Synthesis of Calcium Carbonate Polymorphs in the Presence of Methionine

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Osteogenesis relates to biomineralization of $CaCO_3$ deposition doped with multiple amino acids. The crystallization of $CaCO_3$ in the presence of methionine with extremely unusual, ginger-shaped morphology is reported in this article. We show that unusual ginger-shaped $CaCO_3$ vaterite crystals selectively grow from the edge of rhombohedral calcite crystals with certain degree of preferred orientation. The unusual morphology and the growth process of the ginger-shaped vaterite $CaCO_3$ crystals were discussed with emphasis on methionine mediated selective binding and oriented aggregation/attachment of vaterite nanocrystals. Moreover, crystallization of $CaCO_3$ in the presence of methionine, a classified nonpolar amino acid, revealed atypical polymorphic selectivity, where vaterite is the major component. With all these in regards, this study further confirmed that nature of amino acid is crucial in achieving the desired selectivity in polymorph nucleation, as well controlling the crystalline nature and morphology of the $CaCO_3$ crystals.

Keywords: Ginger-shaped, Calcite, Vaterite, Methionine, Amino acid, Crystal growth.

INTRODUCTION

Osteogenesis relates to biomineralization of $CaCO_3$ deposition doped with multiple amino acids. The unique properties and rich variety of morphologies associated with biominerals have prompted the development of biomimetic synthesis of inorganic materials with complex form. The outperformed mechanical property associated with biominerals/biocomposites is essentially inherent from their highly complex, hierar-chical orderings with size range from nanometer to centimeter scales. Control over structures and morphologies always stand for fundamental concern in biomimetic synthesis of inorganic materials[1,2]. As one of the most abundant minerals in human body, calcium carbonate is an attractive model mineral for the studies of biomimetic synthesis. Over the past decades, great endeavor has been devoted to the control over biomimetic polymorph and morphologies of calcium carbonate, motivated by the fact that varieties of morphologies of calcium carbonate have been achieved in the presence of diverse organic substan-ces[3-9]. It is believed that protein plays a critical role in dictating the polymorphism and morphologies of $CaCO_3$ crystals in the biological system. Amino acids, major components of proteins, shown to effectively prompt the formation and growth of vaterite, the least thermal dynamically stable polymorph of $CaCO_3$ crystals in nature but essential polymorph

in human body[10-14]. In particular, the systematic studies of Kai *et al.*[15] seemed point to the direct connection of polarity and acidity of amino acids with the proportion of vaterite in the calcite-vaterite mixture of $CaCO_3$ crystals. It is also worth to mention that in Kai *et al.*[15] studies, amino acids with nonpolar polarity led to almost 100 % calcite in the final form of $CaCO_3$ crystals.

Methionine is an α-amino acid with the chemical formula $HO_2CCH(NH_2)CH_2CH_2SCH_3$. This essential amino acid is classified as nonpolar. Together with cysteine, methionine is one of two sulfur-containing proteinogenic amino acids. As an essential amino acid, methionine is not synthesized in humans. Hence we must ingest methionine or methionine-containing proteins. Methionine assists in the breakdown of fats and thereby prevents the build-up of fat in the arteries, as well as assisting with the digestive system and removing heavy metals from the body. Methionine is also a great antioxidant as the sulfur it supplies inactivates free radicals. Methionine is also one of the three amino acids needed by the body to manufacture creatine monohydrate, a compound essential for energy production and muscle building.

In this paper, we outline our findings in characterization and understanding of crystallization of $CaCO_3$ in the presence of methionine with extremely unusual, highly-porous, gringer-shaped morphology. The growth process of the unusual ginger-

shaped $CaCO_3$ crystals could be described as selective growth of vaterite crystal from the edge of rhombohedral calcite crystals with certain degree of preferred orientation. The unusual morphology and the growth process of the ginger-shaped vaterite $CaCO_3$ crystals were discussed with emphasis on methionine mediated selective binding and oriented aggregation/attachment of vaterite nanocrystals. Moreover, in contrast with previous report in which calcite was the major component in the presence of typical nonpolar amino acids, crystallization of $CaCO_3$ in the presence of methionine, a classified nonpolar amino acid, results in vaterite as the major component in $CaCO_3$ crystals[15].

EXPERIMENTAL

A typical synthesis of $CaCO_3$ crystals was carried out according to the following procedure: Calcium chloride (10 mL, 0.1 mol/L) solution, dissolved with additional 10 mg methionine, was slowly dripped into K_2CO_3 (10 mL, 0.1 mol/L) solution at a fixed speed of 0.5 mL/min. The precipitation reaction was carried out at 37 °C under continuous stirring with a constant rate of 150 rpm. After the completion of mixing of the reagent solutions, the reaction system was allowed to incubate for certain period of time (0, 5, 30 and 60 min) before quenching of the reaction. The obtained $CaCO_3$ crystals were filtered and rinsed extensively with distilled water, then dried in a desiccator at 70 °C for 48 h and used for later morphological and structural characterization.

RESULTS AND DISCUSSION

The morphology of obtained $CaCO_3$ was examined on FEL COMPANY (XL30ESEM-FEG) scanning electron microscopy (SEM). Fig. 1 presents typical SEM images of the obtained $CaCO_3$ crystals after 0.5 h incubation. The lower magnification image (Fig. 1a) indicates that $CaCO_3$ crystals exhibit unique ginger-shaped morphology. Observation in higher magnification image (Fig. 1a and 1b) reveals that ginger-shaped crystals appeared selectively "grow up" from the edge of layered-rhombohedral crystals, which are presumably the typical morphology of calcite crystals. The ginger-shaped crystals stretch out from layered-rhombohedral crystals with average length of 1 μm and diameter of 300 nm (Fig. 1c and 1d). Morphological and structural characterization of $CaCO_3$ crystals in the controlled experiment, *e.g.*, in the absence of methionine, revealed only the presence of layered-rhombohedral crystals-thermodynamically stable calcite crystals (Fig. 2a, SEM and 2b X-ray diffraction [XRD]). This result highly suggested that the presence of methionine in the reaction system is crucial in dictating the formation of unusual ginger-shaped $CaCO_3$ crystals.

To determine the crystal structure and polymorph of the ginger-shaped $CaCO_3$ crystals, powder XRD studies were performed with SHIMADZU-6000 X-ray diffractometer using Cu-Kα (γ = 1.5418 Å) radiation. Typical XRD pattern of Ginge-shaped $CaCO_3$ crystals, as shown in Fig. 2b, revealed
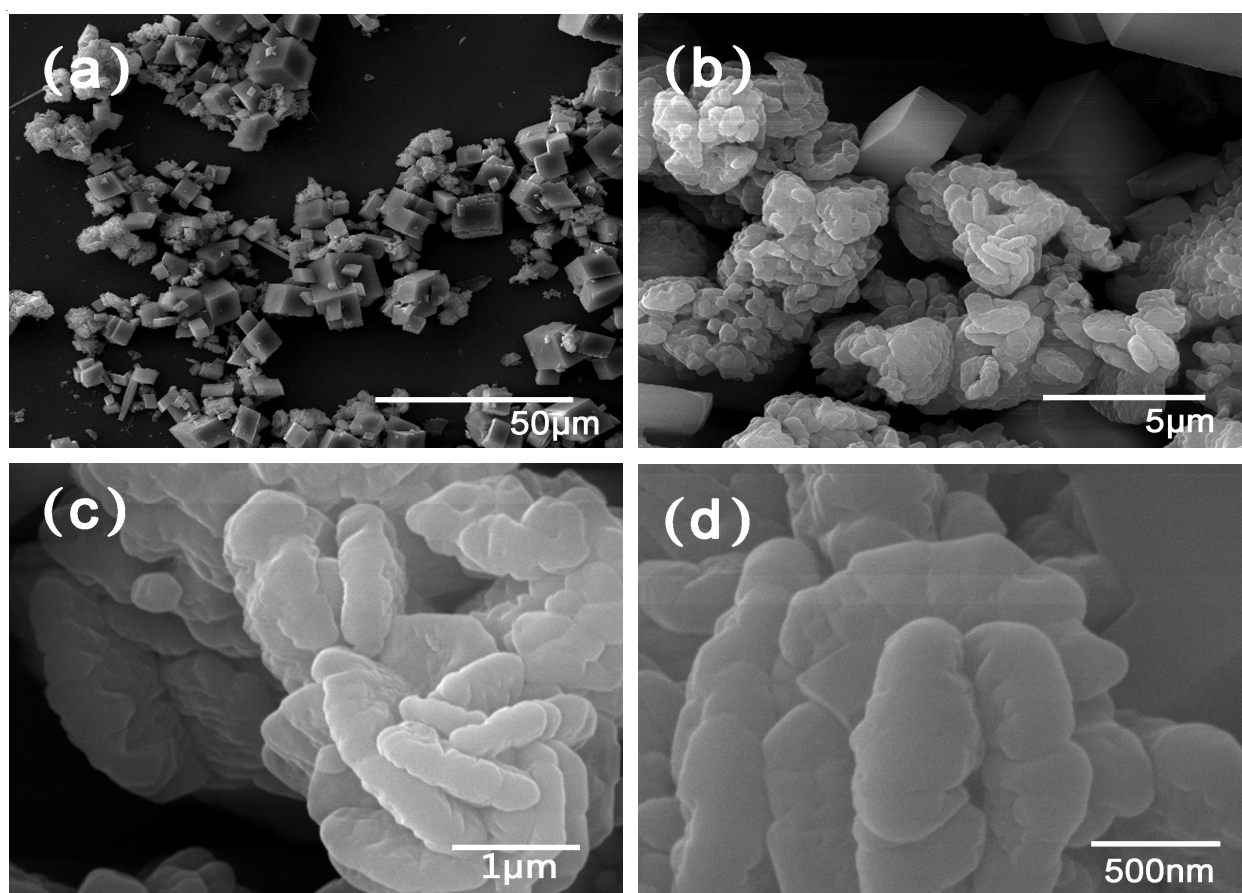

Fig. 1. SEM images of $CaCO_3$ crystals obtained in the presence of methionine after 0.5 h incubation; (a) Low magnification image, (b, c, d) high magnification images
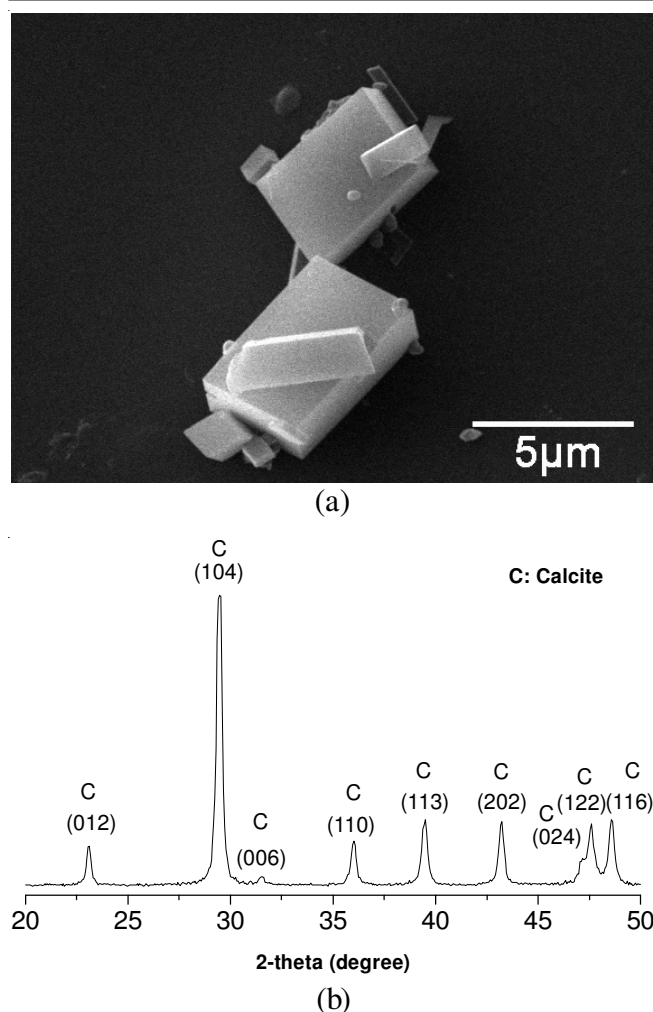
(a)

(b)

Fig. 2.   SEM images; (a) and XRD profile (b) of CaCO₃ crystals obtained in the absence of methionine after 60 min incubation

the crystals are consist of crystallographic mixtures-coexistence of both vaterite and calcite phases. On the basis of the principal peaks of the two phases [highlighted as (V) for vaterite and (C) for calcite], quantified analysis indicated vaterite forms the major fraction (-88 % in mol) in the crystals. This is *drastically different* with the early studies, in which amino acids with nonpolar polarity lead to almost 100 % calcite in CaCO₃ crystals. As a complementary technique, IR spectrum has been extensively applied in the analysis of the signature of polymorph in CaCO₃ crystals. FT-IR spectrum of CaCO₃ crystals was recorded on an ominic system 2000 in the KBr matrix (in proportion of 1 % CaCO₃ in KBr matrix). FT-IR measurement of CaCO₃ crystals (Fig. 3) exhibited prominent absorption bands at 712, 745, 872, 1080, 1490, 2853 and 2923 cm⁻¹. The relatively strong band at 875 cm⁻¹ and weak band at 712 cm⁻¹ are the characteristic peaks of the calcite phase. The strong band at 1485 cm⁻¹ and weak ones at 1080 cm⁻¹, 745 cm⁻¹ are the signature peaks of vaterite phase. This result confirmed again that the as-prepared ginger-shaped CaCO₃ crystals are the crystallographic mixtures of calcite and vaterite phases and, vaterite phase is the dominated polymorph. The weak and broad absorption bands around 2853 and 2923 cm⁻¹ could be assigned as bending vibrations of CH₃ and CH₂ groups associated with methionine. This implies that the as-prepared

ginge-shaped CaCO₃ crystals still contain small quantity of methionine, the phenomenon has been reported also in early studies. Thermogravimetric analysis confirmed the incorporated methionine in the as-prepared ginger-shaped CaCO₃ crystals is around 1.63 % (Fig. 4).
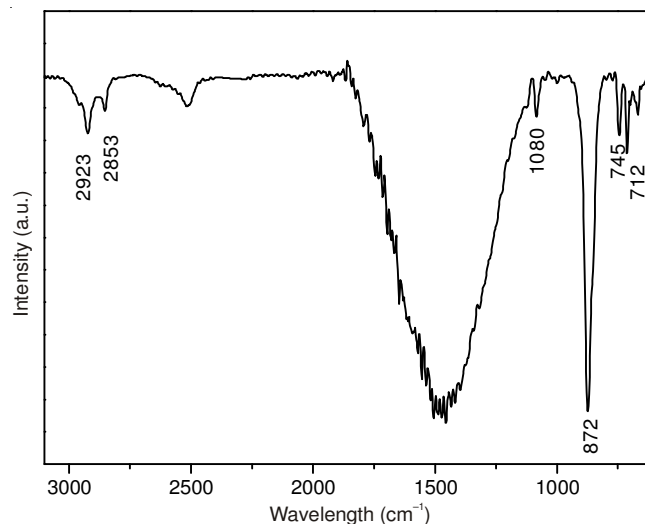

Fig. 3. IR spectra of CaCO₃ obtained in the presence of methionine
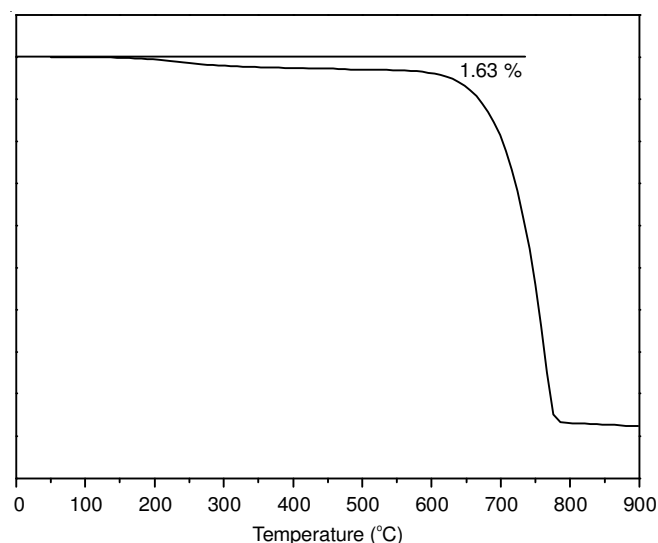

Fig. 4.   Thermogravimetric analysis profile of CaCO₃ crystals obtained in the presence of methionine after 0.5 h incubation

To determine the possible formation mechanism of ginger-shaped CaCO₃ crystals, the CaCO₃ crystals at different growth stages, controlled by tailoring incubation time before quenching the reaction, were collected for structural and morphological examination. SEM observation of CaCO₃ crystals with 0 min incubation time (Fig. 5a) showed loose aggregations of nanocrystals with irregular shape, the average size of the nanocrystals is around 100 nm. Structural characterization of CaCO₃ crystals in this early stage of growth revealed presence of both calcite and vaterite phases with vaterite is the dominated phase (Fig. 6). In considering of inorganic nucleation in biology system, it is generally believed that the role of organic substrate is to lower the activation energy of nucleation. In the cases of multiple polymorphic structures, structure-specific nucleation

leads to nucleation of one which is kinetically un-favored structure in the absence of organic substrate. Results showed here might suggest that nucleation of $CaCO_3$ crystals in the presence of methionine presumably proceeds through structure-specific nucleation, evident as vaterite is the dominated polymorph, but bearing certain degree of nonspecific nucleation characteristics, evident as calcite is still present in the products.

Evolution of crystal morphologies was evident in the SEM examination of $CaCO_3$ crystals after 5 to 60 min incubation (Fig. 5b-5d.). Growth of crystal is well established to follow two classical mechanisms, growth of large crystals at the expense of small crystals-ostwald ripening or formation of colloidal nanoparticles followed by internal reconstruction-aggregation-mediated crystal growth. The disappearance of nanocrystals and show up of rhombohedral $CaCO_3$ crystals (Fig. 5b) the typical morphology of thermodynamically stable calcite phase-strongly implied growth of calcite phase indeed obeys the classical growth mechanism. Moreover, highly unusual ginger-shaped crystals also started to appear after just 5 min incubation, which are presumably to be vaterite phase-aggregation of nanocrystals formed in the early growth stage. As shown in Fig. 1d, the crystals are assembled by interconnected network of nanocrystals, which is consistent with early studies, where vaterite micrometer-sized spherical grains were shown to be constructed with tiny nanometer-sized polycrystals. Interestingly, close examination of SEM images seemed indicate that ginger-shaped crystals appeared selectively "grown up" from

the edge of rhombohedral crystals (Fig. 5b and 5c). This interesting phenomenon can be understood by the following arguments: Rhombohedral crystals of calcite have six crystallographically equivalent facets, in which cationic Ca^{2+} and anionic CO_3^{2-} groups connected *via* a rhombohedral network with minimum surface free energy. Spiral growth of rhombohedral crystals result in the atomic steps on the crystal surfaces with two steps acute to the cleavage plane and two steps obtuse to the cleavage plane. The terrace plane and the step form vicinal surface. Chirally-selected microscopic crystal shape modification of calcite crystal was demonstrated in recent studies because of the specific binding of asp to the cleavage surface-step edge. Given the fact of specific binding of asp to the surface-step edge and in connection with fairly close common feature of molecular orbitals of asp and methionine, results shown here might conceivably be due to methionine-assisted selective binding of vaterite nanocrystals onto the edge of rhombohedral crystals followed by oriented aggregation/attachment growth. The argument seemed valid because of, geometrically, when a high symmetry facet joins the rounded part of a crystal the step line density increases while approaching facet edge. The availability of increased multiple binding sites (surface-step edges) might maximize the binding probability of vaterite nanocrystals, in the assistance of methionine, to the facet edge of rhombohedral crystals; energetically, thermal fluctuation of crystal facet edge might alter the terrace width of vicinal surfaces, this might lead to altered step surface energy in favor for the binding of vaterite nanocrystals in the
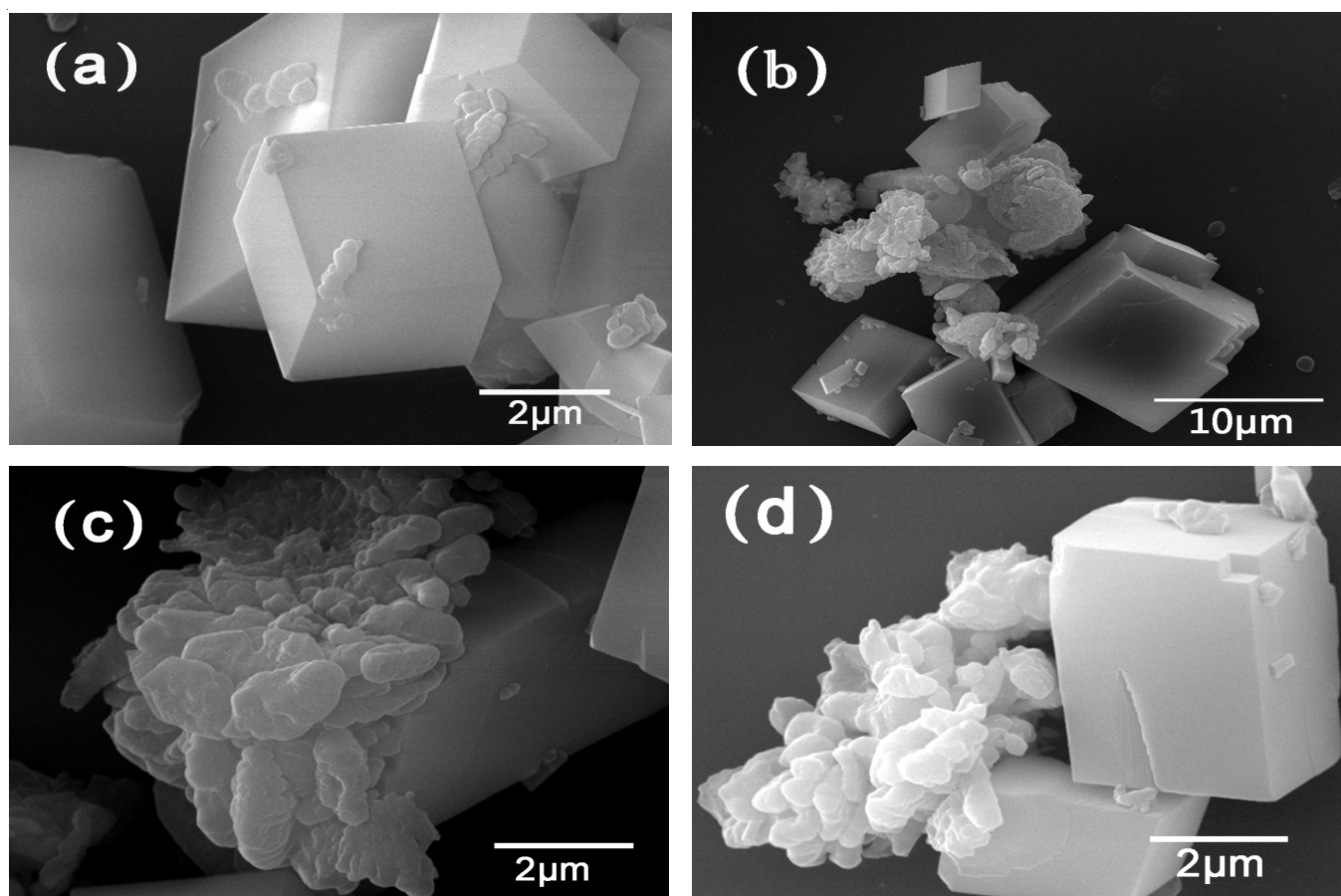

Fig. 5. SEM images of $CaCO_3$ crystal particles grown in the presence of methionine with different incubation times; (a) 0 min (b) 5 min (c) 30 min and (d) 60 min

assistance of methionine. Coexisting of vaterite spherical micro-particles with calcite rhombohedral crystals is the common phenomenon in biomimic synthesis of $CaCO_3$ with amino acids, surfactants, proteins and polymers as substrates. However, phenomenon of selectively binding of vaterite microparticles onto the edge of rhombohedral crystals as showed here in this study is drastically different with the early reports in which vaterite spherical grains appeared either grown separately from or randomly attached to the facet of calcite rhombohedral crystals. Methionine certainly plays its unique role here. Detailed structural and molecular modeling in revealing this particular growth phenomenon is under ongoing studies.

The retaining of the nanoparticle nature (*e.g.* aggregation of nanocrystals) of the resulting ginger-shaped vaterite crystals strongly implied that growth of ginger-shaped vaterite crystals doesn't follow the conventional crystal growth mechanism. Recently, a non-classical crystallization mechanism has become increasingly important in considering biomimetic crystalli-zation in the presence of organic molecules. In contrast to the ion-mediated classical crystallization pathway, non-classical crystallization events are always occurring with nanoparticles as intermediates. Followed by oriented nanoparticle aggrega-tion/attachment and mesoscopic transformation, this non-classical crystallization frequently leads to crystallographically single crystals but with complex morphologies and super-structures. Crystallization and growth of ginger-shaped vaterite crystals seemed fall into this category. Especially, the mechanism of oriented nanoparticle aggregation/attachment possibly plays a key role in dictating the formation of the unusual ginger-shaped morphology of vaterite crystals. As schematics showed in Fig. 5a, followed above arguments, after primary vaterite nanoparticles attached to the edge of calcite crystals, if the vaterite nanoparticle growth obey just randomly fusion-limited growth mechanism, as proposed previously, the vaterite nanoparticle particles would collide with each other to form secondary particles in the shape of spherical microparticles or microparticles with cauliflower shape, which are surely the cases in crystallization of $CaCO_3$ in the presence of certain amino acids. The unusual ginger-shaped vaterite crystal highly suggests, in addition of fusion-limited growth, oriented nanoparticle aggregation/attachment mediated by methionine also occurred during crystal growth, as schematics showed in Fig. 5b. The oriented nanoparticle aggregation/attachment actually tends to align in the direction normal to the calcite facet edge as revealed in Fig. 5a-5c. The trend seemed also quite maintained while multiple aggregated particles building upon the previous low level aggregated particles in order to form the ginger-shaped morphology. The aggregation of vaterite nanocrystals exhibit this anisotropic assembly in the presence of methionine is still not clear. However, it is quite possible that the anisotropic assembly is the consequence of selective desorption of methionine from certain vaterite facet followed by fusion of vaterite nanocrystals resembled as anatase nanocrystals self-organized into pearl necklace structures in the recent studies.

Conversion of vaterite phase to calcite phase is well known commonly occurred in aqueous reaction system. Surprisingly, in the presence of methionine, vaterite phase seemed quite stable and the proportion of vaterite phase to calcite phase

seemed independent of incubation time (Fig. 6). Moreover, early studies seemed indicate that proportion of vaterite phase to calcite phase was only dependent on the concentration of certain amino acid in aqueous reaction system. All these suggested that strong binding of amino acid to the vaterite nanocrystals surface might lead to the reduce of the surface energy, which in turn inhibit the conversion of vaterite phase to the thermodynamically stable calcite phase and, in the meantime, maintain the growth of vaterite crystal and calcite crystal separately and independently. Consequently, binding of amino acid to the surface of vaterite crystals or incorporation into the bulk of vaterite phase to stabilize it is extremely nece-ssary to maintain and interpret the unusual stability of vaterite phase with amino acid as substrate. Result of thermogravi-metric analysis of this study certainly confirmed the presence of methionine, which is also consistent with early studies where $CaCO_3$ vaterite microparticles always shown to have been incorporated with small quantity of amino acid whereas calcite microcrystals shown to have been incorporated very less or no amino acid. The similar conclusion has also been drawn on the study of crystallization of vaterite $CaCO_3$ in the presence of anionic phospholipids.
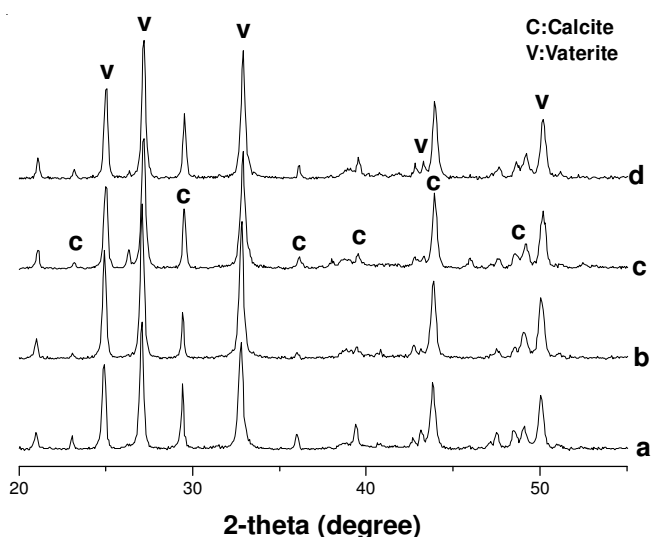

Fig. 6. X-ray diffraction patterns of $CaCO_3$ crystal particles grown in the presence of methionine with different incubation times; (a) 0 min (b) 5 min (c) 30 min and (d) 60 min

Conclusion

We have presented detailed studies on the crystallization of $CaCO_3$ in the presence of methionine with extremely unusual, highly-porous, ginger-shaped morphology. We have shown that the unusual ginger-shaped $CaCO_3$ vaterite crystals tend to selectively grow from the edge of rhombohedral calcite crystals with certain degree of preferred orientation. The unusual morpho-logy and the growth process of the ginger-shaped vaterite $CaCO_3$ crystals were explained on the basis of methionine mediated selective binding and oriented aggregation/attach-ment of vaterite nanocrystals. The atypical polymorphic selec-tivity in favor of vaterite polymorph has been discussed in considering the drastic difference in molecular orbital configu-ration of methionine with typical nonpolar amino acid. This research offers a novel and sample system for the study of

polymorphic selectivity and morphological control in the crystallization of CaCO$_3$ crystal with amino acid as substrate. With all these in regards, this study further confirmed that nature of amino acid is crucial in achieving the desired selectivity in polymorph nucleation, as well controlling the crystalline nature and morphology of the CaCO$_3$ crystals.

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