

A Thermodynamic Analysis of the Amorphous to Crystalline Calcium Phosphate Transformation

J.L. Meyer and E.D. Eanes

Laboratory of Biological Structure, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014, USA

Summary. A thermodynamic analysis of the precipitation of amorphous calcium phosphate (ACP) and its transformation to crystalline apatite has been made. A nearly constant ion product, over a wide variety of conditions, was obtained for a tricalcium phosphate (TCP)-like phase suggesting that the molecular unit which governs the solubility of ACP may be similar in composition to TCP. The introduction of 10% acid phosphate into the formula for the TCP ion product improves the fit of experimental data and results in an invariant ion product. The stability of ACP in solution was found to be dependent upon its thermodynamic instability with respect to an octacalcium phosphate (OCP)-like phase. The dependence of the induction period for the amorphous to crystalline transformation upon the pH and the Ca/P ratio of the solution is best explained by the assumption that an OCP-like phase is initially nucleated on the surfaces of the ACP particles. The events that occur in the immediate post-transition period suggest the hydrolysis of this OCP-like material to an apatitic phase.

Key words: Amorphous calcium phosphate — Thermodynamics — Apatite — Octacalcium phosphate — Tricalcium phosphate.

Introduction

The spontaneous precipitation of amorphous calcium phosphate (ACP) and its subsequent transformation into crystalline apatite have been extensively investigated (Eanes and Posner, 1965; Termine and

Eanes, 1972; Eanes et al., 1973; Boskey and Posner, 1973). These studies, however, have generally focused upon the properties of the solid phase. The composition and structure of the first formed crystalline materials are well understood since they are easily studied by conventional chemical, diffraction, and spectroscopic techniques. The structure of ACP cannot be as easily studied by these methods, however, because of the apparent absence of long-range periodic order. The composition of ACP seems to be invariant over a wide range of conditions (Termine and Eanes, 1972), therefore it is possible that ACP contains some short-range order. Radial distribution measurements tend to confirm this and some postulates have been made as to the structure of ACP (Betts and Posner, 1974).

Although some of the parameters which describe the composition of the solutions in equilibrium with the solid phase have been determined, an extensive thermodynamic analysis of the amorphous to crystalline transformation has not been made. The purpose of this investigation was to obtain additional information concerning the structure and composition of ACP and the factors which influence its transformation by analyzing the thermodynamics of the solution phase in equilibrium with the various solid phases. This would seem to be a reasonable approach since the amorphous-crystalline transition is solution mediated; no transformation occurs in the anhydrous state (Eanes and Posner, 1965). Since the kinetics of the transformation are critically dependent upon the solution composition (Termine and Posner, 1970; Termine et al., 1970; Boskey and Posner, 1973), a study of the system under a wide variety of experimental conditions should give some insight into the thermodynamics of the amorphous to crystalline transition. Attempts were made, when possible, to relate the observations to the presence or absence of well-defined crystalline calcium phosphate phases.

Send offprint requests to J.L. Meyer at the above address

Methods

Reagent grade chemicals were used without further purification. All solutions were prepared with deionized, distilled, carbonate-free water. Calcium phosphate solutions, which would later be induced to precipitate, were prepared by combining measured amounts of concentrated calcium nitrate (78 mM) and potassium dihydrogen phosphate (48 mM) stock solutions with sufficient water in glass vessels to attain a total volume of 600 ml. This produced solutions of pH approximately 5, which were subsaturated. Once thermal equilibrium was attained at 25° C, spontaneous precipitation was induced by the rapid addition of potassium hydroxide (Appx. 2 M). The amount of base added was that required to increase the solution pH, in the presence of the precipitating ACP, to the desired value. Identical results were obtained if the precipitate was formed by combining equal volumes of basic solutions of calcium and phosphate, i.e. forming ACP from a rapidly decreasing rather than increasing solution pH. In either case an equivalent amount of KOH was used. The pH was maintained at the preset level, within ± 0.01 pH units, by means of a Metrohm Combititrator 3-D pH stat which added KOH solution. The pH stat provided a constant trace of base consumed in the reaction versus time of reaction. Nitrogen gas was bubbled through the solution to prevent uptake of carbon dioxide.

The composition of the solution, in equilibrium with the precipitated calcium phosphate phases, was determined at various times during the course of the reaction by withdrawing 5 ml aliquots, removing the solid phase by 0.22 μ Millipore filtration, and analyzing the resultant filtrate for calcium and phosphate. The filtration process required about 10 s. Calcium concentrations were determined by atomic absorption spectrophotometry while phosphate concentrations were determined spectroscopically as the phosphomolybdate complex (Murphy and Riley, 1962).

Larger samples (30 ml) were removed for the collection of precipitates to be characterized by surface area and x-ray diffraction methods. These samples were filtered through 0.22 μ Millipore filters, frozen in liquid nitrogen, and lyophilized. Surface areas were determined by a dynamic nitrogen gas absorption system (Quantasorb-Quantachrome Corp.). A standard 114.6 mm diameter x-ray powder camera employing copper K α radiation was used for the diffraction patterns.

Results

Calcium phosphate was formed in different experiments by spontaneous precipitation at pH 7.40, 7.80, 8.25, 8.75, and 9.25. Initial calcium and phosphate concentrations were adjusted so that approximately equal amounts of calcium and phosphate would remain in solution upon the formation of the relatively large amount of precipitate. This assured that both ions could be measured with reasonable accuracy for the thermodynamic treatment described below. Ionic concentrations were determined at various time intervals throughout the period encompassing the formation of ACP, its transformation to the first crystalline phase, and over a short equilibration period thereafter. The data from these five experiments are plotted in Figures 1–5. Also plotted is the continuous trace of the amount of potassium hydroxide added by the pH stat to the

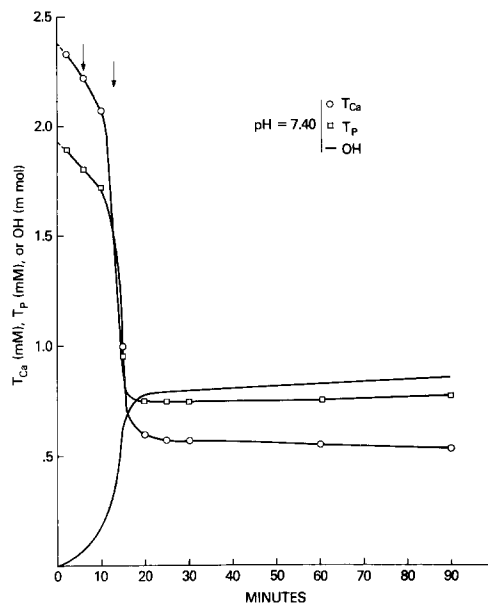


Fig. 1. Variation with time of total calcium (T_{Ca} ; O), total phosphate (T_P ; \square), and base uptake (OH; —) for experiment in which calcium phosphate was spontaneously precipitated at pH 7.40. Initial T_{Ca} and T_P were 9.6 and 7.2 mM, respectively. Arrows signify samples were taken for SSA analysis

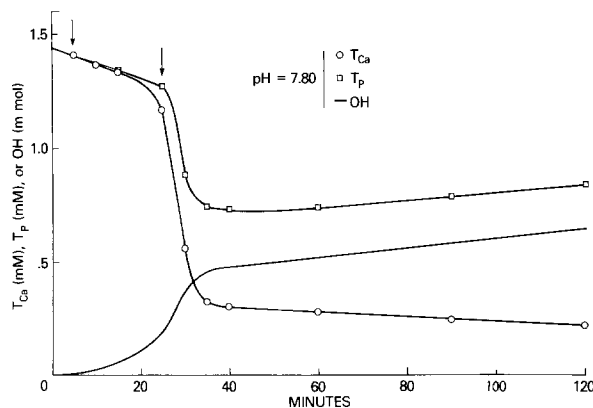


Fig. 2. Variation with time of total calcium (T_{Ca} ; O) total phosphate (T_P ; \square), and base uptake (OH; —) for experiment in which calcium phosphate was spontaneously precipitated at pH 7.80. Initial T_{Ca} and T_P were 10.1 and 7.7 mM, respectively. Arrows signify samples were taken for SSA analysis

solution to maintain constant pH. Solid material for specific surface area (SSA) measurements was isolated from each of the experiments (a) soon after the formation of ACP (approximately 5 min) and (b) at the onset of nucleation. In each of the figures the time of separation is indicated by an arrow.

The data of Figures 1–5 were also used to determine induction periods (I) for the transformation

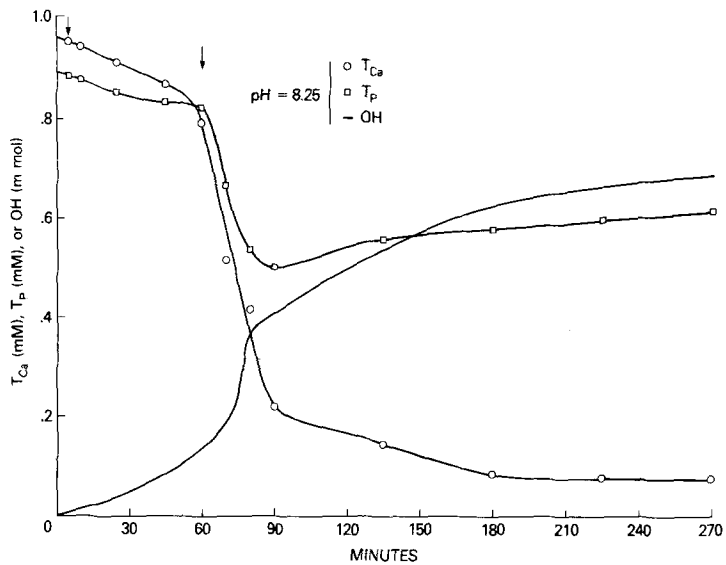


Fig. 3. Variation with time of total calcium (T_{Ca} ; O), total phosphate (T_P ; □), and base uptake (OH; —) for experiment in which calcium phosphate was spontaneously precipitated at pH 8.25. Initial T_{Ca} and T_P were 10.8 and 7.9 mM, respectively. Arrows signify samples were taken for SSA analysis

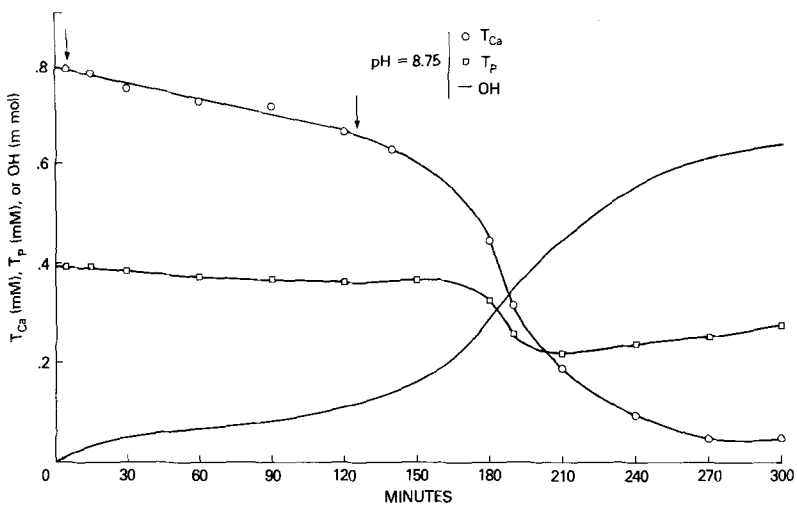


Fig. 4. Variation with time of total calcium (T_{Ca} ; O), total phosphate (T_P ; □), and base uptake (OH; —) for experiments in which calcium phosphate was spontaneously precipitated at pH 8.75. Initial T_{Ca} and T_P were 11.7 and 7.9 mM, respectively. Arrows signify samples were taken for SSA analysis

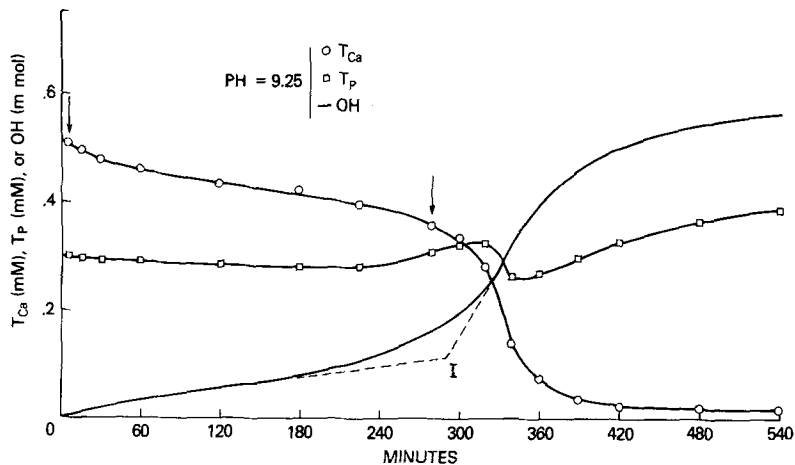


Fig. 5. Variation with time of total calcium (Ca; O), total phosphate (T_P ; □), and base uptake (OH; —) for experiments in which calcium phosphate was spontaneously precipitated at pH 9.25. Initial T_{Ca} and T_P were 11.6 and 7.9 mM, respectively. Arrows signify samples were taken for SSA analysis

of ACP to the first crystalline phase. These were determined, as illustrated by the dotted lines in Figure 5, from the intersection of the tangents to the flat pre-transformation period and to the steepest portion of the base uptake curve. The base uptake curve was chosen for this determination rather than any of the other measured parameters since a continuous, rather than stepwise, curve was obtained. A previous report (Boskey and Posner, 1973) has shown that the break in the rate of base addition correlates with the amorphous to crystalline transition. The data from the surface area measurements are presented in Table 1 and parameters associated with the induction periods are given in Table 2.

Table 1. Specific surface areas of the solid phases isolated from spontaneously precipitated calcium phosphate solutions during the period of amorphous phase stability (ACP) and at the onset of nucleation (Pre-cryst)

pH	7.4	7.8	8.25	8.75	9.25
ACP (m ² /g)	84	85	88	88	88
Pre-cryst (m ² /g)	171	168	162	166	203

Similar experiments, in which only half the amount of ACP was precipitated, were conducted at each of the five values of pH. This was accomplished by using lower initial calcium and phosphate concentrations. The solution profiles in calcium, phosphate, and base uptake were similar and the induction periods at each pH were the same as before. Induction periods were also determined for solutions of similar composition to those in Figures 1–5 (extrapolated to time = 0) but in the absence of precipitated ACP. Under these conditions a metastable solution is produced and precipitation does not occur immediately. The onset of precipitation is easily determined by monitoring the pH of solution with the pH stat. The time periods required for nucleation in the absence of preformed ACP, I^0 , are also included in Table 2 along with computed values of I^0/I . Material isolated from such a “homogeneous”

nucleation experiment at pH 7.4 as soon as the base uptake curve of the pH stat indicated that nucleation had begun showed an apatitic x-ray diffraction pattern.

Free ionic calcium and phosphate activities were calculated for all experiments from the analytically determined total concentrations of calcium, T_{Ca} and phosphate, T_p . The computational procedure involved the mass balance equations:

$$T_{Ca} = [Ca] + [CaH_2PO_4] + [CaHPO_4] + [CaPO_4] \quad (1)$$

and

$$T_p = [PO_4] + [HPO_4] + [H_2PO_4] + [H_3PO_4] + [CaPO_4] + [CaHPO_4] + [CaH_2PO_4] \quad (2)$$

where [] indicates molar concentrations. Ionic charges have been eliminated for simplicity. Since pH is known, substitution of the equilibrium expressions for the calcium phosphate ion pairs and the protonated phosphate species transforms the above relations into two equations in two unknowns, [Ca] and [PO₄], which can be solved by conventional methods. Activity coefficients were estimated from an extended form of the Debye-Hückel equation (Davies, 1962). Since the activity coefficients are dependent upon the concentration terms in Equations (1) and (2), an iterative procedure was used. All computations were performed with the aid of a computer program written in Fortran IV. Thermodynamic values for the ion pair formation constants were those of Chughtai et al. (1968). Values for the acid dissociation constants of H₃PO₄, H₂PO₄, and HPO₄ were taken from the work of Vanderzee and Quist (1961), Bates and Acree (1943), and Bates (1951), respectively.

Activity products were calculated for the solution phase at each point in Figures 1–5 using molecular formulae for the calcium phosphate phases: Ca₅(PO₄)₃OH, hydroxyapatite (HAP); Ca₃(PO₄)₂, tricalcium phosphate (TCP); Ca₄H(PO₄)₃ · 2.5H₂O, octacalcium phosphate (OCP); and CaHPO₄ · 2H₂O, dicalcium phosphate dihydrate (DCPD). The log of the activity product for each of the molecular formulas

Table 2. Induction periods and nucleation parameters for ACP-crystalline formation at variable pH

pH	I^a min	I^{0a} min	I^0/I	$I \times (OCP)^b$ $\times 10^{43}$	$I \times (TCP)$ $\times 10^{25}$	$I \times (HAP)$ $\times 10^{45}$
7.40	13	19	1.4	2.0	7.0	1.4
7.80	26	44	1.7	2.9	17.6	7.9
8.25	62	144	2.3	3.8	50.4	56.5
8.75	166	300	1.8	4.5	166.0	631.1
9.25	290	590	2.0	3.6	303.7	2769.5

^a I and I^0 are induction periods for the formation of crystalline phase in the presence and absence, respectively, of ACP

^b () are ion activity products for the crystalline phase designated

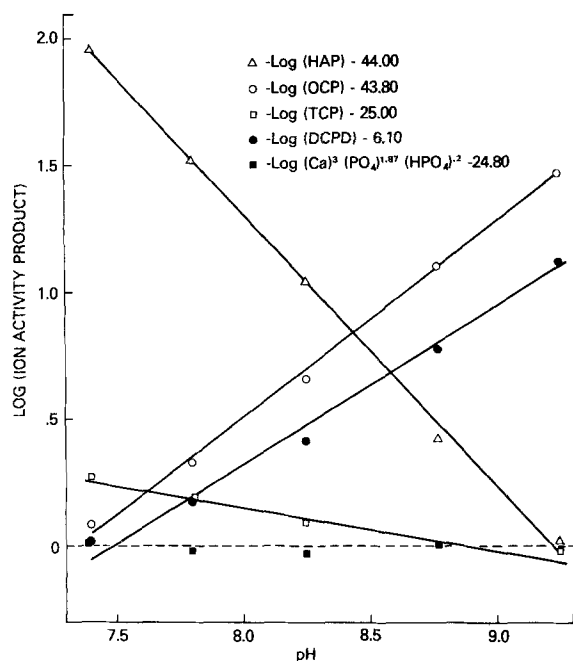


Fig. 6. The dependence of the log of the activity product at $t = 0$ for the calcium phosphate phases HAP (Δ), OCP (\circ), TCP (\square), DCPD (\bullet), and $\text{Ca}_3(\text{PO}_4)_{1.87}(\text{HPO}_4)_{0.2}$ (\blacksquare) on the pH of the precipitating solution. The values are normalized by subtracting 44.00, 43.80, 25.00, 6.10, and 24.80 from the negative logarithms of the above phases, respectively

above is plotted versus the pH of the solution in Figure 6. The concentrations used in these calculations were those extrapolated to time zero in Figures 1–5 although similar correlations were observed at other regions of the curves in which the amorphous phase was present. Identical results were also obtained in those experiments in which lesser amounts of ACP were precipitated. The activity product based on the TCP formula appears to be the least dependent upon solution conditions, e.g. pH and Ca/P ratio. An invariant activity product was empirically obtained for a tricalcium phosphate-like phase containing 9.7% acid phosphate. The composition of the charge neutral phase that meets this criterion is $\text{Ca}_3(\text{PO}_4)_{1.87}(\text{HPO}_4)_{0.2}$, i.e. a precipitate containing approximately one acid phosphate for every nine phosphate ions.

The thermodynamic stability of the experimental solutions with respect to a solution in equilibrium with the well-defined pure crystalline phases HAP, TCP, OCP, and DCPD was determined at each point in the precipitation sequence using the equation:

$$(\Delta G = -2.303RT/n \log (AP_i/AP_s))$$

where AP_i is the ionic activity product at any point in the precipitation experiment and AP_s is the thermo-

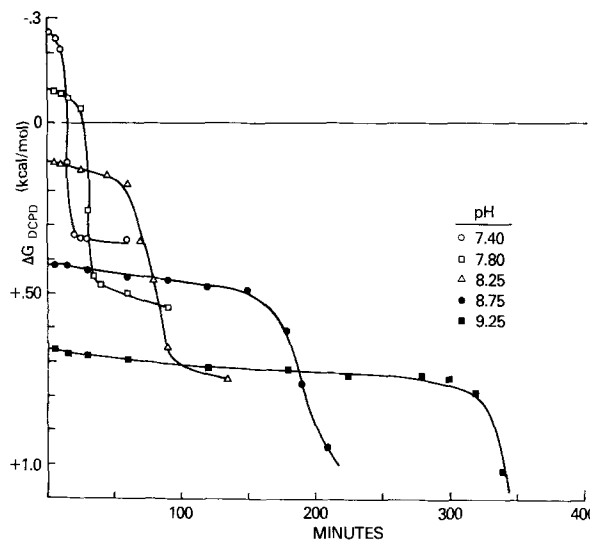


Fig. 7. The thermodynamic stability of the solutions in Figures 1–5 with respect to a solution in equilibrium with pure crystalline DCPD

dynamic solubility product. R and T are the ideal gas constant and absolute temperature, respectively, and n is the number of ionic terms in the activity product expression. The results of these free energy (ΔG) calculations are presented in Figures 7–10. In each figure the data for experiments at each of the values of pH are plotted. The line drawn at $\Delta G = 0$ (except for Fig. 10 for which it is off the scale) signifies thermodynamic equilibrium with that phase. Points at or near the line are solution compositions having the same thermodynamic stability as solutions in equilibrium with that phase. Points above the line (ΔG negative) represent solutions less stable (i.e. supersaturated) and points below the line (ΔG positive) more stable (i.e. subsaturated) than those in thermodynamic equilibrium. The thermodynamic solubility products, at 25°C , used in the calculations for DCPD, TCP, OCP, and HAP were those of Gregory et al. (1970), Gregory et al. (1974), Moreno et al. (1960), and McDowell et al. (1969), respectively. Some of the features of the figures that will be important in future discussions are: (a) the initial free energy with respect to DCPD and OCP decreases with increasing pH; (b) the initial free energy with respect to HAP increases with increasing pH and; (c) the initial free energy with respect to TCP varies the least with pH. Also of interest is the fact that inflections occur in the curves obtained at the lower pH points for solution compositions similar to those in equilibrium with pure crystalline OCP (i.e. $\Delta G = 0$).

Another series of experiments was done at pH 7.4 but with varying initial calcium:phosphate molar

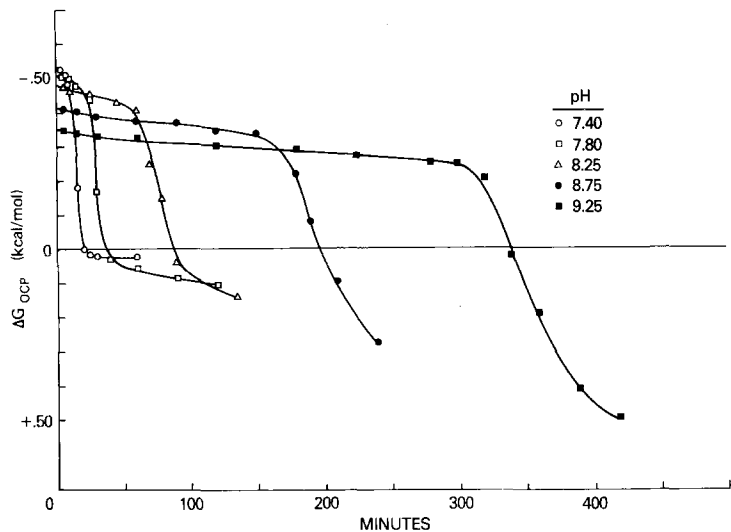


Fig. 8. The thermodynamic stability of the solutions in Figures 1-5 with respect to a solution in equilibrium with pure crystalline OCP

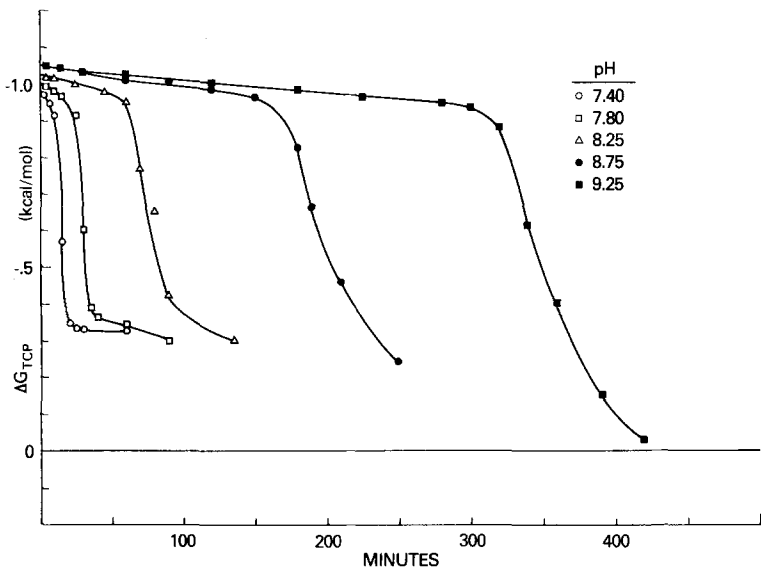


Fig. 9. The thermodynamic stability of the solutions in Figures 1-5 with respect to a solution in equilibrium with pure crystalline TCP

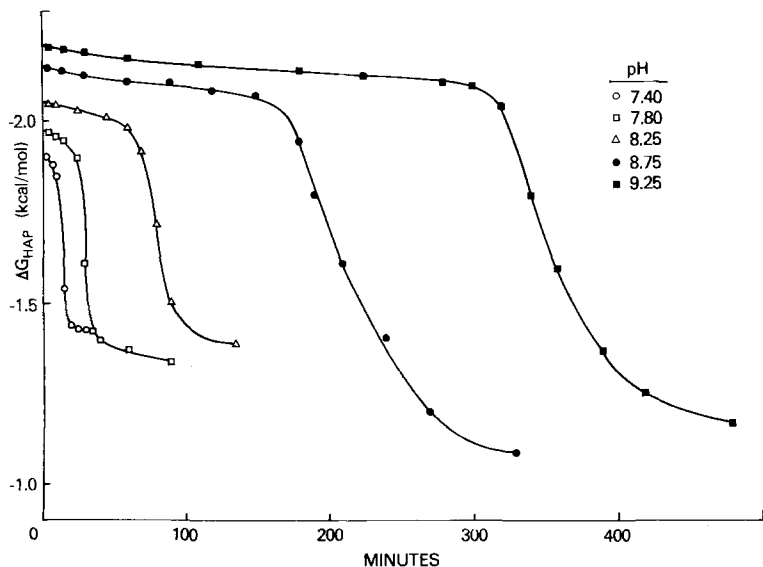


Fig. 10. The thermodynamic stability of the solutions in Figures 1-5 with respect to a solution in equilibrium with pure crystalline HAP

Table 3. Induction periods, activity products, and nucleation parameters for ACP-crystalline phase transformations at variable Ca/P ratios at pH 7.4

Ca/PO ₄ molar	<i>I</i> min	<i>I</i> × (OCP) ^a × 10 ⁴³	<i>I</i> × (TCP) × 10 ²⁵	<i>I</i> × (HAP) × 10 ⁴⁶	−log(DCPD)	−log(OCP)	−log(HAP)	−log(TCP)	−log(Ca) ³ (PO ₄) ^{1.87} (HPO ₄) ^{0.2}
0.69	10	1.8	5.5	9.8	6.12	43.75	46.01	25.26	24.83
1.33	14	2.3	8.2	18.0	6.16	43.78	45.89	25.23	24.81
2.45	19	2.7	11.2	28.1	6.24	43.84	45.83	25.23	24.82
7.08	24	3.0	15.5	53.7	6.34	43.90	45.65	25.19	24.81

^a () are ion activity products for the crystalline phase designated

ratios. The data were treated in the same manner described above and the results are presented in Table 3. The Ca/P ratios in the table are those in solution in equilibrium with the first formed solid phase. Induction periods are seen to increase as the Ca/P ratio increases. Also computed in Tables 2 and 3 are the products of the induction periods for the amorphous to crystalline transformation and the ion activity products for the phases OCP, TCP, and HAP.

Discussion

The characteristics of the amorphous to crystalline transformation are similar to those described earlier (Eanes and Posner, 1965; Termine and Eanes, 1972; Boskey and Posner, 1973). ACP is the first formed solid phase which, after a reproducible induction period, transforms to a microcrystalline phase with an apatitic x-ray diffraction pattern. In terms of the solution chemistry, the stability of the amorphous phase is marked by relatively steady concentrations of calcium and phosphate and the transformation is signaled by a sharp drop in ionic concentrations which accompany the formation of the less soluble crystalline phase. The rate of base addition necessary to maintain constant pH follows the same pattern with a sharp upturn in the curve, from a slow, steady rise, occurring at the onset of the ACP-crystalline transformation. The flatness of the curves in the pretransition period suggests that equilibrium with solid phase occurs rapidly (<5 min) upon precipitation.

The structural unit of the amorphous phase that governs its solubility may be inferred by a molecular formula which gives a constant ionic product under all conditions. A wide range in either pH (7.40–9.25) or starting Ca/P molar ratio (0.69–7.08) at constant pH were used in this study resulting in amorphous precipitates with greatly different solubilities in equilibrium with varying solution stoichiometries. The solubility of ACP decreases rapidly with increasing pH yet the ion activity product for TCP, (Ca²⁺)³(PO₄^{3−})², varies little over the pH range studied. The ion activity product for HAP, (Ca²⁺)⁵(PO₄^{3−})³(OH[−]), increased

greatly and the products for OCP, (Ca²⁺)⁴(H⁺)PO₄^{3−})³, and DCPD, (Ca²⁺)(HPO₄^{2−}), decreased greatly with increasing pH. Based on solution thermodynamic considerations it would appear that of the four calcium phosphate phases known to exist in physiologic-like solutions a molecular unit similar to TCP best described the composition of the amorphous phase. This would appear in agreement with the Ca₉(PO₄)₆ cluster proposed in another study (Betts and Posner, 1974). An invariant ion product is obtained if 0.2 HPO₄^{2−} groups are substituted for PO₄^{3−} in the Ca₃(PO₄)₂ formula resulting in a charge neutral unit of Ca₃(PO₄)_{1.87}(HPO₄)_{0.2}. A phase of this stoichiometry would contain approximately 10% acid phosphate (mole/mole total phosphate) and have a Ca/P molar ratio of 1.45. It is interesting that this is similar to the composition of the solid phase precipitated under similar conditions and washed free of all adsorbed impurities (Termine and Eanes, 1972).

Similar conclusions can be made from the thermodynamic treatment of the data obtained from the experiments with variable Ca/P ratios but constant pH (7.40). Although there is not as much variability in the ion activity products, it is clear that the product for a TCP-like phase is least sensitive to changes in Ca/P ratios. Ion products for HAP increase while products for OCP and DCPD decrease with increasing Ca/P ratio. If 0.2 mole of acid phosphate is substituted into the TCP formula, as described above, the identical invariant ion activity product is obtained.

Earlier studies of the amorphous crystalline transformation (Termine et al., 1970; Boskey and Posner, 1973) have shown that, among other factors, ACP is stabilized by an increase in the pH and/or the Ca/P ratio in solution and the data in Tables 2 and 3 support these observations. Before now a theoretical basis for these phenomena was lacking, however. Since the first formed crystalline phase is always found in contact with the surfaces of the amorphous spherules (Eanes et al., 1973), it is likely that the amorphous crystalline transition is induced by a heterogenous nucleation on the surfaces of the ACP particles. Nucleation, involving an induction period, of a large number of sparingly

soluble salts has been empirically described by an equation of the form $IC^p = \text{constant}$ in which I is the induction period, C is a concentration product term and p is an integer (Van Hook, 1940; Christiansen and Nielsen, 1951; Nielsen, 1955; and Walton, 1965). The product of the induction period and the ionic activity products for OCP, TCP, and HAP, obtained in the pH range 7.40–9.25 (Table 2) suggest that it is the instability with respect to OCP that governs the rate of nucleation of the first crystalline phase. The terms involving OCP vary little with the pH of the solution whereas the terms containing the HAP and TCP ion activity products increase greatly with pH.

Another way of showing that the conclusions above are justified is by examining the free energy data in Figures 8–10. The free energy of the solutions in contact with the amorphous phase provides the driving force for the nucleation phenomena which initiate the amorphous to crystalline transformation. The free energy with respect to equilibrium with pure crystalline OCP is seen to decrease with increasing pH. Since the driving force is smaller at the higher values of pH it is reasonable to expect a longer induction period. If the initially formed crystalline phase had a TCP-like composition, the induction period should have been insensitive to pH since the supersaturation with respect to any phase containing a $\text{Ca}_3(\text{PO}_4)_2$ -like composition would have changed little with pH. Finally, it seems unlikely that the first nucleated crystals are a HAP phase, based on thermodynamic considerations. The free energy with respect to crystalline HAP increases as the pH of the precipitating medium increases yet the induction periods for nucleation become greater. It should be pointed out that although the first formed crystals may have solubilities slightly different, due to size, imperfection or other factors from those of the more well-defined crystalline phases, the relation between induction period and ion activity product remains qualitatively the same no matter what reasonable value for the solubility product is used in the ΔG calculation. The free energy with respect to DCPD was not considered for the calculation of the nucleation parameters since for $\text{pH} > 7.8$ the solubility of DCPD becomes greater than the solutions in equilibrium with ACP (Fig. 7) and it becomes thermodynamically impossible for DCPD to nucleate.

Similar correlations are found in the systems in which the Ca/P ratio was varied. The nucleation term involving the OCP ion activity product appears to vary the least with increasing Ca/P ratios. The participation of TCP and HAP in the initial heterogeneous nucleation step can again be ruled out by using the arguments above.

The possibility that the great increase in induction period with pH was due to a change in the ability of the

preformed ACP spherules to nucleate the crystalline phase is considered unlikely since the chemical and physical composition of ACP seems to vary little within the pH range of this study. The rate of nucleation may be dependent, in general, upon the surface areas of the nucleating agent; however, the surface areas of the first formed ACP particles were found to be unaffected by the pH of precipitation (Table 1). The induction period did not seem to be particularly dependent anyway upon the surface of ACP available to nucleate since the induction periods were the same in experiments in which half the amount of ACP was precipitated. This insensitivity of induction period to the amount of seed available for nucleation has been observed in other epitaxial systems involving delayed heterogeneous nucleation (Meyer et al., 1976; Meyer et al., 1977). The most important consideration for the length of the induction period, then, seems to be the thermodynamic instability of the solution phase.

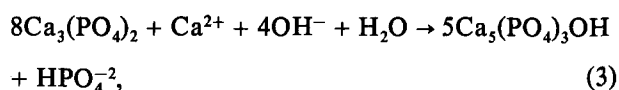
The physical dimensions, as well as the chemical composition, seem to be the same for the first nucleated crystalline phase obtained at all values since the surface areas of the solid material separated from the systems at the onset of nucleation are similar (Table 1). The SSA's are about twice those measured for the pure amorphous phase. The SSA obtained for the pH 9.25 sample is somewhat higher suggesting a more finely divided precipitate as has been observed for other crystalline calcium phosphates prepared at high pH (Meyer and Eanes, unpublished results).

Additional evidence, which supports the conclusion that heterogeneous nucleation upon the ACP particles initiates the amorphous crystalline transformation, is provided by the fact that the induction periods for the formation of a detectable amount of crystalline material are greater in the absence of spontaneously precipitated ACP. The sensitive pH stat method of monitoring precipitation events indicates (Table 2) that approximately twice as much time is required for nucleation in the absence of ACP. Although the solid phase separated from the solution at the onset of nucleation had a characteristic apatitic x-ray diffraction pattern, it is unknown whether it was preceded by a small, undetectable amount of ACP or whether the crystalline phase was nucleated directly from solution as proposed earlier (Boskey and Posner, 1976). Although an apatitic diffraction pattern was obtained, the extreme thinness of the earliest crystals might account for the absence of the characteristic 010 and 020 lines of OCP (Eanes and Meyer, 1977). Unless sufficient development of the crystal lattice occurs in this direction the somewhat diffuse patterns obtained for the microcrystalline phase will not allow distinction between HAP and OCP.

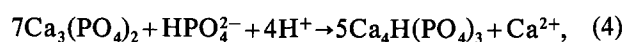
The thermodynamic data obtained in this study

suggest that three distinct consecutive chemical phases are involved in the formation of the first apatitic crystals: (1) an amorphous (to x-rays) phase with possible short range order corresponding to a TCP-like composition containing small amounts of acid phosphate as an integral structural unit; (2) a first nucleated crystalline phase with the thermodynamic properties of an OCP-like phase; and (3) a crystalline phase with the structural characteristics of an apatite and which, if left in the precipitation medium, approaches the thermodynamic stability of hydroxyapatite (Eanes and Meyer, 1977).

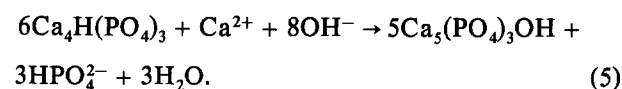
Although the sharp drop in precipitant ion concentration accompanying the transformation of ACP to the less soluble crystalline phase tends to mask whatever subtle chemical changes occur in this step, certain inferences can be made by examining the solution data in Figures 1–5. The conversion of the TCP-like phase to HAP is described by Equation (3) and is consistent with experimental facts. Equation (3) requires



an uptake of calcium and hydroxide ions from solution, and the possibility, if the reaction corresponding to Equation (3) occurs more rapidly than the overall removal of calcium and phosphate from solution, of an increase in solution phosphate. Calcium and hydroxide uptake was noted under all conditions in the pre-transformation period whereas an increase in phosphate concentration was observed at the higher values of pH where the net amount of solid calcium phosphate to be precipitated during the transformation was minimal. Equation (3) describes only the overall process, however, and does not rule out the participation of more acidic intermediate phases. It seems unlikely that the initial transformation is simply ACP to stoichiometric OCP since a balanced reaction such as Equation (4),



requires an uptake of phosphate and hydrogen ions and a release of calcium into solution which is contrary to observed events. The possibility remains, however, that OCP is the first-formed crystalline phase but is so unstable that it begins to convert to HAP via Equation (5).



This precipitation scheme is consistent with the solution

data. The instability of OCP with respect to hydrolysis to HAP has been well established (Brown et al., 1962).

It is apparent that the post-transition period is not a simple ripening and crystal growth of an apatitic phase. Although a consistent uptake of base and a consistent decrease in calcium ion concentration with time were noted in every stage of the reaction and under all conditions studied, solution phosphate concentrations were seen to either level off or increase, depending upon the pH, during the post-transition periods. The free energy (with respect to all crystalline phases) of the systems involves both calcium and phosphate, however, and consistently decreases with time. A possible explanation of the observed post-transition solution chemistry is that a mixture of OCP-like and HAP-like phases is formed during the amorphous to crystalline transition and the hydrolysis of OCP to the thermodynamically more stable HAP, via Equation (5), contributes to the calcium and base uptake and phosphate release by the precipitate. It is interesting that the ion activity products for the solutions in equilibrium with the first formed post-transition phase tend to level off near the solubility product of OCP ($\Delta G = 0$) at the more acidic values of pH at which the stability of OCP is greatest. An additional complicating factor in the post-transition period is an additional inflection in the base uptake, calcium, and phosphate curves (Eanes and Meyer, 1977). This will be reported in more detail in a subsequent publication.

References

- Bates, R.G.: First dissociation constant of phosphoric acid from 0° to 60° C: Limitations of the electromotive force method for moderately strong acids. *J. Res. Nat. Bur. Stand.* **47**, 127–134 (1951)
- Bates, R.G., Acree, S.F.: ΔH values of certain phosphate-chloride mixtures, and the second dissociation constant of phosphoric acid from 0° to 60° C. *J. Res. Nat. Bur. Stand.* **30**, 129–155 (1943)
- Betts, F., Posner, A.S.: An x-ray radial distribution study of amorphous calcium phosphate. *Mat. Res. Bull.* **9**, 353–390 (1974)
- Boskey, A.L., Posner, A.S.: Conversion of amorphous calcium phosphate to microcrystalline hydroxyapatite. A pH dependent, solution-mediated, solid-solid conversion. *J. Phys. Chem.* **77**, 2312–2317 (1973)
- Boskey, A.L., Posner, A.S.: Formation of hydroxyapatite at low super-saturations. *J. Phys. Chem.* **80**, 40–45 (1976)
- Brown, W.E., Smith, J.P., Lehr, J.R., Frazier, A.W.: Crystallographic and chemical relations between octacalcium phosphate and hydroxyapatite. *Nature, Lond.* **196**, 1050–1055 (1962)
- Chughtai, A., Marshall, R., Nancollas, G.H.: Complexes in calcium phosphate solutions. *J. Phys. Chem.* **72**, 208–211 (1968)
- Christiansen, J.A., Nielsen, A.E.: On the kinetics of formation of sparingly soluble salts. *Acta Chem. Scand.* **5**, 673–674 (1951)
- Davies, C.W.: Ion association. London: Butterworth and Co. 1962

- Eanes, E.D., Meyer, J.L.: The maturation of crystalline calcium phosphates in aqueous suspensions at physiological pH. *Calcif. Tiss. Res.* **23**, 259–269 (1977)
- Eanes, E.D., Posner, A.S.: Kinetics and mechanism of conversion of noncrystalline calcium phosphate to crystalline hydroxyapatite. *Trans. N.Y. Acad. Sci.* **28**, 233–241 (1965)
- Eanes, E.D., Termine, J.D., Nysten, M.U.: An electron microscopic study of the formation of amorphous calcium phosphate and its transformation to crystalline apatite. *Calcif. Tiss. Res.* **12**, 143–148 (1973)
- Gregory, T.M., Moreno, E.C., Brown, W.E.: Solubility of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ in the system $\text{Ca}(\text{OH})_2\text{—H}_3\text{PO}_4\text{—H}_2\text{O}$ at 5, 15, 25, and 37.5° C. *J. Res. Natl. Bur. Stand.* **74A**, 461–475 (1970)
- Gregory, T.M., Moreno, E.C., Patel, J.M., Brown, W.E.: Solubility of $\beta\text{-Ca}_3(\text{PO}_4)_2$ in the system $\text{Ca}(\text{OH})_2\text{—H}_3\text{PO}_4\text{—H}_2\text{O}$ at 5, 15, 25 and 37° C. *J. Res. Natl. Bur. Stand.* **78A**, 667–674 (1974)
- McDowell, H., Wallace, B.M., Brown, W.E.: The solubilities of hydroxyapatite at 5, 15, 25, and 37° C, abstracted, IADR Program and Abstracts of Papers, No. 340, 1969
- Meyer, J.L., Bergert, J.H., Smith, L.H.: The epitaxially induced crystal growth of calcium oxalate by crystalline uric acid. *Invest. Urol.* **14**, 115–119 (1976)
- Meyer, J.L., Bergert, J.H., Smith, L.H.: Epitaxial relationships in urolithiasis: the brushite-whewellite system. *Clin. Sci. Mol. Med.* **52**, 143–148 (1977)
- Moreno, E.C., Brown, W.E., Osborn, G.: Stability of dicalcium phosphate dihydrate in aqueous solutions and solubility of octacalcium phosphate. *Soil Science Society of America Proceeding* **21**, 99–102 (1960)
- Murphy, J., Riley, J.P.: A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27**, 31–36 (1962)
- Nielsen, A.E.: The kinetics of electrolyte precipitation. *J. Colloid Sci.* **10**, 576–586 (1955)
- Termine, J.D., Eanes, E.D.: Comparative chemistry of amorphous and apatitic calcium phosphate preparations. *Calcif. Tiss. Res.* **10**, 171–197 (1972)
- Termine, J.D., Posner, A.S.: Calcium phosphate formation in vitro. I. Factors affecting initial phase separation. *Arch. Biochem. Biophys.* **140**, 307–317 (1970)
- Termine, J.D., Peckauskas, R.A., Posner, A.S.: Calcium phosphate formation in vitro. II. Effect of environment on amorphous-crystalline transformation. *Arch. Biochem. Biophys.* **140**, 318–325 (1970)
- Vanderzee, C.E., Quist, A.S.: The third dissociation constant of orthophosphoric acid. *J. Phys. Chem.* **65**, 118–123 (1961)
- Van Hook, A.: The precipitation of silver chromate. *J. Phys. Chem.* **44**, 751–764 (1940)
- Walton, A.G.: Nucleation of crystals from solution. *Science* **149**, 601–607 (1965)

Received April 18 / Revised July 11 / Accepted July 15, 1977