Control of the Production Amount and Polymorphism of Calcium Carbonate by Biomimetic Mineralization

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The cooperation between carbonic anhydrase (CA) or some biomimetic zinc model compound as a catalyst for the conversion from CO_2 to HCO_3^- and poly(L-aspartate) as calcium ion recognition sites induced the aragonite formation of calcium carbonate. The higher CA-arising activity promoted the calcium carbonate production amount.

The marine invertebrate animals of mollusks, coelentera, and zooplanktons have been attmpting the conservation and prosperity of the species on the earth by biomineralization over the several billions of year. The sea organisms are used to make up shell and skeleton during the limited term within their life time. The mineralization was in vitro reproduced using proteins and macromolecules extracted from mollusk shell and investigated from the viewpoint of polymorphs and morphology.[1] The reduced amino acid sequence of nacrein contains two domains of carbonic anhydrase (CA) active sites for CO_2 hydration and the repeated aspartic-acid peptides for Ca^{2+} recognition.[2] An in vitro study on the control of aragonite crystal growth has been carried out by scanning electron microscope using a chitin-silk fibroin assembly containing acidic macromolecules as-extracted from the mollusk shell layer or some artificial acidic polypeptides.[1b,e,g,h] The role of the CA activity in the presence or absence of the Asp/Glu-including polypeptide as Ca^{2+}-recognition sites has never been revealed by the in vitro mineralization using the natural macromolecules.[1] The calcium carbonate formation and its polymorphism depend on pH, but the in vitro mineralization proceeds under the carbon dioxide as well as ammonia generation.[1]

Our research target is to reveal whether the CA-arising activity induced by the zinc-bound H_2O/OH^- has good control over the production amount of calcium carbonate during the limited term; whether the simple addition of poly(L-aspartate) pAsp or pAsp-coated chitin substrate,[3] as a Ca^{2+}-collector, brings about good control over the calcium carbonate polymorphism. The catalytic conversion rate from CO_2 to HCO_3^- has been measured for CA and the zinc model compound L1SZn(OH_2/OH^-)[4] using the CO_2-Veronal indicator method:[5] CA ($\sim$0.1 μM or 150 mg L^{-1}) showed $\Delta\tau^6 = 0.93$ ($\tau_0 = 15$ s, $\tau = 1$ s) for the pH change of 8.5 to 7.5 at 0 °C. For the change of pH 9.0 to pH 8.1, the L1SZn(OH_2/OH^-) ($\sim$50 μM or 30 mg L^{-1}) generated $\Delta\tau$ to be 0.07 ($\tau_0 = 145$ s, $\tau = 135$ s) at 20 °C. The zinc model compound with L1S showed the highest activity among all the other artificial zinc compounds as CA-active models.[4b,7]

The calcification reactions may take place as follows:

$$CO_2 + \text{zinc-bound } H_2O \rightarrow HCO_3^- + H^+$$
$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+$$

in 10 mM $Ca(NO_3)_2$ solution in ca. 0.04 v/v% CO_2[8] atmosphere at 25 °C for 2 days or one day and at pH 8.5 or 9.[9] Figure 1 shows the production amount ΔP^{10} of calcium carbonate in each solution including CA, L1SZn(OH_2/OH^-), or hydrated zinc ion Zn^{2+}(aq) in the absence of pAsp as Ca^{2+}-collector; $[cat]_0$ stands for their initial concentrations. The highest active CA showed the highest ΔP (Figure 1). The faster formation rate of HCO_3^- induces the faster formation rate of $CaCO_3$; the higher CA-arising activity promoted the $CaCO_3$ production amount. The in situ observation using optical microscopy[13] revealed that the CA activity on the conversion from CO_2 to HCO_3^- controlled the production amount of calcium carbonate (Figure 2). In the presence of active CA the number of crystalline $CaCO_3$ particles was significantly increased and their dimensions became smaller on chitin substrate, compared with the less active Zn^{2+}(aq). The crystalline materials formed in the absence of pAsp, as shown in the observed morphologies at higher magnification (Figure 2b) and X-ray diffraction patterns.[11]

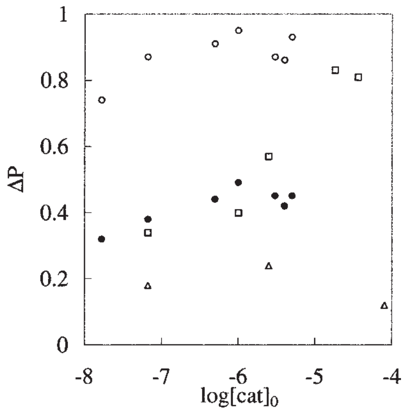

Figure 1. The effect of CA (circles; pH 8.5–7.5; $[cat]_0$: mol L^{-1}) or zinc model compound (L1SZn: squares, pH 9.0–8.1; Zn^{2+}(aq): angles, pH 8.5–7.5; $[cat]_0$: mol L^{-1}) on the production amount of calcium carbonate in the absence of pAsp after calcification for 2 days (open marks) and one day (solid marks) at 25 °C.

The addition of 5×10^{-7} wt% pAsp into the CA solution induced the formation of calcium carbonate polymorphs, aragonite/vaterite and calcite; they were assigned by the observed X-ray diffraction patterns as well as the microscope images (Figure 2).[12] Each crystalline particle at higher magnification was shown to be the in vitro formed spherulites of aragonite/vaterite under the presence of pAsp (Figure 2e). For Zn^{2+}(aq) having less CA-arising activity, the calcium carbonate was much less formed and only made of calcite under the presence of pAsp. The aragonite formation condition needs both CA as an active catalyst for the conversion from CO_2 to HCO_3^- and pAsp as calcium ion recognition sites. The CA-promoted calcium carbonate formation

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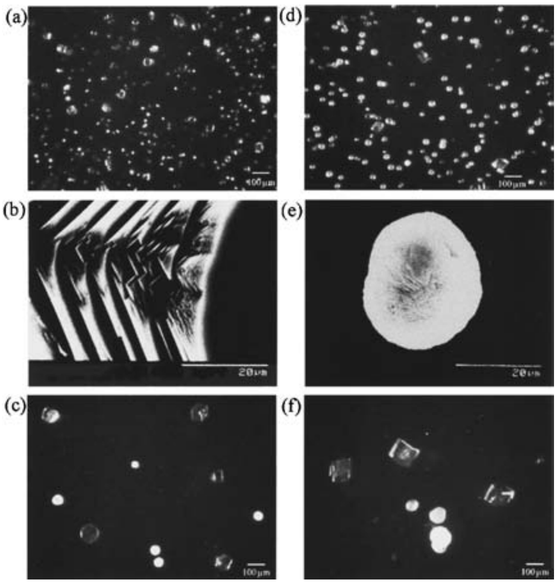

Figure 2. The video optical-microscope images of $CaCO_3$ observed on chitin substrate after calcification for 2 days; for (a) CA or (c) Zn^{2+}(aq) as a catalyst in the absence of pAsp, (d) CA or (f) Zn^{2+}(aq) as a catalyst in the presence of 5×10^{-7} wt% pAsp at pH 8.5–7.9 and 25 °C. The images (b) and (e) at higher magnification of (a) and (d) were observed, respectively by scanning electron microscope.

may be indispensable for the prosperity of marine invertebrate animals.

The aragonite/vaterite is never deposited from inorganic solution, which consists of both Ca^{2+} and HCO_3^-, by the chemical reaction associated with the evolution of carbon dioxide as follows:

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3(\text{calcite}) + CO_2 \uparrow + H_2O$$

at ca. pH 7 and lower than 25 °C.[13]

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References and Notes

1 a) S. Weiner and W. Traub, *Philos. Trans. R. Soc. London, Ser. B*, **304**, 4215 (1984). b) L. Addadi and S. Weiner, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 4110 (1985). c) S. Mann, in "Biomineralization: Chemical and Biochemical Perspectives," ed. by S. Mann, J. Webb, and J. P. Williams, VCH Publishers: New York (1989), p 35. d) L. Addadi and S. Weiner, *Angew. Chem., Int. Ed. Engl.*, **31**, 153 (1992). e) G. Falini, S. Albeck, S. Weiner, and L. Addadi, *Science*, **271**, 67 (1996). f) S. Weiner and L. Addadi, *J. Mater. Chem.*, **7**, 689 (1997). g) Y. Levi, S. Albeck, A. Brack, S. Weiner, and L. Addadi, *Chem.—Eur. J.*, **4**, 389 (1998). h) Q. L. Feng, G. Pu, Y. Pei, F. Z. Cui, H. D. Li, and T. N. Kim, *J. Cryst. Growth*, **216**, 459 (2000).

2 a) T. F. Goreau, *Biol. Bull.*, **116**, 59 (1959). b) H. Miyamoto, T. Miyashita, M. Okushima, S. Nakano, T. Morita, and A. Matsushiro, *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 9657 (1996). c) M. Kono, N. Hayashi, and T. Samata, *Biochem. Biophys. Res. Commun.*, **269**, 213 (2000).

3 The chitin substrate was prepared by immersing a strip of glass into the *N*,*N*-dimethyl-acetamide and *N*-methyl-2-pyrrolidone solution (50/50 : w/w) containing 0.4 wt% chitin: T. Kato, T. Suzuki, and T. Irie, *Chem. Lett.*, **2000**, 186.

4 a) K. Ichikawa, K. Nakata, and M. M. Ibrahim, *Chem. Lett.*, **2000**, 296. b) K. Nakata, N. Shimomura, N. Shiina, M. Izumi, K. Ichikawa, and M. Shiro, *J. Inorg. Biochem.*, **89**, 225 (2002). c) K. Nakata, M. K. Uddin, K. Ogawa, and K. Ichikawa, *Chem. Lett.*, **1997**, 991.

5 S. Y. Yang, M. Tsuzuki, and S. Miyauchi, *Plant Cell Physiol.*, **26**, 25 (1985).

6 $\Delta\tau$ is defined as $(\tau_0 - \tau)/\tau_0$, where τ and τ_0 are the reaction times required for the pH change in the presence and absence of zinc catalysts, respectively.

7 a) P. Woolly, *Nature*, **258**, 677 (1975). b) R. S. Brown, N. J. Curtis, and J. Huguet, *J. Am. Chem. Soc.*, **103**, 6953 (1981). c) R. S. Brown, D. Salmon, N. J. Curtis, and S. Kusuma, *J. Am. Chem. Soc.*, **104**, 3188 (1982). d) H. Slebocka-Tilk, J. L. Concho, Z. Frakman, and R. S. Brown, *J. Am. Chem. Soc.*, **106**, 2421 (1984). e) X. Zhang, R. van Eldik, T. Koike, and E. Kimura, *Inrog. Chem.*, **32**, 5749 (1993). f) X. Zhang and R. van Eldik, *Inrog. Chem.*, **34**, 5606 (1995).

8 The CO_2 concentration in the atmosphere of a closed reaction-container was monitored by a CO_2 controller (TOA): CO_2 was supplied by the decomposition of $(NH_4)_2CO_3$ and the other component NH_3 was eliminated by concentrated H_2SO_4. The pH of aqueous solution was able to be kept not lager than 9 or 8.5. The reaction container was kept in an incubator (TABAI) at 15 or 25 °C.

9 The pH adjusted to 8.5 or 9.0 using AMPSO buffer just before starting the calcification was changed to ca. 7.5 or 8 after mineralization during one day or two days.

10 ΔP is estimated from $(P_0 - P)/P_0$, where P and P_0 are the calcium ion concentration ($mg\,L^{-1}$) in aqueous solution after calcification for one day or two days and the initial concentration, respectively. The calcium concentration was determined using atomic absorption spectrometry.

11 a) P. Swarthmore, Powder diffraction file set 33, International Centre for Diffraction Data: Pennsylvania, (1989). b) P. Swarthmore, Powder diffraction file set 41, International Centre for Diffraction Data: Pennsylvania, (1991).

12 Micrographs were acquired on a Video Microscope in combination with 200× and 650× magnification lens (CHROMA ATE 7310).

13 Y. Kitano, *Bull. Chem. Soc. Jpn.*, **35**, 1980 (1962).