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Influence of Different Templates on Crystal Growth of Calcium Oxalate

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Crystal growth of calcium oxalate in bulk aqueous solution, human serum albumin template, uric acid template and mixed template containing human serum albumin and uric acid was studied in this paper. Calcium oxalate crystals were characterized by scanning electron microscopy, fourier transform infrared spectroscopy and x-ray diffraction spectrum. The results showed that different from manifold crystal morphologies of calcium oxalate formed in bulk aqueous solution, human serum albumin promotes the formation of calcium oxalate trihydrate, uric acid is propitious to the orientation growth paralleled with (020) plane for calcium oxalate monolydrate, but in the induction of mixed template, the orientation growth almost paralleled with $(\bar{1}01)$ plane for calcium oxalate monolydrate. It suggested that different templates promote crystal growth of different calcium oxalate hydrate selectively. Furthermore, it could be concluded that human serum albumin template may be the depressor of kidney stone. But mixed template containing human serum albumin and uric acid may be the accelerant of kidney stone.

Key Words: Calcium oxalate, Crystal growth, Human serum albumin, Uric acid, Kidney stone.

INTRODUCTION

Kidney stone is common throughout the world. Calcium oxalate is a major component of most human kidney stone. Many *in vitro* methods have been used to study the complex processes of nucleation, growth and agglomeration of calcium oxalate in an attempt to elucidate the means by which kidney stone occurs. It's helpful to prophylaxis and cure of kidney stones[1-7].

Kidney stones are made up of inorganic crystal and organic matrix. The major composition of crystals is calcium oxalate[8,9]. Calcium oxalate

crystallizes with very different crystallization kinetics, *e.g.* monodinic monolydrate ($CaC_2O_4 \cdot H_2O$), (COM) tetragonal dihydtate ($CaC_2O_4 \cdot (2+x) H_2O$), $x < 0.5$) (COD) and triclinic trihydrate ($CaC_2O_4 \cdot (3-x)H_2O$), $x < 0.5$) (COT). However, calcium oxalate occurs in stones either as the COM or COD form or as a mixture of the two species. In comparison with them, COT is the thermodynamically least stable phase[10,11] and much easier to be ejected out along urine.

Biomineralization is a complicate process, which is difficult to study in the original situation inside the organism. Thus an important method is to simulate it out of the organism. It's necessary to select the system similar to membrane and the component similar to human urine. In previous work, many systems such as Langmuir-Blodgett films, Langmuir monolayer have been used as the system similar to membrane[12-15]. But in these work, main components of urine have little been considered. Human urine is a complex system and containing many kinds of components such as uric acid, protein and polysaccharide. These play important roles in the formation of kidney stone[16,17]. Thus human serum albumin, uric acid and mixture containing human serum albumin and uric acid were selected as the templates to induce growth of calcium oxalate crystals. The formation mechanism and inhibition of kidney stone were also discussed.Very helpful conclusions were obtained.

EXPERIMENTAL

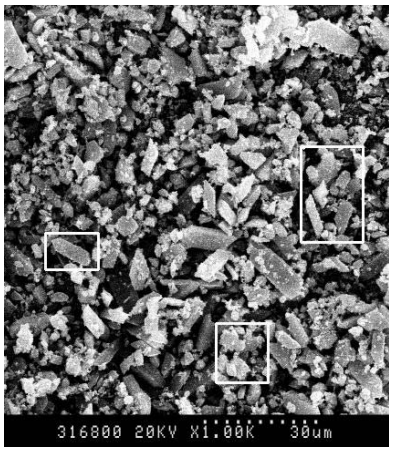
Human serum albumin and uric acid were purchased from Sigma (St. Louis, MO) and used without further purification. Calcium chloride (AR), sodium oxalate (AR) were used as received from Aldrich Chemical Co. (95%). The water was obtained by reversed osmosis, using a Milli RO-Milli Q system (Millipore); its pH and specific resistivity were 6.2 and 18 MΩ, respectively.

Crystal growth of CaC_2O_4 in different template systems: Four kinds of solution were prepared previously, *i.e.* 0.5 mmol/L $Na_2C_2O_4$, 2 % human serum albumin (wt %), 2 % uric acid (wt %) and mixture containing 2 % human serum albumin and 2 % uric acid, which were called solution I, II, III, IV, respectively. Then mixed solution I and solution II, III, IV, respectively and stirred slowly to form system II, system III, system IV. After the mixture was uniformity, four systems (solution I, system II, system III, system IV) were deposited for 12 h. Finally, 0.5 mmol/L $CaCl_2$ was dropped to above four systems by the speed of 6 drop per min, respectively. After the reaction finished, the precipitate obtained from centrifugation was washed with distilled water and absolute ethanol at least five times in order to remove the residual reactants and byproducts. All the products were dried in vacuum oven for 24 h until a constant weight was achieved.

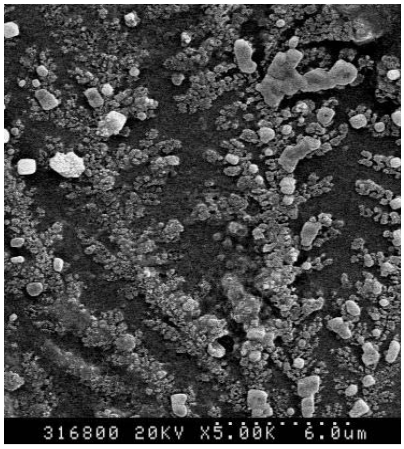
The sizes and morphologies of calcium oxalate crystals were investigated using a S-450 microscope at an accelerating voltage of 20 KV (made in Japan). Calibrated pellets of calcium oxalate (in proportion of 1 % in KBr powder) were performed and recorded with a fourier transform infrared spectrometer Niolet 870 between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. The XRD measurements were performed by a MAP18XAHF X-ray diffractometer at a scanning rate of 4°/min, using a monochromatized CuK_α radiation (λ = 0.154 nm).

RESULTS AND DISCUSSION

Fig. 1 shows crystal morphologies of CaC_2O_4 occurred in different systems. It was seen from Fig.1a that three kinds of crystal morphologies appeared in the bulk aqueous solution. Their cross-sections were hexagonal, rhombic and quadrangular. The mean diagonal size of the first was 11.0 μm, 9.5 μm and 9.5 μm, that of the second was about 6.32 μm, 6.32 μm and the last was around 7.1 μm. Compared with Fig. 1a, Fig. 1b was largely different. Only came out one kind of crystal morphology: quadrangular cross-section CaC_2O_4 crystals. Furthermore, these CaC_2O_4 crystals had inerratic permutation. It formed fractal structure similar to arborization as a whole. It's apparent that the appearance of the phenomena was connected with the induction of human serum albumin as the template. It's well-known that fractural structure of protein has been confirmed. When human serum albumin was as the template to induce crystal growth of CaC_2O_4, carboxyls of human serum albumin could attract Ca^{2+} in some special points and Ca^{2+} could attract $C_2O_4^{2-}$ ulteriorly.



(a)



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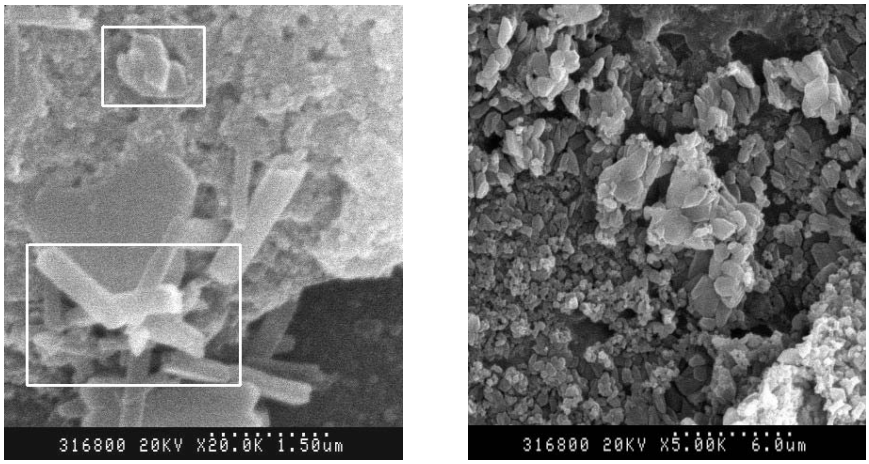

(c) (d)

Fig. 1. The morphologies of CaC_2O_4 crystals gained from different systems (a) Bulk aqueous solution (b) human serum albumin template (c) Uric acid template (d) Mixed template containing human serum albumin and uric acid

Formation of CaC_2O_4 crystals produced in some special points. So fractal structure could be observed in SEM. When crystal growth of CaC_2O_4 was induced by uric acid, two kinds of crystal morphologies of CaC_2O_4 appeared (Fig. 1c). A lot of claviform crystals and little of hexagonal cross-section crystal could be observed. It implied that the induction of uric acid was different from that of human serum albumin. The existence of uric acid may promote the formation of claviform CaC_2O_4 crystals. Finally, when mixed template containing human serum albumin and uric acid was used to induce crystal growth, SEM was shown in Fig. 1d. It could only be seen hexagonal cross-section crystal. Obviously, the mixed template promotes orientation growth of CaC_2O_4 crystal paralleled with special plane.

Infrared spectra of CaC_2O_4 crystals produced in different systems were shown in Fig. 2. It could be seen from infrared spectrum of CaC_2O_4 crystal gained from bulk aqueous solution (Fig. 2a) that the peaks of symmetrical and antisymmetrical oxalate C=O bond stretching located at 1618 and 1320 cm^{-1}, respectively. The O-C-O in plane rocking occurred at 783 cm^{-1} and the -OH stretching of coordinated water was around[14,15] 3000-3500 cm^{-1}. All these peaks were in agreement with the archived data for CaC_2O_4 crystals[16,17].

Fig. 2b-d showed infrared spectra of crystals formed in human serum albumin template, uric acid template and mixed template containing human serum albumin and uric acid, respectively. It could be found that after the addition of different templates, the intensity of antisymmetrical oxalate C=O bond and the full width at medium height of symmetrical oxalate C=O bond became stronger. The change may be attributed to the

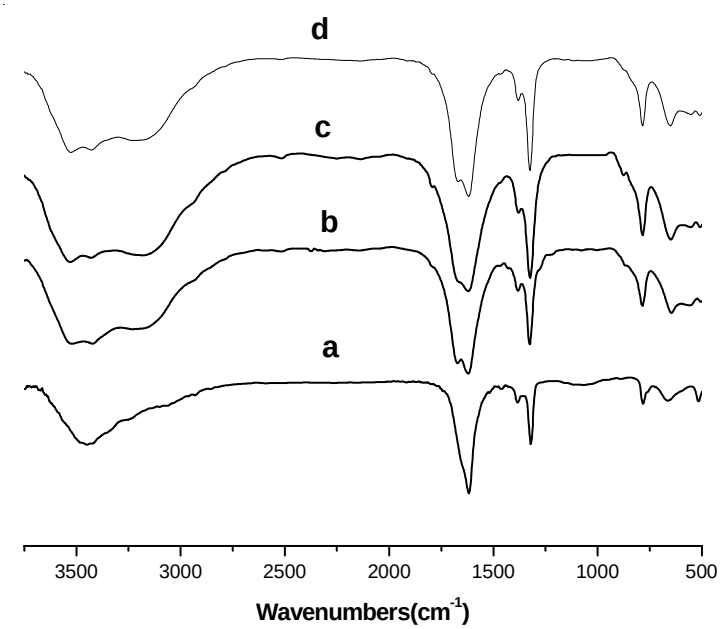

Fig. 2. FT-IR spectra of CaC_2O_4 crystals formed in different systems (a) Bulk aqueous solution (b) human serum albumin template (c) Uric acid template (d) Mixed template containing human serum albumin and uric acid

influence of complex hydrogen bond, such as the interaction among carboxyl group of $C_2O_4^{2-}$, carboxyl group, amino group of human serum albumin or uric acid. These facts suggested that templates containing carboxyl and amino groups had effect on Ca^{2+} or $C_2O_4^{2-}$ in CaC_2O_4 crystal. It had important influence on the microstructure of CaC_2O_4 crystal.

A comparison of the X-ray diffraction patterns for the samples is shown in Fig. 3. XRD patterns of CaC_2O_4 crystals grew in bulk aqueous solution showed more peaks (Fig. 3a). These peaks corresponded to the index of the reflecting planes for ($\bar{1}$ 0 1), (0 2 0), ($\bar{2}$ 0 2), (1 3 0), (1 0 0), (0 0 1) and (1 1 0)[18], which indicated the presence of COM, COD and COT. According to the diffraction peak intensity corresponded to different crystals in XRD patterns, the rate of different crystals in mixed crystals could be calculated. For example, the rate of COM can be calculated using the formula as followings:

$$COM\ \% = I_{COM}/(I_{COM} + I_{COD} + I_{COT})$$

where I is the diffraction peak intensity in XRD patterns. The detailed results are shown in Table-1. Compared with Fig. 3a, the corresponding main diffraction peaks assigned to the (001), (11 0) planes of the COT crystal became stronger. The facts suggested that human serum albumin promotes the crystal growth of COT, which is connected with the

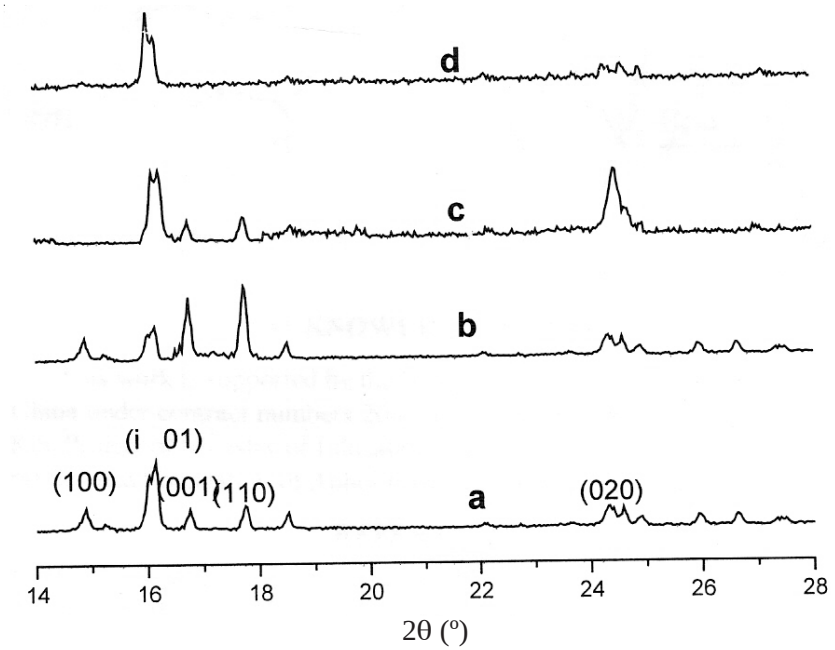

Fig. 3. XRD patterns of crystals produced in different systems (a) Bulk aqueous solution (b) human serum albumin template (c) uric acid template (d) mixture containing human serum albumin and uric acid

TABLE-1
RATE OF DIFFERENT CALCIUM OXALATE HYDRATES IN DIFFERENT SYSTEMS

Additives	COM (%)	COD (%)	COT (%)
Aqueous solution	62.08	12.45	25.47
Human serum albumin	26.67	10.00	63.33
Uric acid	75.86	0.00	24.14
Human serum albumin + Uric acid	93.00	0.00	7.00

induction of human serum albumin as the template. It could decrease the special activation energy for COT crystal nucleation, these crystallization planes of COM could be recognized, which made the crystals have very strong selected catalysis effect on the surface and result in the orientation growth of crystal. COT is difficult to agglomerate inside organism to produce kidney stone, so human serum albumin template may restrain the formation of kidney stone. In Fig. 3c, only diffraction peaks corresponding to COM, COT could be seen, and diffraction peaks assigned to COD disappeared. It's apparent that uric acid inhabited crystal growth of COD. And the number of diffraction peak decreased. In XRD patterns, it only

could be observed peaks corresponded to the index of the reflecting planes for ($\bar{1}$ 0 1), (0 2 0), (1 0 0), (0 0 1). In comparison with Fig. 3a, the intensity of the diffraction peak corresponded to the index of the reflecting plane for (020) became much stronger. The addition of uric acid is propitious to the orientation growth paralleled with (020) plane. It may be related to the induction of uric acid and lead to selective orientation growth of crystals. Electron-rich atoms such as nitrogen atom and oxygen atom existed in uric acid molecule (Fig. 4), hydrogen atom is actually a exposed proton with small radii. Oxygen atom of $C_2O_4^{2-}$ takes the lone pair electrons, which is easier to form hydrogen bond with hydrogen atom in uric acid. So nucleus sites of oxalate may form on uric acid matrix probably. It would make the orientation growth of CaC_2O_4 crystal paralleled with $C_2O_4^{2-}$-rich (020) plane. Different from Fig. 3c, large changes occurred in Fig. 3d. The peak corresponded to the index of the reflecting planes for (1 0 0) disappeared. And the peak corresponded to the index of the reflecting planes for (020) could hardly be investigated. It is obvious that crystal growth of COD, COT and orientation growth of COM crystal paralleled with (020) plane was prohibited when mixed system containing human serum albumin and uric acid was selected as the template. It may be resulted from the interaction between them. On one hand, carboxyl and amido existed in human serum albumin molecule, it could bind with uric acid by hydrogen bond, which would prevent the formation of the hydrogen bond between uric acid and oxalate and restrain the formation of $C_2O_4^{2-}$ nucleus sites. So the peak corresponded to the index of the reflecting plane for (020) could hardly be seen. On the other hand, both human serum albumin and uric acid contain carboxyl, moreover, in reaction system, net negative charges were taken on their surfaces, which could bind Ca^{2+} strongly by static attraction, thereby providing a mechanism to sufficiently concentration calcium ions, mimicking the calcium-rich face and crystals preferentially orient with the ($\bar{1}$ 0 1) face[19] (Fig. 5). COM is the thermodynamically most stable phase and is main component of kidney stone, so mixed template containing uric acid and human serum albumin may perhaps promote the formation of kidney stone.

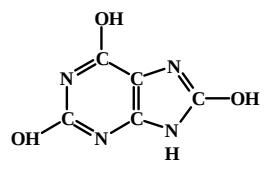

Fig. 4 Structure of uric acid

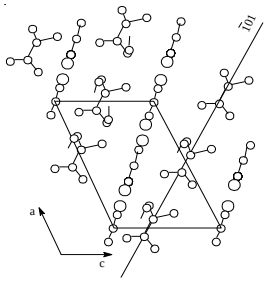

Fig. 5 Calcium oxalate monohydrate crystallographic projection $\bar{1}$ 0 1

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