Calcif Tissue Int (1983) 35:783–790

Calcified Tissue International

An Intermediate State in Hydrolysis of Amorphous Calcium Phosphate

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Summary: The hydrolysis of previously prepared amorphous calcium phosphate (ACP) was studied in a solution "saturated" with ACP; this eliminated the initial consumption of acid due to ACP dissolution. The procedure established that conversion of a high-concentration ACP slurry to an apatite involves two processes: the first process consumes acid and indicates the formation of a more acidic calcium phosphate intermediary with the solubility of octacalcium phosphate (OCP); the second process consumes base and indicates the conversion of the intermediary to apatite and, possibly, direct conversion of ACP to apatite. The thermodynamic analysis of the solution composition data suggests that ACP converts into a nonstoichiometric apatite when the OCP-like intermediary is formed, and a stoichiometric apatite is formed when no OCP-like intermediary is involved.

Key Words: Amorphous calcium phosphate — Apatite — Calcification — Hydrolysis — Octacalcium phosphate.

The initial solid phase that precipitates from a calcium phosphate solution depends on the degree of its supersaturation [1]. In a solution of low supersaturation, hydroxyapatite (OHAp), $Ca_{10}(PO_4)_6(OH)_2$, with Ca/P ratio of 1.67 is obtained without precursor phases [1, 2]. On the other hand, the first solid to form in highly supersaturated solutions is a noncrystalline amorphous calcium phosphate (ACP), approximating $Ca_9(PO_4)_6$ in composition [2]. In a study of the transformation and crystal ripening of ACP that had been precipitated in highly supersaturated and unbuffered media, initially adjusted to pH 7.4, Furedi-Milhofer et al. [3] demonstrated that crystalline precipitates were formed by a two-step precipitation via (1) ACP and (2) a precursor, octacalcium phosphate (OCP), $Ca_8(PO_4)_6H_2 \cdot 5H_2O$, as proposed by Brown et al. [4]. Eanes and Meyer [5–7] further showed that spontaneously precipitated ACP maintained at pH = 7.4 was converted to an OCP-like crystalline phase, which subsequently hydrolyzed into apatite, with unusual inflections in the calcium and phosphate concentrations and the base consumption profiles. Similar inflections have been observed by Nancollas et al. [1] in the growth of calcium phosphate crystals at high supersaturation; these were attributed to the superpositioning of a number of kinetic processes which take place at different rates and with the involvement of more than one calcium phosphate phase in the formation of OHAp. They further showed, through dissolution experiments [1, 8] and the constant composition method [9], that OCP is one of the unstable precursor phases.

The transformation of previously prepared ACP to OHAp in aqueous medium has been described as an autocatalytic conversion process [10, 11]. The dependence of this conversion process on the solution environment has been extensively studied [12, 13]. In all the latter studies [10–13], the conversion of ACP is considered as a single process without an intermediary. The present work, therefore, was undertaken to study the nature of the hydrolysis processes in previously prepared ACP, with emphasis on the formation and effects of an OCP-like intermediary. The results indicate that ACP in a high slurry concentration converts to a more acidic calcium phosphate intermediary with the solubility of OCP, which subsequently hydrolyzes to OHAp.

A method, described here, was developed to study the kinetics of these two processes separately. A thermodynamic analysis was made on the solution-phase data to determine if the solution phase was in quasi-equilibrium with one of the solid phases. The analysis suggests that formation of nonstoichioimetric OHAp is due to the formation of an

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OCP-like intermediary as proposed by us [14]; this mechanism has biological relevance since nonstoichiometric OHAp has been considered as a prototype compound for bone apatite, and there is evidence that OCP [15, 16] may be a precursor in hard tissue formation.

Materials and Methods

Reagent grade materials were used in all experiments. All solutions were prepared with distilled water. The ACP used in this study was prepared by adding 225 ml of 0.335 M $Ca(NO_3)_2$ to 650 ml of vigorously stirring 0.145 M $(NH_4)_2HPO_4$ solution at pH = 9.9. Immediately after the precipitation, the solid was vacuum filtered through a large filter paper (diameter = 25 cm) in 15 min. The precipitates were first thoroughly washed with pH 10 ammonium hydroxide to remove all unreacted and excess ions and then with acetone to remove the water. The samples were lyophilized and stored in a vacuum dessicator.

Hydrolysis of ACP was carried out, with and without maintaining constant pH, by adding ACP powder to a calcium phosphate solution previously "saturated" with respect to ACP, that is, there would be no additional dissolution when the ACP was added to the solution unless a new phase precipitated. Under this system, when ACP converts to a more acidic calcium phosphate, such as OCP or dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD), OH^- and Ca^{2+} ions are released to the solution; when ACP, OCP, or DCPD converts to OHAp, OH^- and Ca^{2+} ions are removed from solution and are incorporated into the apatite phase. Therefore the change of the pH in solution as function of time, or the consumption of acid or base in a pH stat, will give the information about the new phase that is formed. The solutions which are "saturated" with respect to ACP have been reported in two systems: (1) in the dissolution of previously prepared ACP, Blumenthal et al. [12] reported that the maximum calcium and phosphate concentrations at pH 7.4 and 30°C were 1.5 mM and 1 mM, respectively; and (2) in the precipitation of ACP from highly supersaturated solution, Meyer and Eanes [6] reported a solubility product ($((Ca^{2+})^3(PO_4^{3-})^2)$ of $10^{-25.23}$ for ACP at 37°C. Conversion of ACP was studied by us in both of these solutions, and the results were similar. Since we were studying the conversion of previously prepared ACP, we used the former solution (1.5 mM calcium nitrate and 1 mM potassium hydrogen phosphate pH = 7.4) in most of our experiments and refer to it as "saturated calcium phosphate" solution in this report.

A pH stat (Radiometer pH meter 25, Titrator II, Titragraph type SBR 2C) with two syringe burettes (type SBU 1a) was used to maintain a constant pH of 7.4; one titrated 0.1 N HCl and the other titrated 0.1 M KOH. Consumption of acid gives information about the conversion kinetics of ACP to a more acidic calcium phosphate intermediary, and that of base gives information about conversion of this intermediary to OHAp as well as direct conversion of ACP to OHAp. The reactions were carried out under ambient atmosphere at 25°C unless specified otherwise.

Portions of the reaction slurry were withdrawn at regular intervals during the course of ACP conversion and filtered immediately through a Swinnex-13 filter unit (Millipore Corp., Bedford, MA) equipped with 0.22 μm GS filters. The filtrates were analyzed for calcium and phosphate, and the solid by chemical and X-ray analysis. Calcium concentrations were determined by the UV spectrophotometric method using Arsenazo reagent [17]. A miniature calcium electrode (product code 011-020-11S, Ionetics Inc., Costa Mesa, CA), which has a sensitivity of 1% at 1.5 mM range, was used to monitor the calcium concentration in solution continuously during the course of the experiments. Total phosphate was determined by the vanado-molybdate spectrophotometric method [18] or malachite green molybdate reagent of Hess and Derr [19]. The X-ray powder diffraction patterns of the solids were obtained with nickel filtered CuK_α radiation.

Two methods were used to calculate the thermodynamic properties of the solutions from data obtained here and reported in the literature [13].

(1) The degrees to which the various solutions were saturated with respect to different pure calcium phosphate crystals were computed using the Gibbs energy expressions as follows:

$$\Delta G = -\frac{RT}{N} \ln \frac{IP}{K_{sp}}$$

where R is the ideal gas constant, T is the absolute temperature, N is the number of ions in the ion product (IP), K_{sp} is the thermodynamic solubility product. The values of K_{sp} used for DCPD [20], β-tricalcium phosphate ($Ca_3(PO_4)_2$, β-TCP) [21], OCP [22, 23], and OHAp [24] were respectively 2.51×10^{-7}, 1.23×10^{-29}, 8.25×10^{-48}, 2.79×10^{-59} at 25°C; and 2.45×10^{-7}, 0.77×10^{-29}, 5.0×10^{-48}, 2.58×10^{-59} at 30°C. A negative, zero, or positive value of Gibbs free energy signifies that the solution is supersaturated, in equilibrium, or undersaturated with respect to the indicated solid phase, respectively. The ionic products for DCPD, OCP, β-TCP, and OHAp were calculated as follows:

$$IP(DCPD) = (Ca^{2+})(H^+)(PO_4^{3-})$$
$$IP(OCP) = (Ca^{2+})^4(H^+)(PO_4^{3-})^3$$
$$IP(\beta\text{-TCP}) = (Ca^{2+})^3(PO_4^{3-})^2$$
$$IP(OHAp) = (Ca^{2+})^5(PO_4^{3-})^3(OH^-)$$

in which parentheses indicate the ionic activities.
Calcium and phosphate ion activities were calculated from the total concentration by correcting ion pairs and activity coefficients. The activity coefficients were estimated by the extended Debye-Huckel equations [25].

(2) The solid phase in equilibrium with the solution was obtained through the use of the "chemical potential" plot [26,27], $-\log Ca^{2+}(OH^-)^2$ vs. $-\log(H^+)^3(PO_4^{3-})$; this plot can be derived from the expressions defining the solubility products used in the above method. The main advantage of this graphical approach is that the stoichiometry of the solid phases in equilibrium with the solution can be obtained from the slope of the straight line in the plot of the solution phase data without any assumption regarding its stoichiometry.

Results

The "saturated calcium phosphate" solution itself at 25°C, pH = 7.4, was metastable with no change in its pH for 2 h. (In 72 h, the pH decreased from 7.4–7.16 and the Ca and PO_4 concentrations decreased by 9%.) When 64.2 mg of ACP was added to 15 ml of this solution, the pH of the slurry increased from 7.4–7.87 in the first 45 min and then decreased; the calcium concentration in the solution, monitored continuously by a calcium electrode, increased about 5% in the first 2 min and then decreased; the phosphate concentration decreased

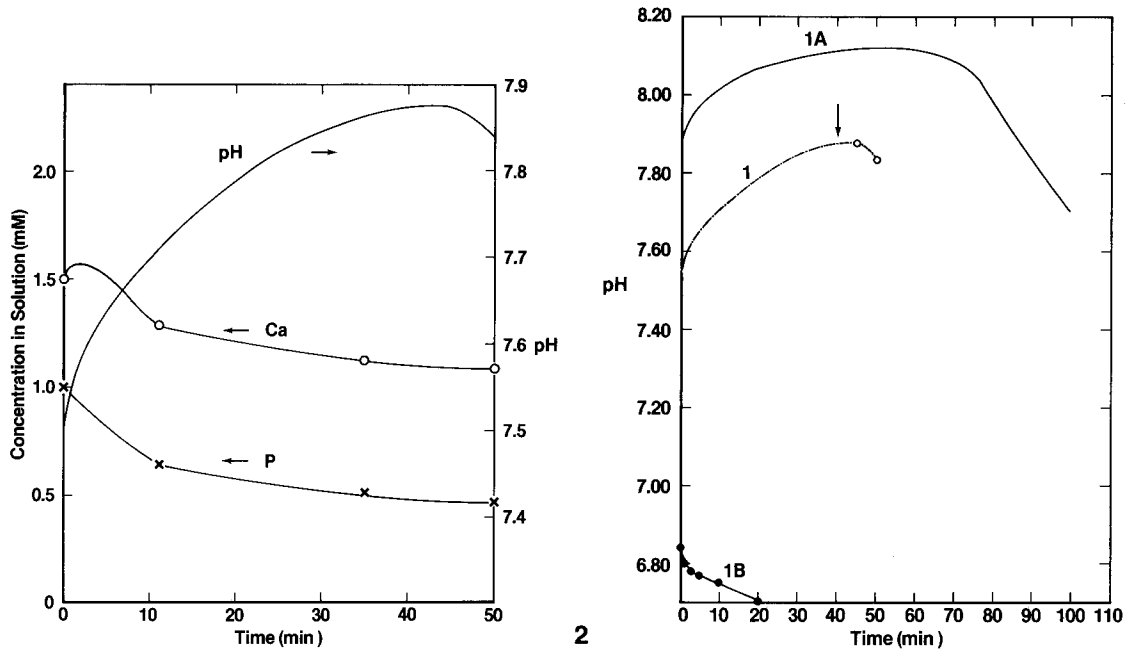


Fig. 1. Variation with time of the pH, calcium, and phosphate concentrations in solution when 64.2 mg ACP was added to 15 ml "saturated calcium phosphate" solution (run 1). The curve for calcium concentration was obtained from the calcium electrode measurements, the points from chemical analysis. **Fig. 2.** The pH of the solution vs. time. Curve 1 (run 1): 64.2 mg ACP added to 15 ml "saturated calcium phosphate" solution (*arrow* indicates the time slurry was centrifuged). Curve *1A* (run 1A): fresh 64.2 mg ACP added to the above supernatant. Curve *1B* (run 1B): fresh "saturated calcium phosphate" solution added to the filtered solid in run 1.

initially, as shown in Fig. 1, and then increased after 2.5 h. The increases in pH and calcium concentration indicate that ACP converted to a more acidic calcium concentration intermediary and released OH^- and Ca^{2+} ions into the solution. The initial decrease in phosphate and the decrease in calcium after 2 min indicate that precipitation of a calcium phosphate was induced by the addition of ACP. These decreases also rule out the possibility that the increase in solution pH was due to the dissolution of ACP. The eventual decrease of pH after 45 min indicates that conversion of the intermediary, and possibly ACP to apatite, took place. A solution containing higher calcium (2.57 mM) and phosphate (1.71 mM) concentrations, under N_2 atmosphere pH 7.4 and 37°C, was also used; the activity product of this solution was reported to be the "solubility" of ACP [6]. This solution was not stable and its pH decreased from 7.4–6.4 in 1 h under N_2 atmosphere and 37°C. When the 160 mg of ACP was added to 25 ml of this solution, the pH of solution increased to a maximum of 7.65 in 6 min and then decreased; this was similar to the results shown in Fig. 1 and indicates that conversion of ACP to a more acidic calcium phosphate intermediary occurred at this temperature also.

The initial increase in pH of the "saturated calcium phosphate" solution upon addition of ACP is not due to dissolution of ACP, but instead is due to formation of an acidic intermediary. This was demonstrated as follows: ACP slurry (64.2 mg ACP in 15 ml "saturated calcium phosphate" solution) was centrifuged and filtered after 40 min reaction when the pH of the slurry approached the maximum indicated by the arrow on curve 1 (run 1), Fig. 2; fresh ACP solid was then added to the supernatant and fresh "saturated calcium phosphate" solution was added to the filtered solid. If the increase in pH had been due to dissolution of ACP, then the pH in the former slurry should not have increased because the solution was already "saturated" with ACP, and the pH in the latter slurry should have increased because of further dissolution of ACP in the fresh solution. The results, shown in Fig. 2, however, indicate the opposite. Curve 1A (run 1A) shows the change in pH of the slurry after the fresh ACP was added to the supernatant; the pH of this slurry increased with time in the first 55 min. This indicates further release of OH^- from the fresh ACP added to the supernatant, and that this ACP converted to a more acidic calcium phosphate intermediary. Curve 1B shows the changes in pH of the slurry when fresh "saturated calcium phosphate" solution was added to the filtered solid (there was a time lag between addition of fresh solution and filtration of the solid); the pH of this new slurry decreased. This

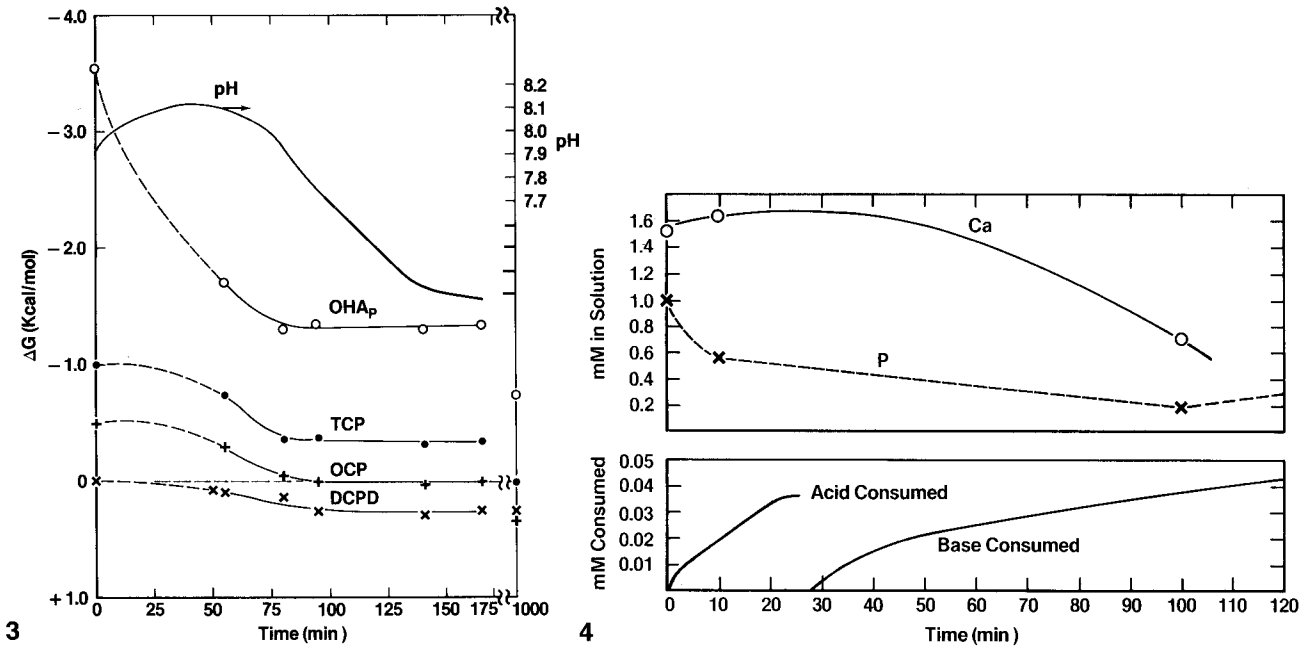


Fig. 3. Time variation in the degree of saturation, ΔG, of run 1A solution at 25°C with respect to the calcium phosphate phases *OHAp* (○), *TCP* (●), *OCP* (+), and *DCPD* (x). The horizontal dashed line at ΔG = 0 is the position at which the solution is saturated with respect to the solid phase. Region above this line represents supersaturation; below this line undersaturation. (1 kcal = 4.184 kjoules.)
Fig. 4. The lower diagram gives the amount of acid and base needed to maintain constant pH at 7.4, 25°C as function of time when 167 mg ACP was added to 25 ml "saturated calcium phosphate" solution. The upper diagram gives the variation of calcium (*Ca*) and phosphate (*P*) concentrations in solution with time during the transformation period. The curve for calcium concentration was obtained from the calcium electrode measurements, the points from chemical analysis.

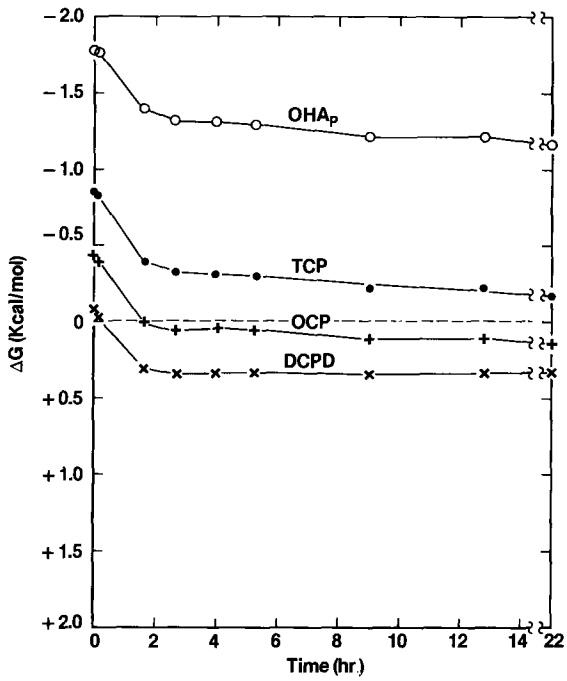
indicates that the previously formed intermediary in the filtered solid converted to apatite and consumed OH^- ions.

The degrees of saturation of the solution in run 1A with respect to pure crystalline OHAp, β-TCP, OCP, and DCPD, as expressed by ΔG, are shown as functions of time in Fig. 3. In the first 3 h, the solution was always supersaturated with respect to OHAp and β-TCP. The solution appeared to be in equilibrium with DCPD at zero time before the ACP was added, and was undersaturated with respect to DCPD after 50 min, making it highly improbable that DCPD could have been the acidic calcium phosphate intermediate phase under the studied condition. Most interestingly, the ΔG curve indicates that the solution was supersaturated with respect to OCP initially, but became saturated with OCP after about 80 min and remained so for at least another 80 min. This shows that the calcium phosphate intermediary which determined the solution properties had the solubility of OCP. We refer to this intermediary as "OCP-like" because, as is often the case for OCP-like materials, the 100 and 200 lines were not present in the X-ray diffraction pattern [5].

The conversion kinetics of 167 mg ACP in 25 ml "saturated calcium phosphate" solution at a constant pH of 7.4 (run 2) is shown in Fig. 4. Clearly, there were two processes: the first one consumed acid and the second one consumed base. In the first process the calcium concentration, monitored continuously by a calcium electrode, increased about 10% and reached its maximum when the consumption of acid reached its maximum, implying that the Ca^{2+} and OH^- ions were released simultaneously from the ACP in the first process. This is in accord with conversion of ACP to an OCP-like intermediary and can be tentatively expressed as follows:

$$Ca_9(PO_4)_6(\text{amorphous}) + 7H_2O \rightarrow Ca_8H_2(PO_4)_6 \cdot 5H_2O(\text{crystal}) + Ca^{2+} + 2OH^- \quad (1)$$

In the first process, the concentration of phosphate decreased; this again rules out the possibility that the increase of Ca^{2+} and OH^- ions was due to the simple dissolution of ACP, and indicates that precipitation from the solution was induced by the addition of ACP. In the second process, which is indicated by the consumption of base, the calcium concentration decreased and phosphate eventually



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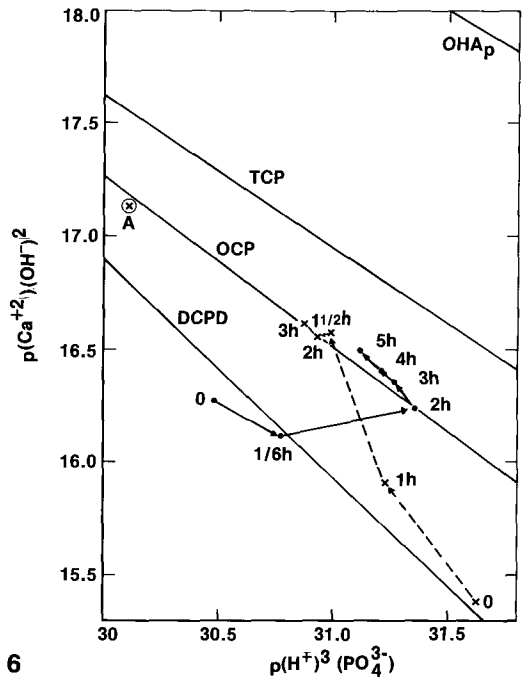

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Fig. 5. Time variation in the degree of saturation, ΔG, of the solution in ACP transformation at 25°C and maintaining constant pH of 7.4 (run 2) with respect to the calcium phosphate phases *OHAp*, *β-TCP*, *OCP*, and *DCPD*. (1 kcal = 4.184 kjoules.) **Fig. 6.** Chemical potential plot of $p(Ca^{2+})(OH^-)^2$ vs. $p(H^+)^3(PO_4^{3-})$ for ACP transformation; the lines for solution in equilibrium with *OHAp*, *β-TCP*, *OCP*, and *DCPD* are shown. The arrows drawn with the broken lines indicate movements of the solution's chemical potential with time in the transformation of the ACP without maintaining constant pH (run 1A); the numbers indicate times of reaction; point A indicates the solution of run 1 after 100 min. The *solid arrows* indicate movements of the chemical potential with time in pH stat study at 25°C and pH = 7.4 (run 2).

increased after 100 min. This indicates that the OCP-like intermediary (eq. 2), and possibly ACP (eq. 3), converted to an apatite approximately as follows:

$$Ca_8H_2(PO_4)_6 \cdot 5H_2O + (2 - x)Ca(OH)_2 \rightarrow Ca_{10-x}H_{(2x-y)}(PO_4)_6(OH)_{(2-y)} \cdot nH_2O + (7 + y - n - 2x)H_2O \quad (2)$$

where $0 \leq x \leq 2$ and $0 \leq y \leq 2$.

$$Ca_9(PO_4)_6(\text{amorphous}) + (1 - x)Ca(OH)_2 + (2x - y + n)H_2O \rightarrow Ca_{10-x}H_{(2x-y)}(PO_4)_6(OH)_{(2-y)} \cdot nH_2O \quad (3)$$

where $0 \leq x \leq 1$ and $0 \leq y \leq 2$.

The reaction was stopped after 24 h, and the final product was nonstoichiometric, Ca/P = 1.56, and apatitic, as indicated by chemical analysis and X-ray diffraction, respectively. The degree of saturation of the solution with respect to the calcium phosphate phases OHAp, β-TCP, OCP, and DCPD in the constant pH study (run 2) is shown in Fig. 5. The solution was supersaturated with respect to OHAp and β-TCP in the transformation period and was undersaturated with respect to DCPD except in the first 10 min. The solution was slightly supersaturated with respect to pure crystalline OCP in the first 2 h, when ACP converted to the intermediary; it became slightly undersaturated with respect to OCP and moved approximately parallel to the OCP line after 2 h. This again implies that the calcium phosphate intermediary which determined the solution properties had solubility approximately that of OCP.

Figure 6 is a chemical potential plot, $p(Ca^{2+})(OH^-)^2$ vs. $p(H^+)^3(PO_4^{3-})$; the lines for solutions saturated with OHAp, β-TCP, OCP, and DCPD are shown and the numbers on the points indicate the times of reaction. The arrows drawn with the broken lines indicate changes of the solution's chemical potentials in the transformation of ACP without maintaining constant pH (run 1A); at zero time the point is below the OCP, β-TCP, and OHAp lines and slightly above the DCPD line, indicating that the solution was supersaturated with respect to OCP, β-TCP, and OHAp, and undersaturated with respect to DCPD. As the transformation proceeded, the solution changed to and along the OCP line, indicating that the solution was nearly saturated with respect to OCP from 1.5 h to 3 h. Point A, the solution of run 1 after 100 min, is also near the OCP line. The solid arrows indicate changes of the solution's

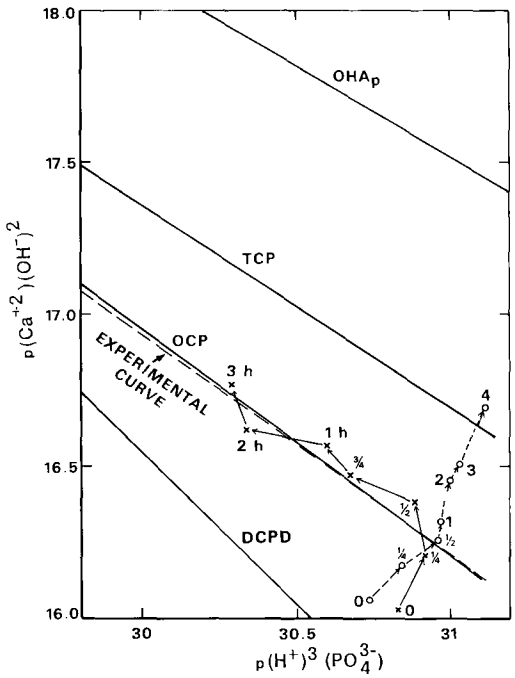

Fig. 7. Chemical potential plot of $p(Ca^{2+})(OH^-)^2$ vs. $p(H^+)^3(PO_4^{3-})$ for ACP transformation reported by Blumenthal et al. [13] at constant pH = 7.4, 30°C. The numbers indicate the times of reaction in the transformation. The *arrows* drawn with the broken lines indicate movements of the solution's chemical potential with time in the low slurry concentration and the *solid arrows* indicate those movements in the high slurry concentration. The experimental curve, indicated by the broken line, is the least squares fit for the solution in high slurry transformation.

chemical potentials in the transformation of ACP maintained at pH = 7.4 (run 2); at zero time the solution was supersaturated with respect to DCPD, OCP, β-TCP, and OHAp; as the transformation proceeded, the solution changed along the saturation line of OCP with slight apparent undersaturation in the period of 3 to 5 h.

Discussion

Our new procedure for studying the conversion of previously prepared ACP in "saturated calcium phosphate" solution confirms that the conversion involves two processes: the formation of a more acidic calcium phosphate intermediary which subsequently converts to apatite. This procedure is also useful in studying the kinetics of these two processes separately and the effects of inhibitors and accelerators of biomineralization on these two processes. A recent report of Williams and Sallis [27] indicates that inhibitors for ACP to OHAp transformation can be grouped into two classes: one class acts mainly as a hydroxyapatite crystal growth inhibitor and the other acts mainly by decreasing the lability of ACP. We believe that these inhibitors may have different effects on the rate of ACP to OCP-like conversion and the rate of OCP-like to OHAp conversion; these effects cause the differences in base consumption, calcium ion depletion, and turbidity.

It has been reported previously that in the transformation of spontaneously precipitated ACP to OHAp, the first crystalline material to form has a solubility similar to OCP [5–7]. It is, therefore, extremely unlikely that the above results indicating that the ACP slurry is in quasi-equilibrium with OCP during the transformation period is a coincidence.

Blumenthal et al. [13] studied the transformation of ACP to OHAp in different slurry concentrations. A thermodynamic analysis of their data made by us is shown in the form of a chemical potential plot (Fig. 7). The equilibrium lines for OHAp, β-TCP, OCP, and DCPD are shown, and the numbers on the points indicate the times of reaction. The arrows drawn with the broken lines indicate changes of the chemical potentials in the transformation of low ACP slurry concentration (50 mg ACP/120 ml) from zero time to 4 h; at zero time the point is below the OCP line, where the solution is supersaturated with respect to OCP. As the transformation proceeded, the chemical potential values changed to above the OCP line in 30 min, where the solution is undersaturated with respect to OCP. The solid arrows indicate changes of the chemical potential values in the high slurry concentration (800 mg ACP/120 ml); at zero time the solution was supersaturated with respect to OCP; as the transformation proceeded, the chemical potentials of the solution changed along the saturation line of OCP indicating the solution was in equilibrium with OCP. The broken line, indicated as the "experimental curve," is the least square fit for the data from 1/4 to 3 h in the high slurry concentration; the slope is −0.725, representing a Ca/P ratio of 8.26/6 for the solid in equilibrium with solution as compared with 8/6 for OCP. The results clearly show that in low ACP slurry concentration the solution was undersaturated with respect to OCP after 30 min, and in high ACP slurry concentration the solution was in quasi-equilibrium with OCP during much of the transformation period.

They further reported that the Ca/P ratios of the final OHAp products depended on the ACP slurry concentration; the low concentration ACP slurry converted to a nearly stoichiometric OHAp with Ca/P ratio of 1.64, and the high concentration ACP slurry converted to a nonstoichiometric apatite with Ca/P ratio of 1.52. The above thermodynamic anal-

ysis of their data therefore indicates that in the transformation of low concentration ACP slurry, the solution was undersaturated with respect to OCP and, hence, there was no OCP-like intermediary except in the first 30 min; the product was nearly stoichiometric OHAp. On the other hand, in high concentration ACP slurry, the solution was in quasi-equilibrium with a material having the solubility of OCP during much of the conversion period, revealing the formation of an OCP-like intermediary; this intermediary then hydrolyzed to a nonstoichiometric apatite.

Based on this analysis, published results [1, 2, 28], and structural considerations, we propose that a stoichiometric OHAp is formed when there is no OCP-like intermediate phase, and a nonstoichiometric apatitic product is formed when an OCP-like intermediate phase occurs. At constant pH, the formation of the OCP-like phase depends on the ion product of the calcium and phosphate in solution. In a highly concentrated ACP slurry with high solid to liquid ratio, the surface area available for dissolution is large, and therefore the dissolution rate is fast, and the calcium phosphate ion products in solution are high; the solution can become supersaturated with respect to OCP and form an OCP-like intermediary, which then hydrolyzes to nonstoichiometric apatite. In an ACP slurry of low concentration, the surface area available for dissolution is small; therefore the dissolution rate is slow, and the calcium phosphate ion products in solution are relatively low. The solution is undersaturated with respect to OCP and direct precipitation of stoichiometric OHAp occurs except possibly in the initial period.

The above mechanism appears to be consistent with the published data; the OHAp obtained from low supersaturation is stoichiometric OHAp with Ca/P ratio of 1.67 [1, 2], whereas the apatite obtained from high supersaturation is nonstoichiometric [28]. Octacalcium phosphate is structurally related to OHAp; it can and usually does hydrolyze *in situ* to apatite [29]. It is during this *in situ* conversion step that the as yet poorly-defined nonstoichiometric apatitic phase can form, which may include incompletely hydrolyzed OCP and/or Ca-deficient apatite [30]. Interlayered structures of OCP and OHAp [29] and "sesquiapatite" [14] have been proposed as some of the possible models for this apatitic product; OCP is a necessary intermediate phase in these two models.

Blumenthal et al. [13] also reported that Ca-deficient OHAp is obtained when excess phosphate is added to solution before the ACP conversion, and attributed the formation of Ca-deficient OHAp to a mass action effect of excess phosphate. According to our model, the solution with the initial addition of phosphate will be supersaturated with respect to OCP at lower calcium concentrations than without additional phosphate, and therefore precipitated the nonstoichiometric apatitic product through an OCP-like intermediary. Using the calcium concentrations reported in Table 3 of reference 13, our calculations indicate that solution was supersaturated with respect to OCP, even in the low concentration ACP slurry, when 1 mM of phosphate was added initially. Therefore, the effect of the initially added phosphate in an ACP slurry, besides the mass action effect proposed by Blumenthal et al. [13], can be to make the solution supersaturated with respect to OCP at the lower calcium concentrations and form an OCP-like intermediary, which then hydrolyzes to nonstoichiometric OHAp.

The X-ray powder diffraction patterns of pure OCP and apatite crystals are very similar; the major differences are in the 100 and 200 lines (at 18.7 and 9.36 Å respectively for OCP). The 100 and 200 lines of OCP are absent in the X-ray patterns of solid samples obtained during the ACP transformation. This, however, does not rule out OCP as the intermediary since the extreme thinness of the effective crystal, twinning, partial hydrolysis, and interlayering [14] could account for their absences. On the other hand, our data from the conversion of high concentration ACP slurries do not establish the presence of an OCP intermediate phase, but they do show that an acidic calcium phosphate intermediary with the approximate solubility and stoichiometry of OCP was present.

Acknowledgment. This investigation was supported by research grant DE 05030 to the American Dental Association Health Foundation Research Unit by the National Institute of Dental Research and is part of the Dental Research Program conducted by the National Bureau of Standards in cooperation with the American Dental Association Health Foundation. We thank Mrs. B. Sieck for technical assistance.

Certain commercial materials and equipment are identified in this paper to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Bureau of Standards or the ADA Health Foundation or that the materials and equipment identified are necessarily the best available for the purpose.

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