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Invited Conference Paper

Phase Transformations in the Spontaneous Precipitation of Calcium Phosphate*

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Recent experimental evidence suggests that octacalcium phosphate (OCP) is a necessary precursor to hydroxyapatite in the amorphous-crystalline transformation that occurs after the spontaneous precipitation of calcium phosphate in the pH range 7.00—9.25. The transformation appears to occur by heterogeneous nucleation of OCP by the initially formed amorphous calcium phosphate (ACP) phase. ACP itself seems to have a well-defined composition with a solubility determining molecular unit. At pH greater than 9.25 the mechanism for the transformation seems to change. At high pH OCP becomes thermodynamically unstable the ACP structure seems to break down.

The spontaneous precipitation of calcium phosphate at neutral and alkaline pH results in the initial formation of a phase which is amorphous with respect to X-ray diffraction and is generally referred to as amorphous calcium phosphate (ACP). After a finite and highly reproducible time, ACP undergoes a transformation to a crystalline phase with many of the chemical and physical properties of the mineral hydroxyapatite (HAP: $\text{Ca}_5(\text{PO}_4)_3\text{OH}$). HAP is the prototype for the crystalline calcium phosphate phase found in the hard tissues of vertebrates¹ and indeed mature crystals prepared by spontaneous precipitation from solution resemble in many respects those found in the skeletal tissues.

In this paper, a summary of the results of recent experiments designed to elucidate the mechanism of the ACP—HAP transformation is presented. Particular attention is given to the initial events in the transformation and to the structure, composition, and chemistry of the intermediate phases. Experimental data in this report are taken from published²⁻⁹ and unpublished studies in this laboratory.

GENERAL

The general features of the amorphous-crystalline transformation, beginning with the spontaneous precipitation of ACP, are presented in Figure 1.

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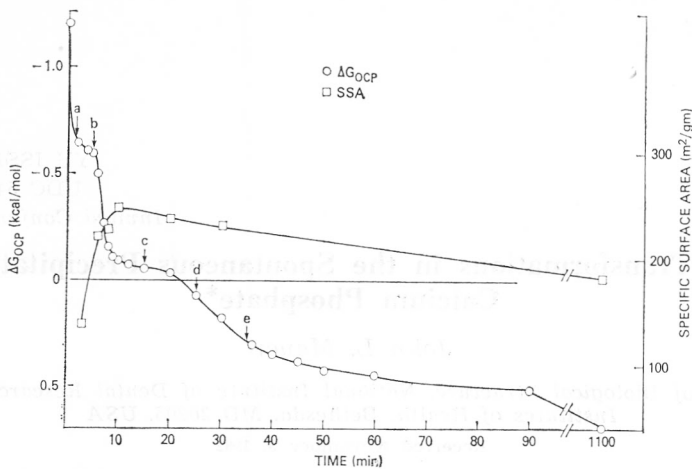


Figure 1. A plot of the free energy of solution, ΔG , with respect to a solution in equilibrium with OCP for a kinetic experiment, performed at pH 7.40 and 37°C, involving the spontaneous precipitation of calcium phosphate. Lettered arrows represent reaction times at which samples were removed for TEM observation (see Figure 3). Also indicated are specific surface areas (SSA) for selected samples.

Plotted is the variation with time of the free energy of the solution phase with respect to a solution in equilibrium with the crystalline calcium phosphate phase, octacalcium phosphate (OCP: $\text{Ca}_4\text{H}(\text{PO}_4)_3$). This term, ΔG_{OCP} , is computed from the general equation:

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

where AP_i is the ionic activity product at any point in the precipitation experiment and AP_s is the thermodynamic solubility product. R and T are the ideal gas constant and absolute temperature, respectively, and n is the number of ionic terms (8 for OCP) in the activity product expression. Points above the $\Delta G = 0$ line represent solutions (in equilibrium with solid phase during the precipitation reaction) which are unstable with respect to the formation of OCP (i. e. supersaturated). Points near this line represent solutions which may be in equilibrium with OCP and those below the line (positive ΔG) indicate solutions in which it is impossible for OCP to form. OCP was chosen arbitrarily for this graph. The free energy of the solution phase with respect to the formation of any other calcium phosphate phase, for which a solubility product is known, could also have been presented. This particular experiment, performed at 37°C and pH 7.40, was initiated by quickly (~ 1 s) increasing the supersaturation of the solution so that at $t = 0$ the solution is greatly supersaturated with respect to the first solid phase to be formed. The kinetic stability of ACP is denoted by the first relatively flat portion of the curve. The transformation of ACP to the first crystalline phase is indicated by the precipitous decrease in ion product (or ΔG) which occurs at $t \cong 6$ min. The composition of the solution phase again becomes rather constant until at $t \cong 20$ min a second inflection in the curve is noted. This discontinuity signifies a secondary solid-solid transformation, this time between crystalline phases. After the second inflection, the free energy of the solution phase is seen to decrease gradually and with no additional apparent discontinuities.

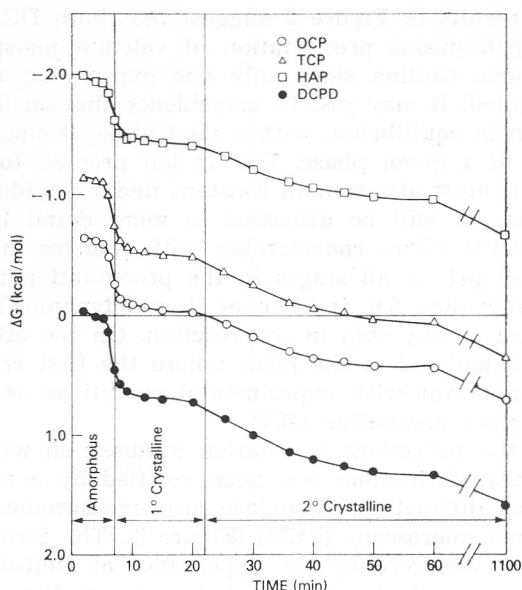


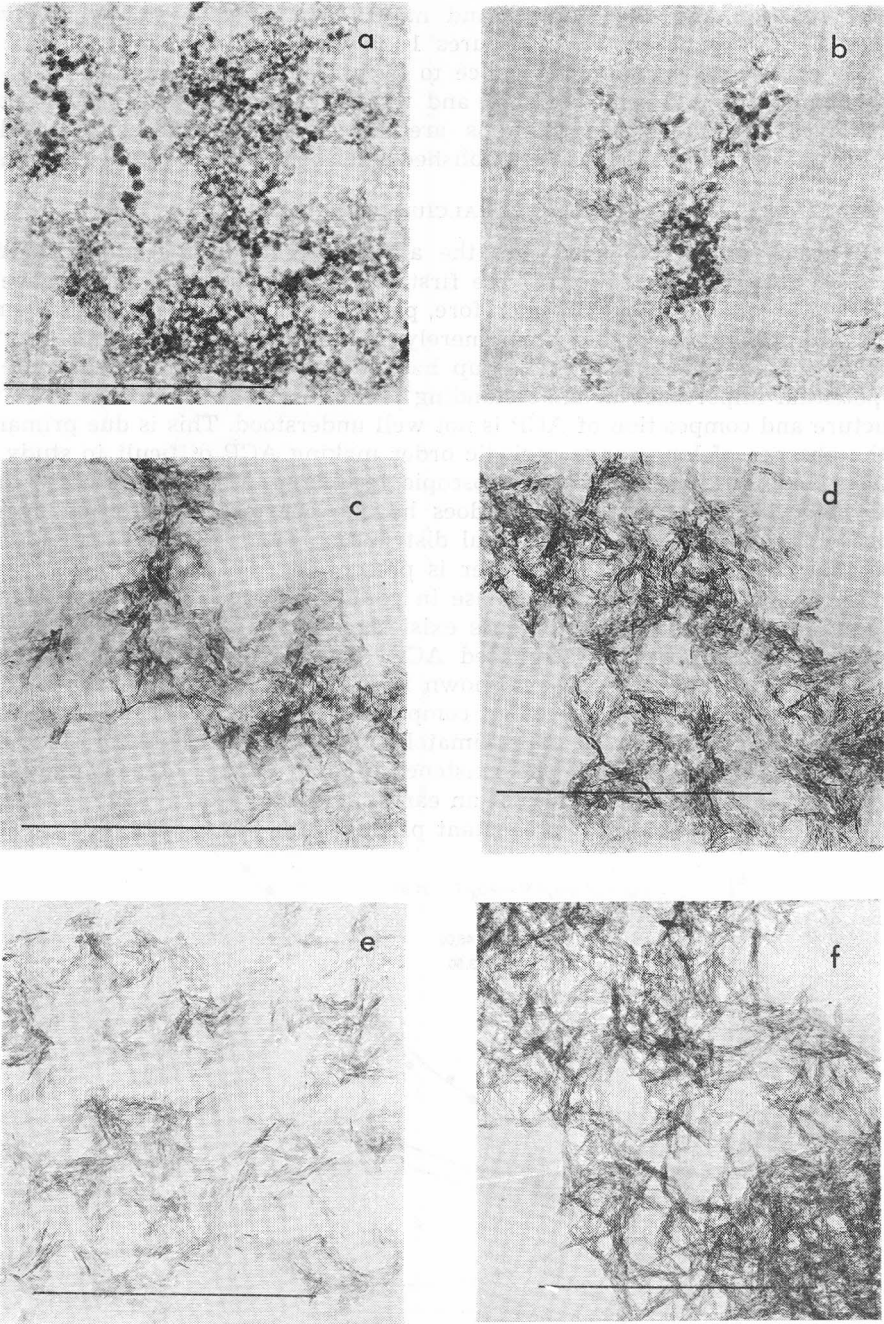
Figure 2. The variation with time of the system free energy, ΔG , with respect to solutions saturated with the calcium phosphate phase OCP, TCP, HAP, and DCPD. The region of stability of the amorphous, primary crystalline (1°), and secondary crystalline (2°) phases are also indicated for this spontaneous precipitation experiment performed at 37°C and pH 7.4.

The variation with time of the system's free energy with respect to other well defined crystalline phase is shown more clearly in Figure 2. Free energy calculations were made using literature values for the solubility product of OCP,¹⁰ HAP,¹¹ TCP¹² (β -tricalcium phosphate; $\text{Ca}_3(\text{PO}_4)_2$), and DCPD¹³ (dicalcium phosphate dihydrate; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) all of which can be formed near neutral pH and which have been observed and identified in biological systems. All four curves show the variation of a given ion product with time and thus have the same shape. The existence and stability of the amorphous, primary crystalline, and secondary crystalline phases are clearly indicated by the two inflections in the curves. The other type of information attainable from Figure 2 is the probability that a particular calcium phosphate phase is present in the system at any point in time. For example, it is apparent that it would be impossible for DCPD to be present in any of the crystalline phases formed under these conditions. However, the ion product for the solution phase in equilibrium with ACP is similar to that of the solubility product of DCPD suggesting that ACP may contain the molecular structure of this crystalline phase as the solubility determining unit. Similarly, the ion product for the solution phase in equilibrium with the first formed crystalline phase is near to that of the solubility product of OCP suggesting that OCP is a precursor phase in the formation of HAP via spontaneous precipitation. After an extended period of time, the solution phase remains supersaturated only with respect to HAP and it would appear that the final stages of the precipitation reactions are best explained by the slow maturation of the secondary crystalline phase to stoichiometric HAP without further involvement of intermediate phases.

Although the results in Figure 2 suggest that both DCPD and OCP are involved in the spontaneous precipitation of calcium phosphate, they must be viewed with some caution since only one experiment under one set of conditions is presented. It may just be coincidence that an ion product calculated for a solution in equilibrium with a solid phase is similar to that of the solubility product of a given phase. For an ion product to be considered a solubility product it must also remain constant under a wide variety of experimental conditions. As will be discussed in more detail later, in fact, the ion product for DCPD varies considerably with changes in solution calcium phosphate ratio and pH, at all stages in the precipitation reaction. Furthermore, at pHs greater than 8.0, it becomes thermodynamically impossible for DCPD to be formed at any step in the reaction. On the other hand, the ion product for OCP, calculated at the point where the first crystalline phase is formed, remains invariant with experimental conditions at a value close to that reported for pure crystalline OCP.

The fact that the inflections in solution composition with time represent actual solid-solid transformations has been verified by a number of techniques such as X-ray diffraction,^{2,14} surface area measurement (Figure 1) and transmission electron microscopy (TEM) (Figure 3). The formation of the first crystalline phase, as observed by the appearance of well-defined diffraction maxima corresponds exactly to the initial drop in solution calcium and phosphate concentrations. A second discontinuity in the variation of lattice *d*-spacings was not observed, however. Similarly, the specific surface area of the solid phase increases sharply during the amorphous-crystalline transformation and shows a gradual decrease with time thereafter with no additional apparent inflections (Figure 1). Direct examination of the solid phase by TEM (Figure 3) confirms the observations made by the other physical and chemical methodology. Sample isolation times are indicated by the lettered arrows in Figure 1. The spherical morphology of freshly-precipitated ACP is shown in Figure 3a. Solid material isolated at the beginning of the first inflection point is a mixture of amorphous and crystalline material (Figure 3b) whereas that isolated after the inflection contains only the plate-like crystalline material (Figure 3c). Crystals isolated after the second inflection (Figure 3d) do not appear to be markedly different from those of the first formed crystalline phase (Figure 3c) but do appear to be somewhat thicker and smaller in their lateral dimensions than the freshly-formed material. This would be consistent with the overall decrease in surface area observed upon aging of the crystals (Figure 1). Crystals isolated later in the experiment (Figure 3e) are comparable to those isolated immediately after the secondary transformation (Figure 3d). In contrast to the platy crystals isolated at pH 7.40, crystals isolated from a pH 10.0 experiment, immediately after the amorphous-crystalline transformation, have a distinctly different morphology that is needle-like. The reason for this change in morphology at high pH is not certain but one difference between the chemistry of the spontaneous precipitation reaction at neutral and high pH is that a secondary inflection is not observed at high pH (vide infra).

In the following sections, the general characteristics of the spontaneous precipitation reaction described above will be examined more critically. Pertinent data, collected over a wide variety of experimental conditions, will be analyzed in some detail in order to draw conclusions about the mechanism



Figures 3a–f. TEM micrographs of solid samples isolated from spontaneous precipitation experiments performed at 37 °C. Figures 3a–e represent the solid phase isolated from a pH 7.40 experiment at the times indicated in Figure 1. Figure 3f represents the crystalline phase obtained from a pH 10.0 experiment immediately after the amorphous-crystalline transformation. The bar is 1 μ m.

of the reaction and the identity and nature of the solid phases involved. Although the results shown in Figures 1—3 were obtained at 37 °C most of the subsequent experimental evidence to be presented was obtained at 25 °C. The chemistry is the same at 25 °C and the lower temperature provides two advantages: (1) The transformations are slower and thus easier to follow kinetically; (2) More extensive published thermodynamic data is available at 25 °C.

AMORPHOUS CALCIUM PHOSPHATE

It seems well established that the amorphous-crystalline transformation is initiated by the nucleation of the first crystalline phase on the surface of ACP.¹⁵ It is possible that ACP, therefore, plays an important role in the overall mineralization process other than merely providing the lattice ions for the final crystalline phase that makes up hard tissue in vertebrates. In spite of its potential importance in understanding the mechanisms of calcification, the structure and composition of ACP is not well understood. This is due primarily to the absence of long-range periodic order making ACP difficult to study by conventional diffraction and spectroscopic techniques. Chemical studies indicate, however, that purified ACP does have a constant composition over a wide range of conditions¹⁶ and radial distribution measurements tend to confirm that, at least, short-range order is present in ACP.¹⁷

An analysis of the solution phase in equilibrium with ACP supports the suggestion that molecular order does exist since a solubility determining unit apparently is formed in precipitated ACP. The existence of a constant ion product over a wide pH range is shown in Figure 4. The best fit of the data assumes a $\text{Ca}_3(\text{PO}_4)_2$ -like molecular composition (not a TCP structural unit) but with the introduction of approximately 10% acid phosphate for structural stability; $\text{Ca}_3(\text{PO}_4)_{1.87}(\text{HPO}_4)_2$. The existence of this amount of acid phosphate in ACP is consistent with results of an earlier chemical study.¹⁶ Similar results (not shown) were obtained at constant pH but with the variation in $\text{Ca} : \text{PO}_4$

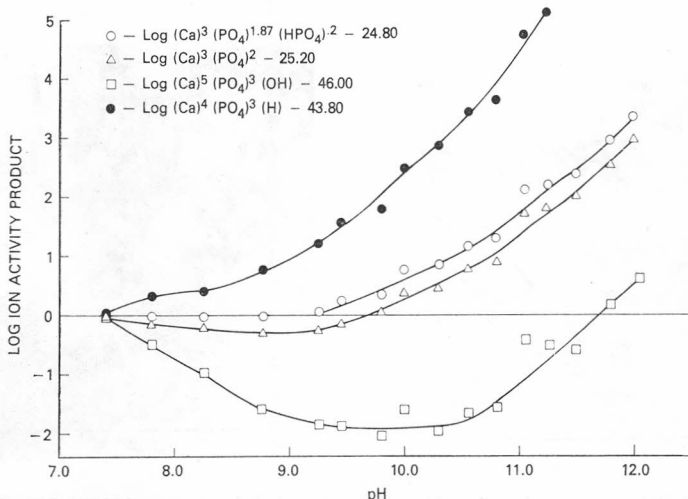


Figure 4. A plot of the log of the ion activity product for the solution phase in equilibrium with freshly formed ACP for various chemical compositions of calcium phosphate as a function of the pH of the precipitation solution, at 25 °C. A constant is subtracted from each product for purposes of presentation.

molar ratio. The integrity of the structure of ACP seems to be maintained in the pH range 7.40—9.25, the region of perhaps most interest in biology. At higher pH the structural unit seems to collapse and it is apparent that no molecular formula, either more acidic or basic than TCP, would result in a constant ion product. Thus, it seems unlikely that a constant ion product could be assigned to any molecular species in the high pH range and it appears that an ACP with a variable structure and/or composition is being formed. This breakdown in structure at high pH is also consistent with the apparent requirement of acid phosphate for the stability of a unique ACP phase.

KINETICS

The kinetics of the amorphous-crystalline transformation can be used to predict the composition of the first formed crystalline phase since the transformation seems to proceed by classical heterogeneous nucleation phenomena.¹⁵ It is well established that nucleation, involving an induction period, can be empirically described by an equation of the form:

$$I C^p = \text{Constant}$$

in which I is the induction period for the nucleation, C is the ionic concentration product of the nucleated phase, and p is an integer, usually one.¹⁸⁻²¹

A plot of $\log IC$ vs pH is presented in Figure 5 using ionic concentration products for three crystalline phases HAP, TCP, and OCP, which can be formed in this pH range. DCPD is not considered a possible component of the first formed crystalline phase, since it is thermodynamically unstable in this pH range. It is obvious that, in the pH range 7.40—9.25, only a nucleating species with the composition of OCP is consistent with the empirical nucleation equation. An identical, invariant, IC product was obtained for experiments performed at constant pH but variable $\text{Ca}:\text{PO}_4$ molar ratios (not shown). It should be emphasized, however, that even though this relationship is valid

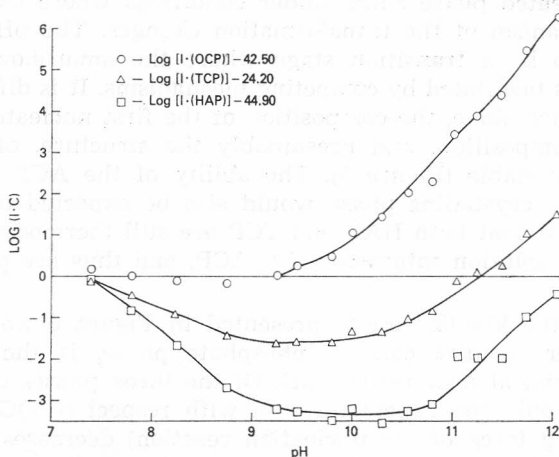


Figure 5. A plot of the log of the product of the induction period for the amorphous crystalline transformation (I) and the ionic concentration product (C) for selected crystalline calcium phosphate phases as a function of solution pH at 25 °C. (ACP), (TCP), and (HAP) represent ion activity products for the phases enclosed in the parentheses. A constant is subtracted from each product for purposes of presentation.

for this system, it is not certain that the first nucleated material is crystalline OCP but only a material with the chemical composition of OCP. At high pH it is obvious that the empirical relationship does not hold for any of the three molecular formulae considered.

The system is further complicated by the fact that, although the induction period increases with increasing pH at the lower pH values, the induction period for the amorphous-crystalline transformation begins to sharply decrease with increasing pH at $\text{pH} > 10.25$ (Figure 6). As shown in Figure 6 this

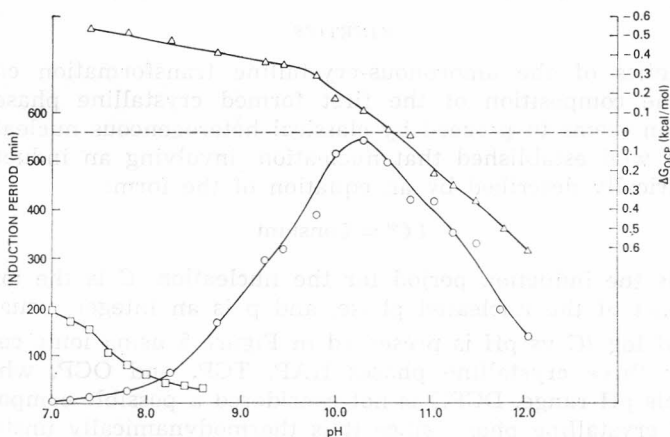


Figure 6. A plot of the variation with pH of solution, at 25°C of the induction period for the amorphous-crystalline (O) and crystalline-crystalline (□) transformations. Also represented is the free energy, ΔG , of the solutions with respect to the formation of OCP (Δ).

reversal occurs at about the same pH that OCP becomes thermodynamically unstable. This would seem to be additional circumstantial evidence that OCP is the first nucleated phase since, under conditions where OCP becomes unstable, the mechanism of the transformation changes. The pH interval 9.25—10.25 seems to be a transition stage where the amorphous to crystalline transformation is facilitated by competing mechanisms. It is difficult to predict, on kinetic evidence alone, the composition of the first nucleated phase at high pH, since the composition, and presumably the structure, of the nucleating agent, ACP, is variable (Figure 5). The ability of the ACP, formed at high pH, to nucleate a crystalline phase would also be expected to vary with pH. It should be noted that both HAP and TCP are still thermodynamically stable at high pH, in a solution saturated with ACP, and thus are possible first-nucleated phases.

Intuitively, the kinetic results presented in Figure 6 would predict that OCP, or another «acidic» calcium phosphate phase, is the first nucleated crystalline material at near neutral pH. Of the three phases considered, HAP, TCP, and OCP, only the supersaturation with respect to OCP (i. e., the free energy or driving force of the nucleation reaction) decreases with increasing pH (Figure 4). Only a decrease in free energy is consistent with an increase in induction period. Secondly, assuming that the secondary crystalline-crystalline transformation observed in Figures 1 and 2 is the hydrolysis of an acidic phase, like OCP, to a more basic phase, the time required for this

transformation to occur should then decrease with increasing pH. This is exactly what is observed as is also shown in Figure 6. The induction period for the completion of the secondary transformation decreased in the pH range 7.00—8.60 and became immeasurably small at higher pHs.

THERMODYNAMICS

The kinetic analysis of the amorphous-crystalline transformation suggests that the first-formed crystalline phase has a composition similar to OCP. As indicated in Figures 1 and 2, at 37 °C and pH 7.4, it also appears that this phase has a solubility similar to that of pure crystalline OCP. The results of a more complete thermodynamic analysis of the solution phase, in equilibrium with the first formed crystalline phase, supports this suggestion. Free energy data, collected at 25 °C and a variety of pHs, for solutions analyzed

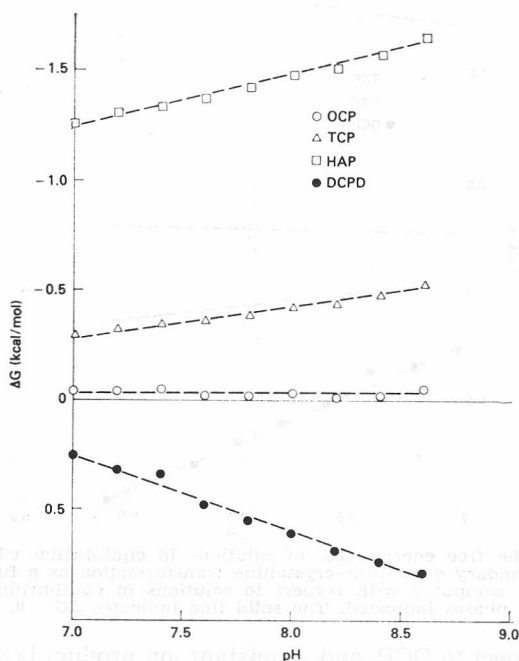


Figure 7. A plot of the free energy, ΔG , of solutions in equilibrium with solid phase immediately after the amorphous-crystalline transformation as a function of pH at 25 °C. The free energies are computed with respect to solutions in equilibrium with the crystalline phases indicated. The solid line indicates $\Delta G = 0$.

immediately after the amorphous-crystalline transformation are shown in Figure 7. The computation of the free energy of the solutions compared to solutions in equilibrium with pure crystalline phase utilized literature solubility data obtained at 25 °C for OCP²², HAP²³, and DCPD.¹³ It is clearly seen that the system, in the pH range 7.0—8.6, has an invariant free energy similar to that of OCP which is strong suggestive evidence that the first-formed phase is indeed OCP. The solubility of this phase is slightly greater than that reported for mature OCP which would be expected for freshly precipitated cry-

stals. In addition, the ion activity product is invariant over the entire range, another requirement for a true solubility product. As seen in Figure 7, none of the other crystalline phases meets these requirements. The solutions are considerably supersaturated with respect to HAP and TCP and undersaturated with respect to DCPD over the entire pH range. Furthermore, a constant ion product is not obtained. Free energy data could not be calculated for higher pHs since the intermediate crystalline phase becomes very unstable at $\text{pH} > 8.6$ (i. e., see Figure 6).

A similar thermodynamic analysis was applied to the solutions, in equilibrium with solid phase, at the end of the secondary crystalline-crystalline transformation (Figure 8). At this point, the solutions are generally under-

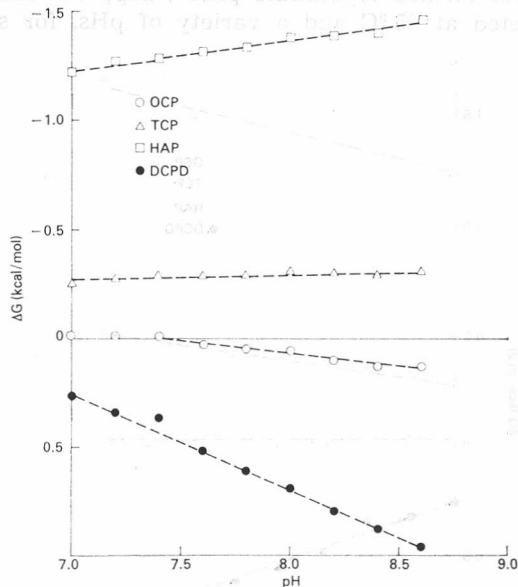


Figure 8. A plot of the free energy, ΔG , of solutions in equilibrium with solid phase at the completion of the secondary crystalline-crystalline transformation as a function of pH at 25 °C. The free energies are computed with respect to solutions in equilibrium with the crystalline phases indicated. The solid line indicates $\Delta G = 0$.

saturated with respect to OCP, and a constant ion product is no longer obtained. A constant ion product is obtained, however, for a solid with the composition $\text{Ca}_3(\text{PO}_4)_2$ but with a solubility greater than crystalline β -tricalcium phosphate. Since no other phase changes have been observed subsequent to the secondary crystalline-crystalline transformation and since HAP is the ultimate solid phase, it is assumed that the material present in the system, at the completion of the final crystalline transformation, is a defect hydroxylapatite (DHA) with a chemical composition similar to that of $\text{Ca}_3(\text{PO}_4)_2$.

CHEMISTRY

The thermodynamic analysis of the spontaneous precipitation of calcium phosphate suggests that OCP is a necessary precursor phase to HAP, at least at $\text{pH} < 9.25$, and that the initial apatitic phase has a TCP-like composition. One way to determine if this assumption is correct is to measure the hydroxyl

content of the precipitates isolated at various stages of the reaction. This has been done, by a recently developed method,⁶ and typical results are shown in Figure 9 for experiments performed at pH 8.25 and 25 °C. As expected, ACP

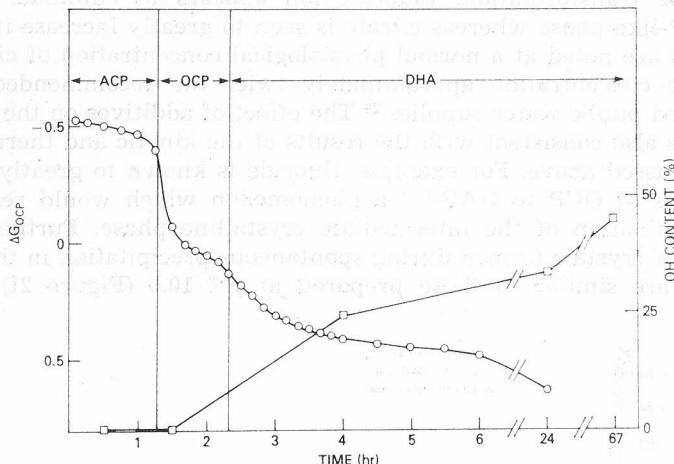
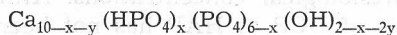


Figure 9. A plot of the hydroxyl content (\square) of solid materials isolated at various times from spontaneous precipitation experiments performed at pH 8.25 and 25 °C. Also plotted is the free energy, ΔG , of the solution phase with respect to a solution in equilibrium with OCP (\circ). Indicated in the figure are the regions of stability of the ACP, OCP, and DHA phases under the conditions of this experiment.

contains no measurable hydroxyl and as predicted, the first-formed crystalline phase, labeled the OCP region in the Figure, also contains no hydroxyl. Only after the crystalline-crystalline transformation of the DHA does the hydroxyl content gradually increase with maturation of the DHA phase, consistent with the suggestion above that the initially formed apatitic phase contains no hydroxyl ions. As suggested in Figure 9 and as observed in a number of other crystal preparations, the hydroxyl content of the solution precipitated apatites asymptotically levels off, with maturation, at about 50% of the hydroxyl content of stoichiometric HAP. Complete chemical analyses on a large number of isolated samples with variable calcium : phosphate ratios and hydroxyl contents have shown that a general chemical formula can, in fact, be employed to describe the chemical composition of the DHAs. This formula,



proposed by Kuhl and Nebergall,²⁴ predicts two types of calcium vacancies. The first or x -type, is coupled to the loss of a hydroxyl and the addition of a hydrogen ion to the lattice and seems to be dependent upon the conditions of preparation. The second, or y -type, is electrically compensated by two vacant hydroxyl positions and seems to be insensitive to solution environment. For unknown structural reasons, it appears that it is the y -type vacancy, accounting for approximately 0.5 calciums per unit cell, which limits the incorporation of hydroxyl to 50% of the theoretical amount.

The influence of chemical species of biological interest on the spontaneous precipitation of calcium phosphate has provided additional insight into the mechanism of the overall precipitation process and into the possibilities for

biological control of the mineralization process. For example, both citrate and fluoride (Figure 10) have a slight inhibitory effect on the amorphous-crystalline transformation but have markedly different effects on the secondary crystalline-crystalline transformation. Fluoride ion appears to eliminate the intermediate OCP-like phase whereas citrate is seen to greatly increase its stability. These effects are noted at a normal physiological concentration of citrate²⁵ and at a fluoride concentration approximately twice the recommended optimum for fluoridated public water supplies.²⁶ The effect of additives on the precipitation process is also consistent with the results of the kinetic and thermodynamic analyses discussed above. For example, fluoride is known to greatly accelerate the hydrolysis of OCP to HAP^{27,28} a phenomenon which would result in the observed elimination of the intermediate crystalline phase. Furthermore, the morphology of crystals formed during spontaneous precipitation in the presence of fluoride⁵ are similar to those prepared at pH 10.0 (Figure 2f) where an

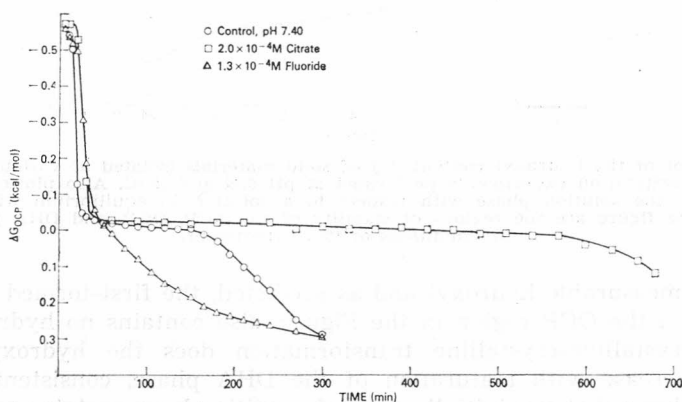


Figure 10. A plot of the free energy of solution, ΔG , with respect to a solution in equilibrium with OCP for kinetic spontaneous precipitation experiments performed at pH 7.40 and 25 °C in the absence and presence of 0.2 mM citrate and 0.13 mM fluoride ion.

intermediate crystalline phase also is not involved. In fact, a platy morphology is usually associated with OCP²⁹ and the retention of this morphology in apatite samples (i. e., Figure 6e) is also consistent with OCP as an intermediate phase. Preliminary results with magnesium ion⁵ indicate that it has an effect similar to citrate at physiological concentrations. Although the influence of citrate on the hydrolysis of OCP to HAP has not been reported it is known that magnesium retards this reaction.²⁸ This is also consistent with the concept of OCP as a necessary intermediate crystalline phase. The marked effects of these substances on the kinetics of the reactions and the morphology of the products provides the means for biological systems to regulate the formation of mineral phase in vivo by altering the chemical environment in which the crystals are formed.

CONCLUSIONS

The overall kinetic and thermodynamic features of the amorphous-crystalline transformation are illustrated in Figure 11, a chemical potential diagram which presents solubility isotherms as diagonal lines computed from solubility products for the well characterized calcium phosphate phases known to be

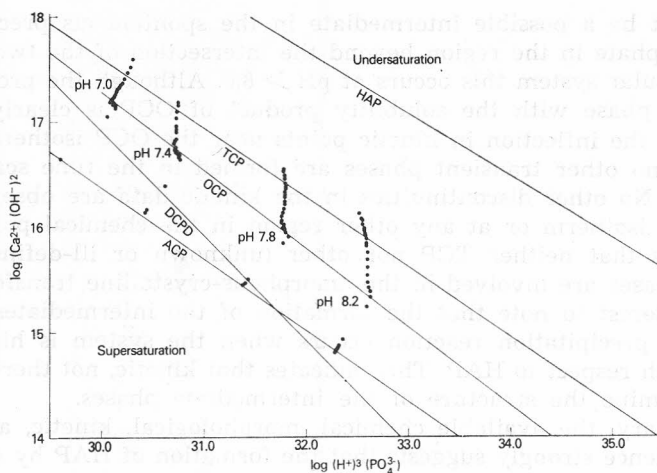


Figure 11. A chemical potential diagram for calcium phosphate computed from the published solubility products for HAP, TCP, OCP, and DCPD and the constant ion product obtained for ACP. Also indicated are experimental points, obtained from four kinetic experiments, at 25 °C and pHs 7.0, 7.4, 7.8, and 8.2, involving the spontaneous precipitation of calcium phosphate. Experimental chemical potentials, within a given experiment, were obtained at 10–15 minute intervals. The spontaneous precipitation reaction begins at the chemical potential of ACP. It is seen that subsequent points are concentrated along the chemical potential line for OCP, particularly at the lower pHs.

formed in physiological systems, HAP, TCP, OCP, and DCPD. Also included in this figure is the solubility isotherm computed from the constant ion activity product for ACP. Plotting experimental data on this type of graph is a convenient means to determine the thermodynamic relationship between a given solution and the formation of various solid phases. For example, an experimental point above an isotherm indicates that the solution is undersaturated with respect to that phase. An experimental point below an isotherm indicates that the solution is supersaturated with respect to that and all other thermodynamically more stable phases. Points near a line suggest that the system may be in equilibrium with the solid phase corresponding to the isotherm.

Data from four different spontaneous precipitation experiments, performed at pH 7.0, 7.4, 7.8, and 8.2, are also plotted in Figure 11. The kinetic as well as the thermodynamic characteristics of each experiment can be noted since all the points were determined at intervals of ten-fifteen minutes. For example at pH 7.0 the first experimental point is located on the ACP isotherm but a number (13) of subsequent points lie near the OCP isotherm. The more points near a given isotherm the longer the system is in apparent equilibrium with that phase. Thus OCP is obviously more stable at pH 7.0 than at pH 8.2 where only a slight inflection is noted in the vicinity of the OCP isotherm. Conversely, the number of points near the ACP isotherm increases from pH 7.0 (1 point) to 8.2 (5 points) consistent with the observed increase in the induction period for the amorphous-crystalline transformation with increasing pH in this range (Figure 6). Other general features of the spontaneous precipitation reaction can be noted by inspection of Figure 11. For example, it becomes clear that ACP becomes thermodynamically more stable than DCPD with increasing pH. Since ACP is the first-formed solid phase, Figure 11 shows conclusively that

DCPD cannot be a possible intermediate in the spontaneous precipitation of calcium phosphate in the region beyond the intersection of the two isotherms. In this particular system this occurs at $\text{pH} > 8.0$. Although the presence of an intermediate phase with the solubility product of OCP is clearly shown in Figure 11 by the inflection in kinetic points near the OCP isotherm, it would appear that no other transient phases are formed in the time scale of these experiments. No other discontinuities in the kinetic data are observed, either near another isotherm or at any other region in the chemical potential plot. This suggests that neither TCP nor other (unknown or ill-defined) calcium phosphate phases are involved in the amorphous-crystalline transformation. It is also of interest to note that the formation of the intermediates (ACP and OCP) in the precipitation reaction occurs when the system is highly supersaturated with respect to HAP. This indicates that kinetic, not thermodynamic, factors determine the structure of the intermediate phases.

In summary, the available chemical, morphological, kinetic, and thermodynamic evidence strongly suggests that the formation of HAP by spontaneous precipitation from aqueous solution in the pH range 7.0—9.25, proceeds via the nucleation of an OCP-like crystalline phase by the initially formed ACP. The ACP formed under these conditions appears to have a constant composition and a well defined structural unit. The OCP-like phase then undergoes a crystalline-crystalline transformation into an apatitic phase with a TCP stoichiometry which is defective in both calcium and hydroxyl positions. Further maturation of the apatitic phase proceeds by the introduction of additional calcium and hydroxyls into the lattice, without the involvement of other intermediate phases, although it would appear that a truly stoichiometric HAP cannot be formed from aqueous solutions at near physiological conditions.

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SAŽETAK

Fazna pretvorba pri spontanom taloženju kalcij-fosfata

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Nedavno prikupljen eksperimentalni materijal upućuje na to da je oktakalcij-fosfat (OCP) nuždan prekursor hidroksiapatita pri amorfnom/kristalnoj pretvorbi, do koje dolazi nakon spontanog taloženja kalcij fosfata u području $7,00 < \text{pH} < 9,25$. Do pretvorbe dolazi uslijed heterogene nukleacije OCP na inicijalno stvorenomu, amorfnom kalcij-fosfatu (ACP). Čini se da i sam ACP ima dobro definiran sastav s molekulskom jedinkom koja određuje topljivost. Mehanizam transformacije mijenja se pri $\text{pH} > 9,25$. Pri visokim pH, OCP postaje termodinamički nestabilan, a struktura ACP se razara.