium Symposium, 1988]. In the present study, we did not observe a transformation of OCP to b-TCMP using similar conditions of temperature and solution composition as those with DCPD-to-b-TCMP or DCP-to-b-TCMP transformations. From these observations, we conclude that the presence of b-TCMP in human dental calculi is largely due to its direct formation and/or hydrolysis of DCPD crystals which form initially. In future studies, we shall try to hydrolyze OCP in solutions containing much higher levels of Mg/Ca than those used in this study.

P-T. Cheng: At pH 7, 37°C, OCP was shown to transform to HAP in the presence of Mg and Ca [Cheng, etal (1988) Magnesium 7: 123-132]. What were the conditions in your experiments where you did not observe transformation?

Authors: The conditions for our experiments in which we did not observe OCP-to-AP transformation in the presence of Mg^{2+} were 60°C, pH 7.5, Mg/Ca in solution, 0.2. Perhaps the difference in our observations is due to the much higher solution phosphate concentration in your system. In our system, for this particular experiment, the only source of phosphate ions would be the dissolving OCP crystals. However, the presence of Mg appeared to inhibit the dissolution of OCP to provide the phosphate ions necessary for the formation of AP.

P-T. Cheng: Do the authors think that the observed 'end-to-end' and 'side-to-side' arrangementments for the apatite crystallites are specific for the preparation process (hydrolysing OCP)? Have they observed similar arrangements in apatite crystallites prepared in other ways, e.g., from ACP? Do TEM micrographs of OCP crystals show micro-domains or other defects before hydrolysis?

Authors: We cannot give answers to your first and second questions because we have not investigated apatite crystals obtained by hydrolysis of ACP. For your third question, Dr. Daculsi (and other people who have tried to do it) observed that OCP crystals are very unstable under the electron beam and therefore it would be difficult to distinguish between beam damage and inherent defects in the OCP crystals before hydrolysis.