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Influence of Different Biomolecules on Crystal Growth of Calcium Oxalate in Microemulsion

WENJIN YUE* and GUANGJUN NIE
Department of Biochemical Engineering, Anhui University of Technology and Science, Wuhu 241000, P.R. China
Tel: (86)(553)5688311; E-mail: yuewenjin_79@163.com

Crystal growth of calcium oxalate in reverse microemulsion of *p*-octyl polyethylene glycol phenylether (op)/iso-octyl alcohol (IOA)/cyclohexane/water and above microemulsion containing different biomolecules, such as protease, glucan, carboxyl methyl chitosans were studied. Calcium oxalate crystals were characterized by transmits electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) spectrum. The results indicated that different crystallization types of the crystals, which were calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and calcium oxalate trihydrate (COT),existed in reverse microemulsion, while protease promotes the formation of COM, glucan and carboxyl methyl chitosans could promote the formation of COT, especially is carboxyl methyl chitosans. It could prohibit the formation of COM, COD strongly. These facts suggested that different biomolecules promote crystal growth of different calcium oxalate hydrate selectively. Furthermore, it could be concluded that carboxyl methyl chitosans may be depressor of urinary stone in future.

Key Words: Calcium oxalate, Crystal growth, Protease, Glucan, Carboxyl methyl chitosans.

INTRODUCTION

Urinary stone is common throughout the world. Many people suffer from the disease of urinary stone. It is made up of inorganic crystal and organic matrix. The major composition of crystals is calcium oxalate. Calcium oxalate crystallizes with different crystallization kinetics, *e.g.*, monoclinic monohydrate ($CaC_2O_4 \cdot H_2O$, calcium oxalate monohydrate, COM), tetragonal dehydrate ($CaC_2O_4 \cdot (2+x)H_2O$, $x < 0.5$, calcium oxalate dihydrate, COD) and triclinic trihydrate ($CaC_2O_4 \cdot (3-x)H_2O$, $x < 0.5$, calcium oxalate trihydrate, COT)[1-6]. 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 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[7-12]. Thus an important method is to simulate it out of the organism. Ordered molecular films, including monolayer, LB film,

microemulsion, micelle, *etc.*, were used as the template to induce crystal growth is important methods to simulate bimineralization process[13-15]. Reverse microemulsion consisted of micron-size water pools dispersed within a continuous oil phase. The water solubilized in reverse microemulsion, in any respects, is similar to the interfacial water presented near the biological membranes. So it's a possible method of simulating biomineralization to induce crystal growth of calcium oxalate in microemulsion. Besides this, an important composition i.e. matrix should be considered. The matrix of urinary stone is made up of protein, enzyme, polysaccharide, *etc.*, these play important roles in the formation of urinary stone. Thus three matrixes, *i.e.*, protease, glucan, carboxyl methyl chitosans were selected as the matrix to induce crystal growth of calcium oxalate in reverse microemulsion.

EXPERIMENTAL

Sodium oxalate, anhydrous calcium chloride, acetone, cydohexane and anhydrous alcohol were all of analytical and *p*-octyl polyethylene glycol phenylether (op) and iso-octyl alcohol (IOA) were used of chemical purity. Protease, glucan, carboxyl methyl polysaccharides were biochemical reagents. They were produced by Aldrich Chemical Company and used without further purification. All solutions were prepared with double deionized water.

Crystal growth of CaC_2O_4 in different microemulsion systems: 0.01 mol/L $CaCl_2$ or 0.01 mol/L $Na_2C_2O_4$ aqueous solutions were added into the organic phases consisted of 26.7 wt % OP, 17.8 wt % of IOA and 55.6 wt % cyclohexane to form two types of reverse microemulsions and stirred slowly for 0.5 h to prepare microemulsion containing CaC_2O_4. After airproffed the mixed microemulsion and deposited for 24 h, part of microemulsion [Microemulsion(I)] was taken out for determination of TEM, the rest was dropped double deionized water slowly to make the microemulsion destroyed. The precipitate obtained from centrifugation was washed with distilled water and absolute ethanol at least five times to remove the surfactants, residual reactants and byproducts. All the products were dried in vacuum over for 24 h until a constant weight was achieved, which was called crystal (I). For the preparation of microemulsions (II)-(IV) and crystals (II)-(IV), same procedures were microemulsion (I) and microemulsions (II)-(IV) was replaced by $CaCl_2$ solutions containing certain concentration (3 mg/mL) of protease, glucan, carboxyl methyl chitosans, respectively.

Characterization: The sizes and morphologies of CaC_2O_4 crystals were investigated by TEOL-TEM-100sx transmits electron microscope (TEM). The microemulsions containing the products were deposited onto carbon film supported by copper grids and evaporated in air at room temperature. Calibrated pellets of CaC_2O_4 (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 Cu Kα radiation (λ = 0.154 nm).

RESULTS AND DISCUSSION

TEM: The morphologies of CaC_2O_4 crystals occurred in different systems were shown in Fig. 1. The micrograph of crystal which produced in reverse microemulsion (I) was exhibited in Fig. 1a. The morphologies were hexagonal with an average diagonal size of 60 nm × 60 nm × 90 nm, quadrilateral whose size was about 50 nm × 80 nm and the sphere-like forms. In comparison with Fig. 1a, in Fig. 1b, the morphology of the crystals gained from microemulsion (II) was mostly hexagonal crystals. The average diagonal size was around 469 nm, 335 nm, 335 nm. It was seen that the morphology of crystal was single and the size of crystal was larger after the addition of enzyme. It is suggested that protease promote the formation of hexagonal crystals. When crystal growth of CaC_2O_4 was induced in microemulsion (III), the micrograph of the gained crystal (III) was shown in Fig. 1c. Compared to Fig. 1b, the morphology changed from hexagonal crystal to irregular transitional crystals, such as ellipsoid, round crystals. Obviously, crystal morphologies changed apparently after the addition of glucan. Then, with the addition of carboxyl methyl polysaccharides, the crystal morphology was different (shown in Fig. 1d). It was seen that most of them were quadrilateral crystals and a little of transitional crystals and hardly hexagonal crystals. The results showed that carboxyl methyl chitosans could prohibit the formation of hexagonal crystals. So it could be concluded that different additive could promote or prohibit the formation of different crystal morphology selectivity.

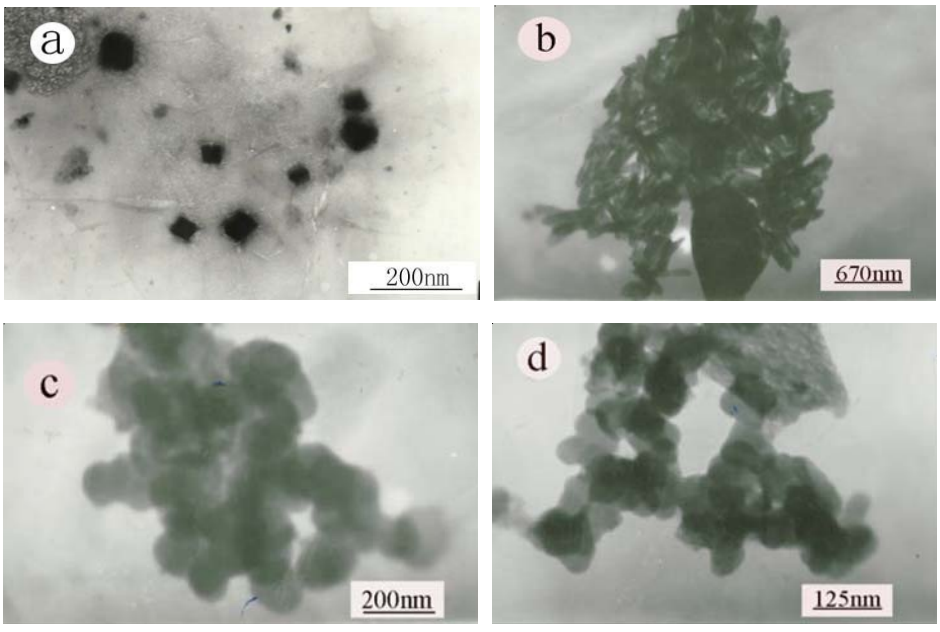

Fig. 1. Morphologies of CaC_2O_4 crystals gained from different systems, (a-d) Microemulsions (I)–(V), respectively

FT-IR spectra: 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 microemulsion (I) (Fig. 2a) that the peaks of symmetrical and asymmetrical oxalte C=O bond stretching located at 1620 and 1318 cm^{-1}, the weak water viberation peaks were around 948 and 665 cm^{-1} in the fingerprint region and the -OH stretching of coordinated water was around 3500-3000 cm^{-1}. Fig. 2b-d showed infrared spectra of crystal (II)-(IV), respectively. It could be found that after the addition of different biomolecules, the intensity of asymmetrical 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 complex hydrogen bond, such as the interaction among carboxyl group of $C_2O_4^{2-}$, carboxyl group, amino group of protease, glucan or carboxyl methyl chitosans. These facts suggested that these biomolecule had important influence on the microstructure of CaC_2O_4 crystal.

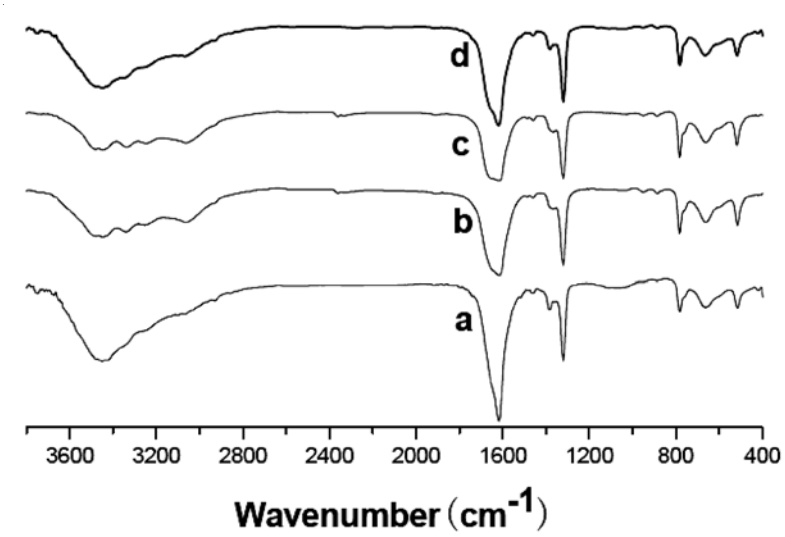

Fig. 2. FT-IR spectra of CaC_2O_4 crystals grown in different systems, (a-d) Microemulsions (I)–(V), respectively

XRD: XRD patterns of CaC_2O_4 crystal produced in different systems were shown in Fig. 3a-d, respectively. XRD patterns of CaC_2O_4 crystals grew in microemulsion (I) showed more peaks (shown in Fig. 3a). These peaks corresponded to the index of the reflection planes for (1 0 1), (0 2 0), (2 0 2), C(1 3 0), (1 0 0), (0 0 1) and (1 1 0), which indicated the presence of COM, COD and COT. According to the diffraction peak intensity corresponded to different crystal in XRD patterns, the rate of different crystal in mixed crystals could be calculated. For example, the rate of COM can be calculated using the formula as followings: COM % = $I_{COM}/(I_{COM} + I_{COD} + I_{COT})$ (I is the diffraction peak intensity in XRD patterns).

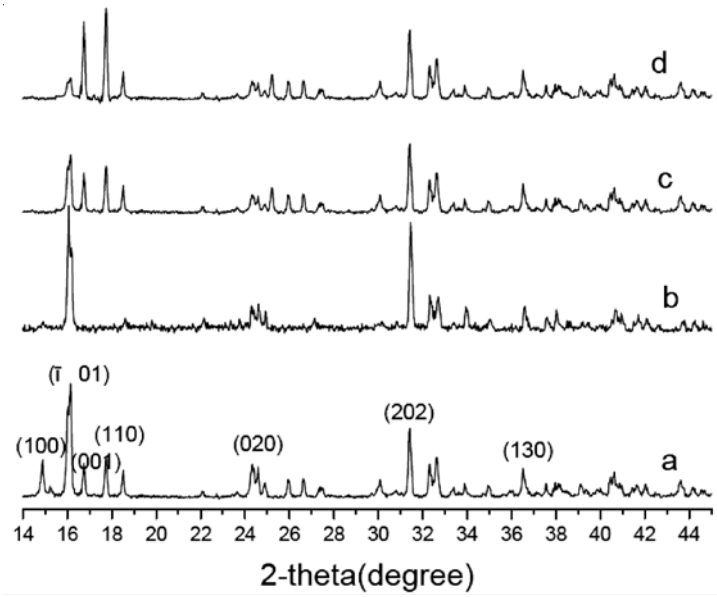

Fig. 3. XRD patterns of crystals produced in different systems, (a-d) Microemulsions (I)–(V), respectively

The detailed result is shown in Table-1.Compared with Fig. 3a, the corresponding main diffraction peaks are located at 0.593, 0.365, 0.297 and 0.248 nm in Fig. 3b, which could be assigned to the (1 0 1), (0 2 0), (2 0 2) and (1 3 0) planes of the COM crystal, respectively. While the diffraction peak corresponded to the index of the reflecting plane for (1 0 0), (0 0 1), (1 1 0) disappeared. These facts suggested that these crystallization planes of COM could be recognized and the energy for CaC_2O_4 crystal nucleation at water/oil interface. The nuclear energy had a strong selected catalytic effect on the surface of COM crystal, it resulted in the crystal oriented growth. It demonstrated that the protease as the matrix could promote the crystal growth of COM. When glucan was added into the reverse microemulsion, the XRD patterns of CaC_2O_4 was shown in Fig. 3c. Compared with Fig. 3a, the corresponding main diffraction peaks assigned to the (0 0 1), (1 1 0) planes of the COT crystal became stronger, while that diffraction peaks assigned to the (1 0 1), (0 2 0) planes of the COM crystal became weaker, and the diffraction peak assigned to (1 0 0) plane of COD crystal disappeared, which is resulted from the induction of glucan. It suggested that glucan could promote the formation of COT. In comparison with it, Fig. 3d showed largely different. Stronger peaks assigned to (0 0 1), (1 1 0) planes of the COT crystal and weaker peaks assigned to (1 0 1), (0 2 0), (2 0 2) planes of the COM crystal could be observed. Especially orientation growth of (COM) plane was strongly inhabited. It may be related to the induction of carboxyl methyl chitosan. From above facts, we can conclude that protease promote the formation of COM and inhabit the crystal growth of COD, COT completely. But

glucan and carboxyl methyl chitosans especially carboxyl methyl chitosans could inhabit the formation of COM and promote the formation of COT. COT is difficult to agglomerate inside organism to produce urinary stone, so carboxyl methyl chitosans may become depressor probably in future.

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

Microemulsion	COM (%)	COD (%)	COT (%)
I	72.88	10.17	16.95
II	100.00	0.00	0.00
III	66.67	0.00	33.33
IV	44.23	0.00	55.77

Conclusion

Different from crystal growth of CaC_2O_4 in reverse microemulsion, protease, glucan, carboxyl methyl chitosans could decrease free energy of some special lattice plane and make orientation growth of CaC_2O_4 crystal paralleled with different lattice planes, which promote different calcium oxalate hydrate to form. For example, protease promotes the formation of COM. But glucan and carboxyl methyl chitosans especially carboxyl methyl chitosans facilitate crystal growth of COT. From the experimental results, it could be concluded that different biomolecules promote crystal growth of different calcium oxalate hydrate selectively.

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