JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 8 (1997) 247–250

Crystal morphology and texture in calcium oxalate monohydrate renal calculi

A. MILLAN*
RIM, Laboratory of Solid State Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

The morphology of calcium oxalate monohydrate crystals in renal calculi has been studied. Morphology modifications with respect to equilibrium morphology have been explained from the structure of (1 0 0) crystal faces and the effect of polyelectrolytes present in urine. The texture of the stones has been considered as the result of both primary aggregation and crystal aggregation produced by anionic macromolecules. The arrangement in concentric layers of crystals with a radial disposition has been related to a process of intense nucleation induced by anionic macromolecules and typical polycrystalline growth on films.

1. Introduction

It is not necessary to reinforce the importance of stone formation in the kidneys in medical care [1]. Consequently with its importance, this subject has aroused much research attention. However, this attention has rarely been focussed on the observation of crystal morphology and even less often to relate this morphology with crystal structure. However, crystal morphology can give very useful information about the conditions in which the crystals were grown and therefore about the mechanisms of formation of the calculi.

2. COM crystal structure

Calcium oxalate monohydrate (whewellite) may adopt three crystalline structures, one of which is a product of the dehydration of calcium oxalate dihydrate [2]. The other two are polymorhps with a transition temperature of 45 °C and they only differ by a slight distortion of the oxalate ions in the low temperature modification [3, 4]. The oxalate ions are practically planar and they are surrounded by six coplanar calcium ions in the first sphere of coordination occupying two types of positions in contact with either two (chelato bond) or one (single bond) oxygen atom. Viewed along the [1 0 0] direction, the crystal structure can be described as a stacking of alternating layers composed of only oxalate ions or oxalate and calcium ions in proportion 1:2, respectively (Fig. 1). The convention in [4] has been followed here for the indexing of crystal faces.

3. Morphology of calcium oxalate monohydrate crystals

A crystal in equilibrium with a medium will adopt the morphology that renders the lowest free energy. During growth, when not in equilibrium, the morphology of crystals may be influenced by kinetic factors, surface defects, grooves, corner and/or edge effects or the presence of foreign substances that bind preferentially to certain crystal faces.

According to our calculations by applying the Hartman–Perdock theory, the most important forms in COM crystals should be: {1 0 0}, {0 1 0}, {0 0 1}, {0 2 1} and {1 2 −1}. COM crystals grown slowly from pure solutions or in gels [3–7] are bounded by these faces. A drawing representative of this morphology is presented in Fig. 2a. Although the term is not exact, it is referred to here as the equilibrium morphology.

Crystals grown from solutions change their shape from needles to platelets and then to prisms before adopting the equilibrium morphology [7]. High ionic strength in combination with either high calcium concentration or the presence of citrate [7] produces tabular habit modification (Fig. 2b).

Crystals grown at moderate temperatures appear normally as four-part (1 0 0) penetration twins and they form aggregates. COM crystal agglomeration takes place mainly by primary mechanisms consisting in the nucleation of new crystals on surface defects (specially on edges and corners) of the existing crystals. In this manner, aggregates become increasingly compact as precipitation proceeds [8].

Mucoproteins [7], vesicle membranes [9] and anionic lipids [10] can induce nucleation of calcium oxalate metastable solutions. They produce morphology changes such as flatenning of the crystals along the [1 0 0] direction and rounding of the side faces (Fig. 2c) and in addition they generate agglomerates with a typical rosette arrangement. They consist of a piling of crystal halfs emerging from about the middle of a (1 0 0) face of a father crystal in a fan arrangement. After a long period in contact with the solution, COM crystals finally adopt the equilibrium morphology.

---

*Present address: Site Carnot, Rambla de la Thermodynamique, 66100 Perpignan, France

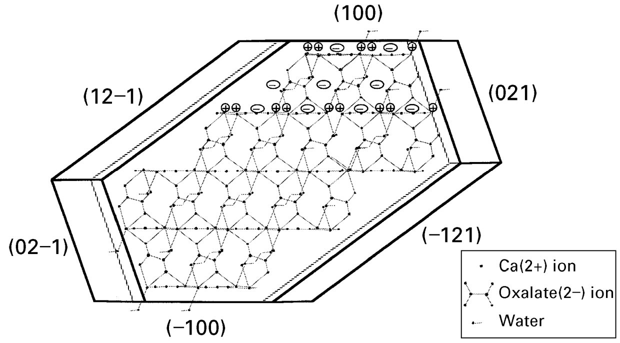

Figure 1 Projection of COM crystal structure along the *b* crystallographic axis.

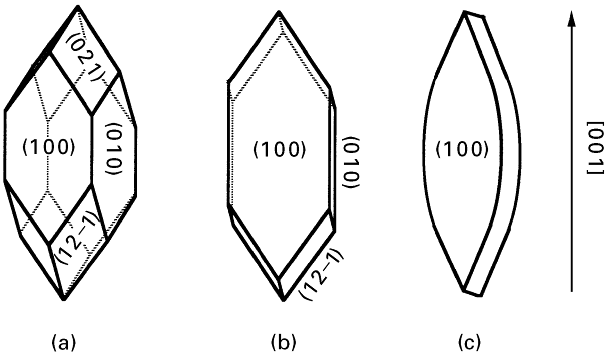

Figure 2 (a) Typical COM morphology. (b) COM tabular crystal. (c) COM crystal with rounded edges.

Morphology modifications can be explained from the structure of the (1 0 0) faces. As can be appreciated from Fig. 1, those faces grow by successive non-stochiometric layers composed of only oxalate ions or oxalate and calcium ions in 1:2 proportion. Polyanions such as citrate or glucosaminoglycans and especially macromolecules with charged side radicals can bind firmly to the flat positively charged layer, retarding the growth of this face and thus yielding a tabular crystal habit. That is the case of charged lipids, membrane vesicles and some polysaccharides and proteins present in urine. Mucoproteins also belong to this group; they are complexes between proteins and polysaccarides, and when they polymerize (and that occurs readily in solution [11]) they link by either glycosidic or glycosamine bonds forming tridimensional nets with lateral residues terminating in carboxilic or sulphate groups. Those groups are very suitable for the binding of molecules to charged surfaces such as COM (1 0 0) faces and they may serve also as nucleation centres of COM crystals [12]. It may be inferred that they can adhere to the crystal surface from one side and use the remaining radicals to nucleate new crystals resulting in the mentioned rosette formations. As they produce other morphology changes such as rounding of the platelets sides, it seems they can also bind to lateral faces. However, surface covering cannot be effective at the acute edges perpendicular to the [0 0 1] direction. Steps could be freely generated at those edges while their advance is obstructed by the macromolecules adsorbed on the flat surfaces producing the elongation of the crystals along the [0 0 1] direction and serrated or oval side faces.

4. Polynuclear growth on films

The texture of layers composed of a number of crystals nucleated upon a substrate is a common subject in chemical vapour deposition (CVD). It is governed by the rule of evolutionary selection [13]: only crystals with the highest growth rate in the direction perpendicular to the substrate survive while the growth of the others is stopped at the faces of the faster growing crystals. Providing a uniform supply of reactants is available over the whole surface, the vertical growth rate of a crystal depends on its orientation with respect to the substrate. In CVD, preferred orientation depends on the angle of incidence of particle beam, probabilities of adsorption and desorption of molecules on each crystal face, and the coefficient of surface diffusion. The case of stone development is simpler as the liquid ensures a homogeneous composition throughout the surface, and COM crystal growth is not limited by diffusion. Thus, stone development can be likened to the limiting case considered by Van der Drift [13] for infinite surface diffusion along a substrate for which the preferred orientation is determined by the shape of isolated crystals grown at identical conditions. That coincides with the direction from the centre of the crystal to the farthest point. For COM crystals, whether polyhedral, tabular or oval, this is always the [0 0 1] direction or close to it. According to mean field theory and tridimensional simulations of cubic and conic crystals [14], the average projected edge length (ξ) should decrease exponentially with time (t),

$$\xi = t^p \tag{1}$$

with exponent p approximately equal to 0.4. t is defined in dv^{-1} units, where d is the distance between the nearest-neighbour crystals and v the linear growth rate. The number of surving crystals on the surface (N) would be proportional to the square root of ξ:

$$N \propto \xi^{-2} \tag{2}$$

5. Experimental procedures

Fourteen spontaneously thrown stones from 10 idopathic stone formers were observed by scanning electron microscopy (SEM) equipped with an energy dispersive (EDAX) analytical device. The stones were first dried in a dessicator and then they were broken into two halfs by an incision with a blade in such a way that the crystals on the broken surface were not erased by the blade. The stone halfs were examined by stereoscopic microscope and then covered with a gold layer.

The stones had a diameter ranging from 2 to 5 mm with a central core of 0.5 to 2 mm.

The analysis with EDAX at high voltage (15 kV) showed the presence of Ca over the whole stone surface, however at low voltage (5 kV) the Ca peak disappeared. This was taken as evidence of the presence of a thin organic layer covering the surface of the crystals.

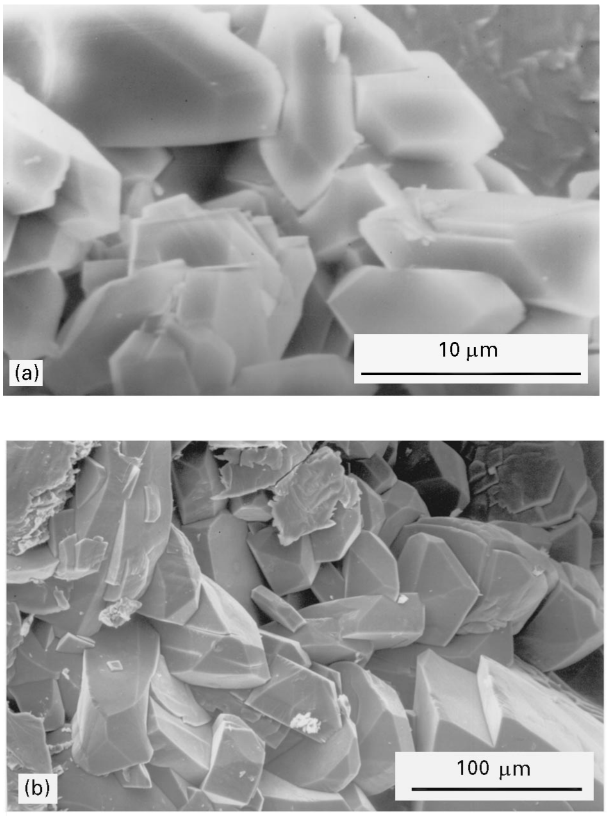

Figure 3 (a) COM crystal aggregate grown from pure solutions at 37 °C. (b) COM crystals in a stone of type II.

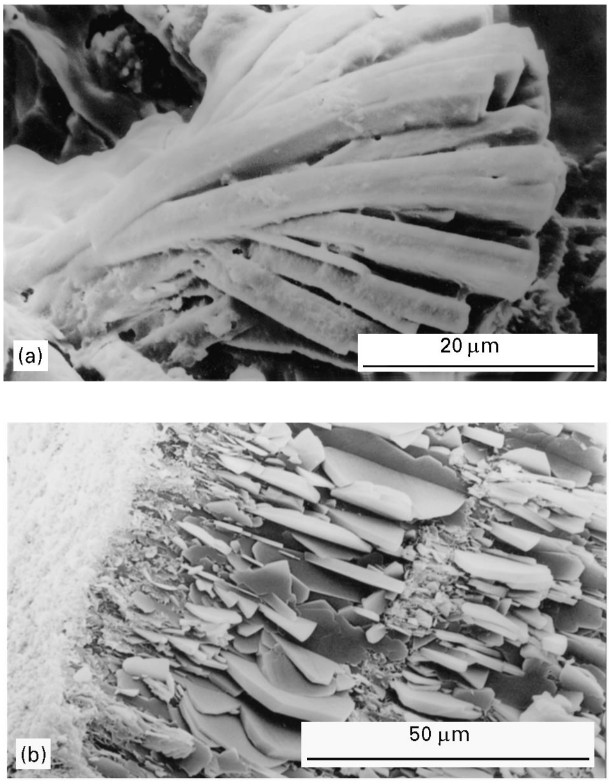

Figure 4 (a) COM crystals in the core of a stone of type I. (b) Shell of a stone of type I.

6. Results and discussion

According to their location in the kidney, the COM calculi studied here have been classified as type I when originating in the renal papillae or type II when coming from the renal calix.

Stones of type II (Fig. 3a) were composed of penetration twin crystals with a morphology not far from that of equilibrium (Fig. 3b). They were more faceted than crystals grown from gels or pure solutions and they had small $(1\,0\,0)$ faces. The absence of either morphology or habit modification indicates that the crystals were grown in permanent contact with their mother face so that they reached equilibrium morphology. In fact, these calculi were found in the cavities of the renal calix where urine can be easily retained. From the considerable size of the crystals we might guess they were grown at low supersaturation, otherwise an intense process of primary aggregation would have hindered the growth of parent crystals resulting in an increase of compactness and a reduction of crystal size. The texture remained the same within the whole calculi and it resembled very closely that of COM aggregates obtained in semi-batch experiments (Fig. 3a, b). This means that growth conditions did not change much during stone development, and aggregation promoters like mucoproteins were never very abundant. For the production of this type of stone it is enough that a crystal is retained in a cavity of the renal pelvis where urine could also be retained. As urine is normally supersaturated the crystal would keep growing at a higher or lower rate where the primary aggregation phenomenon would give the stone its characteristic texture.

Stones of type I always showed two distinct parts:

The core (Fig. 4a). This was mainly composed of single crystals flattened along the $[1\,0\,0]$ direction and elongated along the $[0\,0\,1]$ direction. $\{0\,1\,0\}$, $\{1\,2\,-1\}$ and, of course, $\{1\,0\,0\}$ forms could be clearly distinguished; $\{0\,2\,1\}$ and $\{0\,0\,1\}$ were also present and other rarer forms could not be indexed because of the compactness of the aggregates. Side faces were often serrated and oval, their curvature being constant within the same specimen, changing from one specimen to another. The crystals had acute edges in the direction of elongation or precisely at the ends in the case of oval crystals. These morphology modifications can be produced by either polyanions such as citrate, mucoproteins or other polymeric compounds having charged radicals present in urine, as was shown in Section 3. The oval shape seems to imply direct participation of mucoproteins as other polyanions were unable to produce this effect. The absence of the calcium peak in the EDAX analysis when scanning at low potentials was taken as indicative of covering of the crystal surface by multicharged polymers. Signs of primary aggregation such as crystal intergrowth were always evident and typical crystal formations like those produced *in vitro* by mucoproteins were very abundant. Habit changes are indicative of a short time of contact of the crystals with the mother phase, otherwise, they would have reached their equilibrium polyhedral shape, as happened in *in vitro* experiments [9]. This is quite reasonable as this type of stone grows attached to the walls of the renal papillae where urine cannot be retained except by simple wetting and the crystal environment is constantly renewed. In an extensive crystallographic study of urinary calculi, Lonsdale [15] found the additional forms $\{0\,1\,1\}$, $\{1\,1\,-1\}$ and $\{1\,3\,1\}$, apart from those described here, while $\{0\,2\,1\}$ was not mentioned.

The shell (Fig. 4b). The core was completely surrounded by a film of organic material. An extremely dense population of very small crystals with random orientation enumerated from this film. Away from the organic film the number of crystals decreased and the grains became coarser. The crystal layer ended on a rough surface composed of crystals with their points up. This surface was covered by another film of organic material that gave birth to a new layer of crystals arranged in a similar manner and so on. This description corresponds to the typical pattern of polycrystalline growth found in CVD films and also in some biological structures such as egg shells. The organic material giving birth to each layer was loosely tied to the layer underneath and cleavage between layers could easily be attained. The morphology of the crystals did not change much with respect to that in the core, and was composed of dominant (1 0 0) faces at the top and bottom of platelets elongated along the [0 0 1] directions, bounded laterally by (0 1 0) and (1 2 −1) faces; however, crystal edges were neater. The close association of crystals and organic matrix in this type of calculi has been proved by TEM [9, 16]. From the discussions of Sections 3 and 4, calculi shell development can be explained as follows: once an organic film has covered the nucleus, a large number of crystals with random orientations are nucleated on it. As the crystals grow larger, those having their [0 0 1] axis forming wide angles with the perpendicular of the film progressively collapse so that finally only crystals with their [0 0 1] axis perpendicular to the film survive. This process gives place to the typical arrangement of the shell consisting of concentric layers of parallel crystals in a radial disposition. Following polycrystalline growth theories either the coarsening of the crystals on the surface or the proportion of crystals surviving give an estimate of the development time of a layer. For instance, considering a crystal growth rate of $10^{-12.3}\,\mathrm{m\,s^{-1}}$, measured on a seed of high crystalline perfection [6] at relative supersaturation equal to 2 and a reasonable nucleation density of 1 particle/$\mu\mathrm{m}^2$, the first layer of the calculi in Fig. 4, would develop in two months.

The evolution of the core into a shell requires the presence of a significant amount of organic material at some stage of the stone development. This accumulation of organic material has often been explained by a period of stopped growth. As normal urinary levels of organic material are low, that would mean a drastic drop in calcium or/and oxalate concentrations down to saturation values for long periods. These concentration drops have no physiological foundation, in fact, the variations in urinary contents of calcium and oxalate over long periods have been found to be small, and the calcium oxalate ionic product always exceeds the saturation value [17]. Layer growth initiation could be better accounted for by an abnormal periodic excretion of organic material, perhaps caused by friction of the sharp stone surface against the kidney walls (crystals are always pointing outwards). Extensive damage observed in the urothelium in rat kidneys after inducing stone formation supports this hypothesis [18].

7. Conclusions

Stones of type II appeared to be formed by primary crystal aggregation. They develop in permanent contact with a media poor in organic material. Crystals in stones of type I have a tabular habit due to the binding of polyelectrolytes to (1 0 0) crystal faces. Polycarboxilic acids, glucosaminoglycans, mucoproteins, charged lipids or any other material having several anionic radicals can play this role. Linear or planar polyelectrolytes could reduce crystal growth rate while preventing aggregation to some extent. In contrast, tridimensional polyelectrolytes could promote aggregation by acting as nucleation centres for several crystals. The texture of the stones can be considered to be the result of crystal aggregation produced in two ways: by a primary mechanism and by the action of anionic macromolecules. The arrangement in concentric layers of crystals with a radial disposition can be related to the large presence of anionic macromolecules that set up typical polycrystalline growth on films.

References

1. H. J. SCHNEIDER, in "Urolithiasis: etiology, diagnosis", edited by H. J. Schneider (Springer-Verlag, Berlin, 1985).
2. G. SCHUBERT and B. ZIEMER, *Cryst. Res. Technol.* **16** (1981) 1025.
3. S. DEGANELLO and O. E. PIRO, *N. Jb. Miner. Mh.* **H.2** (1981) 81.
4. V. TAZZOLI and C. DOMENEGHETTI, *Amer. Mineral.* **65** (1980) 327.
5. A. M. CODY and R. D. CODY, *J. Cryst. Growth* **83** (1987) 485.
6. M. FRANCHINI-ANGELA and D. AQUILANO, *Phys. Chem. Minerals* **10** (1984) 114.
7. A. MILLAN, *J. Crystal Growth*, submitted.
8. A. MILLAN, F. GRASES, O. SOHNEL and F. GRASES, *Cryst. Res. Technol.* **28** (1992) 31.
9. S. R. KHAN, O. W. PATRICK and P. A. GLENTON, *J. Cryst. Growth* **134** (1993) 267.
10. S. R. KHAN, P. N. SHERVOCK and R. L. HACKETT, *J. Urol.* **139** (1988) 418.
11. D. S. SCURR and W. G. ROBERTSON, *ibid.* **136** (1986) 505.
12. F. GRASES, L. MASAROVA, O. SOHNEL and A. COSTA-BAUZA, *Brit. J. Urol.* **70** (1992) 240.
13. A. VAN DER DRIFT, *Philips Res. Repts.* **22** (1967) 267.
14. J. M. THIJSSEN, *Phys. Rev.* **22** (1995) 267.
15. K. LONSDALE, *Nature* **217** (1968) 56.
16. H. IWATA, S. NISHIO, A. WAKATSUKI, K. OCHI and M. TAKEUCHI, *J. Urol.* **133** (1985) 334.
17. B. GOLDWASSER, J. L. WEINERTH and C. C. CARSON III, *ibid.* **135** (1986) 1.
18. R. L. HACKETT, P. N. SHEVOK and S. R. KHAN, *ibid.* **144** (1990) 1535.

Received 21 May
and accepted 11 July 1996