Crystallization of Calcium Oxalate in Liposome Solutions of Different Carboxylates

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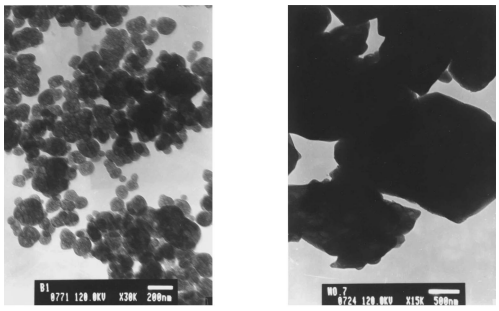
The effects of tri-, di- and monocarboxylate on the growth of nanoparticles of calcium oxalate (CaOxa) were first investigated in liposome system. Sodium acetate (NaAc) only induced calcium oxalate monohydrate (COM), while sodium tartrate (Na_2tart) and sodium citrate (Na_3cit) could induce calcium oxalate dihydrate (COD) with a preferential growth of (213) crystal face. The promotion effectiveness to COD followed this order: Na_3cit > Na_2tart > NaAc.

Urolithiasis constitutes a serious health problem that affects a significant section of mankind. Between 3 and 14% of the population, depending on the geographical region, suffer from this illness.[1] The recurrence rate is about 50% in 5 to 10 years in America and more than 80% in China.[2] Calcium oxalate (CaOxa) is the most frequent crystalline phase in human stones and occurs in more than 80%.

There are many reports about the crystallization of CaOxa in aqueous solution,[3] diluted[4] or undiluted urines,[5] and artificial urines.[6] However, common aqueous solutions are much different from those in biological systems. Urinary stones are usually formed within membrane-bound microspace, and the nucleation and growth of urinary stones are regulated by organic matrix.[2,6] So in the recent years, some ordered systems were designed to mimic the formation of CaOxa stone especially with Langmuir monolayer as a model system.[7] There is no report about the deposition behavior of CaOxa inside liposome. Liposome has advantages of providing confined microspace and organic matrix.[8,9] In this paper, the effects of tricarboxylate, sodium citrate (Na_3cit), dicarboxylate, sodium tartrate (Na_2tart), and monocarboxylate, sodium acetate (NaAc), on the growth of nanoparticles of CaOxa were studied in lecithin (PC)-H_2O ordered liposome system.

Egg yolk lecithin (PC, Sigma) was first dissolved in chloroform. After the organic solvent was volatilized at room temperature, 10.0 ml aqueous 10 mmol/L $CaCl_2$ and a certain amount of the carboxylate additive were added. The final concentration of PC in the $CaCl_2$ solution was 5.0 mg/ml. The solution was sonicated for 20 min in order to form the liposomes. Dynamic laser scattering showed the diameter of the liposomes to be about 80–100 nm and the liposomes were stable. The quantitative titration results of the Ca^{2+} ions outside the liposomes after osmosis as well as the determination of the electrical potentials of the original $CaCl_2$ solution and the liposomes indicated that about 48% of $CaCl_2$ was present inside the liposomes. Then 10.0 ml 10 mmol/L K_2Oxa solution was added while the mixture was stirred. After 30 min of reaction, a drop of the suspension was examined by transmission electron microscopy (TEM). The rest solution was let to stand for 3 h. Then the product was centrifuged, washed with $CHCl_3$, and dried over night under vacuum. The morphology and properties of the products were analyzed by powder X-ray diffraction (XRD). All experiments were carried out at pH = 5.8 at 25 ± 1 °C.

Figure 1 shows the TEM images of CaOxa crystals grown in the liposome system and in aqueous solution. The size of CaOxa crystals grown in liposomes (about 100 nm) is much smaller than that in aqueous solution (about 1500–2000 nm). The different results are due to the ordered array of lecithin head groups in liposomes providing an interface for CaOxa crystallization.[8] Judging from the bilayer phase diagram of egg PC, the bilayer consists of liquid and liquid crystals in 90% water.[9] The coexistence of the lipid membrane and aqueous solution lowers the activation energy of nucleation (heterogeneous nucleation). These factors promote the nucleation and may induce nucleation at many sites at the interface between liposome membrane and aqueous solution. When the nucleation occurs simultaneously at many sites on the membrane surface of the liposomes, homogeneous CaOxa nanoparticles (about 100 nm) were formed. However, the nucleating interface is missing in aqueous solution, so larger and inhomogeneous CaOxa solids were formed.



(a) (b)

Figure 1. TEM images of CaOxa crystals grown a) in liposome (the bar: 200 nm) and b) in pure water (the bar: 500 nm).

The XRD patterns of CaOxa crystals after adding different kinds of carboxylates were listed in Table 1. In the undoped experimental setup (Figure 2d) and in the presence of NaAc (Figure 2c) in liposomes, all the CaOxa crystals are monohydrate (COM). The corresponding main diffraction peaks located at 0.365, 0.236, and 0.182 nm, which are assigned to (020), (130), and (040) planes of COM crystal, respectively. This means that there is no effect of NaAc on the phase composition and morphology of CaOxa crystals in liposomes. The crystallization of CaOxa in liposomes is much different from that in aqueous solution. In the latter case, the strongest diffraction peak of COM was due to the archived ($\bar{1}$01) face with d spacing of 0.593 nm (Figure 2e). However, a preferential alignment of the (020) and (130) planes parallel to the surface of the membrane was induced in the liposomes. The ($\bar{1}$01) crystal faces of COM are character-

Table 1. Effects of different carboxylates on the phase compositions of CaOxa crystals in liposomes and in pure water

PC / mg/ml	NaAc / mM	Na_2tart / mM	Na_3cit / mM	I_M/I_D[a]
5.0	0.60			∞
5.0	3.3			∞
5.0		0.60		19.0
5.0		3.3		1.9
5.0			0.60	2.4
5.0			3.3	0
5.0				∞
0		0.60		∞
0			0.60	5.0
0				∞

[a]I_M/I_D is the ratio of the strongest diffraction peak of COM at 0.365 nm and the strongest peak of COD at 0.224 nm.

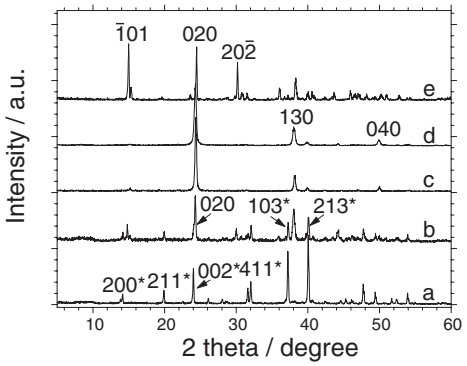


Figure 2. Powder X-ray diffraction patterns of CaOxa crystals grown in the presence of 3.3 mmol/L Na_3cit (a), Na_2tart (b) and NaAc (c), and without additive (d) in the liposomes, and in pure water (e). The crystal faces with asterisk are of COD and without asterisk are of COM.

ized by oxalate ions emerging oblique to the faces with a dense pattern of complexed calcium ions.[8] That is, there is a positively-charged surface for ($\bar{1}$01) crystal faces of COM. Since the surface of the liposome membrane is negatively-charged, a strong interaction occurs with the ($\bar{1}$01) crystal face of COM, resulting in the growth inhibition of this face and the preferential growth of other faces.

However, the addition of Na_2tart (Figure 2b) and Na_3cit (Figure 2a) significantly alters the crystal habits as revealed by XRD. In addition to the characteristic peaks assigned to COM, new diffraction peaks appeared at 0.618, 0.442, 0.368, 0.278, 0.241, and 0.224 nm, which are assignable to the crystal faces (200), (211), (002), (411), (103), and (213) of calcium oxalate dihydrate (COD), respectively. The strongest diffraction peak of COD grown in liposomes was (213) face with *d* spacing of 0.224 nm. The fraction of COD increased as increasing the concentration of Na_3cit or Na_2tart (Table 1). When the concentration of the carboxylates increased from 0.60 to 3.3 mmol/L, I_M/I_D (I_M/I_D is the ratio of the characteristic diffraction peak of COM at 0.365 nm and COD at 0.224 nm)[10] values decreased from 19.0 to 1.9 in the case of Na_2tart and from 2.4 to 0 in the case of Na_3cit.

In order to prove that the liposome membranes play a role in inducing COD formation, the crystallization of CaOxa from aqueous solution without PC in the presence of Na_2tart or Na_3cit was comparatively investigated. It can be seen from Table 1 that the ability of Na_2tart and Na_3cit to induce COD in aqueous solution is weaker than that in liposomes. In aqueous solution, COD can be induced only when the concentrations of Na_2tart and Na_3cit were larger than 1.0 and 0.55 mmol/L, respectively. In comparison, COD is formed in liposome when the concentrations of Na_2tart and Na_3cit are larger than 0.55 and 0.35 mmol/L, respectively. That is, the membrane/water interfaces provided by the liposomes have enhanced the ability of Na_2tart or Na_3cit to induce COD. Some special effect of $tart^{2-}$ and cit^{3-} ions on the crystal growth of COD may be occurred on the surface of lecithin bilayer. There may be a good correspondence between the COM crystal lattice and the group $^{-}$COO–CR^1(OH)–CR^{2}H–COO^{-} (for $tart^{2-}$: $R^1 = H$, $R^2 = OH$; cit^{3-}: $R^1 = CH_2COO^{-}$, $R^2 = H$) in citrate and tartrate. Thus, they can inhibit the pre-critical nuclei of COM and favor COD. Computer modeling[11,12] also confirmed the structural match between the di- or tricarboxylate molecule and calcium spacing in the crystal lattice. It can be seen from Table 1 that the capacity of inducing COD and that of inhibiting COM follows this order: Na_3cit > Na_2tart > NaAc.

COM has more affinity for renal tubule cell membrane surface than COD.[2] Theoretical calculations also suggested this conclusion.[11] So if any additive can induce more COD, this additive reagent may act as an inhibitor for urinary stones. This result may have potential application to suppress CaOxa crystallization directly and maybe useful in stone therapy.

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References

1 F. Grases and A. Llobera, *Micron*, **29**, 105 (1998).
2 J.-M. Ouyang, *Chem. Bull.*, **65**, 326 (2002).
3 S. Guo and M. D. Ward, *Langmuir*, **18**, 4284 (2002).
4 T. Bretherton and A. Rodgers, *J. Cryst. Growth*, **192**, 448 (1998).
5 P. K. Grover and R. L. Ryall, *Clin. Sci.*, **92**, 205 (1997).
6 N. Laube, B. Mohr, and A. Hesse, *J. Cryst. Growth*, **233**, 367 (2001).
7 R. Backov, C. M. Lee, S. R. Khan, C. Mingotaud, G. E. Fanucci, and D. R. Talham, *Langmuir*, **16**, 6013 (2000).
8 S. R. Khan, P. O. Whalen, and P. A. Glenton, *J. Cryst. Growth*, **134**, 211 (1993).
9 Q. L. Feng, Q. H. Chen, H. Wang, and F. Z. Cui, *J. Cryst. Growth*, **186**, 245 (1998).
10 M. Yuzawa, K. Tozuka, and A. Tokue, *Urol. Res.*, **26**, 83 (1998).
11 M. Sikiric and N. Filipovic-Vincekovic, *J. Colloid Interface Sci.*, **212**, 384 (1999).
12 D. E. Fleming, W. Bronswijk, and R. L. Ryall, *Clin. Sci*, **101**, 159 (2001).