

## Interfacial Interactions between Polymerized 10,12-Pentacosadiynoic Acid LB Film and Calcium Carbonate Crystals

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Sizes, shapes and crystallographic directions of inorganic crystals formed by biomineralization processes are highly regulated. Generally it is believed that the regulation is due to interfacial interactions between inorganic materials and organic biopolymers. We attempted to control phase switching and crystallographic directions of calcium carbonate crystals on organic molecular assemblies. A thin-film of polymerized 10,12-pentacosadiynoic acid was prepared by Langmuir-Blodgett (LB) method and was soaked in supersaturated calcium bicarbonate solution containing magnesium ion. After soaking, water-drop shaped precipitates of about 10  $\mu\text{m}$  in size were formed on the film surface. The precipitates were calcite single crystals containing magnesium. Calcite *c*-axes in the precipitates were perpendicular to the polymerization direction of the LB film. Aragonite precipitates, that are generally expected to grow from the magnesium containing solutions, formed only when foreign particles acted as nuclei. These results indicate that the regulated features of inorganic/organic interfaces can control the polymorphism and crystallographic directions of inorganic materials.

Key words: calcium carbonate, crystal growth, LB film, biomineralization

### 1. INTRODUCTION

Biomineralization processes, or the biological syntheses of inorganic solids, yield materials with highly regulated structures under moderate conditions of temperature and pressure. In the processes, an organism creates proper organic matrices, and the inorganic crystals successively precipitate onto the matrices due to the chemical interaction at the inorganic/organic hetero-interface. The nucleation, growth and organization of biominerals are mediated by the supramolecular systems, and it is believed that the interfacial interactions can control crystallographic properties of inorganic solids such as phases, sizes, morphologies and orientations. Many current and evolving technologies require synthetic strategies that provide control over such the crystallographic properties. Therefore, understanding of the molecular interactions at the inorganic/organic interfaces is very important aspect of materials science [1].

Calcium carbonate and calcium phosphate are often utilized as the inorganic substances in biological organs. In the process of calcium carbonate formation in living bodies, the phase switching between calcite and aragonite, morphologies and crystallographic directions are controlled by organic polymers or membranes. However the controlling mechanism is still obscure, because the interfaces between inorganic/organic materials in living bodies possess such small and tangled structures that they cannot be studied directly. A model system that can reproduce the

inorganic/organic interfaces with regulated structure is required for the study on the interactions. We employed Langmuir-Blodgett (LB) method to obtain adequate artificial molecular assemblage. In our previous works, it was reported that LB films with carboxyl groups induced nucleation and crystal growth of calcium phosphate in a body environment [2, 3]. In the present study, we fabricated an organic thin-film with regulated molecular alignment by the LB method and studied the crystal growth process of calcium carbonate on the film. Surrounding environments also influence the phase, size and morphology of the resultant inorganic crystals. As to calcium carbonate crystals, many studies have shown that some additives own the ability to influence the crystallographic features [4, 5]. In the present paper, an attempt to control over the nucleation and growth of calcium carbonate crystals by the organic template and additives is reported.

### 2. MATERIALS AND METHODS

#### 2.1 Preparation of polymerized LB film

Polymerized LB films of 10,12-pentacosadiynoic acid (PDA:  $\text{CH}_3(\text{CH}_2)_{11}\text{C}\equiv\text{CC}\equiv\text{C}(\text{CH}_2)_8\text{COOH}$ ), a 25-C long chain with a diacetylene functionality, were prepared as reported in [6, 7]. Monomeric PDA was dissolved in chloroform with concentration of 1 mM, and the solution obtained was spread on a subphase of ion-exchanged distilled water at 18°C. After chloroform was evaporated, the residual organic film was slowly compressed up to a surface pressure needed

for the ordering of the organic molecules, typically  $20 \text{ mN}\cdot\text{m}^{-1}$ , and allowed to equilibrate for 30 minutes. The organic thin film was polymerized in-situ with 254 nm UV light. Polymerized PDA film was transferred onto the hydrophobic fused silica substrates or carbon micro grids supported by copper meshes using a horizontal lifting method.

## 2.2 Preparation of supersaturated calcium carbonate solutions

Aqueous solutions supersaturated with respect to calcium carbonate were prepared using the method by Kitano [8] with some modifications. Carbon dioxide gas was bubbled into a stirred suspension of calcium bicarbonate for 3 hours. The excess calcium carbonate particles were removed by filtration. A further period of bubbling (0.5 hour) was then employed to dissolve any crystal nuclei. The solution containing magnesium ion ( $[\text{Mg}^{2+}]/[\text{Ca}^{2+}] = 10$ ) was obtained by adding the magnesium chloride to the solution. The calcium carbonate polymorph most commonly obtained from the solution is aragonite [9].

## 2.3. Characterizations

Morphology of the precipitates on the LB film was investigated using SEMs (S-4500 and S-5000, HITACHI, Japan) operated at 5-10kV. Detailed crystallographic directions of the precipitates were analyzed using an electron backscatter diffraction (EBSD) system (Phase ID, ThermoNoran, USA) equipped to the SEM (S-4500). Another sample was sonicated in ethanol, and the precipitates and the polymerized films were suspended in the ethanol. A droplet of the suspension was transferred onto a carbon micro grid for TEM observations. The observations were performed using a TEM (JEM-2010, JEOL, Japan) operated at 200 kV equipped with an energy dispersive X-ray spectrometer (EDS).

## 3. RESULTS AND DISCUSSION

Figure 1 shows a TEM image of the polymerized PDA film on the carbon micro grid and the corresponding selected-area electron diffraction (SAED) pattern. Many slits are observed in parallel to the polymerization direction; the film cracked in a process of the directional polymerization of PDA [10]. Figure 2 shows the molecular arrangement in the polymerized film expected from the SAED pattern. The PDA molecules (carboxyl groups) are in slightly distorted hexagonal arrangement. The periodicity along the polymerization direction is 0.54 nm and the distance between neighboring polymers is 0.44 nm. Figure 3 shows a low-magnified SEM image of the polymerized film surface soaked in the magnesium containing supersaturated solution for 3 days. Very large aggregates of needle-like crystals were occasionally observed. These crystals were identified as aragonite using EBSD technique. Although the volume of the aggregates is not small, they were probably induced by some foreign particles, not by the polymerized film, because the density of the aggregates is extremely low. On the other hand, precipitates of about  $10 \mu\text{m}$  in size with a water-drop shape are observed on the surface of the film (Fig. 4). In the figure many parallel cracks are also imaged on the film. The water-drop shaped precipitates are uniformly oriented; their longitudinal

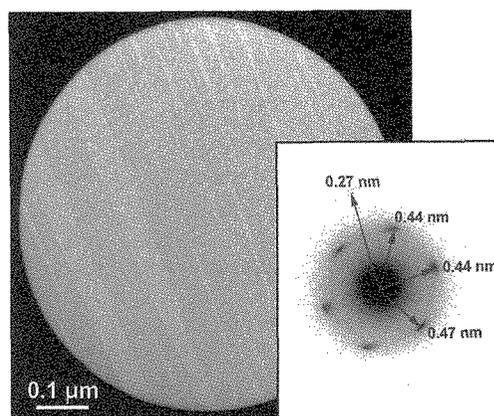


Fig. 1 TEM image and SAED pattern of the polymerized PDA film.

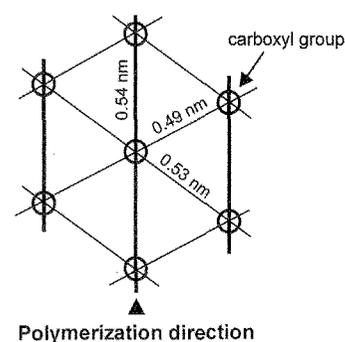


Fig. 2 Polymerized PDA film structure schematic. The open circles represent the positions of PDA molecules that correspond to the positions of carboxyl groups.

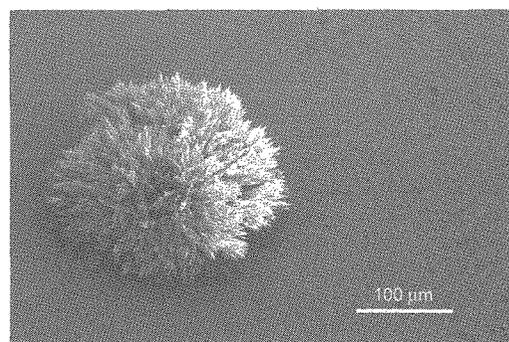


Fig. 3 Low-magnified SEM image of the precipitates grown from the magnesium containing solution. The large aggregate of needle-like crystals is identified as aragonite using EBSD technique.

axes are at a right angle with the cracks. The cracks are identical to the structure observed in the TEM image (Fig. 1) and they correspond to the polymerized structure of the PDA film.

Electron diffraction patterns from the precipitates indicate that they are calcite single crystals, despite their rough surface. The calcite *c*-axis is along the longitudinal direction of the water-drop shape. Therefore, the calcite single crystals are aligned with their *c*-axes perpendicular to the polymerization direction of the PDA film. EDS analyses of the

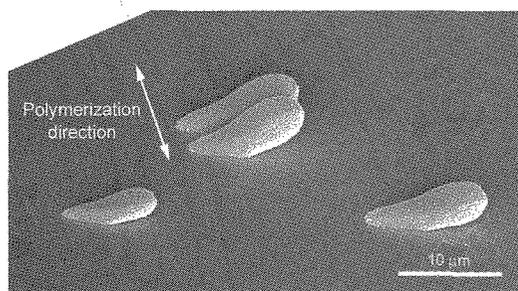


Fig. 4 SEM image of the precipitates grown on the polymerized PDA film. Note all precipitates are aligned with their longitudinal axes perpendicular to the polymerized direction.

specimens showed a considerable amount of magnesium (~30 at.%) in the crystal, indicating that the obtained precipitates are magnesian calcite.

Figure 5 illustrates the result of the combined use of SEM and EBSD techniques on the precipitate. The result revealed that the calcite single crystals adhered to the LB film most frequently with their (014) facets. The directions of the *a*-axes of the calcite precipitates and the polymerization direction of the PDA film were in fairly precise agreement. On the other hand, inclinations of the *c*-axes from the substrate normal had some dispersion around 45°.

Calcite is the only thermodynamically stable phase of calcium carbonate at the ambient temperature. However, as denoted above, previous works reported that the calcium carbonate polymorph expected from the magnesium-rich solution is aragonite. Magnesium ion acts as an inhibitor on the calcite crystal growth and affects the selection of the polymorphs. In the present study, foreign particles on the film probably played the role of nuclei and induced large aggregates of needle-like aragonite crystals. Nevertheless, only the calcite structure was found upon the polymerized PDA film. This result indicates that the initial stage of crystal nucleation determines the polymorph of following crystals growth, and the PDA film may exert a control over the polymorphism through the initial nuclei formation. There is not good agreement between the molecular alignment of the PDA film and the crystal structure of calcite (014) plane. The regulations on crystallographic features are probably not due to geometrical matching but to a stereochemical effect between carboxyl groups and calcite surfaces.

In the experiment, obtained calcite crystals were almost uniformly aligned on the polymerized LB film. Their *a*-axes were along the polymerization direction almost perfectly, whereas the inclination of *c*-axes against the substrates had a little variation. The difference in regulation accuracy between *a*-axes and *c*-axes should be important key to clarifying the stereochemical conformations between carboxyl groups of p-PDA film surface and calcite crystals.

#### 4. CONCLUSION

This study indicates that the regulation of structural features in the inorganic/organic interfaces leads to control over the crystallographic properties of inorganic materials. The carboxyl groups with regulated molecular alignment induced uniformly aligned calcite single crystals. The present result also implies the

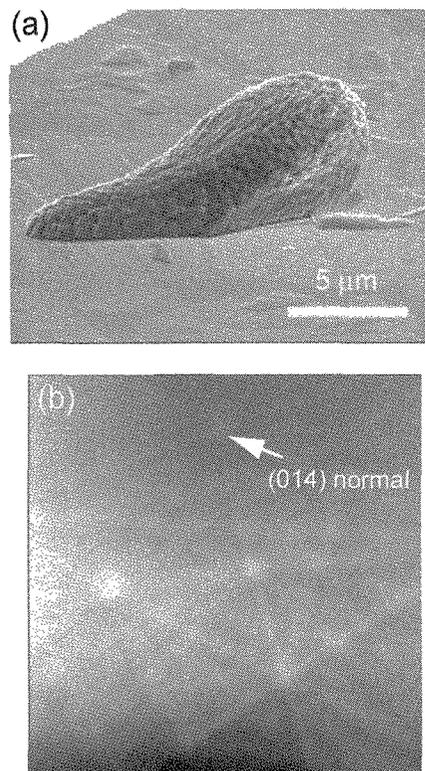


Fig. 5 Results of EBSD analyses on calcite precipitates: (a) SEM image of an analyzed precipitate and (b) obtained EBSD pattern. The EBSD pattern indicates that the calcite precipitate grew with its (014) facet parallel to the substrate.

possibility of polymorphism switching by the interfacial interactions between inorganic materials and organic matrices.

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