Calcif Tissue Int (1997) 61:216–222

Calcified
Tissue
International

Crystal Growth and Molecular Modeling Studies of Inhibition of Struvite by Phosphocitrate

A. Wierzbicki,[1] J. D. Sallis,[2] E. D. Stevens,[3] M. Smith,[2] C. S. Sikes[4]

[1]Department of Chemistry, University of South Alabama, Mobile, Alabama 36688, USA
[2]Department of Biochemistry, University of Tasmania, Hobart, Tasmania 7001, Australia
[3]Department of Chemistry, University of New Orleans, New Orleans, Louisiana 70148, USA
[4]Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688, USA

Received: 20 December 1996 / Accepted: 23 April 1997

Abstract. The inhibition by phosphocitrate of struvite crystal formation and growth has been examined in the present study. Crystal growth in a gel matrix was controlled by phosphocitrate in a dose-dependent manner. The effects of inhibition were followed using scanning electron microscopy, optical microscopy, and single crystal X-ray analysis. The presence of phosphocitrate induced very strong, crystal face specific inhibition of struvite, leading to total cessation of crystal growth when sufficient concentration of the inhibitor was made available. Crystal growth studies and results from molecular modeling indicated strong affinity of phosphocitrate to (101) faces of struvite. This in turn led to an alteration in the expression of these faces and the development of a characteristic arrowhead struvite morphology. Similar changes were not observed in the presence of identical concentrations of citrate, acetohydroxamic acid, and N-sulfo-2 amino tricarballylate (an analog of phosphocitrate), emphasizing the unique interaction of phosphocitrate with the struvite crystal lattice.

Key words: Struvite — Inhibition — Phosphocitrate — Crystal growth — Scanning electron microscopy — X ray analysis — Molecular modeling.

Struvite (magnesium ammonium phosphate: $MgNH_4PO_4 \cdot 6H_2O$) is a complex mineral known to assume a number of natural morphological forms including coffin, wedge, short prismatic, or short tabular forms [1, 2]. The crystallite has been reported to precipitate out in animal urines [3], including human, when a urinary tract infection stimulates the formation of ''mixed stones'' or infection stones. Increasing urinary alkalization results from ammonia being generated by the infecting microorganism and together with the abundant presence of the magnesium and phosphate ions, rapidly generates the large, relatively soft stone material. These stones have struvite as the predominant crystallite with a minor component of carbonate apatite [4]. Struvite stones in smaller species, particularly rodents and felines, can in addition be generated from noninfection sources and may occur together with newberyite ($MgHPO_4$). Blockage of the urinary tract and encrustation of indwelling catheters are two important medical problems that can arise as a consequence of struvite mineralization.

Correspondence to: A. Wierzbicki

Controlling the formation and growth of struvite requires not only eliminating the primary source driving the reaction (e.g., by antibiotic treatment to eliminate infection) but also restricting access of ionic species to the growing face of the crystal. Inhibitors such as pyrophosphate and citrate have been used to slow growth [4], suggesting some form of interaction with the crystallite. Strong inhibition and elimination of the recurrence of crystallites, however, is not seen with these compounds.

Acetohydroxamic acid (AHA) and other urea analogs have been used to treat infection-stone patients as they inhibit urease activity and reduce alkalization [5]. Recent evidence suggests that AHA can induce morphological changes in the struvite crystal [6]. However, the problem remains in that elimination of the formation and growth of stuvite on a preexisting nidus is not assured with such compounds, indicating a need for having more powerful chemical inhibitors available.

Phosphocitrate (PC) is a natural biological compound [7] that does offer the potential for restricting both struvite and newberyite formation *in vivo* [8]. In addition to its ability to control the growth of magnesium crystallites, it has been reported to be an active inhibitor of the formation of hydroxyapatite [9, 10], calcium oxalate [11], calcite, and gypsum [12]. Its activity is partly understood to be derived from a stereochemical interaction of its anionic groups, which at pH 7.4 provide a negative charge of −4.0 [13], with the crystal surfaces. Recent molecular modeling studies of binding of PC with calcium oxalate crystal have revealed that PC interacts strongly with the crystal surface and also becomes incorporated into the growing crystal lattice to inhibit crystal growth [13]. We believe that a similar mode of action occurs with the magnesium crystallites and that a distorted crystal morphology and alteration of crystal size as a result of inhibition by PC should occur. The present study links experimental crystal growth data with computer modeling of the morphology expressed when crystals are formed in the presence of limiting concentrations of PC. The interaction of PC with specific faces of the struvite crystal provides insights into why this particular compound is such a powerful inhibitor of this crystal type.

Materials and Methods

Struvite Growth

Crystals were grown in a gel medium as previously described [4]. Sodium silicate 27% (Sigma Chemical Co., St. Louis) was diluted 10-fold in distilled water and 15 ml was added to glass culture

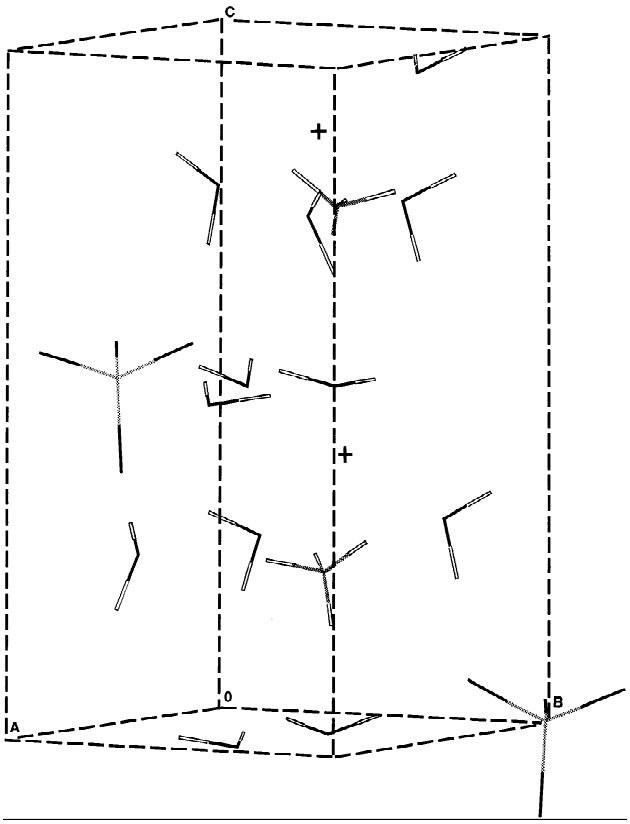

Fig. 1. Unit cell of struvite crystal ($MgNH_4PO_4 \cdot 6H_2O$) determined in ref. [17]. Struvite crystallizes in the orthorhombic space group $Pmn2_1$ with unit cell dimensions a = 6.955 Å, b = 6.142 Å, and c = 11.218 Å. Magnesium ions are represented by crosses, oxygen atoms are drawn as dark sticks, and hydrogen atoms as open sticks.

bottles. Ammonium phosphate (monobasic, 2.5 ml, 0.5 M) was added and the mixture was brought rapidly to pH 7.1 with 4 M acetic acid. The gels were allowed to set for 24 hours. Magnesium acetate (5.0 ml, 0.5 M dissolved in water with or without inhibitor) was then layered on top of the gel. The range of concentrations of PC used in this study varied from 0 to 8.3 mg/ml. After sealing with screw caps, the gels were incubated at 25°C with shaking at 130 oscillations/minute for up to 24 hours. Most data were derived from growth experiments of 10 hours.

Struvite crystals that had settled on top of the gel were gently suspended into the aqueous medium, poured into glass vials, and allowed to settle. The medium was then aspirated off and the crystals were washed once with water and immediately examined by microscopy while crystal surfaces were still smooth. Composition of the crystals was confirmed by IR and chemical analysis.

A gel matrix system was chosen over other systems because it offers the advantage of controlling rate of crystal growth under defined conditions through the slow diffusion of the interacting ionic species, which allows struvite crystals to grow that are large enough for both the scanning electron microscopy (SEM) and X-ray analysis. In the case of struvite, spontaneous homogeneous nucleation will occur when ideal species concentrations are reached in the overlaying medium. Crystals precipitate onto the matrix surface and proceed to grow over time in a controlled manner. We have found that struvite crystals, whether formed in pure aqueous media, in the presence of urine, or developed as described here, all display the same morphological features.

Test Compounds

PC was synthesized and characterized by previously published modified procedures [14, 15]. The monosodium crystalline salt was neutralized to pH 7.1 with NaOH before use.

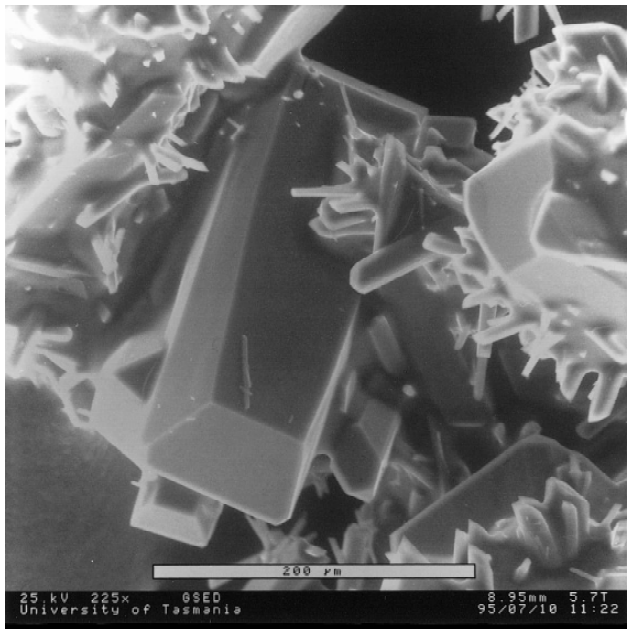

Fig. 2. SEM of typical struvite crystal grown without the inhibitor. Characteristic ''coffin-lid'' shape of the crystal is seen with (001), (101), and (011) faces present. Figure 3 can be used as reference for the labeling of crystal faces. Scale bar is 200 μm.

Crystal Morphology

Crystal structures of control and inhibited forms together with measurements of their respective sizes were obtained using an environmental SEM (ElectroScan Environmental Scanning Electron Microscope 2020 from ElectroScan Corp. of Wilmington, MA). Wet specimens were mounted directly onto stubs and positioned in the electron beam.

Determination of Miller indices of crystal faces of struvite was obtained as follows. A representative crystal of struvite grown with 3 mg/ml of PC was mounted on an Enraf-Nonius CAD4 single crystal automated diffractometer. Crystal orientation and unit cell dimensions were determined from the measured setting angles of 25 reflections. The crystal faces were indexed by orienting the crystal under software control so that each crystal face was placed perpendicular to the X-ray beam. The dimensions of the crystal were measured using a calibrated microscope and the indexing was confirmed by generating a stereographic projection plot of the crystal.

Molecular Modeling

General Procedures. The PC structure has been recently characterized using the *ab initio* methods of computational chemistry (Gaussian92/DFT from Gaussian Inc. of Pittsburgh PA) [13]. This structure agreed remarkably well with the subsequent X-ray analysis [13]. Computational geometry optimizations and charge determination for PC were as previously described [13]. Since for PC in the pH range of 6–8, one of the P-OH, a central COOH (β), one outer COOH (α), and as the pH approaches 8, the second P-OH, are deprotonated, single point calculations were run using SPARTAN (SPARTAN Version 4.0 from Wavefunction Inc. Irvine, CA), an electronic structure computation program to determine the electrostatic charges from the electrostatic potential for a four valent anion of PC [16].

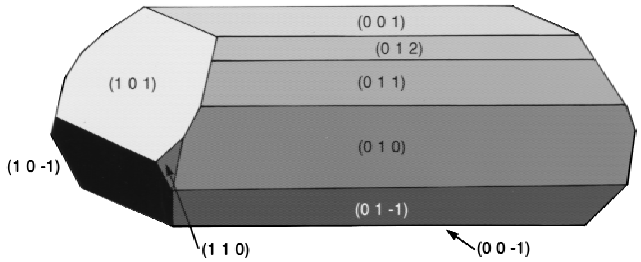

Fig. 3. Computer model of struvite control crystal morphology. Possible, frequently occurring faces are drawn. Not all faces indicated on this figure were expressed in the crystals observed experimentally in this paper.

Models of crystal surfaces of struvite were prepared from the data provided by the X-ray crystallography and neutron diffraction analysis [17] using materials research software Cerius,[2] Version 2.0 molecular modeling software for materials research (Molecular Simulations Inc. of San Diego, CA.). Several slabs of struvite crystal parallel to (011), (012), and (101) surfaces were prepared. Molecular and atomic charges of the surfaces were determined using the charge equilibration method [18]. The general Universal 1.01 force field parameters of Cerius[2] were used throughout the calculations.

Modeling of PC Binding to Struvite. Using Cerius[2] molecular modeling software, the PC inhibitor molecule in the initial conformation was positioned near the struvite surface within the electrostatic interaction range, and the conjugate gradient method of Cerius[2] for the energy optimization procedure was applied. The minimization procedure takes into account the contributions from electrostatic forces, van der Waals forces, hydrogen bonds, bond angles, and dihedral angles to the total energy of the system. Starting from the initial position of PC-surface system, the total energy was minimized yielding the energy and geometry of the most favorable position of PC on the struvite crystal surface. During the minimization, the coordinates of crystal lattice atoms were kept fixed and the inhibitor molecule was allowed to translate, rotate, and adopt any conformation on the struvite surface. The binding energy was determined as the difference between the energy of the PC-struvite system and the sum of the PC and struvite energies when separated beyond the interaction distance.

Influence of Hydration. The effect of solvent was anaylzed by introducing an explicit hydration shell around the inhibitor molecule and repeating the minimization procedure. The final configuration of the inhibitor-crystal system was essentially unaffected by the presence of the solvent since the electrostatic interaction between the struvite surface and the inhibitor was the driving force determining the final geometry of the system. The water molecules were accommodated around the inhibitor, modifying the energy of attraction of the molecule to the surface, but having no major effect on mechanism of binding to the surface [13]. For this reason further consideration was only given to the inhibitor surface system, using a constant dielectric model with dielectric constant equal to unity. To assess the contribution of electrostatic interactions into the binding energy, the procedure of optimization of binding between PC and struvite was repeated for a dielectric constant equal to six, more closely representing the dielectric environment surrounding the growing crystal.

Results and Discussion

Struvite crystallizes in the orthorhombic space group $Pm2_1$ with unit cell dimensions a = 6.955 Å, b = 6.142 Å, and c = 11.218 Å [17]. The unit cell of struvite crystal used for the purpose of modeling is shown in Figure 1.

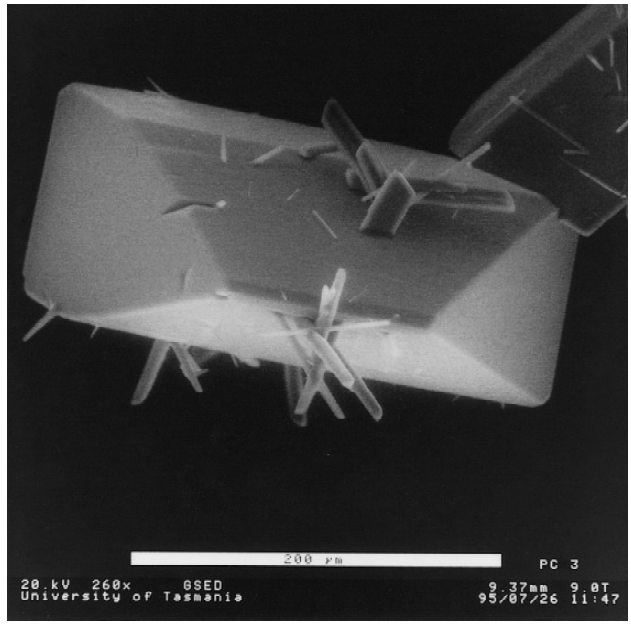

Fig. 4. SEM of struvite grown in the presence of 3 mg/ml of PC, showing well-developed (011) and (101) faces. Figure 6 can be used as the reference for the labeling of crystal faces seen in this figure. Scale bar is 200 µm.

In the absence of an inhibitor, struvites form the well-described ''coffin lid'' shape as they grow along the [100] axis of the crystal [1, 2]. Crystals grown in the gel medium were relatively large, reaching up to 400 µm. Figure 2 shows an SEM image of a representative control crystal of struvite. To identify crystal faces of struvite we used morphology modeling software of Cerius[2]. Figure 3 depicts a computer model of a struvite control crystal morphology showing the most frequently occurring faces [1, 2]. Only some of these faces are expressed in our control system (Fig. 2).

Modeling of struvite morphology was complicated by the fact that struvite belongs to a class of hemimorphic crystals [2], for which the crystal habit does not reflect the full symmetry group of the crystal. This effect cannot be accounted for at the level of currently existing software, so modeling predictions need to be closely correlated with the assignment of faces obtained from X-ray crystallography. The occurrence of different sizes of symmetry-related faces of struvite has been explained using the arguments of surface polarization [2]. The hemimorphic character of struvite is clearly seen in Figure 2 where smaller (001) faces are accompanied by larger (00-1) faces. Also clearly seen are (011) faces with a significantly smaller (01-1). This crystal morphology is completed by a set of (101) faces, again accompanied by much smaller symmetry-related (1 0-1) faces.

Figure 4 shows struvite crystals grown in the presence of an initial amount of 3 mg/ml of PC. Gradual addition of inhibitor induces changes in the crystal morphology that reflect the interactions between the inhibitor and the crystal surfaces at a molecular level and can provide an important insight into the nature of the inhibition mechanism. These changes can be clearly identified using morphology modeling tools since morphological changes at this dosage of the inhibitor are gradual as well. An important issue that had to be solved, however, was whether the faces terminating the top of the coffin lid and converging at the (001) faces, or

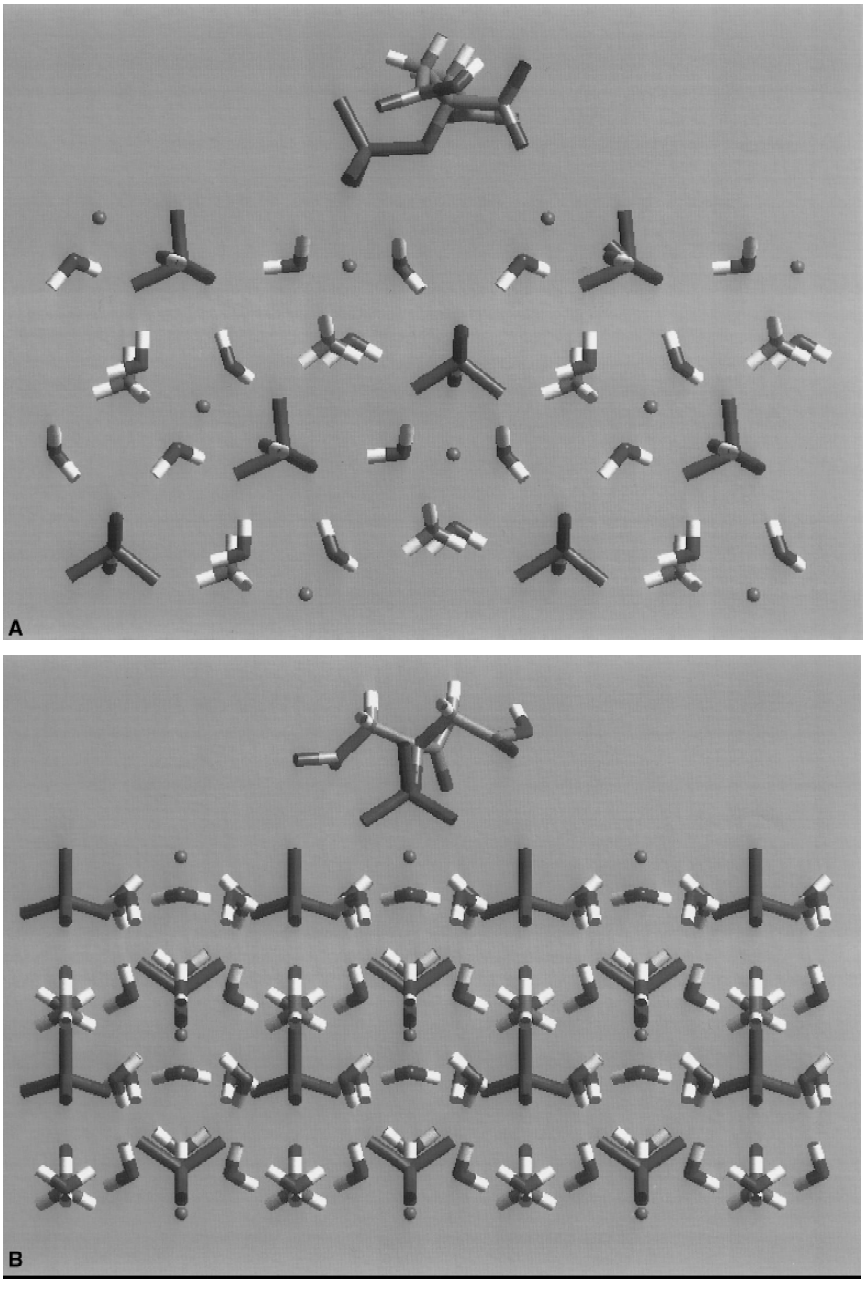

Fig. 5. Computer model of optimized geometry of interactions between phosphocitrate molecule interacting with (011) faces of struvite. Magnesium ions are drawn as small dark spheres, oxygens are dark cylinders and hydrogens are white. The phosphate group of phosphocitrate is in excellent register with the phosphate ions of struvite (011) face below. **(A)** The surface is viewed parallel to the [100] axis. All negatively charged groups of phosphocitrate are participating in binding to the surface. **(B)** View rotated 90° with respect to **(A).**

entirely displacing them, belonged to the (011) or (012) family of faces. Both faces are reported to be expressed in natural and synthetic struvites [1, 2], with the (011) family of faces being expressed more frequently. This issue was resolved by performing a series of morphology simulations of growth of the (011) and (012) faces and carefully comparing the results with SEM images of struvite grown with the inhibitor at the amount of 3 mg/ml of PC. This analysis clearly indicated that the faces seen in Figure 4 belong to the (011) family of faces. In Figure 4, clear reduction to almost total disappearance of (001) faces occurs. Crystals were still elongated along [100] direction and (101) faces were clearly expressed. Also, disparity between (011) and (01-1) was decreased as the (01-1) faces grew in size. Some crystals showed emerging (010) faces. Crystals were still large (300 μm) as the amount of the inhibitor was not significantly affecting the crystal sizes, only modifying the morphology.

In order to understand the preference of PC binding to (011) over (012) and to elucidate the molecular mechanism of inhibition of struvite crystals by PC, we modeled binding of PC to these faces of struvite. The relative binding energies to specific crystal faces indicate the potential for stereospecific recognition and binding between an inhibitor and crystal surface [13, 19]. Inhibitor binding to a specific crystal face in turn leads to an enhanced expression of this face, as it is stabilized and its relative growth rate is decreased, thus modifying crystal morphology. Analysis of PC binding to (011) and (012) faces revealed that PC was not attracted to (012) planes of struvite. Relatively low density of magnesium ions in this plane and the presence of negatively charged phosphate groups in the near vicinity of magnesium ions resulted in the lack of attraction between the PC and (012) faces, thus explaining the absence of (012) faces in struvite morphology as seen in Figure 4. In contrast,

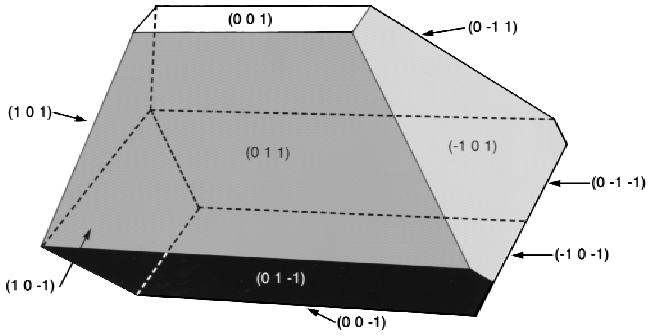

Fig. 6. Single crystal X-ray-derived indexation of struvite crystal grown in the presence of 3 mg/ml of PC. See text for the details.

binding of PC to (011) was relatively strong and utilized both the phosphate group of PC and all negatively charged groups of PC, resulting in a binding energy of −471 kcal/mol (−71 kcal/mol for a dielectric constant equal 6).

Figure 5 shows a computer model of binding of PC to (011) face of struvite. The phosphate group of PC positioned itself in perfect register with the underlying (011) surface of struvite, assuming the position of the incoming phosphate ions of struvite, if crystal growth were to continue in the absence of the inhibitor. This affinity of PC to the (011) family of faces was reflected in modification of crystal morphology seen in Figure 4.

The sizes of struvite crystals grown in the presence of 3 mg/ml of PC were 200–300 μm, similar to these depicted in Figure 4. This was just large enough to afford X-ray assignment of crystal faces. This analysis entirely confirmed the assignment based on morphological and molecular modeling arguments. Figure 6 shows the single-crystal, X-ray: derived morphology for the struvite crystals grown in the presence of 3 mg/ml of PC.

Increasing the concentration of PC to 6 mg/ml resulted in a dramatic modification of both crystal morphology and size (Fig. 7). Characteristic arrowhead morphology now dominated the crystal appearances. The crystals were much smaller in size, reaching dimensions of 100 μm. Modeling revealed that this arrowhead morphology was dictated by dominating (101) faces that frequently converged to form the tip of the arrowhead. Faces (001) were virtually nonexistent. Faces (00-1) were present, often showing twinning parallel to these faces. Faces (011) were well developed with symmetry-related (01-1) faces present as well. The (010) faces were also clearly discernible.

Figure 8 shows the model of calculated morphology of struvite crystal grown in the presence of 6 mg/ml of PC. The crystal faces were indexed using the X-ray Enraf-Nonius CAD4 single crystal automated diffractometer software. To understand the dramatic morphology change of struvite crystals induced by addition of 6 mg/ml of PC, we modeled binding of PC to (101) planes of struvite. As seen in Figure 9, a slab of struvite cut parallel to the (101) surface contains planes rich in magnesium ions coordinated with water molecules. These planes provide a local, positively charged environment perfectly suitable for the binding of the negatively charged PC anions. The PC binding energy to (101) was much stronger than binding to (011), and it was equal to −1403 kcal/mol (−232 kcal/mol for a dielectric constant of 6). Figure 9 clearly shows that the phosphate group positions itself in place of the phosphate ion of the crystal, participating in the completion of the crystal coordination

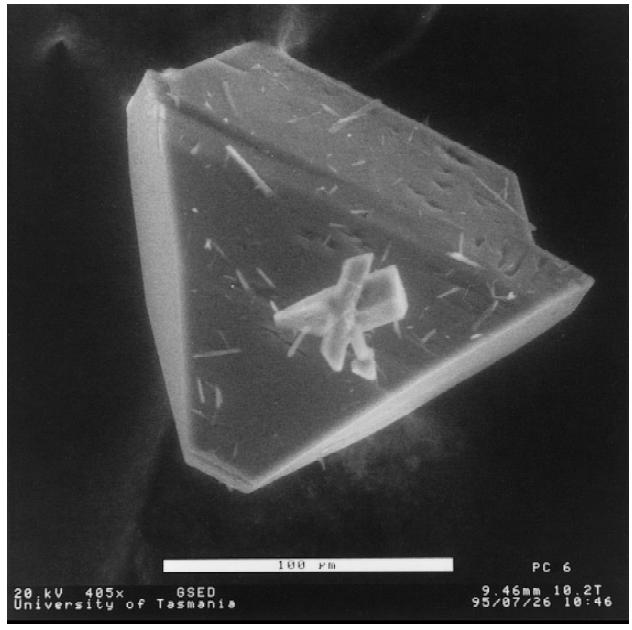

Fig. 7. SEM of struvite grown in the presence of 6 mg/ml of PC, clearly showing the arrow-head morphology of the crystal with dominating, sloping (101) faces. Figure 8 can be used as reference for the labeling of crystal faces. Crystals are much smaller, scale bar is 100 μm.

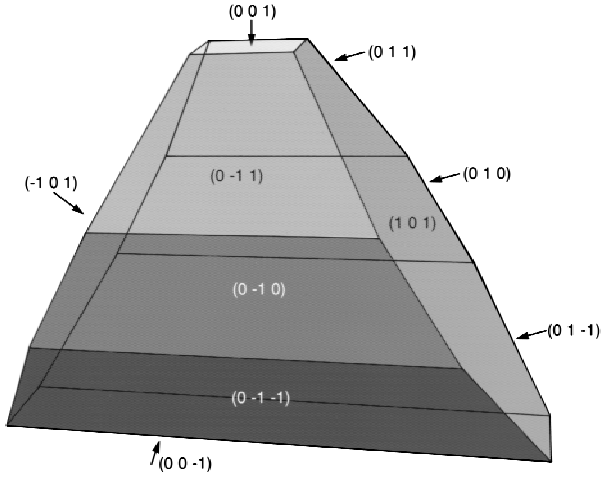

Fig. 8. Calculated crystal morphology of struvite grown in the presence of 6 mg/ml of PC.

polyhedra disrupted during cleavage along (101) plane. PC binding to (101) also involved full participation of negatively charged carboxylate groups.

Binding of PC to (101) and (011) planes was an essential factor in the inhibitory activity of PC. Further addition of PC up to 8.3 mg/ml resulted in even greater reduction of crystal size and number. In fact, no crystals were formed at the highest dose of 8.3 mg/ml of PC.

In additional studies (data not shown), we observed that neither citrate nor an analog of phosphocitrate, N-sulfo-2 amino tricarballylate (SAT), induced comparable change to struvite morphology and size when investigated at levels of 8.3 mg/ml, equivalent to the highest concentration of PC

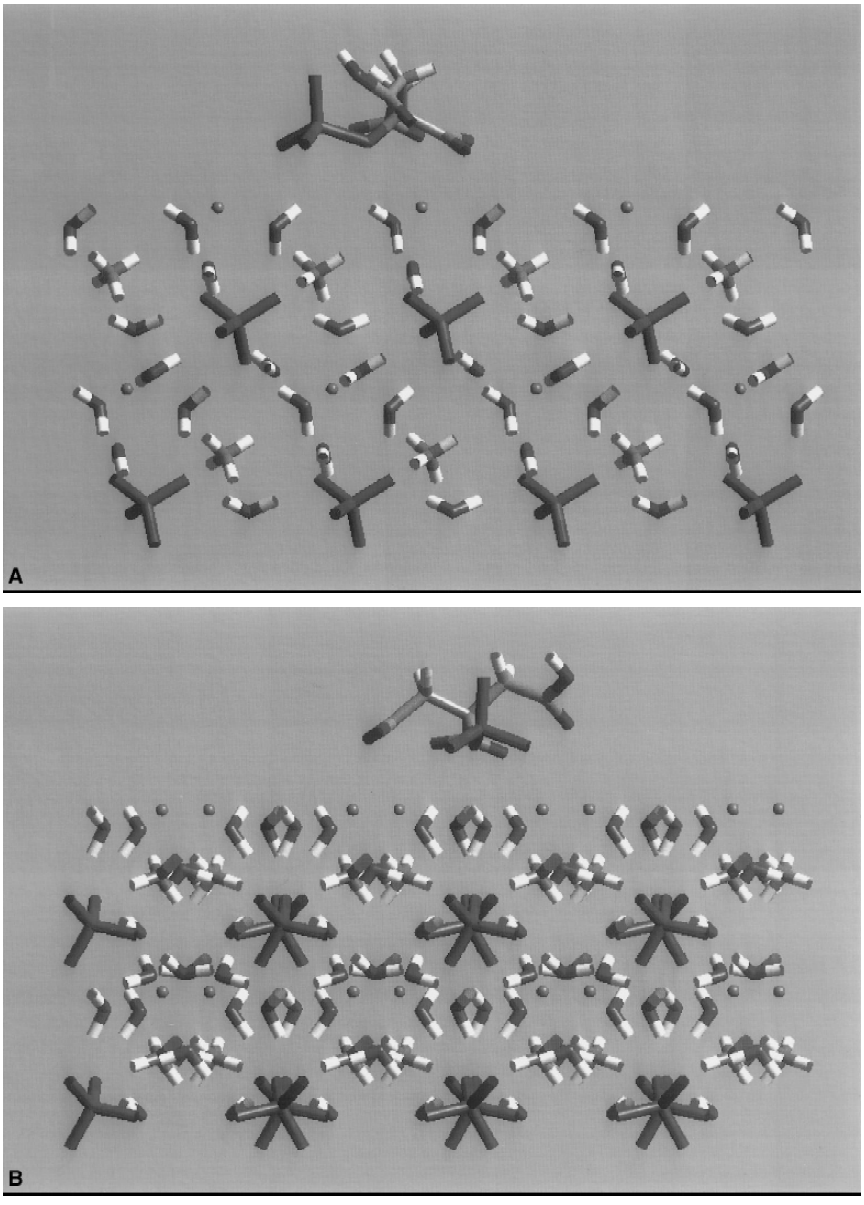

Fig. 9. Computer model of PC interacting with (101) faces of struvite with atoms colored as in Figure 5. **(A)** Viewed in the direction parallel to the [010] axis of struvite crystal. The phosphate group of phosphocitrate is in perfect register with the phosphate groups of the surface. All negatively charged groups of phosphocitrate are particpating in binding to (101) surface. **(B)** View rotated 90° with respect to **(A).**

used in this study. This finding is interesting as both compounds are only slightly less negatively charged than PC. Also, acetohydroxamic acid seemed incapable of significantly affecting struvite growth, although the crystals did appear less bulky and more plate-like, elongating along the [100] direction and sometimes forming characteristic X-shape crystals. This shape seemed to originate from two struvite crystals growing over each other, each of them elongated along its own [100] direction.

Conclusions

In this study we performed crystal growth and molecular modeling studies of PC inhibition of struvite crystals. Strong interference by PC to the molecular structure of the growing crystal resulted in significant modification of crystal morphology, and crystals were much smaller in size and fewer in number. It seems likely that PC not only had a strong affinity for the selective growing faces but also an ability to get incorporated into the crystal lattice. In studies with calcium oxalate crystals, we had observed a strong crystal-PC interaction based on a similar mechanism [13].

We postulate that the strength of PC as an inhibitor of struvite derives from the fact that PC carries a negatively charged phosphate group, which is also a component of the crystal lattice and therefore is readily incorporated into the growing crystal surface. In addition, the relatively rigid framework of the PC molecule bound to the crystal surface creates a sufficiently large disturbance of the local binding environment for incoming struvite ions to disrupt crystal growth perpendicular to the binding plane.

We have concluded from both crystal growth experiments and molecular modeling that the adsorption of PC on (101) planes of struvite is of primary importance to its strong inhibitory activity. Incorporation of struvite on these planes leads to the termination of growth along the [100] direction, resulting in the characteristic arrowhead morphol-

ogy, significant dwarfing of the crystal size, and total termination of crystal growth at sufficiently high dosages. The importance of the phosphate group of PC is due to its contribution to the overall stereochemistry and total anionic charge of the molecule. This makes PC unique as an inhibitor for controlling the formation and growth of the struvite crystal that significantly exceeds the inhibitory activity of other struvite inhibitors such as citrate, SAT, and AHA. *In vivo* and *in vitro* evaluations of the potential of PC in testing struvite stone formation and encrustation on catheters are ongoing [8, 20].

Acknowledgments. We are grateful for support from Research Corporation Grant C-3662, National Science Foundation Grant EHR-9108761, and an ARC Small Grant administered through the University of Tasmania.

References

1. Palache C, Berman H, Frondel C (1951) The system of mineralogy of James Dwight Dana and Edward Salisbury Dana, 7th ed, vol II. John Wiley & Sons, New York
2. Abbona F, Boistelle R (1979) Growth morphology and crystal habit of struvite crystals ($MgNH_4PO_4 \cdot 6H_2O$). J Crystal Growth 46:339–354
3. Griffith DP (1978) Struvite stones. Kidney Int 13:372–382
4. Marickar YMF, Koshy P (1987) Scanning electron microscopic study of effect of vaious agents on urine crystal morphology. Scanning Microsc 1:571–577
5. Griffith DP, Gibson JR, Clinton CW, Musher DM (1978) Acetohydroxamic acid: clinical studies of a urease inhibitor in patients with staghorn renal calculi. J Urol 119:9–15
6. Downey JA, Nickel JC, Clapham L, McLean RJC (1992) In vitro inhibition of struvite crystal growth by acetohydroxamic acid. J Urol 70:355–359.
7. Williams G, Sallis JD (1981) The sources of phosphocitrate and its influential role in inhibiting calcium phosphate and calcium oxalate crystallization. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis, clinical and basic research. Plenum Press, New York, pp 569–577
8. Sallis JD, Thomson R, Rees B and Shankar R (1988) Reduction of infection stones in rats by combined antibiotic and phosphocitrate therapy. J Urol 140:1063–1066
9. Tew WP, Mahle C, Benavides J, Howard JE and Lehninger AL (1980) Synthesis and characterization of phosphocitrate, a potent inhibitor of hydroxylapatite growth. Biochemistry 19: 1983–1988
10. Williams G, Sallis JD (1982) Structural factors influencing the ability of compounds to inhibit hydroxyapatite formation. Calc. Tiss. Int. 34:169–177
11. Sallis JD, Parry NFG, Meehan JD, Kamperman H, Anderson ME (1995) Controlling influence of phosphocitrate in vitro and in vivo on calcium oxalate formation and growth. Scanning Microsc 9:127–136
12. Sallis JD, Juckes W, Anderson ME (1995) PC: potential to influence deposition of scaling salts and corrosion. In: Amjad Z (ed) Mineral scale formation and inhibition. Plenum Press, New York, pp 87–98
13. Wierzbicki A, Sikes CS, Sallis JD, Madura JD, Stevens ED, Martin KL (1995) Scanning electron microscopy and molecular modeling of calcium oxalate monohydrate crystal growth inhibition by citrate and phosphocitrate. Calcif Tissue Int 56: 297–304
14. Williams G, Sallis JD (1980) The synthesis of un-labelled and ^{32}P-labelled PC and analytical systems for its identification. Anal Biochem 102:365–373
15. Pankowsky AH, Meehan JD, Sallis JD (1994) Synthesis via a cyclic dioxatrichlorophosphorane of 1,3-dibenzyl-2-phosphonooxy citrate. Tetrahedron Letters 35:927–930
16. Breneman CM, Wiberg KB (1990) Determining atom-centered monopoles from molecular electrostatic potentials: the need for high sampling density in formamide conformational analysis. J Comput Chem 11:361–373
17. Ferraris G (1986) Neutron diffraction study of $MgNH_4PO_4 \cdot 6H_2O$ (struvite) and survey of water molecules donating short hydrogen bonds. Acta Crystallographica B 42:253–258
18. Rappe AK, Goddard WA III (1991) Charge equilibrium for molecular mechanics simulations. J Phys Chem 95:3358–3363
19. Wierzbicki A, Taylor MS, Knight CA, Madura JD, Harrington JP, Sikes CS (1996) Analysis of Shorthorn sculpin antifreeze protein stereospecific binding to (2-1 0) faces of ice. Biophys J 71:8–18
20. Wills LH, Sallis JD, Smith ME (1996) In vitro prevention of struvite encrustation on catheter material with phosphocitrate. In: Pak YC, Resnick MI, Preminger GM (eds) Urolithiasis 1996. Millet the Printer, Inc. Dallas, pp 234–235