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SYMPOSIUM PAPER

Calcium oxalate: calcium phosphate transformations

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Abstract Knowledge of the physical–chemical mechanisms responsible for the crystal growth and dissolution events involved in stone formation might enable the manipulation of thermodynamics in such a way as to increase the solubility of sparingly soluble phases (such as calcium oxalates and phosphates), thereby reducing the driving force for stone formation. This may be accomplished through modification of pH, reduction of supersaturation with respect to nucleating phases, and the presence of key inhibitors. If these modifications are made during the initial stages of crystallite nucleation, they could potentially reduce the participation of phases such as Randall's plaques in stone formation.

Keywords Calcium oxalate · Calcium phosphate · Renal stones · Randall's plaque · Thermodynamics

Renal stones are inorganic crystalline aggregates which contain ~5% organic matrix. Although several combinations of crystals have been identified, the majority of stones are composed of calcium oxalate, predominantly the monohydrate (COM); a small fraction of calcium phosphate phases are also present in these COM stones [1]. An elevated calcium level in patients with calcium oxalate stones raises the degree of saturation with respect to calcium oxalate and calcium phosphate in urine. However, the physical–chemical mechanisms governing the nucleation and crystal growth of these two sparingly soluble calcium-containing phases and the interactions between them have not been fully elucidated. Following food ingestion, changes in urinary pH and flow rate as well as increased urinary calcium and/or phosphate excretion may cause the calcium phosphate concentration products to exceed the solubility values.

The crystal nucleation, growth, and agglomeration of both calcium oxalate and calcium phosphate in urine are strongly influenced by the levels of saturation expressed as a saturation ratio, S, in Eq. 1:

$$S_{\mathrm{HAP}} = \left(\frac{I_{\mathrm{p,HAP}}}{K_{\mathrm{sp,HAP}}}\right)^{1/\nu}, \quad S_{\mathrm{COM}} = \left(\frac{I_{\mathrm{p,COM}}}{K_{\mathrm{sp,COM}}}\right)^{1/\nu} \tag{1}$$

where I_{p} is the ionic activity product, and K_{sp} is the thermodynamic solubility product (Table 1). Usually, precipitation does not occur as soon as the solutions become supersaturated ($S > 1$), but rather the solutions remain metastable until the supersaturations exceed certain critical values which may reflect the presence of inhibitor molecules that delay the formation of solid phases. Thus, for calcium oxalate, this limit may be as high as 10S before spontaneous crystallization occurs. The driving forces (relative supersaturations) for nucleation, σ, are given by Eq. 2 in terms of the activities, α, of the ions:

$$\sigma_{\mathrm{HAP}} = \left(\frac{(\alpha_{\mathrm{Ca^{2+}}}{}^{10})(\alpha_{\mathrm{PO_4^{3-}}})^6(\alpha_{\mathrm{OH^-}})^2}{K_{\mathrm{sp,HAP}}}\right)^{1/18} - 1,$$

$$\sigma_{\mathrm{COM}} = \left(\frac{(\alpha_{\mathrm{Ca^{2+}}})(\alpha_{\mathrm{C_2O_4^{2-}}})}{K_{\mathrm{sp,COM}}}\right)^{1/2} - 1 \tag{2}$$

It is first necessary to consider the possible calcium phosphate polymorphs and calcium oxalate hydrates that may form under typical renal tract conditions. The solubilities

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Table 1 Solubility product constant for physiological phosphate and oxalate biominerals

Phase	Formula	K_{sp} (at 37.0°C)
DCPD	$CaHPO_4 \cdot 2H_2O$	2.32E–07
OCP	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	5.01E–50
TCP	$Ca_3(PO_4)_2$	2.83E–30
HAP	$Ca_{10}(PO_4)_6(OH)_2$	2.35E–59
COM	$CaC_2O_4 \cdot H_2O$	2.28E–09
COD	$CaC_2O_4 \cdot 2H_2O$	4.95E–09
COT	$CaC_2O_4 \cdot 3H_2O$	7.19E–09

of the most important calcium phosphate phases are plotted as a function of pH shown in Fig. 1. It can be seen that brushite (DCPD, dicalcium phosphate dihydrate) is the phase of highest solubility and, following Ostwald's rule of stages, may form first as the least stable phase [2]. In the slightly acidic aqueous environment of urine, brushite may be the thermodynamically most stable calcium phosphate phase that forms [1, 3]. However, in vivo, DCPD may sequentially transform to the other calcium phosphate phases of greater stability (Fig. 1) until the formation of the thermodynamically most stable phase, hydroxyapatite (HAP), is favored [4]. In addition to these well-characterized calcium phosphates, amorphous calcium phosphate (ACP) has been shown to be an increasingly important participant in the precipitation of calcium phosphates under physiological conditions. ACP can be considered to be a hydrated tri-calcium phosphate, $Ca_3(PO_4)_2$, which may form as nano-sized assemblies in most supersaturated solutions of calcium phosphate at near physiological conditions of pH and ionic strength. These will undergo association to form amorphous assemblies of increasing size and stability until they are transformed to mature crystallites of HAP.

The calcium phosphate phases observed in many renal stones may therefore consist of at least five possible phases: DCPD, OCP, HAP, and a number of ACPs. Transformations between these phases are sensitive to the pH changes that undoubtedly occur in vivo. Randal's plaque is a readily available source of phosphate for interacting with the calcium oxalate hydrates found in most renal stones. For the calcium oxalate component, there is also possible transformation involving mono- (COM), di- (COD) and tri- (COT) hydrates. In terms of Ostwald's rule of stages, COT as the most soluble and least stable phase will form first, followed by COD, and then COM, the least soluble and therefore most frequently observed calcium oxalate phase in kidney stones. Frequently, the nucleation and growth of the thermodynamically most stable COM may be inhibited by a urinary component such as osteopontin (OPN), thereby kinetically stabilizing the more soluble COD. In

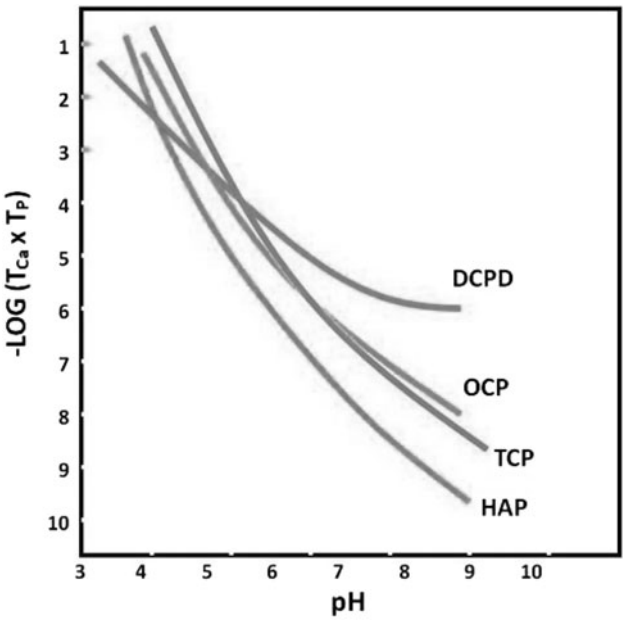

Fig. 1 Solubility of biologically relevant calcium phosphate phases as a function of pH

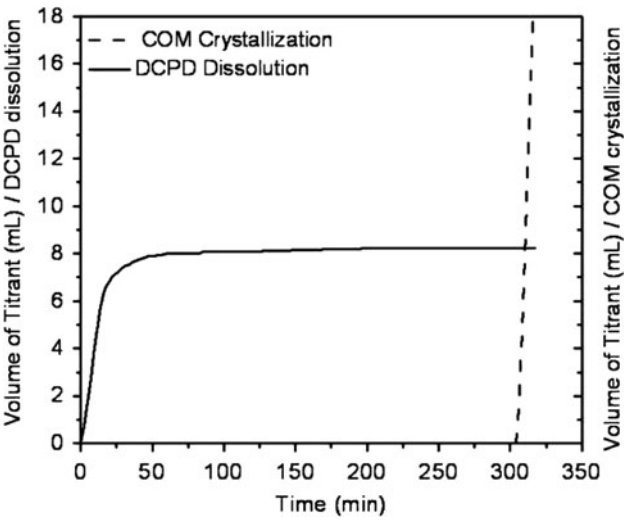

Fig. 2 DCC crystallization plots in a mixed solution of calcium phosphate ($\sigma_{DCPD} = -0.330$) and oxalate ($\sigma_{COM} = 0.326$). XRD data (not shown) indicate that the crystallites after nucleation and growth in the absence of phosphate are pure COM

mixed oxalate/phosphate systems, since the solubility of DCPD ($K_{sp} = 2.32 \times 10^{-7}$ M) is considerably greater than that of COM ($K_{sp} = 2.28 \times 10^{-9}$ M), there is an appreciable thermodynamic driving force for conversion of DCPD to COM. This has been demonstrated kinetically in Fig. 2 which shows the results of a typical dual constant composition experiment in which a mixed solution undersaturated in DCPD but supersaturated in COM comes into contact with a DCPD surface mimicking Randall's plaque.

At time zero, following the introduction of DCPD crystals, the dissolution of DCPD takes place at a rate given by the slope of the curve in Fig. 2; these changes are monitored potentiometrically, and the volume of the titrant needed to maintain the solution composition during the DCPD dissolution reaction is recorded. After 300 min, the dissolution of DCPD has resulted in an increase in calcium concentration which in turn has raised the supersaturation with respect to COM; subsequent COM nucleation then began at 305 min, and the precipitation of this phase occurred rapidly. It should be noted that a new set of titrants, controlled by calcium and pH electrodes, containing both calcium and oxalate ions, is substituted at this stage in order to maintain constant solution composition for crystal growth of COM. In the net result, DCPD is therefore converted to COM, the more stable phase. It is more likely that the phosphates observed in renal stones are a result of this preferred dissolution of brushite, followed by the heterogeneous nucleation and growth of calcium oxalates on the brushite crystals. This is strikingly demonstrated in Fig. 3; thus, brushite crystals may serve as an effective substrate for the heterogeneous nucleation of COM at a level of supersaturation for COM that would not otherwise have led to spontaneous precipitation. The nucleated COM crystallites aggregate to form concretions that could ultimately evolve into kidney stones. Moreover, the COM crystallites will be expected to have brushite cores as is frequently encountered in kidney stones. This series of precipitations constitutes a thermodynamically favored transformation.

The possibility of changing the nature of the calcium oxalate hydrate from COM to COD would be an attractive method for dealing with stone formation in vivo since COD crystallites are much more readily dispersed than COM. COD, however, is less thermodynamically stable than COM, and we must therefore consider the possibility of a contra-thermodynamic change. In Fig. 4, the relative supersaturations of calcium phosphates and COM phases are plotted as a function of pH. It can be seen that the supersaturation with respect to HAP rises dramatically as the pH is increased by very small increments. In contrast, the supersaturation with respect to OCP, COM, and DCPD remain almost unchanged. If HAP dissolved under slightly acidic urine conditions, the resulting pH increase will markedly increase σ_{HAP} resulting in a greater tendency toward re-nucleation of this phase. In contrast, σ_{COM} will remain relatively constant or possibly decrease due to the consumption of calcium with increased HAP formation; this reduction in calcium concentration would ultimately limit the formation of COM. Another route for reversing thermodynamics is to consider the possibility that proteins and other macromolecules present in urine may preferentially inhibit the formation of the more thermodynamically stable hydrate, COM, thus stabilizing more soluble hydrates such as COD. This would constitute an assisted contra-thermodynamic transformation of COM to calcium phosphate phases. A test of these mechanisms still awaits experimental verification.

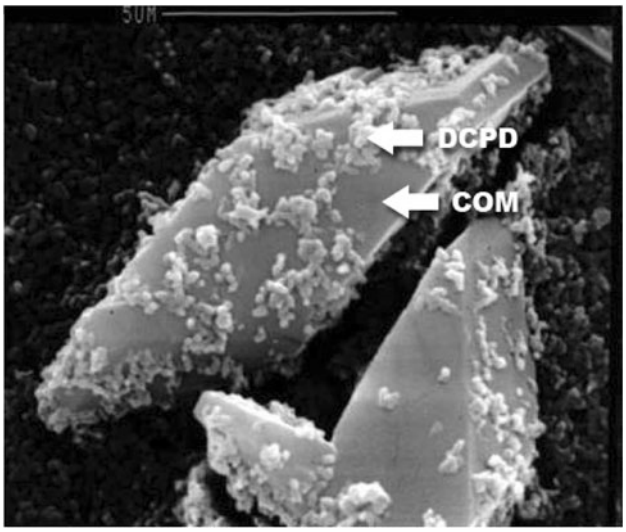

Fig. 3 In a solution supersaturated with respect to COM, added crystallites of DCPD induce the formation of large COM crystals to which they adhere

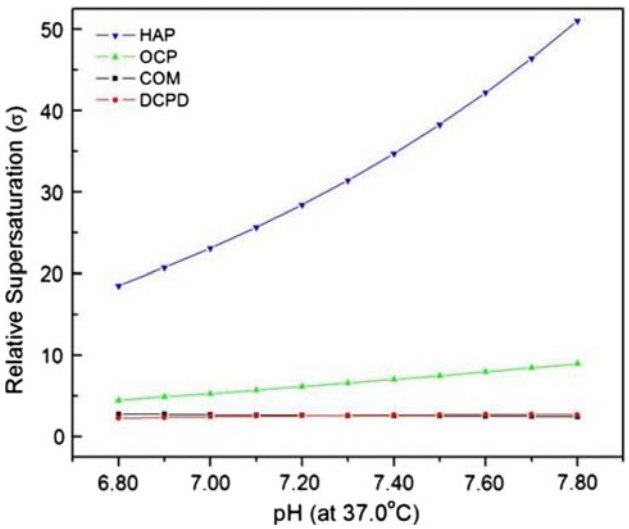

Fig. 4 Influence of pH on the relative supersaturation, σ, of mineral phases

Clearly, Randal's plaques may be intimately involved in solid phase transformations during kidney stone formation. These plaques are sites of interstitial crystal deposition at or near the papillae tips [5]. It is interesting to note that the initial site of formation of these plaques has been reported to be in the basement membranes of the thin loops of Henle where the calcium phosphate supersaturation is greatest. At

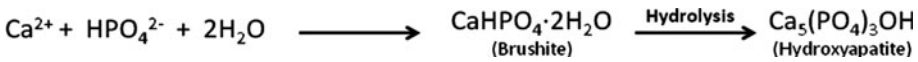

Fig. 5 Simple reaction scheme for the formation of brushite crystals and their subsequent transformation to hydroxyapatite in the basement of the loop of Henle

these slightly acidic conditions, the more likely calcium phosphate renal phase to form would be brushite (Fig. 5). Since brushite is less thermodynamically stable than hydroxyapatite, the resulting conversion to hydroxyapatite over time will account for the presence of HAP in Randal's plaques. However, the increase in pH also raises the relative supersaturation with respect to HAP, thus consuming calcium ions, and decreasing the driving force for COM nucleation and growth. In nature, therefore, biomineralization of the thermodynamically stable phase can be induced by another precipitated inorganic phase which may direct the course of crystallization such as aggregation. In this manner, it is possible to effect a contra-thermodynamic transformation of calcium oxalate to calcium phosphate. Such a process would also be aided by the action of inhibitors that would preferentially reduce the rate of the thermodynamically preferred phase.

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