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An Understanding of Renal Stone Development in a Mixed Oxalate–Phosphate System

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Abstract

The in vivo formation of calcium oxalate concretions having calcium phosphate nuclei is simulated in an in vitro (37 °C, pH 6.0) dual constant composition (DCC) system undersaturated ($\sigma_{\text{DCPD}} = -0.330$) with respect to brushite (DCPD, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and slightly supersaturated ($\sigma_{\text{COM}} = 0.328$) with respect to calcium oxalate monohydrate (COM, $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$). The brushite dissolution provides calcium ions that raise the COM supersaturation, which is heterogeneously nucleated either on or near the surface of the dissolving calcium phosphate crystals. The COM crystallites may then aggregate, simulating kidney stone formation. Interestingly, two intermediate phases, anhydrous dicalcium phosphate (monetite, CaHPO_4) and calcium oxalate trihydrate (COT), are also detected by X-ray diffraction during this brushite–COM transformation. In support of clinical observations, the results of these studies demonstrate the participation of calcium phosphate phases in COM crystallization providing a possible physical chemical mechanism for kidney stone formation.

Introduction

Studies of abnormal biomineralization processes as in the formation of urinary stones are of considerable importance.¹ More than 50% of calcium oxalate stones contain variable amounts of phosphate, usually apatite, in a “nuclear” location.^{2–5} There is evidence that Randall’s plaques originate as a precipitation of calcium phosphate (CaP) either in the loop of Henle or in the distal tubule.^{6–8} Plaque is interstitial and composed of apatite. Much evidence has shown that apatite is more soluble than brushite at low pH and less soluble at high pH. Brushite, therefore, should be able to transform spontaneously to apatite at high pH, which can possibly explain why apatite is the most likely calcium phosphate salt detected in a unique region of the kidney.^{6–8} It is postulated that brushite can induce the heterogeneous crystallization of

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Supporting Information Available: Materials and methods for DCC crystallization kinetics. SEM characterizations of forming crystallites. X-ray powder diffraction (XRD) analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

CaOx^{9–11} and that supersaturation with respect to brushite may be the fundamental abnormality in calcium oxalate stone formers.¹² Tiselius suggested that crystals of CaP that form high in the nephron undergo dissolution when they are exposed to acidic urine in the collecting duct.¹³ Akbarieh and Tawashi reported that the surface transformation of COT to COM occurs only in the urines of stone formers.¹⁴ Clearly, a better understanding of the relationship between the crystallization and CaP → CaOx phase transformation and their progression to stones is needed. In this letter, a dual constant composition (DCC)¹⁵ method has been used to maintain solution conditions, under-saturated with respect to brushite (CaHPO₄ · 2H₂O, DCPD) and slightly supersaturated with respect to calcium oxalate monohydrate (COM), similar to those in different regions of the urinary tract.¹⁶

The reaction solutions contained calcium, phosphate, and oxalate concentrations of 1.00, 8.00, and 0.08 mM, respectively, at pH 6.0, 37 °C, and 0.15 M (NaCl) ionic strength. The constant composition (CC) method was used to study, individually, concomitant DCPD dissolution and COM nucleation reactions. DCC reactions were initiated by the introduction of known amounts of dry DCPD seed crystals into the reaction solutions. The driving force for either the dissolution of DCPD in undersaturated solution or the crystal growth of COM in supersaturated solutions is due to the change in the Gibbs free energy from the reaction solutions to equilibrium. The relative undersaturation of DCPD and the relative supersaturation of COM are defined by eq 1

$$\sigma = S - 1 = \left[\frac{IP}{K_{sp}} \right]^{1/2} - 1 \quad (1)$$

The ionic products (IPs) are $IP_{DCPD} = a_{Ca^{2+}} \cdot a_{HPO_4^{2-}}$ and $IP_{COM} = a_{Ca^{2+}} \cdot a_{C_2O_4^{2-}}$, in which $a_{Ca^{2+}}$, $a_{HPO_4^{2-}}$, and $a_{C_2O_4^{2-}}$ are the activities of Ca²⁺, HPO₄^{2−}, and C₂O₄^{2−} ions. The solubility activity products, K_{sp} , of DCPD and COM at 37.0 °C are 2.32×10^{-7} and 2.20×10^{-9} mol² L^{−2}, respectively.

Using CC, DCPD dissolution and COM nucleation were monitored separately in the absence of either oxalate or phosphate, respectively. DCPD dissolution was initiated by the addition of 22.5 mg of DCPD seed crystals, and typical plots of titrant volume as a function of time for the dissolution are shown in Figure 1a.^{17–20} The dissolution rate decreased with time during the reaction and eventually approached zero with no detectable dissolution even though the DCPD crystals, which were confirmed by X-ray diffraction (Figure 1a inset), remained in the under-saturated solutions. This result can be rationalized by the following argument. Dissolution is usually assumed to be a diffusion-controlled process with the transport of lattice ions away from the crystal surface as the rate-limiting step. Figure 1b shows that the dissolution of DCPD seed crystal began with the creation of unit pits and continued with the spreading of their stepwaves. However, only the larger pits (of size greater than a critical value r^*) are active, with stepwaves contributing to dissolution with spreading velocities decreasing with decreasing crystallite size^{17–22}

$$R(r) \approx R_{\infty} \left(1 - \frac{r^*}{r} \right) \quad (2)$$

In eq 2, R_{∞} is the velocity of dissolution steps at $r \rightarrow \infty$, and r^* is the critical radius of nuclei. When the dissolving crystallite size is of the same order as r^* , no large active pits can be present

on the surface. The low R values and density of active pits on the small crystallite surfaces account for dissolution rates that are too low to be detected experimentally.

It is well known that homogeneous nucleation in a pure supersaturated solution may not occur immediately following its preparation. Rather, a period of time, the “induction time” τ , elapses before new crystallites can persist in the supersaturated solutions. Although the induction period is a complex quantity, if the simplifying assumption is made that τ is dependent only on the nucleation process, then it can be described by eq 3,²³

$$\ln \tau \propto \left\{ C_1 + C_2 \frac{\gamma_{\text{SL}}^3}{k^3 T^3 [\ln(1+\sigma)]^2} \right\} \quad (3)$$

where C_1 and C_2 are independent constants and γ_{SL} is the interfacial energy. Heterogeneous crystallization including the epitaxial overgrowth of one crystalline phase upon another can reduce the critical supersaturation at which the nuclei reach critical size and thereby τ . In COM nucleation experiments, the metastable reaction solutions remained crystal-free for 3600 ± 100 ($n = 4$) min (Figure 2, solid black line). XRD spectra confirmed that the crystalline phase that formed was pure COM (Figure 2 inset).

Using DCC, both DCPD dissolution and COM nucleation were monitored simultaneously. The red and green curves in Figure 2 represent the brushite dissolution and growth of COM, respectively. In the presence of DCPD, the nucleation of COM was promoted, and the induction period was reduced to 300 ± 20 ($n = 4$) min. This suggests that the spontaneous dissolution of DCPD produced a relatively high supersaturation with respect to calcium oxalate by increasing the local concentration of calcium at the surfaces of the DCPD crystallites. The nucleation of calcium oxalate could thus take place either by epitaxis on the surface of the dissolving DCPD crystals or by precipitation near these surfaces. It can be seen (Figure 2) that under the same solution conditions the DCC and CC curves of the DCPD dissolution are identical, indicating that the reaction is not altered in the presence of oxalate.

Figure 3 shows the X-ray diffraction patterns of the solid-phase samples, indicated in Figure 2 as stars A, B, and C, separated by filtration at different stages. An unexpected calcium phosphate phase, monetite (calcium hydrogen phosphate, CaHPO_4 , Figure 3b) was produced during the dissolution of DCPD in the presence of oxalate. Because this phase was not present in the seed material (Figure 3a), it must be regarded as an intermediate phase formed during the DCPD dissolution. It is interesting to note that Duff reported that the removal of the water of hydration from the crystal lattice of brushite resulted in brushite–monetite transformation in the presence of fluoride ions.²³ At the DCPD–solution interface, continuous dissolution at $\sigma = -0.330$ enhances the concentrations of Ca^{2+} and HPO_4^{2-} ions on the dissolving crystallite surfaces. A slow dissolution for DCPD faces will be followed by a surface nucleation mechanism due to its relatively high surface energy.²⁴ Thus, a new thermodynamic equilibrium could be reached with monetite as a precipitate. This phenomenon is consistent with the work of Berényi, who suggested that monetite crystals may appear first, later transforming into apatite during the formation of the calculi.²⁶

The solid phases sampled at 300 min in the DCC experiment (Figure 3c) consisted of 72.8% calcium oxalate monohydrate with 13.6% monetite and 13.5% calcium oxalate trihydrate (COT).^{27,28} If the nucleation and growth of calcium oxalate follows the Ostwald–Lussac law, then COT would be expected to be the hydromorph most likely to form as an initial crystal nucleus (eq 4). Our previous study^{18–20} showed that the dissolution rates of the calcium oxalate hydrates differ markedly, with first-order rate constants, k , at relatively high undersaturation

in the order $k(\text{COT}) > k(\text{COD}) > k(\text{COM})$; transformation of the higher hydrates to COM was considerably more rapid for COT than for COD. Therefore, it is possible that COT nucleates initially, followed by a period of crystal agglomeration in which a solid-state transformation from COT to COM takes place (eq 5). Crystals of calcium oxalate monohydrate are then in contact with a slightly supersaturated calcium oxalate solution and consequently undergo solution growth according to eq 6.

In the area of urolithiasis, some investigators believe that COT is the most unstable polymorph and if it did form it would rapidly convert to COM. However, Heijnen et al. have shown that COT does persist and can be found in about 30% of all stones where COM is the principal constituent.^{29–31} Calcium oxalate dihydrate (COD) is not a factor in the present investigation, and no evidence has been found to suggest that this phase forms as a result of transformation from less stable higher hydrates.³²

In conclusion, the present studies clearly demonstrate that brushite crystals may serve as a very effective substrate for the heterogeneous nucleation of COM at a level of supersaturation below that required for spontaneous precipitation. During brushite dissolution, monetite is also observed by X-ray diffraction. It is demonstrated that phase transformations of DCPD to monetite and COT to COM occur under these in vitro conditions. The study successfully simulates the formation of calcium oxalate stones having calcium phosphate nidi and also indicates that heterogeneously nucleated/aggregated calcium oxalate either on or near the surface of the dissolving calcium phosphate crystals can lead to kidney stone formation.

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Figure 1. DCPD dissolution. (a) CC plot of titrant volume against time of DCPD dissolution in the absence of oxalate. X-ray diffraction patterns (inset) show that the undissolved phase is pure DCPD. (b) AFM image of DCPD dissolution in the absence of oxalate.

Figure 2.

DCC crystallization plots in a mixed solution of calcium phosphate ($\sigma_{\text{brushite}} = -0.330$, red line) and oxalate ($\sigma_{\text{COM}} = 0.326$, green line). Stars mark the different stages at which the solid samples were taken. The black line shows a typical COM crystallization CC plot of titrant volume as a function of time in the absence of DCPD/phosphate. XRD patterns (inset) indicate that the crystallites after nucleation and growth in the absence of phosphate are pure COM.

Figure 3.

Comparison of the X-ray diffraction patterns for (a) DCPD seeds, a pure phase without other forms of calcium phosphate, (b) following DCPD dissolution in the presence of oxalate. The DCC experimental results showed the presence of an unexpected calcium phosphate phase, monetite (CaHPO_4). (c) Samples taken after 300 min of the DCC experiment showed calcium oxalate monohydrate with trace amounts of monetite and calcium oxalate trihydrate (COT).