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Heterogeneous Nucleation with Urate, Calcium Phosphate and Calcium Oxalate (39485)

CHARLES Y. C. PAK,[1] YASAFUMI HAYASHI, AND LEROY H. ARNOLD

Section on Mineral Metabolism, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Renal stones frequently occur as mixtures of two or more mineral phases (1, 2). It has been suggested that the formation of such mixed stones is initiated by heterogeneous nucleation (3-5). This process allows the crystal nidus to cause "overgrowth" of crystals of different chemical composition (6).

Several forms of heterogeneous nucleation have been reported. Brushite ($CaHPO_4 \cdot 2H_2O$), the predominant crystal phase formed in urine upon induction by Ca^{2+} (7) or by organic matrix (8) at pH <6.9, facilitated the nucleation of calcium (Ca) oxalate (9). Meyer *et al.* (5) have shown that both hydroxyapatite and brushite are capable of inducing heterogeneous nucleation of Ca oxalate. Seeds of monosodium urate monohydrate have been shown to induce heterogeneous nucleation of both Ca phosphate and Ca oxalate (3, 4). This process could account for the formation of Ca-containing renal stones in patients with hyperuricosuria, who present no abnormalities of Ca metabolism (10).

The following studies will examine on a physicochemical basis the heterogeneous nucleation which occurs with Ca oxalate monohydrate, uric acid, monosodium urate monohydrate, brushite, and hydroxyapatite.

Materials and methods. The general procedure for the examination of heterogeneous nucleation is as follows. The precipitant (or "seed" of the solid phase) was added to a solution which was metastably supersaturated (9) with respect to the precipitate. Without seeding, such a solution did not allow spontaneous precipitation during the period of the study. The formation of the precipitate was assessed from the change in filtrate concentration of the constituent ions.

The ambient solution was obtained by filtration through 0.05-μm Millipore filters. The following seeds were utilized: brushite (Mallinkrodt), hydroxyapatite (L-apatite) (11), Ca oxalate monohydrate (12), uric acid (Sigma) (3), and monosodium urate monohydrate. Monosodium urate monohydrate was prepared as follows. Ten grams of uric acid were dissolved in 2 liters of hot, boiling 0.031 NaOH solution; pH was kept at 7.2 by titration with HCl or NaOH. The precipitate, which formed upon cooling to room temperature, was "aged" in the same medium at 6°C for 1 week. It was separated by filtration through 0.45-μm Millipore filter, washed with distilled water, and dried at 80°C for 1 day. Direct chemical and X-ray diffraction analysis disclosed pure preparation of monosodium urate monohydrate. The crystal consisted mostly of needles, 8-12 μm in length.

The studies were performed at 37°C under constant stirring with Teflon-coated magnetic bars with the use of freshly-cleaned, new Pyrex glassware (7) at several pHs. Each experiment was run two or three times; results were acceptable only if replicate values agreed within 5%. In the figures presented here, each point represents the mean of replicate experiments.

Heterogeneous nucleation of urate. It was tested for seeds of brushite, hydroxyapatite, and Ca oxalate monohydrate at pH 5.3, 5.7, and 6.7. The test solution at pH 5.3 contained 150 mmole sodium (Na), 50 mmole acetate and 154 mg uric acid/liter. The solution at pH 5.7 had 150 mmole Na, 50 mmole acetate and 360 mg uric acid/liter. The solution at pH 6.7 contained 150 mmole Na, 5 mmole cacodylate and 2560 mg uric acid/liter. These solutions were approximately twofold saturated with respect

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[1] All correspondence should be addressed to Dr. Pak. Supported by UPHS Grant 1-R01-AM16061 and by a grant from Burroughs-Wellcome Co.

to uric acid, since their uric acid concentrations were approximately twice the solubility of uric acid, measured 2 hr after incubation in similar aqueous solutions (13). Heterogeneous nucleation was induced by adding to each solution seeds of brushite, hydroxyapatite or Ca oxalate monohydrate at a solid:solution ratio of 2:1 (mg:ml). The filtrates, obtained at various times after seeding for 2 hr, were assayed for uric acid. The heterogeneous nucleation of uric acid or urate was shown by the fractional change in the filtrate concentration of uric acid.

After completion of the study, the precipitates were collected by filtration in 0.05-μm Millipore filters, and washed in distilled water. They were treated in hot, saturated solution of Li_2CO_3 and assayed for Na and uric acid.

Heterogeneous nucleation of Ca phosphate. It was tested for seeds of uric acid, monosodium urate monohydrate, and Ca oxalate monohydrate at pH 5.3, 5.7, 6.7, and 7.4. The solution at pH 5.3 contained 150 mmole Na, 18.9 mmole Ca, and 9.7 mmole phosphorus (P)/liter. The solution at pH 5.7 had 150 mmole Na, 10 mmole Ca, and 9.4 mmole P/liter. The solution at pH 6.7 was 150 m*M*, 1.35 m*M*, and 10 m*M* with respect to Na, Ca and P, respectively. The solution at pH 7.4 contained 150 mmole Na, 0.92 mmole Ca, and 5 mmole P/liter. These solutions were approximately 3.5-fold (7) saturated with respect to brushite. The solid:solution ratio of uric acid seed was 5:1 (mg/ml); that for monosodium urate monohydrate and Ca oxalate was 2:1. Heterogeneous nucleation of Ca phosphate by seeds of uric acid and monosodium urate was examined from the fractional change in the filtrate concentration of Ca over 6–7 hr following seeding. Because the seed of Ca oxalate could undergo partial dissolution, the heterogeneous nucleation of Ca phosphate by Ca oxalate was monitored by the fractional change in the concentration product of Ca and P of the filtrate. Under the conditions of the experiment, the filtrate concentration of Ca did not increase after seeding.

After the completion of the study, the precipitates were treated in 6 *N* HCl solution and assayed for Ca and P.

Heterogeneous nucleation of Ca oxalate. It was tested for seeds of uric acid, monosodium urate monohydrate and hydroxyapatite at pH 5.3, 5.7, 6.7, and 7.4. The solid:solution ratio for uric acid was 5 mg/ml; that for monosodium urate monohydrate and hydroxyapatite was 2 mg/ml. The test solutions for studies with seeds of uric acid or monosodium urate monohydrate contained 150 mmole Na, 5 mmole cacodylate, 0.4 mmole Ca, and 0.4 mmole oxalate (Ox)/liter. It was approximately sixfold saturated with respect to Ca oxalate monohydrate (12). Heterogeneous nucleation of Ca oxalate was measured from the fractional change in the filtrate concentration of oxalate.

Since hydroxyapatite seed could undergo significant dissolution, the following precautions were undertaken. An excess of hydroxyapatite was incubated to steady-state in aqueous solutions containing 150 mmole Na and 5 mmole cacodylate at pH 5.3, 5.7, 6.7, and 7.4. Appropriate amounts of oxalate as oxalic acid were then added to the filtrates. The solutions were therefore saturated with respect to hydroxyapatite and metastably supersaturated with respect to Ca oxalate. The test solutions at pH 5.3 and 5.7 contained 1.46 mmole of Ca and 0.13 mmole oxalate/liter. The solution at pH 6.7 was 0.28 m*M* and 0.43 m*M* with respect to Ca and oxalate, respectively. The solution at pH 7.4 had 0.14 mmole Ca and 2 mmole oxalate/liter. These solutions were approximately six-, six-, five-, and ninefold saturated with respect to Ca oxalate. The heterogeneous nucleation of Ca oxalate by hydroxyapatite was examined from the fractional change in the filtrate concentration product of Ca and oxalate.

The precipitates obtained after the conclusion of the study were treated with 6 *N* HCl solution, and assayed for Ca and oxalate.

Analytical methods. Uric acid was determined by the uricase method (14), Ca by atomic absorption spectrophotometry, P by the method of Fiske and SubbaRow (15), and oxalate by the technique of Hodgkinson and Williams (16).

Results. Heterogeneous nucleation of urate (Fig. 1). Without seeding, the filtrate con-

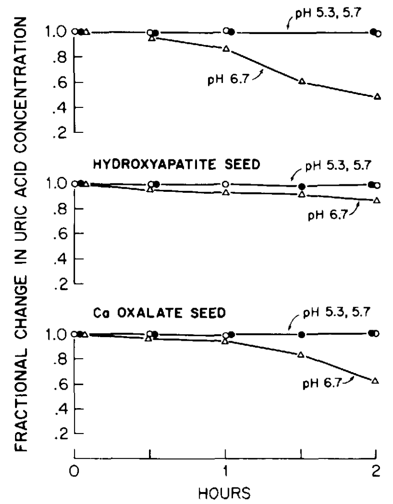

FIG. 1. Heterogeneous nucleation of urate by seeds of brushite, hydroxyapatite, and Ca oxalate. The seeds were added at time zero.

centration of uric acid did not change significantly in any of the test solutions. Seeding of solutions by uric acid (2 mg/ml) caused a prompt fall in filtrate concentrations of uric acid.

Uric acid is relatively stable at pH 5.3 and 5.7; however, it transforms to monosodium urate monohydrate at pH 6.7 in the presence of Na (3, 13, 17). Thus, if heterogeneous nucleation were to occur, the precipitate obtained at pH 5.3 and 5.7 will probably be uric acid, whereas that at pH 6.7, monosodium urate monohydrate. Seeding by brushite, hydroxyapatite, or Ca oxalate (indicated by time zero in the figure) did not cause crystallization of uric acid at pH 5.3 and 5.7, since the filtrate concentration of uric acid did not change. However, at pH 6.7, all three seeds caused a substantial fall in the filtrate concentration of uric acid. The analysis of precipitates disclosed presence in equimolar amounts of Na and uric acid. The results suggest that brushite, hydroxyapatite, and Ca oxalate induce heterogeneous nucleation of monosodium urate, but not of uric acid.

Heterogeneous nucleation of Ca phosphate (Fig. 2). Without seeding, there was no change in the filtrate concentrations of Ca and P in any of the solutions. When these solutions were seeded by brushite or hydroxyapatite (2 mg/ml), the filtrate concentrations of Ca and P decreased promptly. The results indicated that these solutions were metastably supersaturated with respect to Ca phosphate.

At pH 5.3, uric acid seeds were ineffective in inducing heterogeneous nucleation of Ca phosphate, since the filtrate concentration of Ca did not change. Since the pK$_1$ for uric acid has been reported to be 5.47 (17) and the principal cation in solution is Na$^+$, uric acid seeds probably underwent partial transformation into monosodium urate at pH 5.7, 6.7, and 7.4 (13). At these pHs, the filtrate concentration of Ca declined after an initial lag period. The results indicate that monosodium urate, not uric acid, induces heterogeneous nucleation of Ca phosphate. This conclusion was supported by the demonstration that seeding by monosodium urate monohydrate causes a prompt fall in the filtrate concentration of Ca at all pHs. The analysis of the precipitates obtained at pH 5.3, 5.7, and 6.7 revealed presence in equimolar amounts of Ca and P, a finding which indicated the formation of dicalcium phosphate or brushite (7, 18). At pH 7.4, the Ca:P molar ratio of the precipitates was 1.4 ± 0.1 SD. The results suggested conversion of dicalcium phosphate toward apatite (7, 18).

The seed of Ca oxalate monohydrate was effective in inducing heterogeneous nuclea-

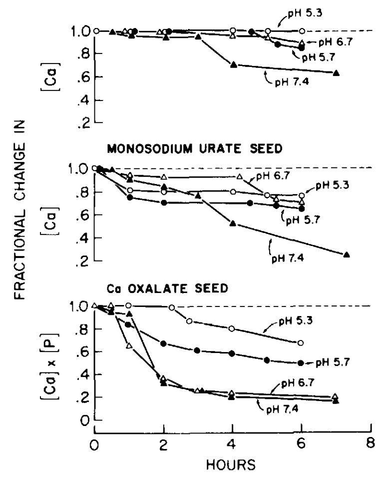

FIG. 2. Heterogeneous nucleation of Ca phosphate by seeds of uric acid, monosodium urate monohydrate, and Ca oxalate. The seeds were added at time zero. Brackets indicate molar concentrations.

tion of Ca phosphate at all pHs, since the concentration product of Ca and P of the filtrate decreased following seeding. However, Ca oxalate did not cause precipitation of Ca phosphate from solutions which were less supersaturated (approximately 2.5-fold saturated rather than 3.5-fold) with respect to brushite.

Heterogeneous nucleation of Ca oxalate (Fig. 3). Without seeding, filtrate concentrations of Ca and oxalate did not change in any of the solutions. The addition of Ca oxalate monohydrate (2 mg/ml) produced a rapid decline in the filtrate concentrations of Ca and oxalate.

Uric acid seed was incapable of inducing precipitation of Ca oxalate at pH 5.3 and 5.7, since the filtrate concentration of Ca did not change. However, at higher pHs in which the conversion of uric acid into monosodium urate may take place (17), seeding by uric acid caused a substantial decrease in the filtrate concentration of oxalate after a lag period of 3-4 hr. The seed of monosodium urate monohydrate was effective in lowering the filtrate concentrations of oxalate at all pHs. This decrease was less prominent at lower pHs, where monosodium urate is less stable (17). The Ca:Ox molar ratio or the precipitates closely approximated one. The results suggested that monosodium urate monohydrate induces heterogeneous nucleation of Ca oxalate, whereas uric acid does not.

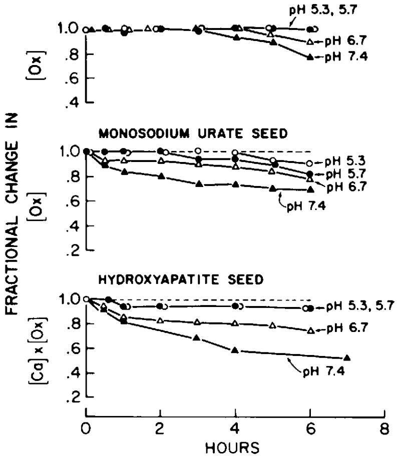

FIG. 3. Heterogeneous nucleation of Ca oxalate by seeds of uric acid, monosodium urate, and hydroxyapatite. The seeds were added at time zero.

Hydroxyapatite seed was effective at all pH's in causing heterogeneous nucleation of Ca oxalate, since the concentration product of Ca and oxalate of the filtrate declined following seeding.

Specificity of heterogeneous nucleation. Identical results were obtained when studies were carried out in siliconized glassware rather than in Pyrex glassware. Ground glass powder (5 mg/ml) did not provoke heterogeneous nucleation in any of the experiments. Although the solid:solution ratio of the precipitants was high, smaller amounts of precipitants often evoked heterogeneous nucleation, though usually to a lesser degree.

Heterogeneous nucleation of Ca oxalate by monosodium urate monohydrate and hydroxyapatite at pH 6.7 and 7.4 was also observed from solutions which were less supersaturated (3.5-fold rather than sixfold saturated) with respect to Ca oxalate.

Discussion. Heterogeneous nucleation requires demonstration that: (a) the solution under consideration is metastably supersaturated with respect to the precipitate (9), (b) the addition of homogeneous nuclei (identical in chemical composition to the precipitate) causes rapid precipitation, (c) seeding by heterogeneous nuclei (different in chemical composition from that of the precipitate) elicits precipitation, and (d) for heterogeneous nuclei which share a common ion with the precipitate, there is no significant dissolution of the nuclei and no consequent increase in the activity product with respect to the precipitate. In this study, these requirements were satisfied in the examination of heterogeneous nucleation which occurred with uric acid, monosodium urate monohydrate, calcium phosphates, and calcium oxalate.

One form of heterogeneous nucleation occurred between monosodium urate monohydrate and Ca salts. Thus, seeds of brushite, hydroxyapatite, and Ca oxalate monohydrate initiated precipitation of monosodium urate, but not uric acid. Conversely, the seed of monosodium urate monohydrate elicited nucleation of Ca phosphate and Ca oxalate, whereas the seed of uric acid did not.

Another form of heterogeneous nucleation, which involved Ca phosphates and Ca oxalate, was identified. The seed of hydroxyapatite induced the nucleation of Ca oxalate. The seed of brushite was previously reported to possess similar action (9). Conversely, the seed of Ca oxalate monohydrate induced precipitation of Ca phosphate. The latter finding contradicts the report that Ca oxalate monohydrate is incapable of inducing heterogeneous nucleation of Ca phosphate (9). This discrepancy may be accounted for by the differences in the metasteability of solutions utilized in the two studies. Thus, heterogeneous nucleation of Ca phosphate by Ca oxalate monohydrate could be demonstrated from solutions 3.5-fold saturated with respect to brushite, but not from those which were 2.5-fold saturated.

The various forms of heterogeneous nucleation described here could explain the formation of certain renal stones of mixed composition. By serving as *promoters* of nucleation, the heterogeneous nuclei may cause precipitation of mineral constituents from urine even though it may be metastably supersaturated with respect to the phases. The full significance of this study must await a better delineation of the physical chemistry of heterogeneous nucleation with the use of other complementary techniques, such as electron probe analysis (19), X-ray diffraction (1), and scanning electron microscopy (5).

Summary. Heterogeneous nucleation may constitute one of the mechanisms for the formation of renal stones of mixed composition. This process allows heterogeneous nuclei to initiate precipitation from solutions which are metastably supersaturated with respect to the precipitate. The seed of monosodium urate monohydrate, but not of uric acid, was capable of provoking nucleation of Ca phosphate and Ca oxalate. Conversely, seeds of brushite, hydroxyapatite, and Ca oxalate caused nucleation of monosodium urate, but not of uric acid. The seed of hydroxyapatite initiated precipitation of Ca oxalate, and vice versa. These physicochemical experiments provide a basis for the explanation of the formation of mixed stones.

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