

Crystallographic analyses of exoskeletal calcium carbonate in *Armadilidium vulgare* (Crustacea, Isopoda)

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Calcium carbonate (CaCO_3) is one of the most major biominerals, found in many organisms such as mollusks, arthropods, and echinoderms. Previously, it is thought that biotic CaCO_3 crystals are precipitated from a supersaturated solution directly. However, in recent years, existence of amorphous calcium carbonate (ACC) as a precursor phase of calcite or aragonite has been suggested in some organisms, but this is still not certain. In this study, we have investigated a terrestrial isopod, *Armadilidium vulgare*, because previous works indicated by Raman spectroscopy that at least a portion of calcite is transformed from ACC in its exoskeleton. We mainly investigated crystallography of calcium carbonates, using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) with Electron Backscatter Diffraction (EBSD), Transmission Electron Microscopy (TEM) to understand the characteristics ACC-mediated calcite. Focused Ion Beam (FIB) was used for the preparation of TEM samples.

FIB-TEM observation indicated that the outermost layer (distal layer) of the exocuticle consists of well-crystallized platy calcite single crystals of ~ 0.5 micron thick and several micron in width. SEM-EBSD analyses of the distal layer indicated that scale-like domains on the surface of the exocuticle correspond to calcite single crystals. The c -axes of calcite in the distal layer are basically parallel to the anteroposterior axis, but varied from the complete parallelism to standing by $\sim 50^\circ$ from the surface. On the other hand, the inner layer (transition zone) of the exocuticle contains spindle-shaped crystals of ~ 0.5 micron and organic matrices. Electron diffraction (ED) indicated that the crystals are also calcite. TEM imaging, ED and Energy Dispersive X-ray Spectroscopy (EDS) analyses suggested that the endocuticle under the transition zone does not contain crystalline calcium carbonates, but ACC and organic matrices. TEM-EDS analyses indicated that calcite in the distal layer was almost free of phosphorous which can act as an inhibitor of the crystallization of ACC, whereas a certain amount of phosphorous was detected in the transition zone. Phosphorous is the most abundant in the endocuticle, probably to stabilize ACC.

From these results, it is inferred that calcite in the distal layer was precipitated on organic matrices from solution directly, and that in the transition zone was crystallized from the ACC precursor.

Keywords: calcite, ACC, SEM-EBSD, FIB-TEM, phase transition

Pressure-induced crystallization of amorphous calcium carbonate

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Amorphous calcium carbonate (hereafter ACC, $\text{CaCO}_3 \cdot n\text{H}_2\text{O}$), a metastable phase of calcium carbonate, contains variable amounts of water molecules and is a precursor of crystalline calcium carbonate, such as calcite, aragonite and vaterite. ACC immediately crystallizes in an aqueous solution or a humid condition. In nature, ACC stably exists only in biominerals, produced by living organisms. Crystals with complicated shapes are formed via ACC in biomineralization. Understanding crystallization of ACC will contribute to well understandings of the fundamental properties of ACC such as stability, structure, and mechanism of biomineralization. It is already known that ACC crystallizes by heating (e.g. Koga et al., 1998). On the other hand, stability of ACC at high pressure is unknown. In general, it is known that some amorphous materials show structural changes and pressure-induced crystallization by pressure. This study newly investigated pressure response of ACC at room temperature.

ACC was synthesized by the following procedure; aqueous solutions of 0.1 M CaCl_2 and 0.1 M Na_2CO_3 at 0 degree C were mixed, and the obtained suspension was filtered and dried in vacuum condition at room temperature. Vacuum drying was conducted using a diaphragm pump (about 10^2 Pa) for one day. The water content of ACC was estimated from water loss in TG-DTA. The compression experiment on ACC was conducted using a piston-cylinder and a hydraulic press. The applied pressure was kept in the range of 0.08-0.8 GPa for 10 min in the each run. The appearance of crystalline phases of calcium carbonate was characterized by power XRD patterns of as-grown ACC and the samples recovered from high pressure.

Chemical formula of the obtained ACC was $\text{CaCO}_3 \cdot 1.5\text{H}_2\text{O}$, which is consistent with previous studies. XRD patterns of the ACC ($n = 1.5$) and its pressured samples demonstrated that ACC transforms to crystalline calcium carbonate (calcite and vaterite) at pressures greater than 0.2 GPa at room temperature. The crystallization pressure of ACC is the lowest in amorphous materials reported before: for instance, amorphous alloys, silicon, and so on.

It is expected that the properties, including pressure response, of ACC depend on its water content. For comparing pressure response between ACCs with different water contents, ACC with less water content was necessary to be synthesized. We tested the possibility that the water content of ACC can be controlled by changing vacuum condition. For this purpose, additional evacuation for another day was applied to the ACC ($\text{CaCO}_3 \cdot 1.5\text{H}_2\text{O}$) using a turbo-molecular pump (about 10^{-3} Pa). As a result, water content of the obtained ACC was substantially decreased ($\text{CaCO}_3 \cdot 0.6\text{H}_2\text{O}$). XRD patterns of the ACC ($n = 0.6$) and its pressured samples indicated that ACC ($n = 0.6$) crystallized at pressure over 0.6 GPa, which is notably higher than the transition pressure of ACC ($n = 1.5$). These results suggest that the water contained in ACC plays an important role in pressure-induced crystallization of ACC.

Keywords: amorphous calcium carbonate, pressure-induced crystallization

Quantitative XRD analysis of {110} twin density in biotic aragonites

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{110} twin densities in biotic aragonite have been estimated quantitatively from the peak widths of specific reflections in powder X-ray diffraction (XRD) patterns, as well as direct confirmation of the twins using transmission electron microscopy (TEM). Influence of the twin density on the peak widths in the XRD pattern was simulated using DIFFaX program, regarding (110) twin as interstratification of two types of aragonite unit layers with mirrored relationship. The simulation suggested that the twin density can be estimated from the difference of the peak widths between 111 and 021, or between 221 and 211 reflections.

Biotic aragonite in the crossed-lamellar microstructure (three species) and nacreous microstructure (four species) of molluscan shells, fish otoliths (two species), and a coral were investigated. The XRD analyses indicated that aragonite crystals in the crossed-lamellar microstructure of the three species contain high density of the twins, which is consistent with the TEM examination. On the other hand, aragonite in the nacre of the four species showed almost no difference of the peak widths between the paired reflections, indicating low twin densities. The results for the fish otoliths were varied between the species. Such variation of the twin density in biotic aragonites may reflect different schemes of crystal growth in biomineralization.

Keywords: {110} twin, aragonite, biomineralization, DIFFaX, TEM, X-ray diffraction

Influence of intracrystalline organic macromolecules in shells on the crystal growth of calcite

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Biominerals often possess well-regulated structures with superior properties. Such superiority may be ascribed to the fact that biominerals are not pure inorganic crystals because they contain a certain amount of organic matter. Among the organic matter, "intracrystalline" organic macromolecules such as proteins and polysaccharides have been studied recently, owing to their important roles during crystal growth. Nanoscopic investigation is essential to understand the interaction between crystals and intracrystalline organic macromolecules because the sizes of the organic macromolecules estimated from their molecular weights are several to dozens nanometers.

We are investigating mollusk shells, especially focusing on the relationships between calcite crystals, which is one of the polymorphs of calcium carbonate, and intracrystalline organic macromolecules. Transmission electron microscopy (TEM) with electron energy-loss spectroscopy (EELS) identifies the intracrystalline organic macromolecules, and X-ray diffraction (XRD) evaluates macroscopic properties of the crystals. Using these nanoscopic and macroscopic techniques, we revealed that the distribution of the intracrystalline organic macromolecules affects the microstructure of the crystals. In the prismatic structure in a pearl oyster (*Pinctada fucata*), which is composed of columnar calcite crystals, intracrystalline organic macromolecules are distributed inhomogeneously, which causes small misorientations and local lattice strain. On the other hand, the organic macromolecules in the prisms in a pen shell (*Atrina pectinata*) are distributed homogeneously in the crystals, and local lattice strain is rarely observed. We thought such difference is originated from the different affinities of the organic macromolecules to the calcite crystals. Therefore we conducted calcium carbonate crystallization *in vitro* with EDTA (ethylenediaminetetraacetic acid) soluble extract from the prisms. It was found that the organic macromolecules in the extract from *P. fucata* and *A. pectinata* are incorporated differently in calcite. Furthermore, we are characterizing the organic molecules in the extract using molecular biological techniques to consider their interaction with their host calcite crystals.

Keywords: organic macromolecules, calcite, biomineral, TEM

Dynamics of organic molecule adsorption on calcium carbonate calcite surface: a molecular dynamics study

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Crystal growth of CaCO_3 in organisms is controlled by organic molecules, such as polymers, proteins and peptides. Elucidating the mechanism of the CaCO_3 growth control by organic molecules is of particular importance for understanding of the mechanism of biomineralization, crystal morphology technologies and the development of novel organic/inorganic hybrid materials. However, it is quite difficult to analyze the structure and dynamics of water and organic molecules at a CaCO_3 crystal surface in detail by experimental means. Thus, our understanding of the mechanism of CaCO_3 crystal growth control by organic molecules is still poor.

Recently, we have started computer simulation studies on the mechanisms of CaCO_3 crystal growth and the control of it by impurities. One of them is a molecular dynamics (MD) simulation study of the adsorption of aspartic acid (ASP) on the surface of CaCO_3 calcite. At the presentation, I will talk mainly about this MD simulation study.

The simulation was carried out for both (104) and (110) faces of CaCO_3 calcite. Intermolecular interactions acting on CaCO_3 and H_2O are calculated using simple Ca^{2+} , CO_3^{2-} and H_2O models in which the Coulomb potentials between charges and the short-range interactions between atoms are assumed. The intermolecular interactions acting on ASP was calculated using the CHARMM force field. Temperature was set at 298 K.

Simulation results indicated the formation of a few H_2O molecular layers on the (104) face. In each layers, H_2O molecules were arranged in an ordered state. However, such layers did not appear on the (110) face. It proved that the formation of the layers strongly influences the adsorption conformation of ASP at the calcite surface. For the (104) face, ASP did not adsorb directly onto the face, but a H_2O molecular layer was sandwiched between the adsorbed ASP and the face. Moreover, careful analysis of the free energy of ASP adsorption suggested the existence of several metastable adsorption conformations at the face. Details of the results will be shown at the presentation.

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Keywords: biomineralization, computer simulation, crystal growth, surface and interface, calcite, aspartic acid

Preparation of Core-Shell Type Organic/Metal Hybridized Nanocrystals and Their Optical Properties

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Organic nanocrystals (NCs) are in the mesoscopic phase between a single molecule and the corresponding bulk crystals, and are expected to exhibit peculiar optical properties, depending on crystal size and shape. Well-defined, i.e., size- and shape-controlled, organic NCs could be prepared successfully from π -conjugated organic and polymer compounds by developing the reprecipitation method. Interestingly, organic NCs exhibited the size-dependence of linear optical properties (extinction and fluorescent emission spectra), which is speculated to be caused not by so-called quantum size effect in semiconductor quantum dots but by thermal softening of nanocrystal lattice, due to increase in specific surface area with decreasing crystal size. Namely, the effective π -conjugation length would decrease, owing to disordered and strained nanocrystal lattice, and then the band gap (or HOMO-LUMO gap) would widen with decreasing crystal size.

In this presentation, recent progress on hybridized organic NCs and ordered array structure of encapsulated organic NCs will be introduced in detail for optically functional materials toward next-generation organic device application. In other word, hybridization is one of the main and important objectives in current material science. Our attention is now focused on core-shell type hybridized organic NCs, which seem to be the best suited nanostructure for the occurrence of novel optoelectronic properties and photonic function induced by core-shell interface interaction. Polydiacetylene (PDA) NCs (core) and Ag nanoparticles (NPs) (shell) hybridized NCs could be fabricated reproducibly by the development of visible-light-driven photocatalytic reduction method. PDA is one of the most promising candidates in organic nonlinear optical (NLO) materials, which shows the large third-order NLO susceptibility and highly speed of optical response in femto-second order. Actually, the value of the susceptibility from PDA NCs (core) has been multiplied evidently, due to the enhancement effect of optically electric field induced by localized surface plasmon resonance from Ag NPs (shell). The present reduction process could proceed easily, being independent of core size and shape, when the redox potential of metal ion (or metal complex) should be located between conduction band and valence band in π -conjugated polymer such as PDA. So some various hybridized NCs could be produced in the similar manner, for example, rod-like and fibrous PDA NCs, polyalkylthiophene NPs as a core material, and Pt NPs and Au NPs as a shell material. But, the subsequent chemical reduction process was performed at the same time, when Au NPs (shell) is prepared.

On the other hand, it should be necessary to arrange and integrate organic NCs, including hybridized ones, on a substrate so as to receive and transmit input- and output-information signals by electronically and/or optically accessing to organic devices. So, encapsulations of organic NCs, patterned substrate, and tapered cell method have been employed suitably to fabricate and control ordered array structure of organic NCs on a substrate. First, mono-dispersed polystyrene microsphere (PSMS) was used as a model of encapsulated (and hybridized) NCs, and some beautiful ordered array structure could be formed on the patterned substrate by optimizing the size of PSMS and pattern design. Especially, so-called Kagome Structure composed of PSMSs could be fabricated for the first time, which structure is said to provide the confinement effect of light propagation and a kind of photonic crystals. In addition, it has become possible to build-up the order array structure consisted of encapsulated semiconductor NPs and PDA NCs produced by emulsion polymerization process for optoelectronics and photonics devices application.

Finally, the future scope in the relevant fields of optoelectronics and photonics will be discussed in brief.

Keywords: Organic Nanocrystal, Hybridized Nanocrystal, Reprecipitation Method, Nonlinear Optical Property, Localized Surface Plasmon Resonance, Visible-Light-Driven Photocatalytic Reduction Method

Crystal Growth in Colloids Due to Charge Induced Crystallization

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Submicron sized charged colloidal particles dispersed in water self assemble into ordered crystal structures at sufficiently strong electrostatic interparticle interactions. Here we report the charge induced crystallization in dilute aqueous dispersions of colloidal silica by varying pH, and unidirectional crystal growths of the silica colloids under gradients of pH and temperature.

Keywords: colloid, charged colloidal particle, colloidal crystal, crystal growth, solid liquid interface, silica particle

Molecular Dynamics Study of Aqueous solutions: solubility calculation and crystallization process

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Aqueous solutions are fundamental to chemistry, biology, and geology. When an aqueous solution is in high pressure and high temperature conditions, the aqueous solution becomes supercritical. It is known that the behavior of supercritical region is different from that of ambient region; however, the experimental study is very difficult. Molecular Dynamics (MD) is useful tool to observe a system in such conditions. Solubility which is one of most significant properties can be calculated with MD. Solubility calculation with MD has mainly two approaches: the free energy calculation and the direct calculation, which are thermodynamic approach and kinetic approach, respectively. The thermodynamic approach, the free energy calculation, calculates separately the values of chemical potential in solution phase and solid phase. This approach has less calculation cost and less information. The kinetic approach, the direct calculation, employs salt-solution combined system. This approach has much information which includes relaxation process. However, there is no knowledge whether two approaches produce the same solubility value or not. Thus, it is, of essential importance, to test by using two different approaches. We firstly have calculated the solubility of ambient system with the two approaches and concluded two approach had good agreement. This work will contribute to the computational chemistry field as well as solubility in supercritical region. The study at supercritical conditions is undergoing.

Keywords: sodium chloride, solubility, crystallization, molecular dynamics, supercritical solution, interface of solid

Growth rate and specific surface free energy of synthesized quartz single crystal

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The specific surface free energy of quartz single crystal was determined by contact angle of water and formamide droplets on the crystal surface, and compared with the morphology of the crystal. The growth rate of the quartz crystal can be regarded as a function of the experimentally obtained specific surface free energy. The distribution of the specific surface free energy indicates the existence of negative step free energy.

Keywords: crystal growth, crystal surface, free energy

In-situ observation of crystal growth from undercooled liquids using containerless technique

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We performed in-situ observation of crystal growth from the undercooled liquids of alloys and oxides using high-energy x-ray diffraction combined with the container-less technique . Using the method, we can see the various phase transformation from the undercooled liquids to the stable crystalline phases. From the observation results we discuss about the crystal growth mechanism from the undercooled liquids from the viewpoint of the correlation between liquid and crystal structures.

Keywords: crystal growth, supercooled liquids, levitation technique