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SELECTION OF CRYSTAL FACE OF CALCIUM OXALATE MONOHYDRATE BENEATH DPPC MONOLAYERS IN THE PRESENCE OF CHONDROITIN SULFATE A

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The crystal face selection of calcium oxalate monohydrate (COM) at dipalmitoylphosphatidylcholine (DPPC) monolayers was investigated in the presence of chondroitin sulfate A (C_4S). The ($\bar{1}01$) face was doubly strengthened and the (010) face was doubly weakened in the presence of C_4S at DPPC monolayers. This was due to the simultaneous interaction of the Ca^{2+}-rich ($\bar{1}01$) crystal face of COM with both the polyanionic polysaccharide C_4S and the negatively charged sites of phosphate groups of the DPPC monolayers.

Keywords: calcium oxalate monohydrate; chondroitin sulfate; DPPC; monolayer

INTRODUCTION

Urinary stones are comprised primarily of inorganic crystals that are mixed with an organic matrix that accounts for about 2% of the total mass [1,2]. Calcium oxalate (CaOxa) is a major inorganic component of urinary stones [3]. The organic matrix is made of carbohydrates such as chondroitin sulfate A (C_4S; Scheme 1), lipids such as dipalmitoylphosphatidylcholine (DPPC), and proteinaceous materials [4]. The microscopic origins underlying aggregation and attachment to cells, however, have not been examined directly. Up to now, most of the reports about the investigations of CaOxa were carried out in aqueous solution systems [5–7]. However, the conditions for crystallization of CaOxa in common aqueous solutions are

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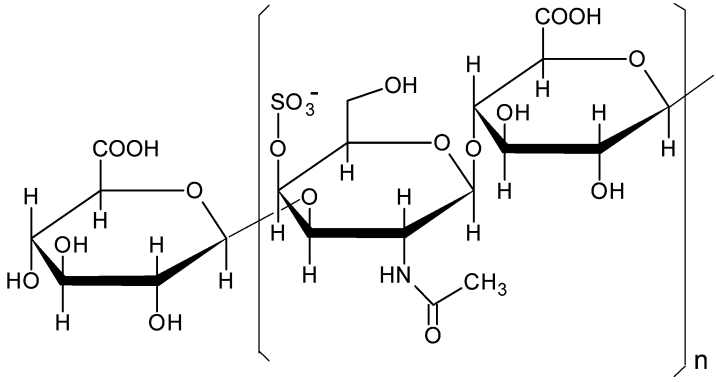

SCHEME 1 The molecular structure of chondroitin sulfate A.

much different from those in biological systems, and the mechanisms about the formation of urinary stones are still poorly understood. Thus in the past decade some ordered systems such as Langmuir monolayers [3,8–13] and vesicles [2,14] were designed as model systems to mimic the formation of calcium oxalate stones.

It has been proposed that the organic matrix may play a significant role in stone formation, including providing sites for the initial crystal nucleation event. C_4S is a well-known organic matrix found in urine and kidney stones. However, the interactions between CaOxa crystals and C_4S, as well as their effects on the crystal nucleation and growth process, is not fully understood. In this work crystallization of CaOxa crystals was investigated at DPPC monolayer in the presence of C_4S. This system will be closer to the real mineralization circumstances than those in aqueous solutions or at monolayers without additives.

METHODS AND EXPERIMENTS

DPPC and C_4S were purchased from Sigma. Milli-Q water with a resistance of 18.2 MΩ cm was used for subphases and for preparation of all solutions. Stock solutions of 10 mM calcium chloride, 10 mM sodium oxalate, and 5.0 mg/ml C_4S were prepared using a TrisHCl buffer (pH 6.0) containing 10 mM sodium chloride and were filtered through a 0.22 μm Millipore filter. pH measurements were done by a combination of glass/saturated calomel electrode standardized before and after each experiment with buffer solutions at 25°C. The formation of DPPC monolayer and the crystallization of CaOxa were according to reference [3,8,9]. After the chloroform had

evaporated on the surface of subphase of 0.22 mmol/l CaOxa, the monolayer was compressed to 10 mN/m. The transfer of CaOxa crystals grown beneath the Langmuir monolayers to solid supports for analysis by scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) was accomplished by carefully draining the subphase from the trough to lower the monolayer onto a substrate that had been placed in the subphase before the monolayer was applied. All work was carried out in a dust-free box at a temperature of 25°C. The experiments were repeated twice at least.

Measurements of surface pressure-area (π-A) isotherms were carried out with a KSV Instruments model 3000 LB system. SEM was performed using a Philips XL-30 ESEM scanning electron microscope, operating at 10 kV. XRD results were recorded on a D/max-γA X-ray diffractometer (Japan), using Ni-filtered Cu-K_α radiation ($\lambda = 0.154$ nm), the scanning rate was 2° min^{-1}. The divergence and scattering slit was at 1° for $5^\circ < 2\theta < 60^\circ$.

RESULTS AND DISCUSSION

Figure 1 shows the SEM images of calcium oxalate crystals grown at DPPC monolayer in the presence of 0.01, 0.1, and 0.50 mg/ml of C_4S and in the absence of C_4S, respectively. X-ray powder diffraction (XRD; Figure 2) and Fourier transform infrared (FTIR) spectroscopy confirmed these crystals were calcium oxalate monohydrate (COM). In the XRD patterns, the corresponding main diffraction peaks are located at 0.593, 0.365, 0.297, and 0.198 nm, which assigned to the $(\bar{1}01)$, (010), $(\bar{2}02)$, and $(\bar{3}03)$ crystal planes of COM [1,3], respectively.

The morphology of COM was remarkably affected by the concentration of C_4S. With the increase of the concentration of C_4S from 0 to 0.5 mg/ml, COM changes from the tridimensional hexagonal prisms (Figure 1a) to the much elongated hexagonal slice crystals (Figure 1d).

When COM grows beneath DPPC monolayers, this monolayer can act as a templating for COM growth, and it has the potential to form an arrangement that favors the incipient crystal face. This can lead to high selectivity for a specific crystal orientation, or even a specific crystal polymorph, as nucleation takes place when a crystal face recognizes the organic interface [8].

Comparing the XRD results of COM crystals grown in presence of different concentration of C_4S, the selectively growing crystal face of COM crystals under control of DPPC monolayers and additives was identified. Beneath DPPC monolayers in the absence of C_4S, the relative intensity of the (020) and $(\bar{1}01)$ diffractive faces are nearly identical (Figure 2a). However, the XRD pattern shows a very strong $(\bar{1}01)$ diffraction peak (Figures 2b, 2c, and 2d) in the presence of C_4S. This suggests that the $(\bar{1}01)$ face was the

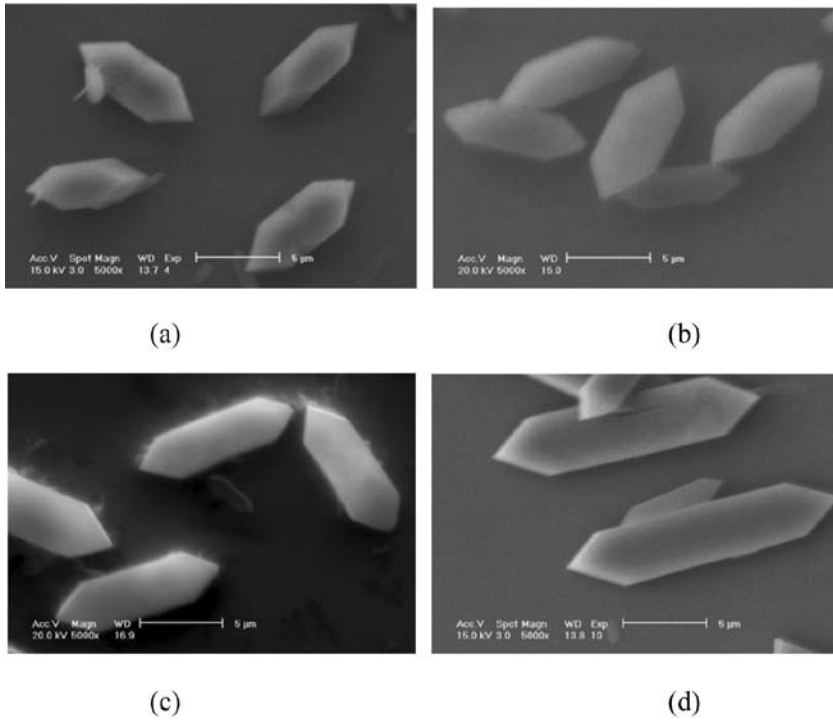

FIGURE 1 SEM images of COM crystals grown at DPPC monolayers at 10 mN/m (a) in the absence, (b) in the presence of 0.01, (c) 0.10, and (d) 0.5 mg/ml C_4S.

mostly exposed face for these crystals. As the concentration of C_4S increases from 0.01 to 0.50 mg/ml, the diffractive lines assigned to $(\bar{1}01)$ face of COM became more and more strong, and those assigned to (010) face became more and more weak. This results in the growth of the quasi two-dimensional COM crystals with the shape of elongated hexagonal slices.

The $(\bar{1}01)$ crystal face of COM is characterized by oxalate ions emerging oblique to the faces with a dense pattern of complexed calcium ions exposed [15,16]. That is, $(\bar{1}01)$ is a calcium ion-rich face, and there is a positively charged surface for $(\bar{1}01)$ crystal face of COM crystals. Since the headgroups of DPPC monolayers are phosphate groups and are negatively charged, DPPC can interact strongly with the densely packed Ca^{2+} ions within the $(\bar{1}01)$ face by electrostatic attraction and then block this face for its further growth while stabilizing the face at the same time. In contrast, the (010) face is nearly neutral and contains an alternative arrangement of Ca^{2+} ions and Oxa^{2-} anions. The Oxa^{2-} anions lie on

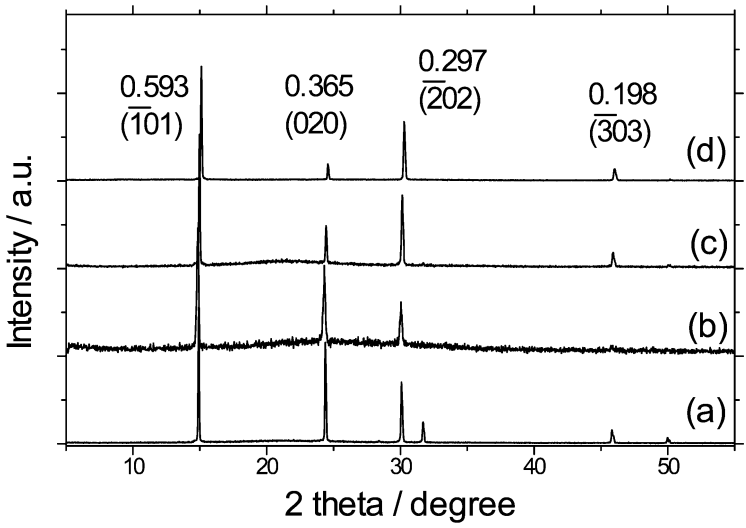

FIGURE 2 XRD patterns of COM crystals grown under DPPC monolayers (a) in the absence, (b) in the presence of 0.01, (c) 0.10, and (d) 0.5 mg/ml C_4S.

the surface with an orientation perpendicular to the (010) face. In this situation, it could be less favorable for the functional groups to interact with this face, leading to a relatively faster growth rate than that of the ($\bar{1}$01) face.

On the other hand, C_4S is a linear polysaccharide polyanionic ions [17]. Each unit has a negative carboxylic group and a negative sulfate group. The morphological studies on COM have suggested that C_4S binds to the crystal surfaces in a regular fashion rather than at random [18]. The binding sites or growth sites seem to be calcium sites on the faces matching negatively charged side groups on the C_4S chains. Actually, Ca^{2+} ions can generate the sulfate salt of C_4S, and they interact with other polar groups in the chondroitin sulfate.

At DPPC monolayers, and in the presence of C_4S, the Ca^{2+}-rich ($\bar{1}$01) crystal face of COM interacted simultaneously with both the polyanionic polysaccharide C_4S and the negative phosphate headgroups sites of DPPC monolayers. It resulted in the ($\bar{1}$01) face of COM being doubly strengthened and the (010) face being doubly weakened. Since the increase of the concentration of C_4S further increases this interaction, so the ($\bar{1}$01) face of COM was also further strengthened.

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GRAPHICAL ABSTRACT

The ($\bar{1}$01) face of COM crystals was doubly strengthened and the (010) face was doubly weakened in the presence of urinary macromolecule chondroitin sulfate A (C_4S) at DPPC monolayers. This was due to the simultaneous interaction of the Ca^{2+}-rich ($\bar{1}$01) crystal face of COM, with both the polyanionic polysaccharide C_4S and the negatively charged sites of phosphate groups of the DPPC monolayers.