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Morphological Evaluation and Film Formation with Iso-Oriented Calcite Crystals Using Binary Poly(Acrylic Acid)

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The influence of poly(acrylic acid) (PAA) on the morphological evolution of calcite crystals depended on the concentration and molecular weight of the polyelectrolyte in an aqueous solution. A low-molecular-weight PAA (Mw 2000) weakly suppressed the regular crystal growth and miniaturized calcite grains as a suppressant. High-molecular-weight PAA (Mw 90 000 or 250 000) drastically decreased the grain size as a strong suppressant and promoted the formation of thin films as a template on the surface of a glass substrate. The difference in the performance of the PAAs was ascribed to the mobility and conformation derived from the polymer chain length. In a binary polymer system (Mw 2000 and 250 000), lozenge-shaped films consisting of iso-oriented nanoscale crystal grains were produced through nucleation with the high-molecular-weight PAA anchored to a glass substrate and controlled growth with the mixture of low- and high-molecular-weight PAAs.

Introduction

In recent years, material synthesis that mimics biomineralization has attracted a considerable amount of attention because of its potential for achieving highly tailored architecture consisting of organic−inorganic composites through ecological processing.[1−5] The morphological study on crystal growth of calcium carbonate, which is a typical biomineral observed in shells and corals, is important for understanding the essence of structural control in biological functions. The formation of calcium carbonate films, which is similar to nacre in shells, is especially interesting in this field. On a glass plate inserted in a living abalone shell, layered calcium carbonate films were built as nacre.[6] In nonbiological solutions containing poly(acrylic acid) (PAA), poly(glutamic acid) (PGu), and poly(aspartic acid) (PAsp), calcium carbonate grew as a film on various surfaces.[7−17] The results of the previous works suggest that the coexistence of specific organic electrolytes induces the film formation of the calcium carbonate crystals. However, the presence of organic functional surfaces, such as a porphyrin monolayer with carboxy groups,[9] polysaccharides,[10−16] and PVA,[17] was usually required for the promotion of the nucleation of calcium carbonate. Although the interaction between the polyelectrolytes and the carbonate crystal is important for morphological evolution, the detailed roles of PAA and PAsp have not been sufficiently clarified. Moreover, the crystallographic orientation of the calcium carbonate films has not been controlled by the addition of synthetic organic molecules, whereas the orientation of biological products is commonly regulated, such as that of aragonite plates in nacre.

This work is an investigation of the influence of PAA molecules on the crystal growth of calcium carbonate by changing the concentration and average molecular weight of the coexisting electrolytes. We found that the influence of the polyelectrolyte depends on the molecular weight. Finally, the formation of lozenge-shaped calcite films consisting of iso-oriented crystal grains was successfully achieved by the combination of low- and high-molecular-weight PAAs. The mechanism of the morphological changes is discussed from the viewpoint of the specific interactions of the polyelectrolytes and crystal faces.

Experimental Section

Calcium carbonate crystals were prepared from a 10 or 20 mM calcium chloride solution supersaturated by adding carbon dioxide generated by the decomposition of ammonium carbon-

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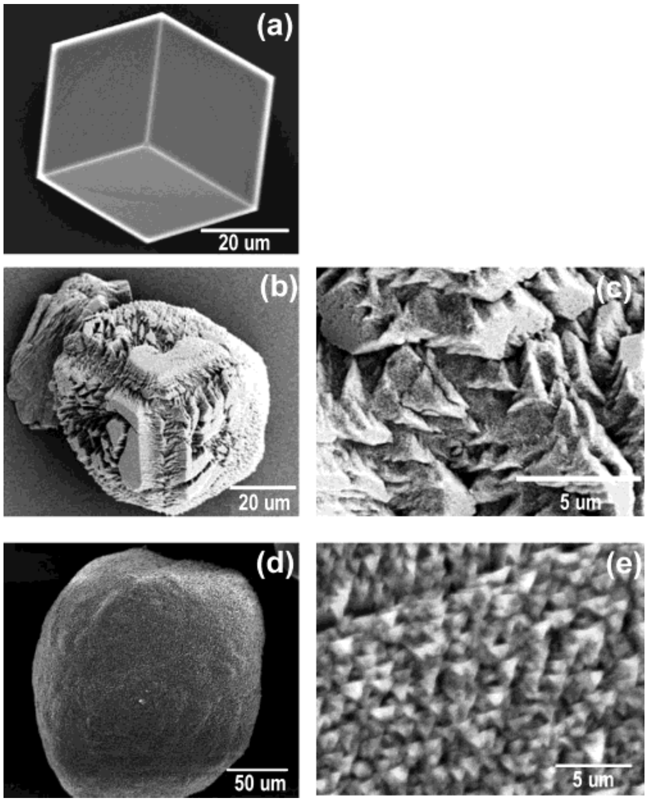

Figure 1. SEM images of calcite obtained in the absence (a) and in the presence of PAA2k: (b) C_{Ca} 10 mM, C_{PAA} 2.4 × 10^{-3} wt % (0.33 mM) for 3 days; (c) an enlarged image of (b); (d) C_{Ca} 20 mM, C_{PAA} 7.2 × 10^{-2} wt % (10 mM) for 3 days; and (e) an enlarged image of (d).

ate. Vessels containing 100 cm^3 of aqueous solution dissolving calcium chloride and coexisting electrolyte were covered with a polymer film having several pinholes and then placed in a ca. 5-dm^3 desiccator with 15 g of ammonium carbonate at room temperature. Glass slides used as substrates for heterogeneous nucleation were treated in a mixture of ethanol and potassium hydroxide aqueous solution, washed with purified water, and then immersed in a calcium chloride solution. We set the substrate upright in a vessel to prevent deposition of precipitates formed through homogeneous nucleation. Three kinds of PAAs with different average molecular weights (2000 (2k, Sigma-Aldrich), 90 000 (90k, Polyscience), and 250 000 (250k, Sigma-Aldrich)) were used as coexisting electrolytes. The concentration of PAAs (C_{PAA}) varied from 2.4 × 10^{-3} to 2.4 × 10^{-1} wt %. The molar concentration of carboxy groups contained in the PAA chains was estimated to vary from 0.33 to 30 mM. After a certain period between 1 and 6 days, the glass substrates were removed from the solution, washed with purified water, and dried at room temperature. The overall morphology of crystals grown on the substrates was monitored using a field-emission scanning electron microscope (SEM) and their structure was observed by means of a field-emission transmission electron microscope (TEM). X-ray diffractometry (XRD) and thermogravimetry were performed with a Rigaku RAD-C system using Cu Kα radiation and a Seiko Instruments TG/DTA6200, respectively.

Results and Discussion

In the absence of PAAs, obtained calcium carbonate was a mixture of calcite crystals bounded by the {10.4} faces (Figure 1a) and petal-like clusters of vaterite (not shown). The presence of PAA2k suppressed the formation of the vaterite clusters and drastically changed the morphology of calcite crystals. The interaction with carboxyl groups of the PAA molecules decreased the activity of the Ca^{2+} ions and inhibited the formation of vaterite, which is more soluble than calcite. The precipitation of calcite was also inhibited by the addition of a large amount of PAA2k (C_{PAA} > 1.44 × 10^{-1} wt %). When the influence of the additives was relatively weak (C_{PAA} 2.4 × 10^{-3} wt % (0.33 mM)), we obtained large {10.4} rhombohedra whose surfaces were rough and exhibited many trenches (Figure 1b) and triangular hillocks (Figure 1c). The size of the hillocks separated by the trenches was approximately 2−4 μm. As the influence of PAA2k increased (C_{PAA} 7.2 × 10^{-2} wt % (10 mM)), rounded crystals were obtained (Figure 1d). Triangular small grains of less than 1 μm which were arranged with the same orientation were found on the rounded crystals (Figure 1e). These facts suggest that the presence of PAA2k molecules suppressed the regular crystal growth through adsorption on specific surfaces, such as the {10.4} and the planes parallel to the *c* axis and then induced the formation of small grains on the basal crystal.

A small amount of high-weight PAAs (PAA90k and PAA250k) provided almost the same effects as that of low-weight one (PAA2k). With the addition of 2.4 × 10^{-3} wt % (0.33 mM) of PAA90k, rhombohedra with rough {10.4} surfaces were obtained on a substrate as shown in Figure 2a. However, the morphological change with an increase in the concentration of PAA90k was quite different from that with PAA2k. The deformation of half-rhombohedra was intensified as the concentration of PAAs increased (Figure 2b and c). Finally, planar films were formed on a glass substrate with 7.2 × 10^{-2} wt % (10 mM) of PAA90k (Figure 2d). In this case, the concentration of the carboxy groups was almost equal to that of calcium ions in the solution.

The morphological evolution with PAA250k was fundamentally the same as that with PAA90k. When the influence of PAA250k was relatively small (C_{PAA} 2.4 × 10^{-3} wt % (0.33 mM)), we also observed rhombohedra with rough surfaces containing small triangles (Figure 3a and b). The direction of the rhombohedron edges suggests that the *c* axis of calcite was perpendicular to the substrate. Planar films of calcium carbonate were grown on a substrate with PAAs of 7.2 × 10^{-2} wt % (10 mM) (Figure 3c). Extremely small grains were found in the films by SEM observation (Figure 3d), and crystallites of calcite with a size of 5−10 nm were recognized in a TEM image (Figure 3e). Since these nanoscale grains were observed in the films, the miniaturization effect of the high-molecular-weight PAAs was greater than that of the low-molecular-weight one. The weight loss on the thermogravimetry indicated the presence of PAAs in/on the calcite crystals grown in the solutions. The content of PAAs increased with increasing PAA concentration, and 3−4 wt % of the organic compound was contained in the calcite films produced with high-molecular-weight PAAs. This means that the adsorption of PAA chains highly suppressed the regular growth of calcite crystals and induced specific morphological changes. Although the previous reports showed that the formation of PAA-mediated calcium carbonate films required a specific surface,[9−17] such as a porphyrin monolayer, chitin derivatives, and PVA, the presence of PAA90k and 250k induced the film formation even on a glass substrate. Thus, the high-molecular-weight

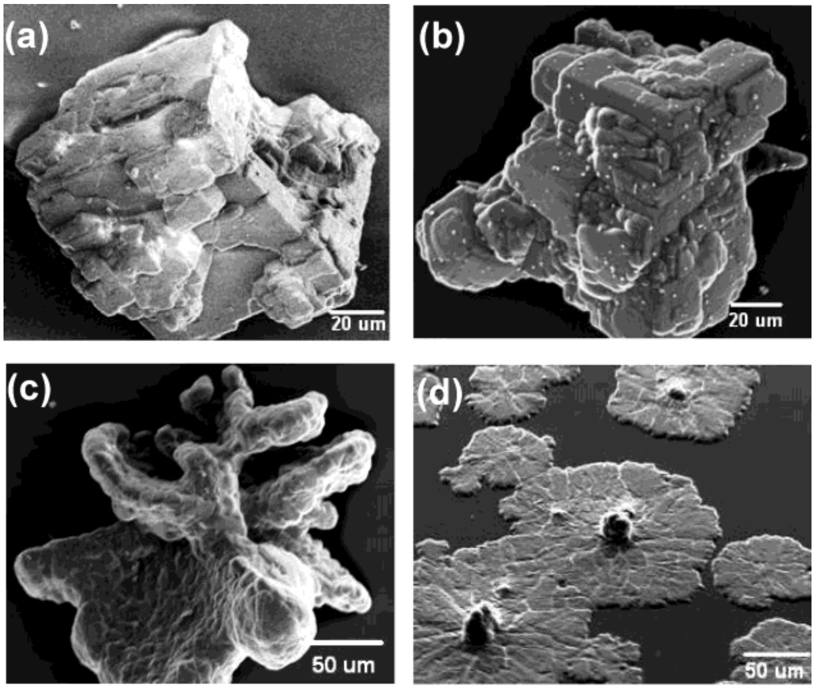

Figure 2. SEM images of calcite obtained in the presence of PAA90k: (a) C_{Ca} 10 mM, C_{PAA} 2.4 × 10^{-3} wt % (0.33 mM) for 3 days; (b) C_{Ca} 10 mM, C_{PAA} 2.4 × 10^{-2} wt % (3.3 mM) for 3 days; (c) and (d) C_{Ca} 10 mM, C_{PAA} 7.2 × 10^{-2} wt % (10 mM) for 3 days.

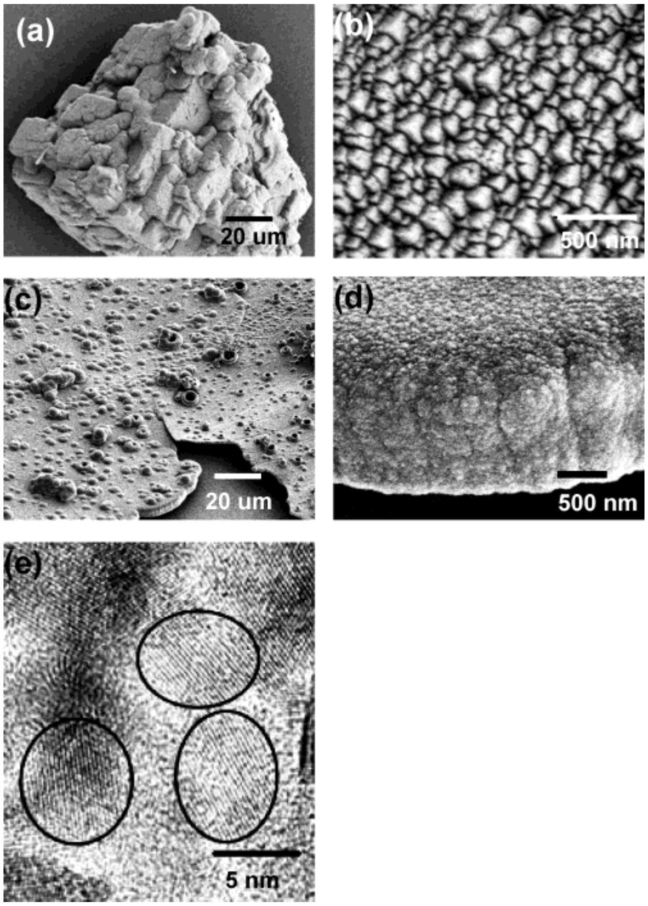

Figure 3. Electron micrographs of calcite obtained in the presence of PAA250k: (a) C_{PAA} 2.4 × 10^{-3} wt % (0.33 mM) for 3 days; (b) an enlarged image of (a); (c) C_{PAA} 7.2 × 10^{-2} wt % (10 mM) for 3 days; (d) an enlarged image of (c); (e) TEM image for C_{PAA} 7.2 × 10^{-2} wt % (10 mM). The lattice spacing was assigned to the (00.6) of calcite planes (d = 2.845 Å).

PAAs are deduced to be anchored to the glass surface where they promote the nucleation of calcite crystals. In these cases, however, the specific orientation of the crystal grains was not recognized in the calcite films.

The combination of low- and high-molecular-weight PAAs induced the formation of a new type of morphology. As shown in Figure 4a, lozenge-shaped calcite films with definite edges were obtained in 20 mM $CaCl_2$ solutions containing 7.2 × 10^{-2} wt % (10 mM) PAA250k and 2.4 × 10^{-3} wt % (0.33 × 10^{-1} mM) PAA2k. The formation of rounded crystals, as shown in Figure 1d, occurred with an increase in the relative concentration of PAA2k with respect to that of PAA250k in this system. Although the outline of the lozenge-shaped films exhibited regular crystallographic habits, the size of the crystal grains observed on the surface was less than 100 nm (Figure 4b). Most of the parallelograms showed the angles of 104° (Figure 4c) and 120° (Figure 4d), corresponding to the shape of the {10.4} and {00.1} faces, respectively. Moreover, the XRD peaks due to the (10.4) and (00.6) planes of calcite were distinct for these samples (Figure 5). These facts indicate that the crystal grains composing the lozenge-shaped films were three-dimensionally arranged with a specific crystallographic orientation in which the ⟨10.4⟩ or [00.1] direction was perpendicular to the substrate. The crystallographic orientation of the lozenge-shaped films was confirmed by the additional growth of calcite in the absence of PAAs. As shown in Figure 6, we observed two types of epitaxial growth in the directions of ⟨10.4⟩ (a) and [00.1] (b) on the surface of the films. The growth indicates the presence of two types of lozenge-shaped films having a specific direction.

As shown in a TEM photograph (Figure 7), there are lattice images of the 11.0 planes in the nanocrystals in the films. Thus, the [00.1] direction of all crystal grains in the films was arranged perpendicularly to the substrate. However, the [11.0] directions of the lattice (arrows) were slightly shifted at the grain boundaries (white broken lines). This suggests that the films were

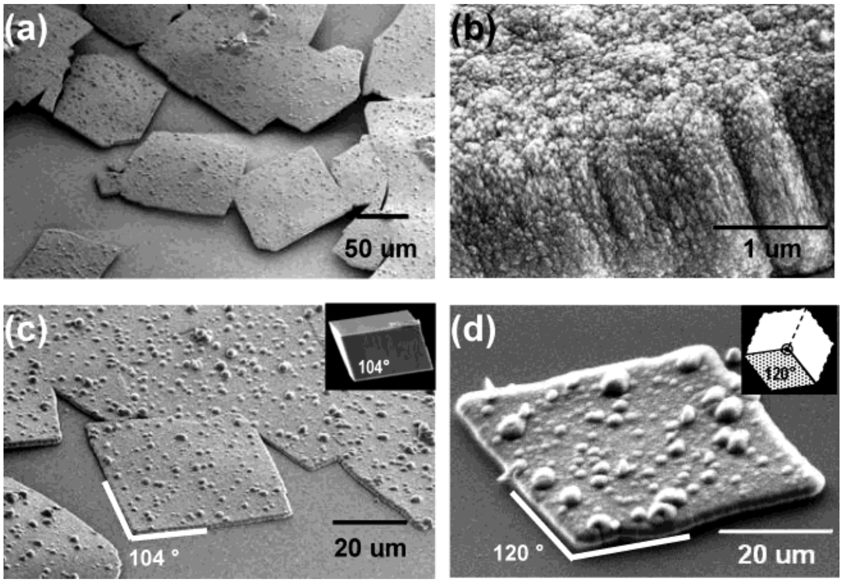

Figure 4. Electron micrographs of calcite obtained in the presence of 7.2×10^{-2} wt % PAA250k and 2.4×10^{-3} wt % PAA2k: lozenge-shaped films (a); enlargement of the surface (b); a film with the angle between the lozenge edges of 104° (c) and 120° (d). Insets in (c) and (d) are a photograph of a calcite single-crystal bounded by the {10.4} faces and an illustration of the top view from [00.1] of the trigonal crystal system, respectively.

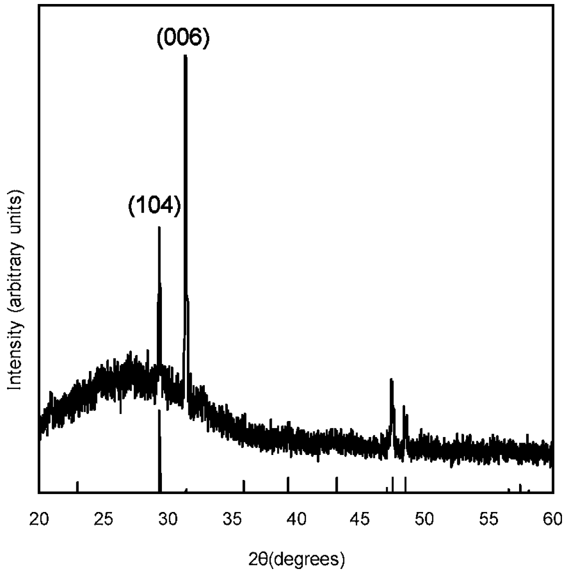

Figure 5. Typical XRD pattern of the lozenge-shaped films. The bar chart shows the standard pattern of calcite (JCPDS 5-0586).

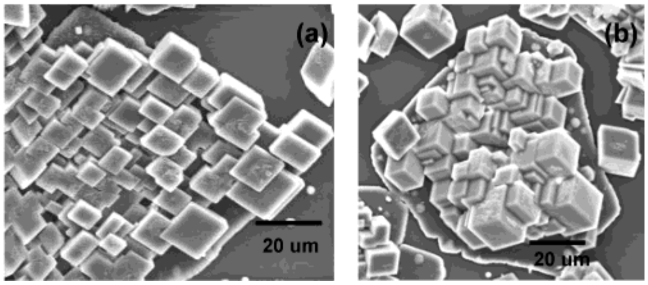

Figure 6. SEM images for additional growth of calcite on the lozenge-shaped films in the absence of any additives. Calcite crystals grew either with the {10.4} faces parallel to the film (a) or with the [00.1] direction perpendicular to the film (b).

composed of a mosaic texture of nanoscale crystal grains with weak crystallographic interaction.

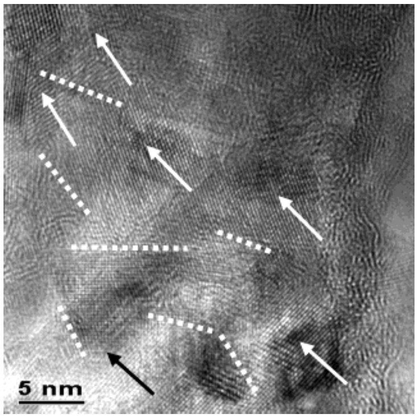

Figure 7. TEM image of the lozenge-shaped films. The lattice equidistances were assigned to the calcite 11.0 planes (d = 2.495 Å). Arrows and broken lines indicate the [11.0] direction and the grain boundary, respectively.

It is inferred that the presence of high-molecular-weight PAAs induced the oriented nucleation on the glass surface. However, the strong suppression effect of the high-molecular-weight PAAs miniaturized the crystal grains as a growth unit. In consequence, the films consisting of the nanoscale grains exhibited indefinite forms, as shown in Figures 2 and 3. The coexistence of low-molecular-weight PAA weakened the suppression of the crystal growth and promoted the interconnection between the crystal grains. Thus, the lamellas showing definite habits were produced through the assembly of iso-oriented grains along a specific crystallographic orientation.

As reported in previous papers, calcium carbonate films have been obtained in the presence of PAA2k on an organic functional surface, such as a Langmuir monolayer with carboxy groups,[9] polysaccharides,[10−16] and PVA.[17] We prepared disc-shaped films of calcite on a chitosan surface in solutions containing PAA2k (Figure 8). This fact indicates that low-molecular-weight PAA anchored to the functional surface and induced the

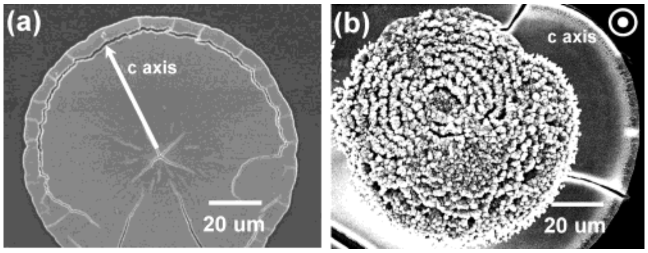

Figure 8. SEM images of calcite produced on a chitosan surface with 10 mM $CaCl_2$ and 2.4×10^{-3} wt % PAA2k: (a) 9 h, and (b) 24 h.

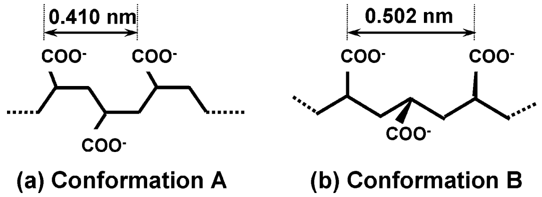

Figure 9. Conformations of carboxy groups of PAA.

nucleation of calcite. According to XRD analysis, calcite crystals grew with their [00.1] axis parallel to the surface in the early stage (Figure 8a), and the axis was perpendicular to the surface in the latter stage (Figure 8b). However, the three-dimensional order of the grains observed in Figure 4 was not achieved in the discoid films.

In the case of actual biomineralization, amorphous calcium carbonate (ACC) exists as a precursor of biomnieral.[18] The formation of crystalline films through the amorphous phase was proposed in vitro using synthetic polymers.[8,9] Although the presence of ACC was not recognized in our products, a similar phenomenon may occur in the solution. On the assumption that ACC is present prior to the crystal formation, high-molecular weight PAA promotes the production of the amorphous phase on a substrate and low-molecular weight PAA could induce it by the assistance of chitosan.

The conformations of PAA molecules are classified into two types, as shown in Figure 9. The distance between the equivalent carboxy groups is 0.410 nm in conformation A and 0.502 nm in conformation B by a geometric calculation. The distances between aligned calcium sites on the {10.4} planes of calcite are 0.405 and 0.499 nm. The basal (00.1) plane of calcite consists of calcium sites with an interval of 0.499 nm (Figure 10). Thus, the PAA chains basically adsorbed on these planes and suppressed the crystal growth. Thus, the triangular grains were observed on the rhombohedra when the influence of PAAs was relatively weak.

As the concentration of PAAs increased, the predominant effects of PAA molecules on the crystal growth of calcite depended on the molecular weight. The low-molecular-weight PAA (PAA2k) only decreased the size of the crystal grains and deformed the clusters of the triangular grains. In this case, the polyelectrolyte behaved as a weak suppressant for the calcite crystal because the molecules were reversibly attached on a surface due to their high mobility in water. On the other hand, the high-molecular-weight PAAs (PAA90k and PAA250k) anchored to the surface of a glass substrate and would promote the oriented nucleation of calcite grains as a template. The agreement of the distances of the carboxy groups (0.502 nm) of conformation B and the calcium sites (0.499 nm) of the (00.1) and {10.4} planes induces the oriented nucleation of calcite (Figure 10). However, the high-molecular-weight PAAs drastically miniaturized the growth units as a strong suppressant and caused the formation of a film consisting of stacked nanoscale crystal grains. The coexistence of PAA2k weakened the suppression with PAA250k and improved the interconnection between the crystal grains. Thus, lozenge-shaped films were produced through the three-dimensional arrangement of the oriented nanoscale crystal grains. The formation of iso-oriented grains indicates that the high-molecular-weight PAAs anchoring to a glass surface provide the specific arrangement of carboxy groups, which was highly suitable to the nucleation of the (00.1) and {10.4} faces of calcite. Although the (00.1) face is unstable and usually shows a kinked character, a flat face was achieved by strong adsorption of PAA. Low-molecular-weight PAA anchoring to a chitosan substrate promoted the formation of a film because PAA molecules were attached on the chitosan surface regardless of their molecular weight.[14] Nonionic hydroxyl groups on the chitosan surface are effective in the attachment of PAA molecules through hydrogen bonds. However, the orientation of the crystal grains on PAA250k on a glass slide was better than that on PAA2k on the chitosan surface. This suggests that the arrangement of the carboxy groups in the former had a higher regularity than that of the latter. The regularity of the arrangement of carboxy groups would be improved with the folding of relatively long polymer chains on the surface.

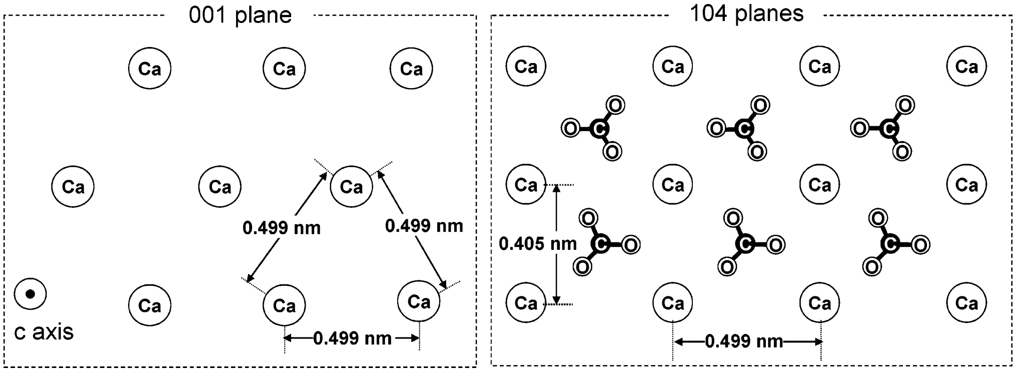

Figure 10. Distances of calcium sites on the 001 and 104 planes.

Conclusions

We found that PAA molecules with carboxy groups behave as a suppressant and template for the crystallization of calcium carbonate. High-molecular-weight PAAs anchored to a glass surface promoted the oriented nucleation of calcite crystals. A moderate suppression effect was achieved by the combination of low- and high-molecular-weight PAAs. Lozenge-shaped films consisting of iso-oriented crystal grains were successfully produced using a binary poly(acrylic acid) (PAA) system. The clarification of the specific interaction between polyelectrolytes and the different crystal faces would be useful for understanding the morphological evolution of the biomineralization and control of the microstructure of functional materials produced by biomimetic processing.

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