Dynamic observation of polymorphic phase transition of calcium oxalate crystals; A novel system to elucidate the process and kinetics of urinary stones formation

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Calcium oxalate (CaC_2O_4, after this referred to as CaOx), one of the main components of urinary stones, has three types of hydrates. They are monohydrate (COM, stable phase), dihydrate (COD, metastable phase), and trihydrate (COT, metastable phase). We have observed traces of phase transition from COD to COM, but the phase transition process and rate are poorly understood. In this study, we crystallized COM, COD, and COT and performed *in situ* observation of the solvent-mediated phase transition from COT and COD to COM.

CaOx supersaturated solution (0.5 mM $CaCl_2$, 150 mM NaCl, 5.0 mM $Na_2C_2O_4$) was prepared in a 1 ml vial and stood at 4°C for 24 hours to obtain COM, COD and COT crystals. Then, the temperature of the solution was changed to 37°C, and the phase transition of the crystals was observed, focusing on COM, COD, and COT crystals, which were close to each other. We identified crystal phases by crystal shapes and Raman spectra.

COT crystals completely dissolved about 16 h after the start of the experiment, after which COD crystals began to dissolve. Finally, the COD crystals wholly dissolved in about 43 h. On the other hand, the stable phase COM crystals continued to grow during COT and COD crystals dissolution. The growth rate of COM crystals was 0.92 mm/h when COT crystals were present and decreased to 0.02 mm/h after the disappearance of COT crystals, indicating that the dissolution of COT crystals serves as an adequate solute supply to COM crystals.

We succeeded *in-situ* observations of solvent-mediated phase transitions from COD and COT crystals to COM crystals. As a result, the presence of COT crystals sustains the growth of COM crystals for a long time during the solvent-mediated phase transition. Since COT crystals rapidly undergo a phase transition and disappear quickly, COT crystals were not found in collected stones and have not been considered important. However, still, it possibly plays an essential role in maintaining and accelerating the rate of stone formation in the body. Furthermore, this method enables us to evaluate the effects of specific urine conditions, including proteins, on the kinetics of the stone deposition and phase transition. Combining this method and the observation of pathological deposits in stones will give us new insights to clarify the stone formation.