

Calcium-Binding Membrane Influence on Polymorph and Morphology of Calcium Carbonate Formed by Biomimetic Mineralization

Kazuhiko Ichikawa* **and Noriyuki Shimomura**

Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, N10W5, Sapporo 060-0810

Received June 9, 2005; E-mail: ichikawa@ees.hokudai.ac.jp

The typical conclusions can be drawn as follows: (1) Since vaterite was only formed on calcium-binding poly(L-aspartate)-coated surface between 15 and 35 °C, the carbonic anhydrase (CA)-arising activity of zinc array (i.e., zinc-coordinated imidazolin-terminated silane monolayer, as shown in *Chem. Eur. J.* **2003**, *9*, 3235) prevailed the temperature influence on the polymorph of calcium carbonate. Time evolution of calcium carbonate formation was enhanced by the zinc array. (2) The calcium carbonate crystallized only on imidazolin- and sulfonate-terminated silane membranes has shown a number of microspheres of 1 μm more or less in diameter. (3) The higher temperature and higher pH induced aragonite formation at the sulfonate-terminated Nafion membrane in the presence of the above zinc array. (4) The observed morphology of aragonite or vaterite was similar to the spherulitic aragonite or vaterite formed by biological mineralization in marine organisms.

Marine invertebrate animals, such as mollusk, reef-building coral, and zooplankton foraminifer have been keeping the preservation and prosperity of their own species through the biomineralization process for over around five hundred million years. The mineralization was in vitro carried out in the presence of macromolecules extracted mainly from mollusk shells to reveal the polymorph and morphology of the formed calcium carbonate.[1] The amino acid residue sequence of the organic matrix protein nacrein extracted from the nacreous layer showed the CO_2 hydration-accelerating carbonic anhydrase (CA) homologous domain and the Gly–X–Asn repeat domain as Ca^{2+}-binding centers (X stands for Asp and rarely Glu).[2] Aragonite crystal growth was performed in the presence of acidic macromolecule that was extracted from the nacreous shell layer and mollusk shells.[3] The role of the CA domain has, however, received little attention in the above-mentioned mineralization experiments. The influence of the CA-arising domain on calcification was, on the other hand, studied by means of biomimetic mineralization; the polymorph of the calcium carbonate formed at the Ca^{2+}-binding substrate showed vaterite in the presence of the zinc-two-dimensionally coordinated silane monolayer (zinc array).[4] The aragonite and vaterite were never formed from the HCO_3^-- and Ca^{2+}-saturated inorganic solution at lower than 25 °C and pH 7–8.[5]

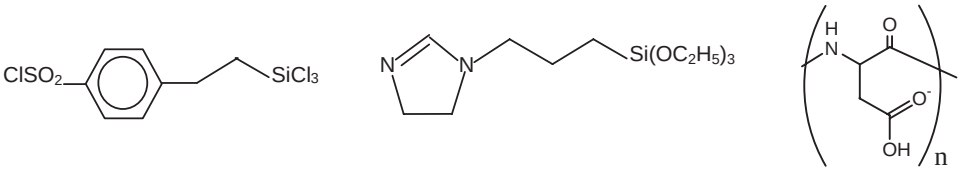
The intrinsic organic matrix in the calcium carbonate mineral formed by marine animals was isolated from reef-building coral skeletons or fossil zooplanktonic foraminifera.[6–8] The amino acid composition of intracrystalline organic matter in coral skeletons was analyzed to have relatively high amounts of Asp, Glu, Gly, and Ala.[6] The deduced amino acid sequence of the major protein extracted from the calcified exoskeleton of scleractinian coral showed tandem repeats but did not exhibit a significant Ca^{2+}-binding domain.[7] The organic matrix of foraminifera consisted of Asp and/or Glu-highly-including Gly, Ala, and Ser-including domains,[8] as seen in corals and mollusks. The marine organisms are used to keep macromolecules in their shells and skeletons on the way to biological mineralization. The intrinsic protein for the calcium carbonate formation is of Ca^{2+}-binding Asp and/or Glu, and may be constructed by β-pleated sheet structures, since the high fraction of amino acid components Gly, Ala, and Ser is similar to fibrous structural proteins such as silk-fibroin.

Our research has been to in vitro carry out calcification by mimicking biological mineralization. This work is to reveal in vitro whether the CA-arising activity of the above zinc array[4] prevails the temperature effect on polymorphic formation of calcium carbonate or not, and whether the calcium carbonate amount produced from carbon dioxide depends on the zinc array activity or not. The second target in this work related to the biomimetic mineralization is to design a membrane that consists of zinc- as well as calcium-binding sites in nanospace. A zinc- and calcium-simultaneously-binding membrane was prepared from sulfonate- and imidazolin-terminated silane molecules, since the intrinsic protein, the nacrein related to mollusk biomineralization, consists of a CO_2 hydration-accelerating center and calcium-binding Gly–X–Asn (X = Asp, Glu, or Asn) domains.[2] In the previous work, the calcification and the hydration of carbon dioxide were carried out at the separated substrates.[4] The third target is to reveal the polymorph/morphology of the formed calcium carbonate at the calcium-binding Nafion membrane under the coexistence of the zinc array. The calcium carbonate can be formed by exposing a number of HCO_3 molecules to carboxylate/sulfonate-legated calcium ions.

Experimental

Materials. 2-(4-Chlorosulfonylphenyl)ethyltrichlorosilane (**ClSuSi**) and 3-(2-imidazolin-1-yl)propyltriethoxysilane (**ImSi**) were obtained from Gelest and Fluka, respectively, poly(L-aspartate) (MW 35400; **pAsp**) (Chart 1) from Sigma, chitin from

Published on the web April 7, 2006; DOI: 10.1246/bcsj.79.580



ClSuSi **ImSi** **pAsp**

Chart 1.

Seikagaku Kogyo, and $Zn(NO_3)_2 \cdot 6H_2O$/$Ca(NO_3)_2 \cdot 4H_2O$/$(NH_4)_2CO_3$ from Wako. These commercial reagents were used as obtained without further purification.

Preparation of Homosilane or Mixed Silane-Coated Mica (ImSi[4] or ImSuSi Substrate). It is difficult to coat a silane monolayer on a mica surface, since the mica surface is inert to react with silane molecules because of few silanols (–SiOH) present. We can induce silanols on the mica surface by exposing it to water vapor plasma[9] or by immersing it in a sodium ethoxide ($NaOC_2H_5$) solution of anhydrous ethanol.[4,10] In this work, freshly stripped mica (Nisshin EM) was immersed in an anhydrous ethanol solution of 30 v/v % $NaOC_2H_5$, at 50 °C for around 10 min, to form silanols on the mica surface. The possible mechanism of –SiOH formation on the mica surface is as follows: The reaction of $NaOC_2H_5$ with Si–O–Si along the cleavage plane of mica induces Si–ONa and Si–OC_2H_5. At the last step, they were hydrolyzed to silanols, respectively. It may be worth pointing out that the mica has hydroxide ions along its own cleavage plane and they were observed by using a reflection Fourier transform infrared (FT-IR) spectrometer (Bio-Rad Laboratories FTS-60A/896; shown as Supporting Information 1).

The activated mica having silanols was immersed in 1 mM **ImSi**[4] or 0.5 mM **ImSi** + 0.5 mM **ClSuSi**-containing hexane to form silanols by hydrolyzing the ethoxysilyl and chlorosilyl groups. The silanols of neighboring molecules react with each other to polymerize and form a two-dimensional network of siloxane bonds (–O–Si–O–).[11] The remaining silanols of silanes can form siloxane bonds with the silanols already formed on the mica surface by immersing it in the sodium ethoxide solution of anhydrous ethanol. The sulfonate-terminated membrane of the **ImSuSi** surface was prepared by hydrolyzing chlorosulfonyl groups of **ClSuSi**.[12] The imidazolin-terminated monolayer formed on the mica surface (**ImSi** substrate) was revealed by the observed AFM images.[4,10] The reflection FT-IR spectrometer observed the imidazolin-terminated monolayer at the mica surface (shown as Supporting Information 1). The **ImSi** or **ClSuSi** silanes were fixed to the mica surface by siloxane bonds formed not only between the silane silanols and the silanols supplied from the mica surface, but also among the silane silanols.

Preparation of Zinc-Coordinated ImSi or ImSuSi Substrate. The zinc array substrate was prepared by immersing the **ImSi** or **ImSuSi** substrate in an aqueous solution of 5 mM $Zn(NO_3)_2$. The images of the two-dimensional array of zinc ions legated by a couple of imidazolins (zinc array) and the force-displacement curves between zinc array and imidazolins were measured with the aid of an **ImSi**-modified cantilever by using chemical force microscopy, while the zinc array on the **ImSi** substrate showed carbon dioxide hydration acceleration.[4] The zinc-coordinated **ImSuSi** substrate immersed in an aqueous calcium solution has the zinc-coordinated centers and calcium-binding domains.

Preparation of Chitin-Coated Glass Substrate (Chitin Substrate) and pAsp Self-Assembled Chitin Substrate (pAsp-Coated Substrate). The chitin substrate was prepared by spin coating (spincoater, ACT-300A, Active) a 0.4% wt chitin solution of *N*,*N*-dimethylacetamide and 1-methyl-2-pyrrolidone (50/50: w/w) on a strip of glass.[4] The chitin substrate was immersed in an aqueous **pAsp** solution and the **pAsp** self-assembled chitin substrate (**pAsp**-coated substrate) provided the calcium-binding sites.

Nafion Membrane as Ca^{2+}-Recognizing Substrate. Nafion is a dry perfluoronic acid membrane that consists of a hydrophobic fluorocarbon backbone, a hydrophilic ionic region of sulfonic acid/sulfonate group, and an interfacial region. The Nafion structure consists of (a) a fluorocarbon backbone $–(CF_2CF_2)_m$-$(CFCF_2)_nO–$, (b) sulfonate exchange site ($–SO_3^-H^+$), and (c) side chain material $–CF_2CF_3CFOCF_2CF_2–$ associated with void volume.[13,14] The surface morphology of a Nafion-117 membrane was characterized by tapping mode atomic force microscopy; the surface showed a number of nodules or spherical grains of around 10 nm in diameter.[13] The energy dispersive X-ray analyzer revealed elements, e.g., F, C, S, and O present in the Nafion membrane.[14] The reflection FT-IR spectra showed a broad band between 1450 and 1350 cm^{-1} that is due to a $–SO_3H$ group and the sharp intense peak centered at around 1350 cm^{-1} is due to the CF_2 backbone (shown as Supporting Information 2).[15] The Nafion-117 membrane immersed in an aqueous solution has calcium-binding domains that consist of sulfonates. In order to clean up the perfluorosulfonic acid ionomer membrane, it was immersed in aqueous 1 M HCl for 24 h and washed in deionized water.

Formation Reaction of Calcium Carbonate. The calcification reaction was carried out by using carbon dioxide supplied by $(NH_4)_2CO_3$ decomposition in an aqueous solution of 10 mM $Ca(NO_3)_2$ (1) under the coexistence of the **pAsp**-coated substrate and zinc-coordinated ImSi monolayer, (2) in the presence of a zinc-coordinated **ImSuSi** membrane that can simultaneously recognize calcium and zinc ions, or (3) under the coexistence of a Nafion membrane and zinc-coordinated **ImSi** monolayer. The reaction container was put into an incubator (LNC-111, TABAI) between 15 and 50 °C.

Video Optical Microscopy (VOM) and Scanning Electron Microscopy (SEM). The time evolution of calcium carbonate formation at the **pAsp**-coated chitin has been observed in situ using a VOM (7310, CHROMA) in combination with 200× and 650× magnification lenses. The calcium carbonate formed on (1) the **pAsp**-coated chitin surface,[4] (2) zinc-coordinated **ImSuSi** membrane, or (3) a Nafion-117 membrane was observed at higher magnification using a SEM (2100A instrument HITACHI).

X-ray Diffractometry (XRD). The formed calcium carbonate polymorph on each substrate was assigned using XRD-MiniFlex diffractometer (RIGAKU).

Results and Discussion

It is very interesting to reveal the effect of the carbonic dioxide hydration-accelerating zinc array on calcification kinetics. It was confirmed by using the Veronal indicator method

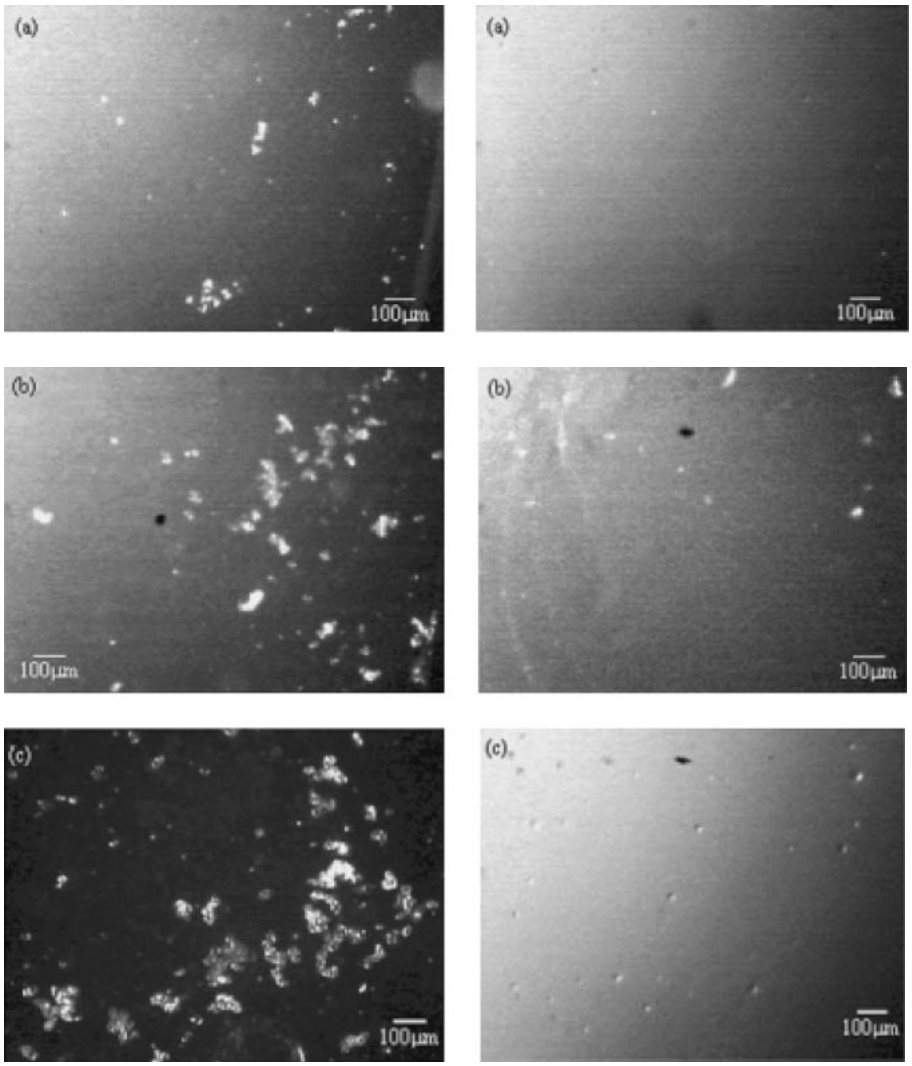


Fig. 1. Time evolution of the calcium carbonate formation at **pAsp**-coated chitin substrate at 15 °C at around pH 8.5 was enhanced by the zinc-coordinated silane monolayer (zinc array); the in-situ observed VOM images were shown after (a) one hour, (b) two hours, and (c) four hours in the presence of the zinc array substrate (left side) and of the **ImSi** substrate free from zinc (right side).

that the tripodal zinc-complex as the carbonic anhydrase active center mimics[16] and the zinc-coordinated silane monolayer[4] has CA-arising CO_2 hydration activity. The time evolution of the calcium carbonate amount produced on the **pAsp**-coated substrate has been observed in situ by using VOM, as shown in Fig. 1. The calcification in an aqueous 5×10^{-5} wt % **pAsp** and 10 mM Ca^{2+} solution has been carried out at 15 °C and around pH 8.5 adjusted just before starting the calcification in the presence of (a) the zinc-coordinated **ImSi** monolayer (Fig. 1, left side) or (b) the **ImSi** monolayer free from zinc (Fig. 1, right side). The in-situ observed VOM images after calcification for 1 (a), 2 (b), and 4 h (c) demonstrated that the zinc array accelerated the calcium carbonate formation. Figure 2 shows powder X-ray diffraction patterns[17] of the calcium carbonate crystallized on **pAsp**-coated chitin surface in the presence of the (a) zinc array, (b) **ImSi** monolayer free from zinc, and (c) mica in an aqueous 5×10^{-5} wt % **pAsp** solution at (1) 15, (2) 25, and (3) 35 °C at around pH 8.5, while the around pH 8.0 was observed after calcification for two days. Since the vaterite was only formed on the **pAsp**-coated chitin surface in the presence of the zinc array at 15–35 °C (a), the carbon dioxide hydration-accelerating zinc array induced the vaterite formation. For the absence of the zinc array, the calcite was formed at 15 and 25 °C and all of the polymorphs of calcite, vaterite, and aragonite simultaneously appeared at 35 °C. The polymorph temperature dependence of the formed calcium carbonate on a **pAsp**-coated substrate in the absence of the zinc array was similar to the polymorphic composition of calcium carbonate crystallized from a saturated Ca^{2+} and HCO_3^- solution at various temperatures.[5] The temperature effect can control the polymorph of the formed calcium carbonate in the absence of the zinc array. Figure 2 demonstrates the superiority of the carbon dioxide hydration enhancement of the zinc array over the temperature effect on calcium carbonate polymorph regulation.

The calcium carbonate formed on the zinc-coordinated **ImSuSi** membrane for 4 days at 25 °C was characteristic of a number of microspheres, i.e., beads of 1 μm more or less in diameter and these beads were located only on the **ImSuSi** membrane islands of around $30 \times 30\,\mu m^2$ (Fig. 3a). The islands

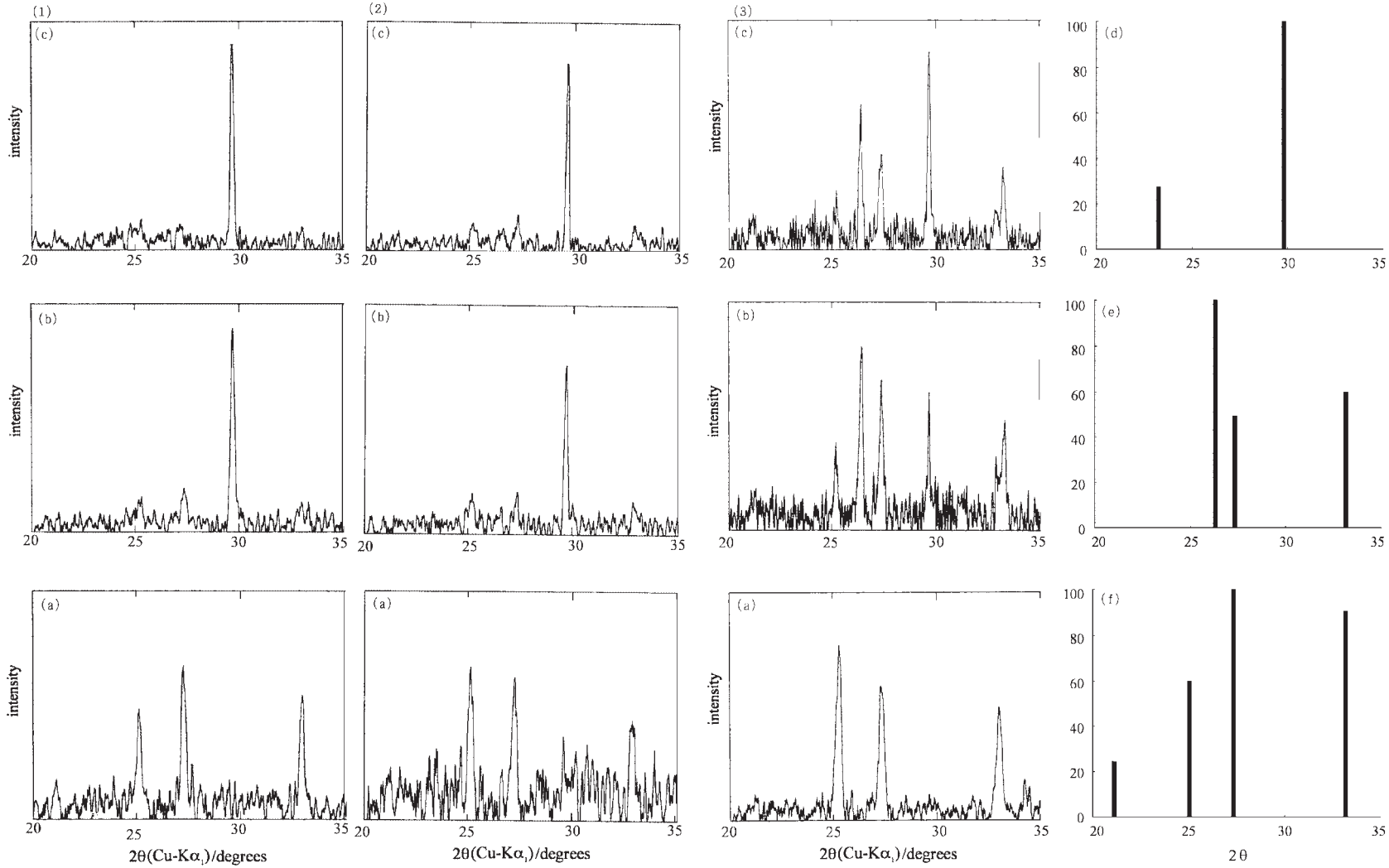


Fig. 2. Powder X-ray diffraction patterns of calcium carbonate crystallized at **pAsp**-coated chitin substrate at (1) 15, (2) 25, and (3) 35 °C, at around pH 8.5: Their patterns were measured for each calcium carbonate formed in the presence of (a) zinc array substrate, (b) **ImSi** substrate free from zinc, and (c) mica substrate. The typical XRD patterns of calcite, aragonite and vaterite are shown in d), e), and f), respectively.

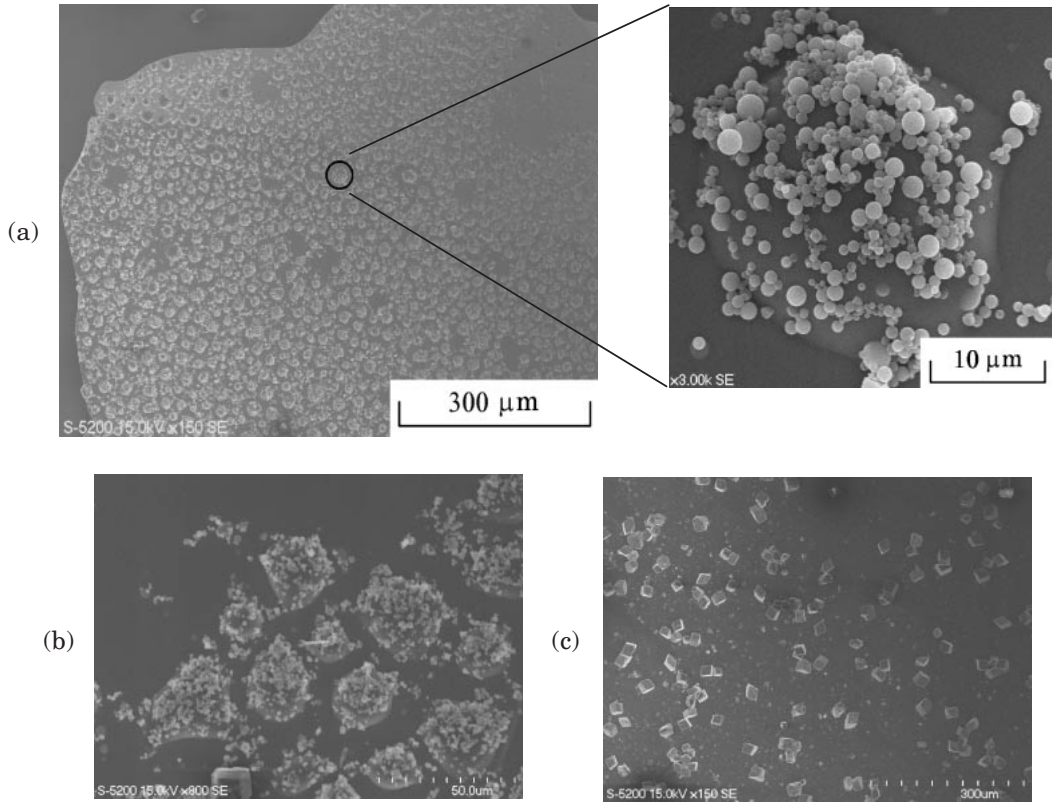


Fig. 3. (a) A number of calcium carbonate microspheres i.e., beads of around 1 μm more or less in diameter were, as shown in the SEM images, formed on sulfonate- and imidazolin-terminated **ImSuSi** membrane islands of around $30 \times 30\,\mu m^2$ at 25 °C. The **ImSuSi** islands extended to around $1 \times 1\,mm^2$ and (b) the regular interval among them was kept by around 10 μm. (c) A number of rectangle calcite species in around $30 \times 20 \times 10\,\mu m^3$ were formed at mica surface free from zinc.

were distributed to around $1 \times 1\,mm^2$ (a) and were separated, by around 10 μm intervals (b). The observed X-ray pattern of these spheres suggested vaterite or/and aragonite. The zinc-coordinated **ImSuSi** membrane consists of both zinc-coordinated centers and calcium-binding domains, and the calcium carbonate-covered **ImSuSi** was formed only on the **ImSuSi** membrane islands. Since each calcium-binding domain is surrounded by zinc-binding centers, a number of **ImSuSi**-including calcium carbonate microspheres must have been formed in each **ImSuSi** membrane island. The thin discs of calcium carbonate showing one mm more or less in diameter were, on the other hand, formed on the **pAsp**-homogeneously-coated substrate in the presence of the zinc-coordinated **ImSi** (zinc array) substrate.[4] The calcium carbonate morphology may be controlled by the calcium-binding domain change caused by zinc coordination. A number of rectangle calcite species of around $30 \times 20 \times 10\,\mu m^3$ were formed at the **ImSuSi**-coated mica surface free from zinc (c). The primary target was to prepare the **ImSuSi** membrane by mimicking the structure of the extracted Nacrein protein from mollusk shells and to form calcium carbonate on the membrane; the protein has a carbon dioxide hydration-enhancing zinc-coordinated center and the calcium-binding domains.[2,4] In this work, the characterization of this membrane was not tried except for the observed membrane islands as shown in the SEM images (Fig. 3). The ratio of zinc-binding sites to calcium-binding sites should be controlled by the composition of the **ImSi** + **ClSuSi** mixture. The ratio effect on the above morphology should be the research target to follow.

The formed calcium carbonate on the sulfonate-terminated Nafion-117 membrane (shown as Supporting Information 2)[14,15] in the presence of the zinc-coordinated **ImSi** substrate at 25 °C is shown in the observed SEM images (Fig. 4). The pH influence of the aqueous calcification solution on the polymorph is as follows: Calcite was formed at a lower pH range from 8.0 to 7.5 (a) and vaterite was formed at a higher pH range from 9.0 to 8.5 (b), associated with a small amount of aragonite and calcite. The magnified SEM images showed the typical morphology of rectangle calcite (c and d).[18,19] The distorted and hollow sphere vaterite (e),[20] and spherulitic aragonite (f).[20,21] The pH of the aqueous calcification solution was decreased by around 0.5 after calcification for 2 or 4 days. The protons are supplied from the biomineralization and biomimetic mineralization reactions CO_2 + zinc-bound $H_2O \rightarrow HCO_3^- + H^+$ and $HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+$.

At around pH 9.5 and 50 °C, calcification on the Nafion membrane produced aragonite including a slight amount of calcite in the presence of the zinc-coordinated **ImSi** substrate (Fig. 5a), as shown in the observed X-ray diffraction pattern (b). A bundle of aragonite needles (c) and a slight amount of rectangle calcite (d) were shown in the enlarged SEM images. The higher pH and temperature brought about the formation of aragonite.

The distorted hollow sphere image in higher magnification (Fig. 4e), which was identified to be vaterite from the observed X-ray diffraction pattern, is similar to spherulitic vaterite crys-

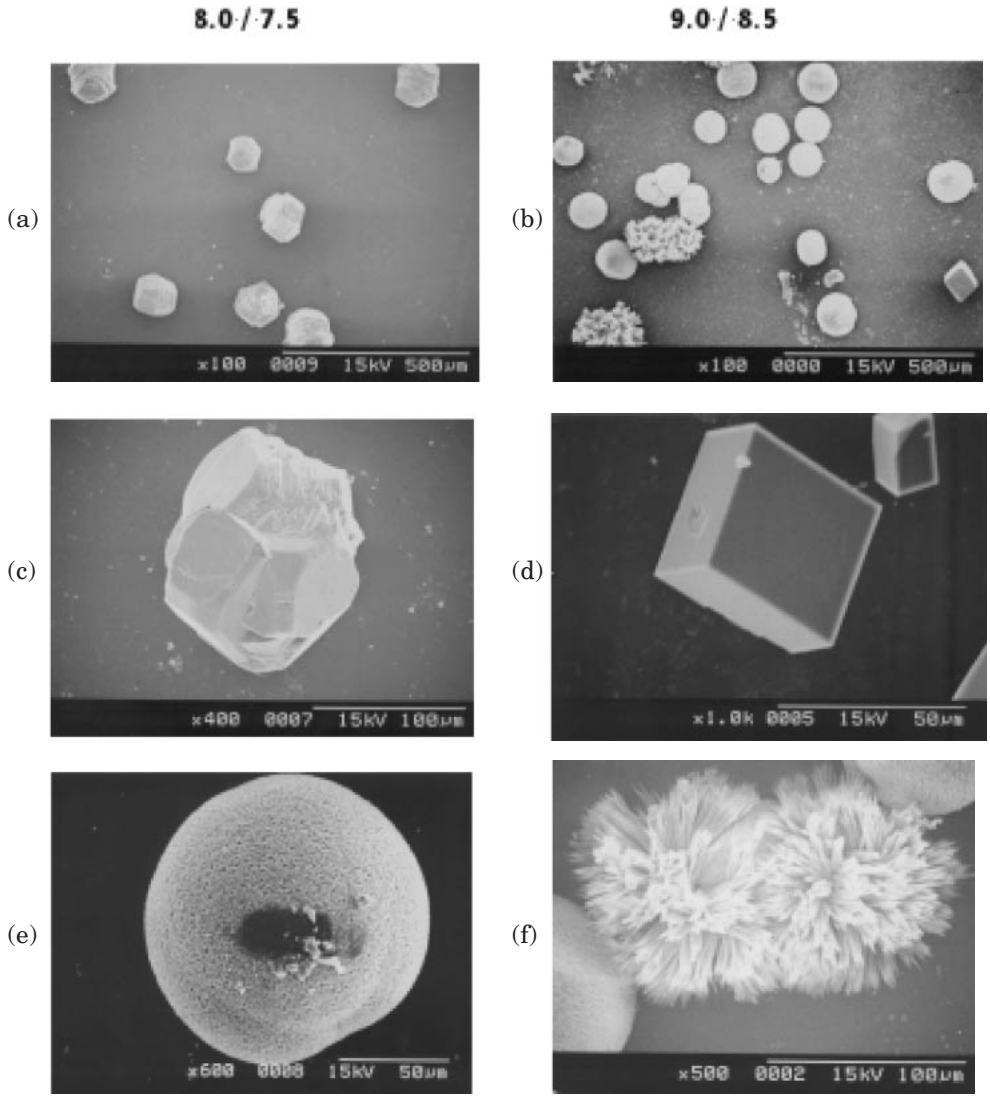


Fig. 4. The observed SEM images show the pH influence on calcium carbonate polymorph/morphology formed on the sulfonate-terminated Nafion membrane at 25 °C in the presence of zinc array substrate (a) at a lower pH range between pH 8.0 and 7.5, and (b) at a higher pH range between pH 9.0 and 8.5. The magnified images (c) at a lower pH and (d) at a higher pH show calcite; at a higher pH, (e) and (f) show a major vaterite and a minor aragonite, respectively.

tals formed by marine organisms.[20] The presence of the CA-arising zinc array induced the rapid calcification (Fig. 1) and controlled to form vaterite (Figs. 2a, 4b, and 4e). The spherulitic shell and scleractinian coral skeleton was produced by many different organisms. The formation of the egg shell is known as the most rapid mineralization process.[22] The calcification at higher pH and higher temperature induced the aragonite formation in the presence of CA-arising zinc array (Fig. 5). The formed aragonite (Figs. 4f and 5) at the Nafion membrane corresponds to the spherulitic aragonite crystals precipitated in aqueous solution inside the shell chamber.[23] Spherulitic mineralization may be practiced by means of rapidly filling a large volume with minerals in marine organisms.

The function group-terminated membrane can provide the selective metals-binding sites on its own surface. A novel approach to prepare the metal-coordinated membrane/monolayer is to control the selective and quantitative substitution reaction, i.e., metal exchange with solvent molecules. The different metals-coordinated membrane will open a novel way to exert some hybrid functions that will simultaneously give rise to catalytic activity and allosteric regulation of the host–guest interaction.

Conclusion

Biomimetic mineralization has been revealed from the view of the morphology and polymorph of the calcium carbonate formed on different calcium-binding substrates. First, the zinc-coordinated imidazolin-terminated and carbon dioxide hydration-enhancing silane monolayer (zinc array)[4] accelerated the calcification at calcium-binding **pAsp**-coated chitin. The carbonic anhydrase-arising zinc array prevailed the temperature influence on calcium carbonate polymorph. Pure vaterite was formed between 15 and 35 °C in the presence of the zinc array. Second, the calcium carbonate crystal formed on imidazolin- as well as sulfonate-terminated membrane were constructed from a number of microspheres of 1 μm more or

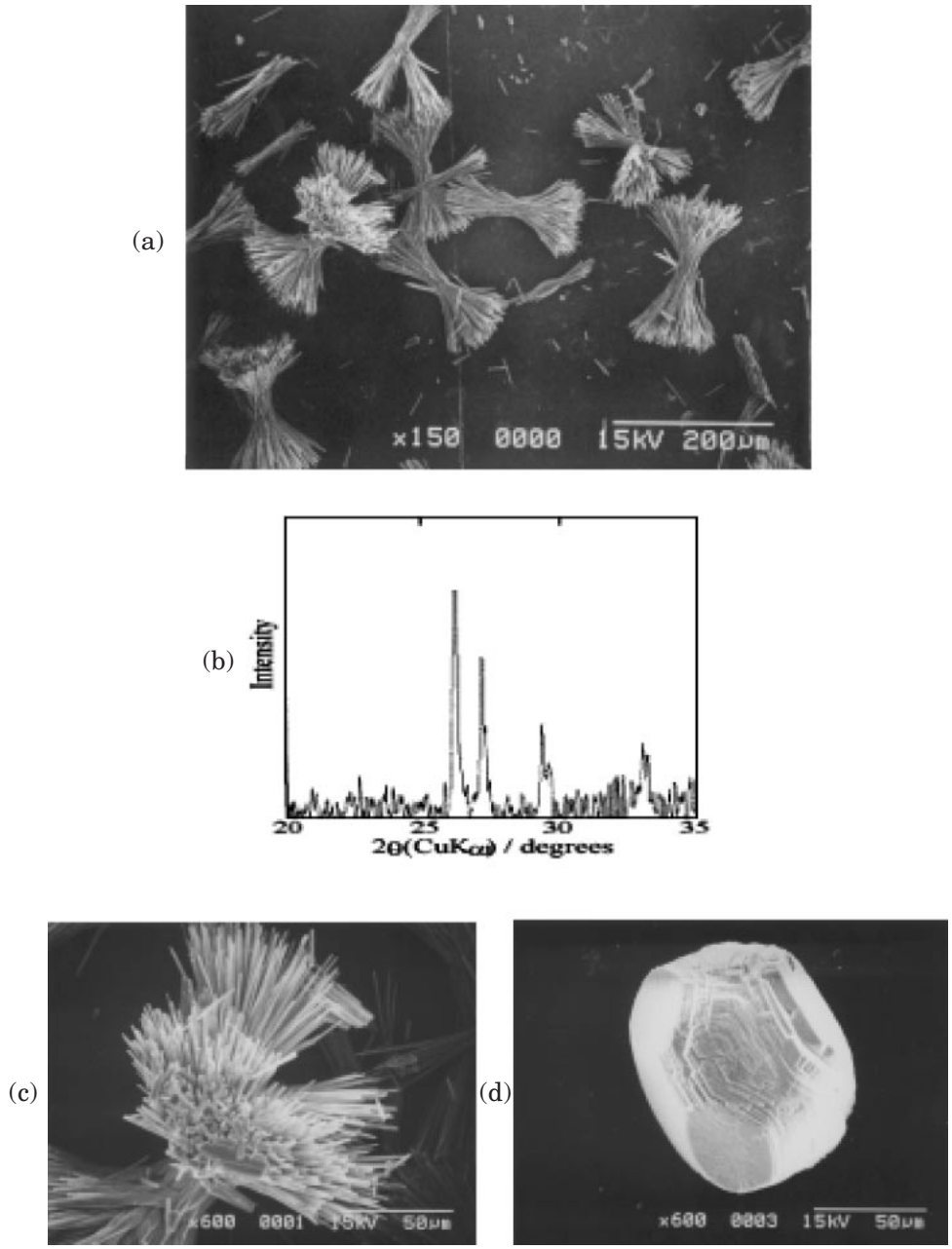


Fig. 5. The calcium carbonate crystals were formed on the sulfonate-terminated Nafion membrane at around pH 9.5 and higher temperature 50 °C in the presence of zinc array substrate. The SEM image (a) shows a large amount of aragonite, and a XRD pattern (b) shows aragonite and calcite. The magnified images (c) and (d) show the large amount of aragonite and the slight amount of calcite, respectively.

less in diameter. Third, higher temperature and higher pH induced superior aragonite formation at the calcium-coordinated sulfonate-terminated Nafion membrane in the presence of the zinc array. The observed morphology of the in vitro formed aragonite or vaterite was similar to the spherulitic crystals precipitated in marine organisms.

K.I. thanks Dr. T. Nakagawa (Matsushita Electric Ind. Co., Ltd.) for his helpful discussion on silane monolayer anchored to the mica surface and Prof. S. Uehara (Grad. School of Sci., Kyushu Univ.) for his displaying the atom array at the cleavage plane of mica. We wish to thank Profs. T. Okuhara and K. Shimazu (Grad. School of Environ. Earth Sci., Hokkaido Univ.) for their hospitality in the utilization of SEM and reflection FT-IR spectrometer instruments.

Supporting Information

The reflection FT-IR spectra of the mica surface and the mica surface coated by imidazolin-terminated silane are shown in Supporting Information 1. The Nafion-117 membrane infrared spectrum is also observed as shown in Supporting Information 2. These materials are available free of charge on the web at: http://www.csj.jp/journals/bcsj/.

References

1 G. Falini, S. Albeck, S. Weiner, L. Addadi, *Science* **1996**, *271*, 67; S. Weiner, L. Addadi, *J. Mater. Chem.* **1997**, *7*, 689; Y. Levi, S. Albeck, A. Brack, L. Addadi, *Chem. Eur. J.* **1998**, *4*, 389.

2 H. Miyamoto, T. Miyashita, M. Okushima, S. Nakano, T. Morita, A. Matsushiro, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9657.

3 T. Samata, N. Hayashi, M. Kono, K. Hasegawa, C. Horita, S. Akera, *FEBS Lett.* **1999**, *462*, 225; M. Kono, N. Hayashi, T. Samata, *Biochem. Biophys. Res. Commun.* **2000**, *269*, 213; A. Matsushiro, T. Miyashita, H. Miyamoto, K. Morimoto, B. Tonomura, A. Tanaka, K. Sato, *Mar. Biotechnol.* **2003**, *5*, 37.

4 K. Ichikawa, N. Shimomura, M. Yamada, N. Ohkubo, *Chem. Eur. J.* **2003**, *9*, 3235; N. Shimomura, N. Ohkubo, K. Ichikawa, *Chem. Lett.* **2002**, 902.

5 J. L. Wray, F. Daniels, *J. Am. Chem. Soc.* **1957**, *79*, 2031; Y. Kitano, *Bull. Chem. Soc. Jpn.* **1962**, *35*, 1980.

6 A. E. Ingalls, C. Lee, E. R. M. Druffel, *Geochim. Cosmochim. Acta* **2003**, *67*, 2827.

7 T. Watanabe, I. Fukuda, K. China, Y. Isa, *Comp. Biochem. Physiol., B* **2003**, *136*, 767.

8 L. L. Robbins, K. Brew, *Geochim. Cosmochim. Acta* **1990**, *54*, 2285.

9 T. Nakagawa, M. Soda, *Jpn. J. Appl. Phys.* **1997**, *36*, 5226; T. Nakagawa, M. Soda, *Jpn. J. Appl. Phys.* **1997**, *36*, 6915.

10 T. Nakagawa, K. Ogawa, T. Kurumizawa, *Langmuir* **1994**, *10*, 525.

11 J. Sagiv, *J. Am. Chem. Soc.* **1980**, *102*, 92.

12 V. V. Tsukruk, V. N. Bliznyuk, *Langmuir* **1998**, *14*, 446.

13 A. Lehmani, S. Durand-Vidal, P. Turq, *J. Appl. Polym. Sci.* **1998**, *68*, 503.

14 R. C. Mercado, F. Moussey, *Biosens. Bioelectron.* **1998**, *13*, 133.

15 P. C. Rieke, N. E. Vanderborgh, *J. Membr. Sci.* **1987**, *32*, 313.

16 K. Nakata, M. K. Uddin, K. Ogawa, K. Ichikawa, *Chem. Lett.* **1997**, 991; K. Nakata, N. Shimomura, N. Shiina, M. Izumi, K. Ichikawa, M. Shiro, *J. Inorg. Biochem.* **2002**, *89*, 255; K. Ichikawa, M. Tarnai, M. K. Uddin, K. Nakata, S. Sato, *J. Inorg. Biochem.* **2002**, *91*, 437; T. Echizen, M. M. Ibrahim, K. Nakata, M. Izumi, K. Ichikawa, M. Shiro, *J. Inorg. Biochem.* **2004**, *98*, 1347.

17 P. Swarthmore, in *Powder Diffraction File Set 33*, International Center for Diffraction Data, Pennsylvania, **1989**; P. Swarthmore, in *Powder Diffraction File Set 41*, International Center for Diffraction Data, Pennsylvania, **1991**.

18 J. M. Didymus, P. Oliver, S. Mann, A. L. DeVries, P. V. Hauschka, P. Wetbroek, *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 2891.

19 J. Küther, R. Seshadri, W. Knoll, W. Tremel, *J. Mater. Chem.* **1998**, *8*, 641.

20 L. Addadi, S. Weiner, *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 153.

21 A. L. Litvin, S. Valiyaveettil, D. L. Kaplan, S. Mann, *Adv. Mater.* **1997**, *9*, 124.

22 H. A. Lowenstam, S. Weiner, in *On Biomineralization*, Oxford University Press, New York, **1989**.

23 H. Mutvei, *Bull. Geol. Inst. Univ. Uppsala* **1972**, *8*, 237.