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Hydroxyapatite Formation from a Hydrated Calcium Monohydrogen Phosphate Precursor*

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Hydrated calcium monohydrogen phosphate is proposed as the logical precursor in the formation of hydroxyapatite and a unifying theory for the formation of low calcium, or defect apatites, is presented. Structural relationships between calcium monohydrogen phosphate dihydrate and hydroxyapatite indicate that either material can provide the atomic arrangement for the epitaxial growth of one on the other. The formation of apatite is presented as the summation of two rate processes: the initial fast formation of amorphous calcium monohydrogen phosphate dihydrate and the subsequent slow formation of crystalline hydroxyapatite from the initial precipitate. Ca/P ratios of calcium phosphates, formed from compositions in the phase region of hydroxyapatite as a function of time, suggest a varying composition of calcium monohydrogen phosphate dihydrate and hydroxyapatite. Hydrated calcium monohydrogen phosphate is proposed on the basis of rate and composition of calcium phosphate formed and on crystallographic data to be a necessary seed for growth of hydroxyapatite in bone and teeth at physiological pH.

Key words: Calcium—Phosphate—Crystals—Epitaxy—Bone—Composition.

Du phosphate de calcium hydraté monohydrogéné est proposé comme précurseur logique dans la formation de l'hydroxyapatite et une théorie expliquant la formation d'apatites défectueux, à contenu faible en calcium, est présentée. Les rapports structuraux entre le phosphate de calcium déhydraté monohydrogéné et l'hydroxyapatite indiquent que l'un ou l'autre de ces produits est susceptible de fournir l'édifice atomique nécessaire à la croissance épitaxique de l'un sur l'autre. La formation d'apatite parait être le résultat de deux processus différents au point de vue cinétique: la formation initiale rapide du phosphate de calcium dihydraté monohydrogéné amorphe et la formation lente consécutive d'hydroxyapatite du précipité initial. Les rapports Ca/P des phosphates de calcium, formés à partir des constituants de solution situés dans la région de la phase d'hydroxyapatite, en fonction du temps, permettent une composition variable du phosphate de calcium monohydrogéné dihydraté et de l'hydroxyapatite. Le calcium de phosphate hydraté monohydrogéné parait être le cristal d'ensemencement nécessaire à la croissance de l'hydroxyapatite dans l'os et les dents à pH physiologique.

Hydriertes Calciummonohydrogenphosphat wird als die logische Vorstufe bei der Bildung von Hydroxyapatit vorgeschlagen, und eine vereinheitlichende Theorie für die Bildung von calciumarmen oder defizienten Apatiten wird vorgelegt. Strukturelle Beziehungen zwischen Dihydrocalciummonohydrogenphosphat und Hydroxyapatit zeigen, daß beide Substanzen die Atomanordnung für das epitaxiale Wachstum des einen auf dem anderen liefern können. Die Apatitbildung wird als die Summe von zwei Vorgängen verschiedener Geschwindigkeit dargelegt: die zu Beginn rasch vor sich gehende Bildung von amorphem Dihydrocalcium-

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monohydrogenphosphat und die nachfolgende langsame Bildung von kristallinem Hydroxyapatit aus dem anfänglichen Niederschlag. Das Verhältnis als Zeitfunktion von Ca/P des Calciumphosphates, welches sich aus Lösungen bildet, deren Zusammensetzung im Gebiete liegt, wo das Hydroxyapatit in der stabilen Phase vorkommt, läßt vermuten, daß diese Calciumphosphate eine unterschiedliche Zusammensetzung von Dihydrocalciummonohydrogenphosphat und Hydroxyapatit haben. Es wird vorgeschlagen, daß das Dihydrocalciummonohydrogenphosphat auf Grund der Bildungsgeschwindigkeit und Zusammensetzung des Calciumphosphates sowie der kristallographischen Werte ein notwendiger Nukleator für das Wachstum von Hydroxyapatit in Knochen und Zähnen bei einem physiologischen pH ist.

Octacalcium phosphate (OCP), $Ca_4H(PO_4)_3$, has been proposed as the precursor to hydroxyapatite (HA), $Ca_5(PO_4)_3OH$ (Brown *et al.*, 1954). More recently, a number of authors [5, 14, 18] have concluded on the basis of precipitation and nucleation studies that neutral, amorphous tricalcium phosphate (TCP), $Ca_3(PO_4)_2$, is formed first and that this then converts to HA. It is now generally accepted that an amorphous form of some calcium phosphate species is precipitated first during the formation of crystalline HA [13]. Wendt and Clarke (1923) were among the first to suggest that $CaHPO_4$ is an unstable precursor to the formation of a more basic calcium phosphate.

Our purpose in this short paper is to propose the hypothesis that hydrated calcium monohydrogen phosphate is the specific precursor for the formation of HA and to present a unifying theory for the formation of low calcium, or defect, apatites. We will show structural relationships between calcium monohydrogen phosphate dihydrate (DCPD), $CaHPO_4 \cdot 2H_2O$, and HA and also describe some of the rate processes taking place in the formation of HA which account for the variety of Ca/P ratios obtained in the synthesis of HA when DCPD is the precursor of HA.

On two surfaces of DCPD, calcium ions have essentially the same geometrical arrangement as corresponding surfaces in HA. These networks of calcium ions can serve as templates for the epitaxial growth of HA. In Fig. 1 the calcium positions in HA are shown in projection (circles) and two surfaces to be matched with surfaces of DCPD are indicated. One surface is parallel to the (010) crystallographic planes and the other is parallel to the (110) planes. In Figs. 2 and 3 the calcium positions in HA are shown in views perpendicular to the surfaces [(010) and (110), respectively] indicated in Fig. 1. In Figs. 2 and 3, calcium positions in DCPD in the (010) and (110) planes, respectively, are shown by crosses. The (010) networks of HA and DCPD, as shown in Fig. 2, have equal surface densities of calcium ions, one calcium every 32.5 Å^2. The (110) surface of DCPD, shown in Fig. 3, is more densely populated with calcium ions than the (110) surface of HA, but the two differ by only 11% in area per calcium atom, as indicated by the dashed (DCPD) as opposed to the solid (HA) rectangular areas. The calcium positions indicated in Fig. 2 lie within 0.1 Å of the plane of the drawing. In Fig. 3, the calcium positions for DCPD are all within 0.5 Å of the plane of the drawing and the positions for HA are all within 1.1 Å (compare with Fig. 1). As can be seen by comparing the circles and crosses in Figs. 2 and 3, there is a strikingly near correspondence of calcium positions on two surfaces of HA and DCPD. Thus it would seem that DCPD would serve as an excellent template for

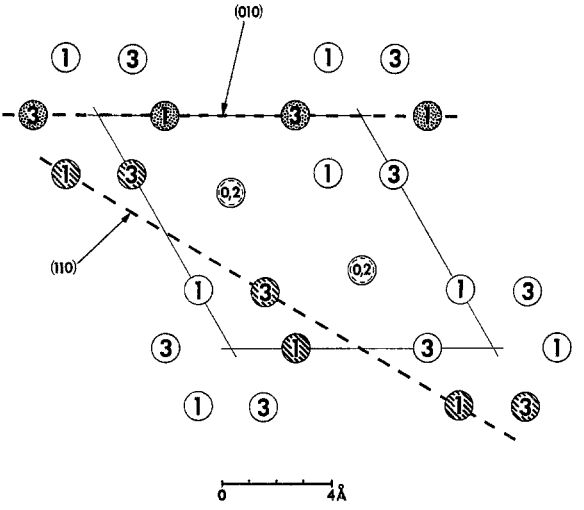

Fig. 1. Ca positions in (HA) as viewed down the c axis. A unit cell is outlined. Levels in the c direction are indicated as 0, 1, 2, or 3 fourths of c. The dashed lines mark possible surfaces parallel to (010) and (110) crystal planes. The dotted and shaded calcium positions are the ones to be considered

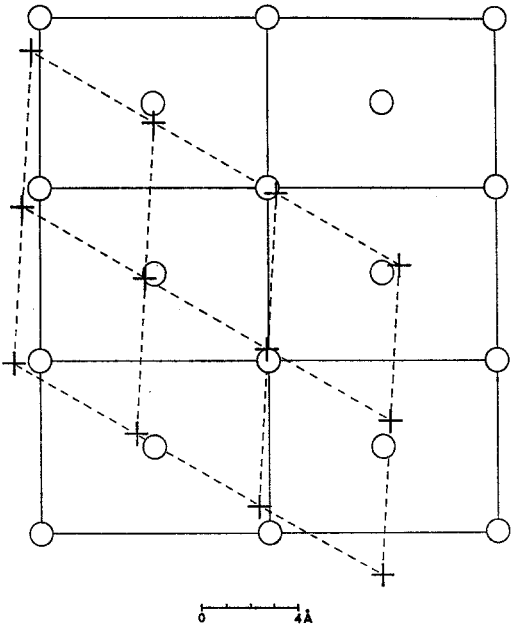

Fig. 2. Circles are Ca positions on the (010) surface of HA (solid line lattice) as shown in Fig. 1 (c is vertical). Crosses are Ca positions on (010) surface of DCPD (dashed line lattice)

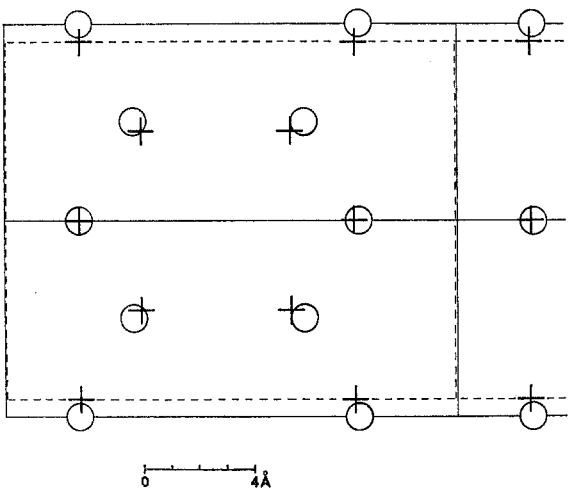

Fig. 3. Circles are Ca positions on the (110) surface of HA (solid line lattice) as shown in Fig. 1 (*c* is vertical). Crosses are Ca positions on (110) surface of DCPD (dashed line lattice)

the growth of HA, since it already has calcium atoms arranged in the same way as they would be arranged in HA. These planes of calcium atoms could easily serve as common boundaries (transition layers) for DCPD and HA. Therefore, depending on the phase required by solution conditions, the phase could change from DCPD to HA, or from HA to DCPD, with little distortion of the crystal structures. Brown *et al.* (1962) discussed the similarities between the OCP and HA structures on a detailed, three-dimensional basis. In an article on epitaxy in urinary calculi and gallstones, Lonsdale [11] pointed out that epitaxial growth of one crystal on another crystal substrate is characterized by a near geometrical fit between the respective crystal networks which are in contact. Structure data were taken from the work of Beevers (1958) for DCPD, and from Kay *et al.* (1964) for HA.

Table. *Calcium phosphate ratios as a function of total reaction time*

Initial mixed concentration (m/l)		pH of reaction	Total reaction time (min)	Molar Ca/P[a] of precipitate	95% Confidence limits
$CaCl_2$	NaH_2PO_4				
1×10^{-2}	1×10^{-2}	7.4	0.50	1.22	1.16–1.30
4×10^{-3}	4×10^{-3}	7.4	14.0	1.33	1.28–1.38
4×10^{-3}	4×10^{-3}	7.4	21.0	1.36	1.31–1.41
4×10^{-3}	4×10^{-3}	7.4	80.0	1.42	1.40–1.44
4×10^{-3}	4×10^{-3}	7.4	410.0	1.46	1.43–1.49

[a] Each ratio is the mean of five separate reactions (see legend Fig. 4) and determinations at each reaction time. Calcium was determined by atomic absorption, phosphorus by the method of Lucena-Conde and Prat [12]. Both precipitates and solutions were analyzed to obtain molar Ca/P of the precipitate.

Experimentally, the rate of formation of HA or DCPD can be followed from base consumption using a pH stat. Under all conditions reported here (Table and Fig. 4) the levels of calcium and phosphate ions added were sufficient to exceed greatly the solubility products of both HA and DCPD. A typical rate curve for the formation of HA is shown by the solid line in Fig. 4. In agreement with other workers, precipitates harvested at approximately 30 sec to 2 min were amorphous by X-ray diffraction standards and were even amorphous by electron diffraction measurements; a precipitate containing small crystallites would appear amorphous. As precipitates were harvested at longer times (further along the solid line curve of Fig. 4), so the crystallinity of the precipitate increased. This has also been shown by Eanes and Posner (1965), who followed the increase in crystallinity of hydroxyapatite by X-ray diffraction. If, in addition to the physical nature of the precipitate formed, the precipitates are analyzed at various points along the time curve, the Ca/P ratio increases as shown in the Table. Since the molar Ca/P ratio of HA is 1.67, of TCP is 1.5, of OCP is 1.33, and of DCPD is 1.0, it is therefore obvious that pure OCP or a mixture of OCP and HA cannot have a Ca/P ratio less than 1.33, whereas DCPD or DCPD and one or more of the other phases mentioned can have a Ca/P ratio of less than 1.33. With our model of DCPD acting as a precursor of HA, a Ca/P ratio of 1.2 at 0.5 min (Table) corresponds to 7 moles of DCPD per 1 mole of HA in the nucleation and formation of DCPD with concurrent nucleation of HA. This could not arise from a mixture of only OCP and HA or from OCP alone. At approximately 14 min, the Ca/P of the precipitate is about 1.33, corresponding to 3.06 moles of DCPD per 1 mole of HA. At a Ca/P ratio of 1.46 at 410 min this would correspond to 1.35 moles of DCPD per 1 mole of HA. Clearly, a ratio of 1.67 corresponds to pure HA. Under slightly different experimental conditions using tris buffer, 0.105 M NaCl, 2×10^{-3} M $CaCl_2$, 1.95×10^{-3} M NaH_2PO_4, pH 7.4, $\mu = .16$, 37°, and 0.22 μ Millipore filter, J. C. Pita (personal communication) has consistently obtained Ca/P ratios in the calcium phosphate precipitate formed of 1.18–1.25 in the first 5 to 30 min after mixing. K. Y. Kim (personal communication) has obtained calcium phosphate precipitates at room temperature which, by infrared and X-ray diffraction, are HA only. However, under identical conditions, he has shown the presence of a weak DCPD peak by X-ray diffraction when short equilibration times were used. Strates *et al.* (1954) using barbital-buffered solutions at 6.9 and 7.4 and $\mu = 0.165$ showed that the Ca/P ratio of the solid was about unity immediately after mixing, but rose rapidly with time to about 1.6; the crystalline phase was HA [15]. In precipitation studies at physiological concentration, Boulet and Marier (1961) also found precipitates formed that were less than 1.3 although they assumed that the lower limit of Ca/P was 1.33 (OCP). They did, however, indicate that there was probably an "association" rather than a simple admixture between the lower and higher Ca/P calcium phosphates formed. Others [9, 14] have shown low Ca/P ratios in the formation of apatite from solutions of calcium and phosphate ions under near physiological conditions. Many authors, we feel, have overlooked the lower ratios of Ca/P because of the length of time required to isolate the precipitate formed from the bulk solution phase, or because of a non-physiological pH (greater than pH 7.6) where the rate of conversion from DCPD to HA was too fast to permit isolation of the low ratio product.

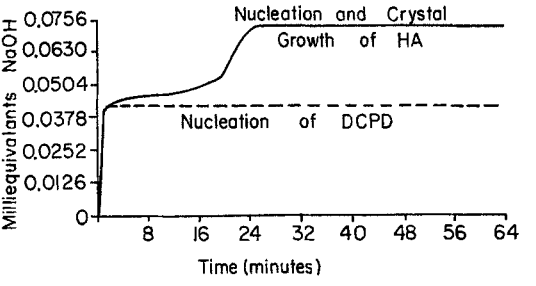

Fig. 4. Consumption of OH^- (NaOH) at constant pH 7.4, 25°, as a function of time when $CaCl_2$ is added to NaH_2PO_4 both preadjusted to pH 7.4, to give an initial mixed concentration of 4.00×10^{-3} M in each at zero time

The simplest way to visualize the formation of HA is that, initially, when homogeneous solutions of calcium and phosphate, each adjusted to pH 7.4, are added together the resultant mixture is supersaturated both with respect to DCPD and to HA. DCPD, however, can form more rapidly than HA; that is, the rate at this point is the controlling factor, and even though DCPD is not the stable phase, DCPD precipitates out immediately. Thus, the first rapid rise in OH^- consumption at constant pH (shown by the dashed curve in Fig. 4) corresponds to the instantaneous nucleation and precipitation of DCPD according to the following reaction:

$$Ca^{2+} + H_2PO_4^- + OH^- \xrightleftharpoons{(H_2O)} \underline{CaHPO_4 \cdot 2H_2O} \downarrow \qquad (1)$$

Simultaneously, HA begins to nucleate heterogeneously and grow on some of the DCPD as already described. As the mass of DCPD, unstable under these solution conditions, converts (by hydrolysis) slowly to HA, some DCPD inevitably becomes buried under HA giving rise to calcium-deficient apatites of a wide range, none of which fit a unique new phase.

Superimposed on the first OH^- consumption (Eq. 1) is the OH^- required for nucleation and crystal growth of HA from DCPD which can be expressed by the following reaction.

$$5CaHPO_4 \cdot 2H_2O + 2OH^- \rightleftharpoons \underline{Ca_5(PO_4)_3OH} \downarrow + 2H_2PO_4^- + 11H_2O \qquad (2)$$

The solid line curve in Fig. 4 shows the summation of OH^- consumption for both reactions (Eqs. 1 and 2). Attempts are currently in progress to identify mathematically and experimentally each of the three separate reaction rates—formation of DCPD, hydrolysis of DCPD, and formation of HA.

The crystallographic arguments for the formation of HA from a DCPD precursor and the chemical evidence in the form of Ca/P ratios which support DCPD as a precursor have been presented. Conception of DCPD as a precursor for HA provides a rationale for the continuously increasing Ca/P ratios that can be obtained. These depend upon the conditions of formation chosen. An explanation why HA is usually the only phase detectable by available physical methods is also provided. Another way of visualizing the amorphous formation of DCPD

is that HA is nucleated on the microcrystallites of DCPD. The apatite, which is stable under these solution conditions, acts as a crystal growth inhibitor through epitaxial covering of the surfaces of the DCPD, while the HA crystals continue to grow in their preferred phase region. On the other hand, the DCPD, which is not covered by HA is unstable under these solution conditions and slowly hydrolyzes with the preferential formation of apatite. It is interesting that live bone contains amorphous calcium phosphate, the greatest amount being found in the growth plate of long bones [16].

Additional evidence for the interrelationship of DCPD and HA has been presented previously by Francis (1965) and by Francis *et al.* (1968) in which the formation of DCPD on hydroxyapatite has been shown by thermodynamic and physical measurements under conditions in which DCPD is the preferred phase and where HA provides the seed for the formation of DCPD on the HA. In studies of the rate of formation of DCPD, G. H. Nancollas (personal communication) has shown that no change occurs in the rate of formation of DCPD under solution conditions where DCPD is the stable phase, regardless of whether HA or DCPD crystals are used as the seeding medium. D. R. Simpson (personal communication) has found continuously-varying amounts of water present in HA formed at low temperature, depending upon the method of formation. Incorporation of varying amounts of DCPD which contains water of crystallization could account for the varying water content seen by Simpson in HA preparations.

We propose that calcium hydroxyapatite formed *in vitro* and also in bone and teeth, is formed from a rapidly precipitated, amorphous, hydrated calcium monohydrogen phosphate (probably DCPD). Small crystallites of DCPD (amorphous to X-ray) then serve as templates or nuclei for the slower growth of HA since on certain exposed surfaces of DCPD calcium ions are already in the same arrangement as they are in HA. The DCPD seeds are then either dissolved or are incorporated into HA crystals. Incorporation would account for various amounts of H_2O and the wide variations in Ca/P ratios found in synthesized HA. Since HA can serve as an epitaxial template for DCPD, solution or *in vivo* changes could bring about the growth of DCPD on HA and vice versa; fluctuations of the solution composition could easily produce sandwich-like crystallites of DCPD-HA-DCPD-HA.

References

1. Beevers, C. A.: The crystal structure of dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$. Acta Cryst. **11**, 273–277 (1958).
2. Boulet, M., Marier, J. R.: Precipitation of calcium phosphates from solutions at near physiological concentrations. Arch. Biochem. **93**, 157–165 (1961).
3. Brown, W. E., Lehr, J. R., Smith, J. P., Frazier, A. W.: Crystallography of octacalcium phosphate. J. Amer. chem. Soc. **79**, 5318–19 (1957).
4. — Smith, J. P., Lehr, J. R., Frazier, A. W.: Crystallographic and chemical relations between octacalcium phosphate and hydroxyapatite. Nature (Lond.) **196**, 1050–55 (1962).
5. Eanes, E. D., Gillessen, I. H., Posner, A. S.: Intermediate states in the precipitation of hydroxyapatite. Nature (Lond.) **208**, 365–67 (1965).
6. — Posner, A. S.: Kinetics and mechanism of conversion of noncrystalline calcium phosphate to crystalline hydroxyapatite. Trans. N.Y. Acad. Sci. **28**, 233–241 (1965).
7. Francis, M. D.: Solubility behavior of dental enamel and other calcium phosphates. Ann. N.Y. Acad. Sci. **131**, 694–712 (1965).

8. Francis, M. D., Gray, J. A., Griebstein, W. J.: The formation and influence of surface phases on calcium phosphate solids. Advanc. Oral Biol. **3**, 83–120 (1968).
9. Furedi-Milhofer, H., Purgaric, B., Brecevic, Lj., Pavkovic, N., Objica, E.: Nucleation of calcium phosphate from solutions at physiological pH. Croat. Chem. Acta **41**, 37–42 (1969).
10. Kay, M. I., Young, R. A., Posner, A. S.: Crystal structure of hydroxyapatite. Nature (Lond.) **204**, 1050–52 (1964).
11. Lonsdale, K.: Epitaxy as a growth factor in urinary calculi and gallstones. Nature (Lond.) **217**, 56–58 (1968).
12. Lucena-Conde, F., Prat, L.: A new reagent for the colorimetric and spectrophotometric determination of phosphorus, arsenic, and germanium. Anal. Chim. Acta **16**, 473–479 (1957).
13. Posner, A. S.: Crystal chemistry of bone mineral. Physiol. Rev. **49**, 760–92 (1969).
14. Schiffman, E., Lavender, D. R., Miller, E. J., Corcoran, B. A.: Amino acids at the nucleating site in mineralizing elastic tissue. Calc. Tiss. Res. **3**, 125–135 (1969).
15. Strates, B. S., Neuman, W. F., Levinskas, J.: The solubility of bone mineral. II. Precipitation of near-neutral solutions of calcium and phosphate. J. Phys. Chim. **61**, 279–282 (1957).
16. Termine, J.D., Wuthier, R.E., Posner, A.S.: Amorphous-crystalline mineral changes during endochondral and periosteal bone formation. Proc. Soc. exp. Biol. (N.Y.) **125**, 4–9 (1967).
17. Walton, A. G., Bodin, W. J., Furedi, H., Schwartz, A.: Nucleation of calcium phosphate from solution. Canad. J. Chem. **45**, 2695–2701 (1967).
18. Weber, J. C., Eanes, E. D., Gerdes, R. J.: Electron microscope study of noncrystalline calcium phosphate. Arch. Biochem. **120**, 723–24 (1967).
19. Wendt, G. L., Clarke, A. H.: An electrometric study of the neutralization of phosphoric acid by calcium hydroxide. J. Amer. chem. Soc. **45**, 881—887 (1923).