The Precipitation of Calcium Phosphates in the Presence of Magnesium

George H. Nancollas, Branko Tomažič,* and Mason Tomson

Chemistry Department, State University of New York at Buffalo, Buffalo, New York U.S.A.

The kinetics of growth of calcium phosphate on hydroxyapatite (HAP) seed crystals was studied at 25 °C and 37 °C and at constant physiological pH in stable supersaturated solutions of calcium phosphate containing various concentrations of magnesium ion. The pH was maintained constant by means of the pH-stat controlled addition of base and the growth was followed by analyzing the solutions for calcium, phosphate and magnesium. Grown material was characterized chemically, by subsequent dissolution kinetic experiments, by infrared spectroscopy and by specific surface area (SSA) measurement. Magnesium ion reduces the overall rate of crystallization by stabilizing the precursor formed initially in the growth runs. Whereas the stoichiometry of the first formed surface phase corresponds to that of octacalcium phosphate (OCP) in the absence of magnesium, lower molar Ca/P ratios, possibly a mixture of dicalcium phosphate dihidrate (DCPD) and OCP are indicated in its presence. The inhibitory effect of magnesium on crystal growth of HAP and DCPD and some other inorganic compounds is described and discussed in terms of adsorption of magnesium ion at active growth sites and stabilization of growth precursor phases.

One of the more important methods for the removal of phosphate from waste waters is by precipitation as calcium phosphate under slightly alkaline conditions[1,2]. In previous studies, it has been shown that the crystallization of the final thermodynamically stable hydroxyapatite phase, $Ca_5(PO_4)_3OH$, (HAP), takes place through the formation of a number of precursors[3-5]. Although there remains considerable uncertainty as to the nature of the phases formed in the early stages of the calcium phosphate precipitation reactions, the results of seeded crystal growth experiments at physiological pH strongly point to the formation of octacalcium phosphate $(Ca_4H(PO_4)_3$, OCP) which undergoes subsequent transformation to the thermodynamically stable HAP[4,5]. At high supersaturation levels, the results of Posner[6] have indicated an approximate composition for the precursor phase corresponding to TCP while Brown[7] has compiled considerable x-ray evidence for the existence of OCP as a possible precursor phase. The suggestion that DCPD may be the precursor was made by Francis[8] from crystallographic data and other workers[9] have found that DCPD will precipitate at pH values as high as 7.0 with a subsequent rapid hydrolysis to HAP.

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* »Ruđer Bošković« Institute, Zagreb, Yugoslavia.

The precipitation of calcium phosphates at concentrations typical of those encountered physiologically is of particular importance. In a recent study[4], it was shown that the overall reaction involves not only the formation of different calcium phosphate phases, but also the concommitant dissolution of a thermodynamically unstable phase, corresponding closely stoichiometrically, with the formation of more basic calcium phosphates. The existence of OCP is formed rapidly in the initial stages of the reaction and, when the solution becomes undersaturated with respect to this phase, it undergoes a dissolution with the formation of more basic calcium phosphates. The existence of OCP was clearly demonstrated by the results of dissolution experiments made with the solid phase formed at different stages of the crystal growth reaction[4,5]. It has also been shown that the formation and dissolution of such intermediate phases in the overall growth of HAP crystals are sensitive to factors such as pH, solid surface/solution ratio, ionic strength and the level of supersaturation with respect to the various possible calcium phosphate phases, dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$ (DCPD), OCP and tricalcium phosphate, $Ca_3(PO_4)_2$ (TCP). Although there still remains considerable uncertainty as to the nature of the phases formed in the early stages of the calcium phospate precipitation reaction, it is possible to identify two distinct regions of concentration and pH in which DCPD forms preferentially upon added calcium phosphate seed materials and in which the growth phase is more basic with a molar calcium to phosphate ratio greater than 1.0[10].

In the precipitation of calcium phosphates in most waste waters there is another complicating factor in understanding the course of the reaction. In addition to other impurities, these waters frequently contain magnesium ions at concentration levels of from 1 to 4 mM. The presence of magnesium ion has been shown to markedly retard the rate of calcium phosphate precipitation so that the concentration of phosphate residual increases[2]. Magnesium ion has also been shown to have an inhibiting influence upon the seeded growth of calcium phosphates and the formation and development of the intermediate precursor phases[11]. A number of investigations have been made of the spontaneous precipitation of calcium phosphates in the presence of magnesium ion. Posner and his co-workers[12,13] found that the induction time for the separation of the initial mineral phase was reduced by the addition of magnesium ion which enhanced the formation of the amorphous calcium phosphate precursor phase while inhibiting its maturation to the more crystalline HAP. Other workers suggested that the magnesium ion inhibits the spontaneous nucleation of calcium phosphate but affects the morphology of the precipitate formed[14], but the results of such studies are very difficult to reproduce. By the time that macroscopic particles are visible in the solutions, most of the interesting phase changes accompanying the precipitation reactions have already occurred. It is difficult to separate the nucleation from the concomitant growth reactions taking place. Variations of the size and size distribution of the particles formed make it very difficult to interpret the results in terms of the kinetics of the reactions taking place.

In an attempt to avoid complications due to concomitant nucleation, studies in our laboratory have centered on the seeded growth of crystals in stable supersaturated solutions of calcium phosphate. The results of such experiments are highly reproducible enabling quantitative studies to be made of the effects of factors such as the level of supersaturation, seed crystal

morphology, and the concentration of added magnesium ions. In order to understand the course of the precipitation reactions, it is important to calculate the levels of supersaturation with respect to each of the possible calcium phosphate phases. This can be expressed as a free energy term given by equation (1)[4]

$$\Delta G = - RT \ln IP/K_{sp} \tag{1}$$

In equation (1), K_{sp} is the thermodynamic solubility product of the phase of interest, and IP corresponds to the activity product in the calcium phosphate solution at any instant. Thus for DCPD and HAP, these values are defined by equations (2) and (3)

$$IP_{DCPD} = [Ca^{2+}] [HPO_4^{2-}] f_2^2 \tag{2}$$

$$IP_{HAP} = [Ca^{2+}]^5 [PO_4^{3-}]^3 [OH^-] f_1 f_2^5 f_3^3 \tag{3}$$

Where f_z is the activity coefficient of a z-valent ion. At pH values less than about 5.6, and at supersaturation levels of DCPD corresponding to ΔG (DCPD) = 0.4 kcal mole^{-1}, the growth of DCPD seed crystals has been shown to follow a kinetic equation in which the rate is proportional to the square of the supersaturation[15]. Under these conditions, no evidence is found for the formation of a more basic calcium phosphate phase despite the fact that the solutions are frequently considerably more supersaturated with respect to phases such as HAP. Clearly kinetic factors are of overriding importance in the precipitation processes.

At physiological pH levels of about 7.4, and at relative supersaturations corresponding to ΔG(HAP) and ΔG(DCPD) of about 15 and 0.3 kcal mole^{-1} respectively, there is little evidence for the formation of DCPD. Crystal growth takes place through the formation of a more basic phase. On addition of seed crystals of HAP to the solutions, the variation of total calcium molar concentration with time is quite different from that for DCPD seed crystals at the lower pH. This is clearly seen in Figure 1. Whereas in the latter case there is a monotonic decrease of calcium concentration as a function of time, at physiological pH there is a marked inflection at about 120 minutes reflecting a more complex kinetic process involving the formation of the precursor phases and their subsequent transformation to the thermodynamically stable HAP. The effect of temperature on the observed inflection is particularly interesting. Some preliminary experiments in our laboratory have shown that at 10 °C the effect was much more pronounced becoming less and less as the temperature was raised until at 37 °C it was no longer detectable.

When seeded crystal growth reactions are made in the presence of foreign ions, these may accelerate the rate of growth owing to the formation of less soluble phases such as fluorapatite. Alternatively, they may retard the rate of growth of crystals as a consequence of increasing the ionic strength of the solution and hence the solubility of the calcium phosphate phases, or by complexing with the calcium or phosphate lattice ions thereby preventing them from precipitating (magnesium ion might be expected to fall into this last category). The foreign ions may also be adsorbed at the growth sites on the crystal surface thus retarding the reaction rate. There are many examples of this last category of additives including various scale inhibitors such as the condensed phosphates, and more recently the organic phosphonates which mar-
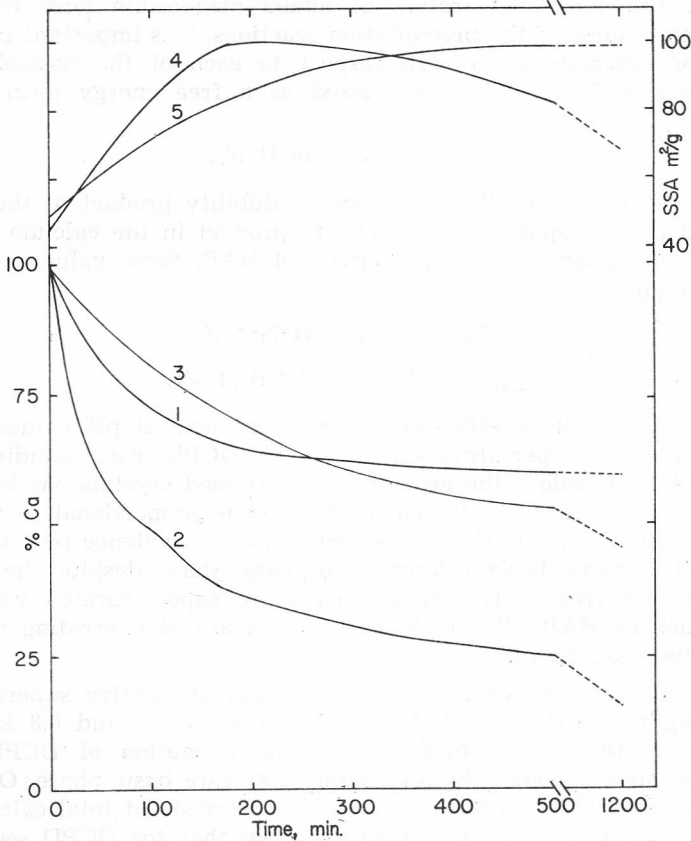
Figure 1. Growth curves 1) DCPD seed crystals in calcium phosphate solution; total calcium = = 3.989 mM total phosphate = 8.0 mM, pH = 5.6. 2) HAP seed, [Ca] = 1.447 mM [P] = 0.900 mM pH = 7.4 3) HAP seed [Ca] = 1.547 mM [P] = 0.944 mM pH = 7.4 [Mg] = 1.0 mM. Specific surface area (righthand ordinate) plotted against time. 4) in the absence of magnesium and 5) in the presence of 1.0 mM. magnesium ion.

kedly retard the growth of calcium phosphate on HAP seed material[16]. Under conditions of spontaneous precipitation, the additives may also supress the development of crystalline phases resulting in the formation of amorphous calcium phosphate.

Experiments have been made at 25 °C and 37 °C with pH = 7.4 and total calcium and total phosphate concentrations 0.1 to 3.2 mM and 0.1 to 2.0 mM respectively. The concentrations of added magnesium ion was 0.1 to 10.0 mM. The stable supersaturated solutions were made by the careful mixing of calcium chloride and phosphoric acid solution and all crystal growth experiments were made in a nitrogen atmospehere to exclude carbon dioxide. Seed material consisted of hydroxyapatite crystals prepared as described previously[3], and the pH was maintained by means of the pH-stat controlled addition of base. The reaction was followed by analyzing the solutions for calcium and phosphate. In addition, samples of the solid phases were prepared for specific surface area and scannnig electron microscopic examination[4,5,11].

In order to obtain information about the surface phases, solid samples withdrawn from the reaction mixtures at known times were also subjected to dissolution kinetic studies in order to determine the stoichiometry of the precipitated surface phases[11]. The results of a typical crystallization experiment in the presence of magnesium ions are shown in Figure 1. It can be seen that the overall rate of crystallization is markedly reduced in the presence of magnesium ion and the characteristic inflection in the growth curve in the absence of magnesium is not observed when this ion is present.

In Table I, the molar calcium to phosphate ratios of the precipitated phase are compared at various times. It can be seen that the presence of magnesium ion has little influence on the stoichiometric ratio for crystal growth on HAP seed. In addition, the $\Delta Ca/\Delta P$ ratio at extended times remains low in the presence of magnesium ion and does not show the general increase which in the absence of magnesium reflects the solid phase transformation to HAP with a 1.67 $\Delta Ca/\Delta P$ molar ratio. This suggests that magnesium stabilizes the calcium phosphate phase or phases formed in the early stages of precipitation reactions, a conclusion which is in accord with the suggestion made by Newesley[18] that magnesium stabilizes TCP in the precipitation of hydroxyapatite.

The precipitation reactions at physiological pH are accompanied by marked changes in the specific surface area (SSA) of the solid phase at various times. These are plotted in Figure 1 and it can be seen that in the absence of magnesium ion there is a pronounced maximum at 120 min. of growth. This indicates that the amorphous precursors to HAP crystallization have very large

TABLE I

Comparison of Precipitation and Dissolution Data in the Absence and Presence of Magnesium*

Time min.	[Mg²⁺] = 0			[Mg²⁺] = 1.0 mM		
	Total Calcium/mM	Deposited $\Delta Ca/\Delta P$	Dissolved** Ca/P after 10 min	Total Calcium/mM	Deposited $\Delta Ca/\Delta P$	Dissolved** Ca/P after 60 min
0	1.372	—		1.547		
30	0.890	1.44	1.33	1.424	1.40	1.20
60	0.771	1.45	—	1.358	1.40	—
100	0.688	1.44	—	1.307	1.39	—
160	0.596	1.48	1.35	1.146	1.51	1.26
270	0.520	1.50	1.37	0.965	1.53	1.30
500	0.403	1.51	1.33	0.827	1.45	1.29
1200	0.243	1.52	1.57	0.710	1.40	1.20

* HAP seed crystals added, 188 mg/l.
** Stoichiometric molar ratio (total calcium : total phospate) of material dissolved from grown solids at various times after 60 min of dissolution in 6 mM potassium chloride solution.

SSA values (approx. 230 m² g⁻¹) and the subsequent decrease in SSA reflects the maturation process with improving crystallinity of the calcium phosphate phases. Such phase changes are also reflected in the scanning electron micrographs of the crystals grown at various times[18]. It can be seen in Figure 1. that in the presence of magnesium, the increase in SSA is much less marked and occurs considerably later (~ 400 min) indicating that the development of the more crystalline OCP is inhibited. It is significant that at all magnesium ion concentrations, no evidence for a decrease of magnesium ion concentration with time reflecting up-take of magnesium by the developing calcium phosphate phases was observed. This result is in agreement with previous findings (eg. ref. 17) that in spontaneous precipitation of calcium phosphates in the presence of magnesium, the magnesium ion is excluded from the precipitates formed.

In order to obtain further chemical information about the nature of the early developing phases in the presence of magnesium, dissolution experiments were also made with solid material extracted from the growth runs at various times[11]. Some values of the molar ratios of calcium to phosphate of the dissolving phase as a function of time are shown in Table I. In the absence of magnesium ion, it can be seen that the molar ratios of the surface phases deposited are close to the value of 1.33, required for OCP. This strongly supports the suggestion that OCP is one of the more important unstable precursors to growth. In the presence of magnesium ion, the molar ratios of dissolved material are considerably lower than the value required for OCP indicating again that magnesium ion may stabilize earlier precursor phases possibly consisting of mixtures of DCPD and OCP or DCPD and TCP[11]. Moreover, the low molar ratios Ca/P for dissolution persist for considerably longer periods in the presence of magnesium ion and no evidence is seen for the observed increase at long times of Ca/P in the absence of magnesium ion reflecting the maturation of the calcium phosphates towards hydroxyapatite.

The growth of DCPD seed crystals in stable supersaturated solutions of calcium phosphate under conditions (pH = 5.60) at which this phase is kinetically favored, has also been studied in the presence of magnesium ion (0.1 — 1.0 mM)[19]. At 1.0 mM, magnesium ion completely inhibits the growth of DCPD crystals without any evidence for its incorporation into the DCPD crystal lattice. At lower concentrations of additive the rate of DCPD growth is reduced from its value in pure supersaturated solution but in all cases the stoichiometry of the precipitated phase is close to the $\Delta Ca/\Delta P = 1.0$ required for pure DCPD. This indicates that magnesium ion may be adsorbed at the crystal surface but is excluded from incorporation as was found in the HAP experiments reported in this paper. Similar results have been obtained for the effect of magnesium ion on another calcium salt, calcium carbonate, and these will be reported elsewhere.

Although many substances such as the polyphosphates, organic phosphonates, and other adsorbates retard the rate of crystallization through adsorption at active growth sites on the crystal surfaces, the influence of magnesium ion on the growth of calcium phosphates appears to be quite different in that it stabilizes the growth precursor phase. Unless careful kinetic studies such as those outlined in the present paper are made, however, it is not possible to distinguish between these different types of additives. Studies of spontaneous precipitation reactions confirm the overall retarding effect of the

magnesium ion, but do not tell us anything about the effect of the additive on the various stages of the reaction. The results of our work[11], confirm the inhibiting effect of magnesium on the overall rate of crystallization. It appears that magnesium ion stabilizes the precursor formed initially in the seeded growth runs. Whereas in the absence of magnesium the stoichiometry of the first formed phase corresponds closely to that of OCP, lower molar Ca/P rations reflecting possibly a mixture of DCPD and OCP are indicated in its presence.

In this study it has been shown that the formation and dissolution of the intermediate calcium phosphate phases in the growth of HAP seed crystals can be influenced by changing the concentration of added magnesium ion. It is clearly desirable to use as many physical methods as possible to characterize the solid phases during the precipitation reactions. Changes in specific surface area following the addition of seed material to the stable supersaturated solutions are readily monitored, and are very sensitive to the presence of magnesium ion. The formation of the precursor phase takes place over a considerably longer period in the presence of magnesium ion and this result is strongly supported by the calcium/phosphate molar ratio of the surface material dissolved from the solid phases at various stages of the reactions. The seeded growth method provides a very useful technique for following details of the crystallization reactions both in the solution and solid phases. Recent studies[20] have shown that the nature of the calcium phosphate phases formed on seed material in stable supersaturated solutions of calcium phosphate is sensitive not only to supersaturation, ionic strength, pH and the presence of added metal ions, but also to the amount of seed material used. When there is a need for secondary nucleation of a new calcium phosphate phase on the seed, the overall process can be described in terms of this secondary nucleation together with the accompanying growth of the active sites already present on the seed crystals. It is hoped that the results of these studies will enable the elucidation of the mechanism of the crystallization of calcium phosphates which is of importance not only in biological systems but also in reactions involving natural water systems.

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SAŽETAK

Taloženje kalcijevih fosfata u prisustvu magnezija

George H. Nancollas, Branko Tomažič i Mason Tomson

Studirana je kinetika taloženja kalcijeva fosfata iz stabilnih prezasićenih otopina kalcijeva fosfata, cijepljenih kristalima hidroksiapatita (HAP) pri temperaturi od 25 °C i 37 °C. Proces taloženja odvijao se pri konstantnoj fiziološkoj vrijednosti pH uz pH-statsko dodavanje baze. U toku reakcije vršene su analize sadržaja kalcija, fosfata i magnezija u otopini. Taložni produkti karakterizirani su kemijski, studijem kinetike otapanja, infracrvenom spektroskopijom te određivanjem specifične površine. Magnezijev ion smanjuje ukupnu brzinu kristalizacije, stabiliziranjem prekursora, koji se stvara na početku reakcije. Prva površinska faza, koja se stvara u odsutnosti magnezija odgovara oktakalcijevu fosfatu (OCP), međutim uz magnezij nastaje faza s nižim molarnim odnosom Ca/P vjerojatno smjesa dikalcijeva fosfata dihidrata (DCPD) i OCP. Inhibitorski efekt magnezija na kristalni rast HAP, DCPD, te još nekih anorganskih spojeva diskutiran je kao posljedica adsorpcije magnezija na aktivnim centrima kristalizacije, te stabilizacije prekursorskih faza.

CHEMISTRY DEPARTMENT
STATE UNIVERSITY OF
NEW YORK AT BUFFALO,
BUFFALO, NEW YORK, U.S.A.