–Paper–

EFFECT OF ORGANIC ACIDS ON THE PRECIPITATION RATE AND POLYMORPHISM OF CALCIUM CARBONATE MINERALS IN SOLUTIONS WITH Mg^{2+} IONS

Motoharu Kawano[a,*] and Misaki Tokonami[b]

[a]*Graduate School of Science and Engineering, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan*
[b]*Department of Earth and Environmental Sciences, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan*

ABSTRACT

Organic acids are abundant biogenic organic molecules in the earth's surface environments. To evaluate the effect of organic acids on the precipitation rate and polymorphism of calcium carbonate ($CaCO_3$) minerals, we conducted precipitation experiments of $CaCO_3$ minerals by the batch method using 100 ml solutions containing citric acid, malic acid, and acetic acid with Mg^{2+} ions at 25°C for 10 days. Each solution contained 5.0 mM Ca^{2+} and Mg^{2+} in addition to 20.0 mM HCO_3^- and 0.01, 0.05, 0.1, 0.5, and 1.0 mM organic acid. In addition, adsorption experiments with these organic acids were conducted on the surfaces of calcite and aragonite to reveal the adsorption affinity of organic acids for $CaCO_3$ minerals. Results confirmed that citric acid significantly decreased the precipitation rate of $CaCO_3$ minerals and strongly favored the formation of calcite as the predominant polymorph by increasing its concentrations. Moreover, malic acid decreased the precipitation rate and favored the formation of calcite to a lesser extent than with citric acid. Acetic acid showed no significant effect on the precipitation rate and polymorphism of $CaCO_3$ minerals. Adsorption experiments revealed that adsorption affinity of organic acids was much higher for the surface of aragonite than that for the calcite surface, and showed adsorption amounts in the following order: citric acid > malic acid > acetic acid. Thus, the effect of organic acids on the precipitation rate and polymorphism of $CaCO_3$ minerals were fundamentally caused by the adsorption of organic acids on the $CaCO_3$ surface. In this process, higher adsorption affinity of the aragonite surface with organic acids led to a greater adsorption, resulting in greater inhibition of aragonite precipitation and favorable formation of calcite as the dominant polymorph.

Key words: Organic acids, Citric acid, Malic acid, Acetic acid, Aragonite, Calcite, Precipitation rate, Polymorphism

INTRODUCTION

The involvement of microbial activity in geochemical processes is an important factor affecting the rate and extent of precipitation and dissolution of minerals in the Earth's surface environments. Calcium carbonate ($CaCO_3$) minerals are major constituents of the Earth's surface and occur mainly as calcite or aragonite, with rare appearances as the following metastable polymorphs: vaterite, calcium carbonate monohydrate, and calcium carbonate hexahydrate. Calcite is the most stable polymorph of $CaCO_3$ minerals and is commonly produced by inorganic precipitation processes in supersaturated solutions, whereas aragonite is less stable than calcite but is abun-

dantly produced by various geochemical factors including microbial interaction (Addadi and Weiner, 1985; Lowenstam and Weiner, 1989; Mann et al., 2007; Pipich et al., 2008). Photosynthetic microbes such as cyanobacteria and green algae are well known to enhance $CaCO_3$ precipitation by increasing the solution pH through the release of OH⁻ during photosynthesis (Riding, 1982; Pentecost and Riding, 1986; De Vrind-de Jong and De Vrind, 1997). A similar enhancement of $CaCO_3$ precipitation by increasing the solution pH occurs through the metabolic activity of chemosynthetic microbes such as sulfate-reducing bacteria (Baumgartner et al., 2006), nitrate-reducing bacteria (Novitsky, 1981), and methanogens (Roberts et al., 2004).

Microbes also inhibit the precipitation rate of $CaCO_3$ minerals and affect the stability of polymorphs by interaction of extracellular organic molecules consisting mainly of negatively charged polymeric substances including polysaccharides

* Corresponding author: Motoharu Kawano, Graduate School of Science and Engineering, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan. e-mail: kawano@sci.kagoshima-u.ac.jp

and proteins (Arp et al., 1999; Kawaguchi and Decho, 2002; Tourney and Ngwenya, 2009; Kawano and Hwang, 2010). These organic molecules are capable of adsorbing on the $CaCO_3$ surface possibly by electrostatic interaction of the negatively charged carboxylic functional group of the substances to positively charged specific surface sites of $CaCO_3$ minerals. This adsorption is considered to block the active growth sites resulting in the inhibition of further growth of the crystals and phase control of the stable polymorph, depending on the adsorption affinity of the substances (Morse et al., 2007; Yang et al., 2008; Decho, 2010; Kawano and Hwang, 2011). However, details on the mechanism for achieving the effect of polymeric molecules on both precipitation rate and polymorphism of $CaCO_3$ minerals remains clear because of the enormous structural diversity of these molecules and uncertainty in their chemical reactivity.

In this study, therefore, we used three biogenic organic acids, citric acid, malic acid, and acetic acid, all which are monomeric molecules with simple structures compared with the extracellular polymeric substances. By using these organic acids, precipitation experiments on $CaCO_3$ minerals were performed to evaluate the effect of these organic acids on the precipitation rate and polymorphism of $CaCO_3$ minerals. Additionally, adsorption experiments with these organic acids on the surface of calcite and aragonite were also conducted to elucidate the interaction mechanism that causes the effect on both precipitation rate and polymorphism of $CaCO_3$ minerals.

EXPERIMENTAL METHODS

Materials

The organic acids used in this study were citric acid, malic acid, and acetic acid. All the reagents were analytical grade and were obtained from Nacalai Tesque Inc. Citric acid is a tricarboxylic acid with dissociation constant (pK) values of the carboxyl functional groups of pK_1 = 3.13, pK_2 = 4.76, and pK_3 = 6.40 (Dawson et al., 1986). Its molecular formula is $C_6H_8O_7$ and molecular weight is 192.1. Malic acid is a dicarboxylic acid with pK values of the carboxyl functional groups of pK_1 = 3.46 and pK_2 = 5.10 (Dawson et al., 1986). Its molecular formula is $C_4H_6O_5$ and molecular weight is 134.1. Acetic acid is a monocarboxylic acid with a pK value of the carboxyl functional group of pK = 4.76 (Dawson et al., 1986). Its molecular formula is $C_2H_4O_2$ and molecular weight is 60.1. The molecular structures of these organic acids are illustrated in Fig. 1.

Precipitation experiments

Precipitation experiments on $CaCO_3$ minerals in solutions containing organic acids were performed by the batch method using 100 ml of glass flasks at 25°C. Prior to conducting the experiments, stock solutions of 100 mM $CaCl_2$, $MgCl_2$, and $NaHCO_3$ were prepared using guaranteed grade reagents (Nacalai Tesque, Inc.). Similarly, stock solutions of 10.0 mM of each organic acid were also prepared by dissolving them in 100 ml deionized water. By using these stock solutions, we prepared three sets of 100 ml solutions containing 0.01, 0.05, 0.1, 0.5, and 1.0 mM citric acid (Ci systems: Ci1 to Ci5), malic acid (Ma systems: Ma1 to Ma5), and acetic acid (Ac
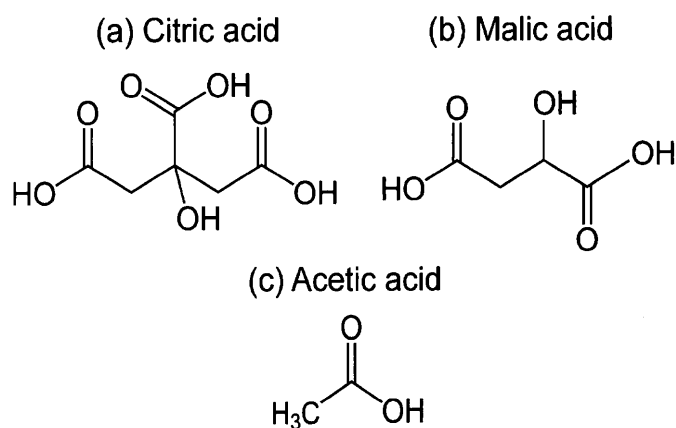

Fig. 1. Molecular structures of citric acid, malic acid, and acetic acid.

systems: Ac1 to Ac5), where each solution contained 5.0 mM Ca^{2+}, 5.0 mM Mg^{2+}, and 20.0 mM HCO_3^- ions. These initial concentrations of 5.0 mM Ca^{2+} and 20.0 mM HCO_3^- ions were used to obtain sufficient amounts of precipitates required for mineral identification by X-ray powder diffraction (XRD). Similarly, the concentration of Mg^{2+} was set to 5.0 mM because it was confirmed that at least 5.0 mM Mg^{2+} ions were necessary for the preferential formation of aragonite with no calcite in the preliminary experiments. A control system (C0) containing the same concentrations of Ca^{2+}, Mg^{2+}, and HCO_3^- ions with no organic acids was also prepared. Throughout the solution preparation, each solution was kept below pH 7.5 by adding 1.0 mM HCl solution to prevent rapid precipitation of $CaCO_3$ minerals, and the solutions were finally adjusted to pH 7.0 by stirring (Table 1). The glass flasks were sealed with aerated caps and incubated at 25°C for 10 days without stirring.

During the precipitation experiments, the solution pH of each flask was measured every day by using a glass electrode;

TABLE 1. Chemistry of initial solutions used in the precipitation experiments.

Run	Ca (mM)	Mg (mM)	HCO₃ (mM)	Organic acid (mM)	Initial pH
C0	5.0	5.0	20.0	–	7.00
Ci1	5.0	5.0	20.0	0.01 mM citric acid	7.00
Ci2	5.0	5.0	20.0	0.05 mM citric acid	7.00
Ci3	5.0	5.0	20.0	0.10 mM citric acid	7.00
Ci4	5.0	5.0	20.0	0.50 mM citric acid	7.00
Ci5	5.0	5.0	20.0	1.00 mM citric acid	7.00
C0	5.0	5.0	20.0	–	7.00
Ma1	5.0	5.0	20.0	0.01 mM malic acid	7.00
Ma2	5.0	5.0	20.0	0.05 mM malic acid	7.00
Ma3	5.0	5.0	20.0	0.10 mM malic acid	7.00
Ma4	5.0	5.0	20.0	0.50 mM malic acid	7.00
Ma5	5.0	5.0	20.0	1.00 mM malic acid	7.00
C0	5.0	5.0	20.0	–	7.00
Ac1	5.0	5.0	20.0	0.01 mM acetic acid	7.00
Ac2	5.0	5.0	20.0	0.05 mM acetic acid	7.00
Ac3	5.0	5.0	20.0	0.10 mM acetic acid	7.00
Ac4	5.0	5.0	20.0	0.50 mM acetic acid	7.00
Ac5	5.0	5.0	20.0	1.00 mM acetic acid	7.00

0.5 ml of solution was centrifuged at 1200 r.p.m. for 5 min, and the supernatant solution was used for measuring Ca^{2+}, Mg^{2+}, HCO_3^-, and organic acid concentrations. The concentrations of these ions were measured by high performance liquid chromatography (HPLC) with a Hitachi instrument (LaChrom Elite) by using a TSKgel IC-Cation I/II HR column for Ca^{2+} and Mg^{2+} ions and a TSKgel OApak-P column for HCO_3^- and organic acids. The solution pH was measured using a METTLER pH meter equipped with a glass electrode. Saturation indices with respect to calcite, aragonite, and $CO_2(g)$ were calculated using the geochemical code of PHREEQC software with the minteq.dat database (Parkhurst and Appelo, 1999). The saturation index is defined as:

$$SI = \log(IAP/K) \tag{1}$$

where IAP is the ion activity product in the systems, and K is the corresponding solubility product. After 10 days of incubation, precipitates in the flasks were washed three times with distilled water, then two times with 90% ethanol, and were dried on a glass slide for XRD analysis. The XRD analysis was conducted with a Rigaku RINT2000 diffractometer using $CuK\alpha$ radiation generated at 40 kV and 30 mA with a scanning speed of $2 \degree$ 2θ/min.

Adsorption experiments

Adsorption experiments with citric acid, malic acid, and acetic acid on the surface of calcite and aragonite were conducted by the batch method, where 0.1 g of calcite or aragonite and 5.0 ml of solution containing 100 μM of each organic acid were placed in 10.0 ml of polypropylene tubes. Five
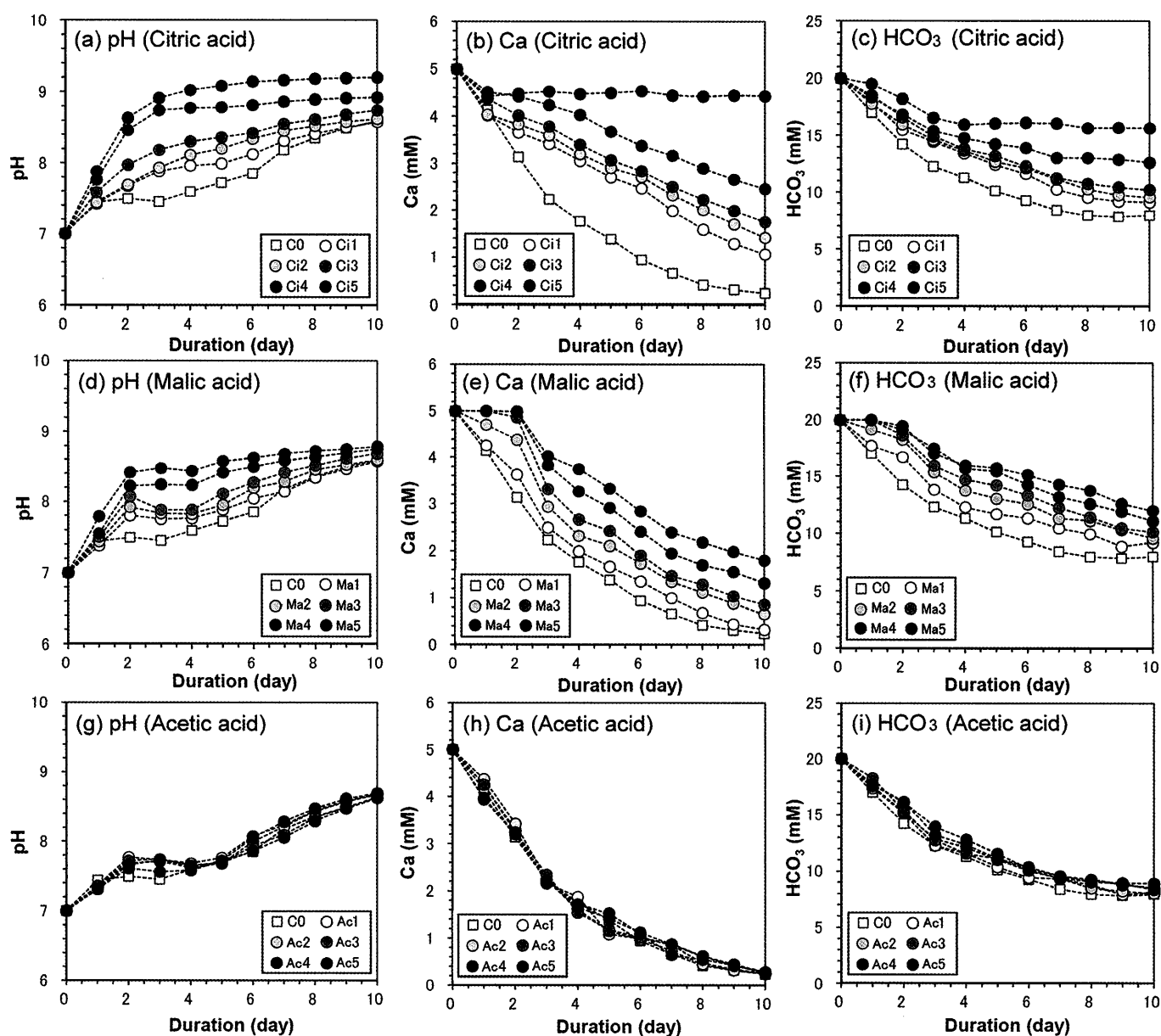

FIG. 2. Variations in solution pH and concentrations of Ca^{2+} and HCO_3^- ions as a function of reaction times for precipitation experiments using citric acid (a, b, and c), malic acid (d, e, and f), and acetic acid (g, h, and i). Concentrations of Mg^{2+} ions remained unchanged throughout the reaction times.

polypropylene tubes with solution pH range of approximately 8 to 10 were prepared for reaction systems of each organic acid. The solution pH of the tubes was adjusted by adding HCl or NaOH solutions. After shaking the solution for 1 hr at 25°C, the supernate solutions were separated by centrifuge and concentrations of organic acids and solution pH were measured by HPLC and a pH meter, respectively, as described above. The calcite used in the adsorption experiments was synthesized using 1.0 L solution containing 20 mM $CaCl_2$ and 40 mM $NaHCO_3$ at 25°C for 5 days with stirring. Similarly, aragonite was synthesized using 1.0 L solution containing 20 mM $CaCl_2$, 40 mM $NaHCO_3$, and 20 mM $MgCl_2$ under the same reaction conditions as those of calcite. After 5 days of the reaction, the precipitates were separated and washed three times with distilled water, then two times with 90% ethanol by centrifuge, and finally dried in vacuum. Before use for the adsorption experiments, the precipitates were confirmed by XRD

to be composed of calcite or aragonite with no impurities. The specific surface areas measured using the BET method with a Shimadzu GEMINI VII 2390 were 0.35 and 1.76 m^2/g for calcite and aragonite, respectively. These specific surface area values were used for normalization of adsorption amounts on the surface of both $CaCO_3$ minerals.

RESULTS

Solution chemistry

During incubation of the three organic systems and the control systems, precipitation of $CaCO_3$ minerals appeared at the beginning of the reaction and continued for 10 days of the reaction period. The solution pH of the Ci system, which was initially adjusted to 7.0, tended to increase up to approximately pH 9.2 mainly due to the degassing of CO_2. The concentrations of Ca^{2+} and HCO_3^- ions decreased with time depending
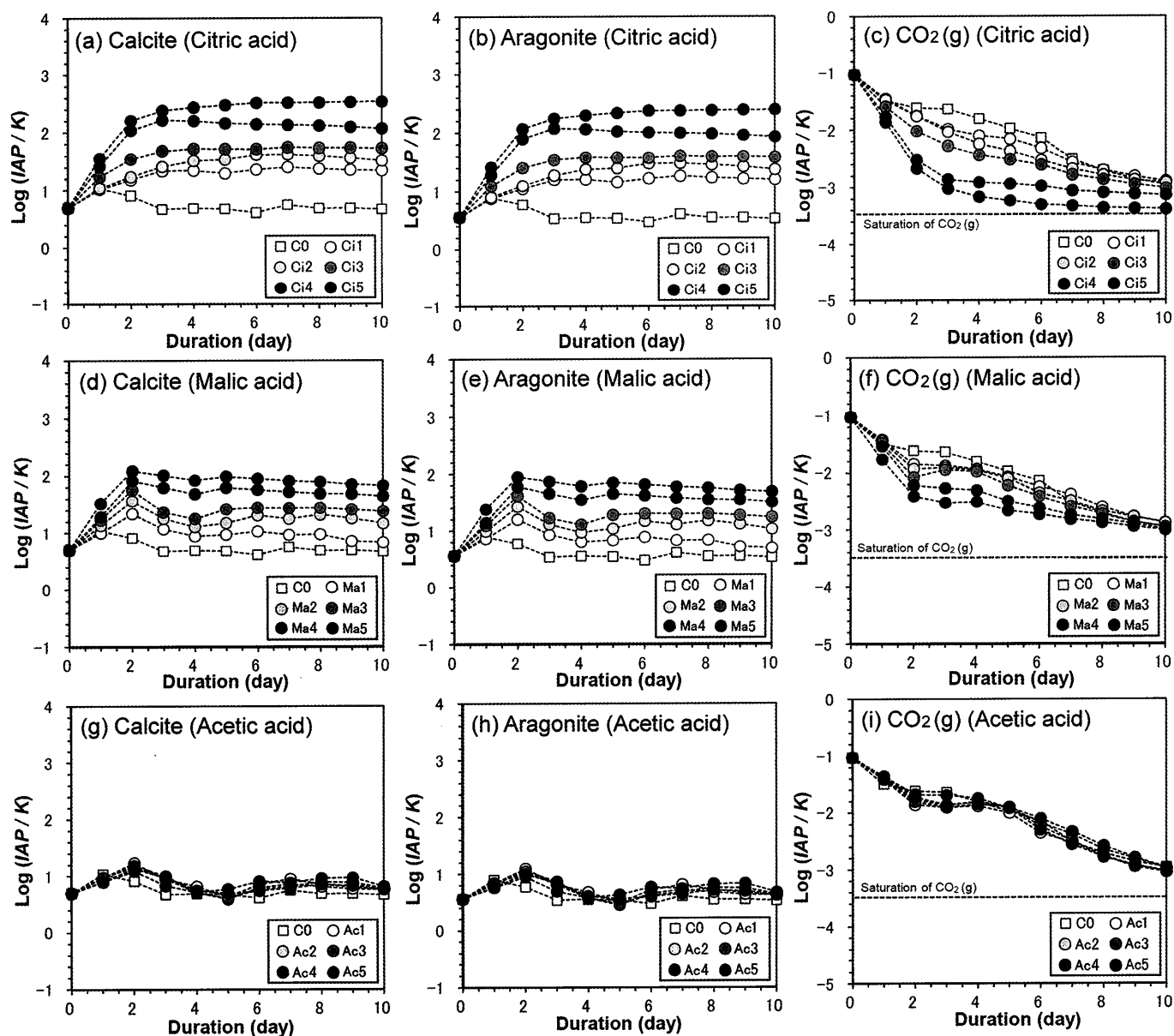

FIG. 3.   Variations in saturation indices ($SI = \log(IAP/K)$) with respect to calcite, aragonite, and $CO_2(g)$ as a function of reaction times for precipitation experiments using citric acid (a, b, and c), malic acid (d, e, and f), and acetic acid (g, h, and i).

on the concentrations of citric acid (Fig. 2a, b, c). The decrease in Ca^{2+} concentrations was due to precipitation of $CaCO_3$ minerals, which also simultaneously consumed HCO_3^- ions to result in a decrease in solution pH. The decreasing rate in the concentration of Ca^{2+} over time tended to slow down with increasing concentrations of citric acid. This result indicates that the rate of $CaCO_3$ precipitation in the Ci system was apparently inhibited by the presence of citric acid ions. Similarly, malic acid also exhibited an increase in solution pH and decrease in Ca^{2+} and HCO_3^- ions to a lesser extent than those in the Ci system (Fig. 2d, e, f), which indicates a smaller inhibition effect of malic acid on $CaCO_3$ precipitation relative to the effect of citric acid. Conversely, no significant variations in pH and concentrations of Ca^{2+} and HCO_3^- ions were observed in the Ac system (Fig. 2g, h, i), which suggests that acetic acid exhibited no inhibition effect on $CaCO_3$ precipitation. For concentrations of Mg^{2+} ions, no significant decrease in concentration was recognized throughout the experiments in these systems.

The saturation indices with respect to calcite, aragonite and $CO_2(g)$ of the organic systems were calculated using the geochemical code of PHREEQC to evaluate the chemical conditions in which $CaCO_3$ precipitation proceeded. The saturation states with respect to calcite and aragonite remained supersaturated throughout the reaction periods in all systems. The *SI* values of the control system ranged from 0.5 to 1.0, whereas those of the Ci system appeared to be much higher at 0.7 to 2.5 (Fig. 3a, b). Therefore, it is apparent that the inhibition of citric acid on $CaCO_3$ precipitation was responsible for the direct interaction of citric acid with the surface of $CaCO_3$ minerals and/or dissolved Ca^{2+} ions. Similarly, the Ma system exhibited higher *SI* values at 0.6 to 2.1 relative to the control system, but lower values than those in the Ci system (Fig. 3d, e). Conversely, the Ac system showed no significant effect of acetic acid on $CaCO_3$ precipitation (Fig. 3g, h). For saturation indices with respect to $CO_2(g)$, the *SI* values decreased over time, while remaining above saturation ($SI = -3.5$) during the reaction periods (Fig. 3c, f, i). This result suggests that the removal of $CO_2(g)$ contributed to the increase in solution pH; therefore, the saturation states remained supersaturated despite the decreases in concentrations of Ca^{2+} and HCO_3^- ions.

Effect on precipitation rate

The results of the precipitation experiments demonstrated that the organic acids tended to inhibit the precipitation rates of $CaCO_3$ minerals. To evaluate the inhibition effect, the rate constant of each precipitation experiment was determined by fitting the Ca^{2+} concentrations with a first-order kinetic model which can be described by:

$$kt = \ln(a_0/a_t) \tag{2}$$

where k is the rate constant (s^{-1}), t is the reaction time (s), and a_0 and a_t are the Ca^{2+} concentrations (M) after 0 and t s, respectively. Figure 4 shows the kt values as a function of reaction times, where linear plots with different slopes giving different values of k can be observed for both systems. This result suggests that $CaCO_3$ precipitation in these systems proceeded with pseudo-first-order reactions. Generally, precipitation of $CaCO_3$ minerals can be expressed as:
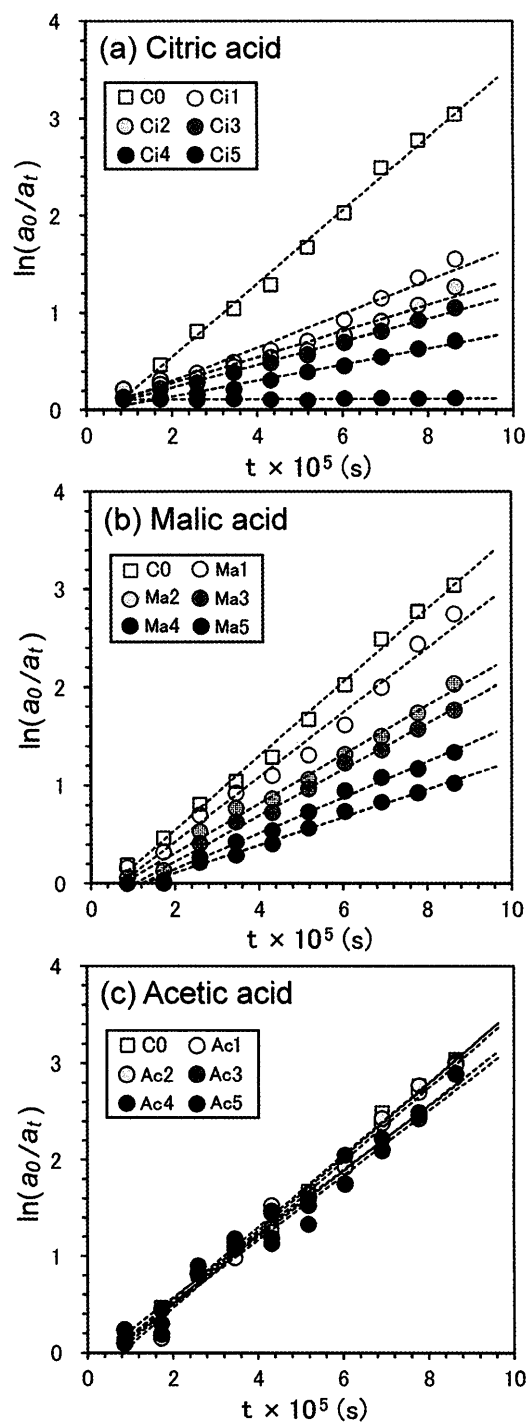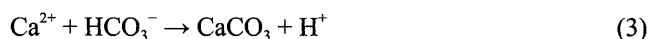

FIG. 4. Plots of kt values of $CaCO_3$ precipitation rates calculated using the first-order kinetic model as a function of reaction times for precipitation experiments using citric acid (a), malic acid (b), and acetic acid (c).

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \tag{3}$$

Thus, the progress of this reaction was controlled by three factors: concentrations of Ca^{2+} and HCO_3^- ions and solution pH. In these reaction systems, increases in solution pH and decrease in HCO_3^- concentrations were observed. Because these two factors are expected to be affected positively and negatively during increases in the precipitation rate, such fac-

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TABLE 2. Rate constants and inhibition factors of $CaCO_3$ precipitations with solution pH after 10 days incubations.

Run	Rate constant (s^{-1})	Inhibition factor	Final pH
C0	0.375×10^{-5}	1.00	8.59
Ci1	0.172×10^{-5}	0.46	8.58
Ci2	0.134×10^{-5}	0.37	8.62
Ci3	0.118×10^{-5}	0.31	8.74
Ci4	0.083×10^{-5}	0.22	8.92
Ci5	0.001×10^{-5}	0.00	9.20
C0	0.375×10^{-5}	1.00	8.59
Ma1	0.329×10^{-5}	0.88	8.57
Ma2	0.250×10^{-5}	0.67	8.59
Ma3	0.235×10^{-5}	0.63	8.68
Ma4	0.183×10^{-5}	0.49	8.75
Ma5	0.142×10^{-5}	0.38	8.79
CO	0.375×10^{-5}	1.00	8.59
Ac1	0.381×10^{-5}	1.02	8.68
Ac2	0.382×10^{-5}	1.02	8.67
Ac3	0.341×10^{-5}	0.91	8.63
Ac4	0.336×10^{-5}	0.90	8.62
Ac5	0.332×10^{-5}	0.89	8.69

tors may have compensated for each other. Consequently, the precipitation rate of $CaCO_3$ minerals in the present systems may have proceeded with pseudo-first-order reactions that were controlled mainly by the concentration of Ca^{2+} ions.

The rate constant of the control system (k_c) and those obtained from the organic systems (k_a) are listed in Table 2 together with the inhibition factors (k_a/k_c) of each system. An inhibition factor of 1.0 translates to no effect on the precipitation rate; however, < 1.0 indicates a decrease in the rate constant corresponding to the values. The inhibition factors of the Ci system were below 1.0, which remarkably decreased to 0.00 with an increase in citric acid concentrations up to 1.0 mM. Similarly, the factors of the Ma and Ac systems decreased to 0.38 and 0.89, respectively, with an increase in the corresponding organic acid concentrations up to 1.0 mM.

Effect on polymorphism

XRD analysis confirmed that only aragonite was precipitated as the dominant polymorph of $CaCO_3$ minerals in the control system containing no organic acids (Fig. 5 C0), which was due to the effect of Mg^{2+} ions (Davis et al., 2000). However, aragonite formation tended to decrease and calcite formation was favored with increasing concentrations of citric acid (Fig. 5a). Notably, only calcite was formed as the dominant $CaCO_3$ polymorph in solutions containing > 0.1 mM citric acid (Fig. 5a Ci3). Thus, it is apparent that citric acid exhibited a substantially greater inhibition effect on aragonite formation than on calcite formation. A similar inhibition effect on aragonite formation was observed in the Ma system (Fig. 5b), although the inhibition effect of malic acid appeared to be less than that of citric acid. For the Ac system, aragonite was formed as the dominant product independent of acetic acid concentration (Fig. 5c), indicating no clear effect of acetic acid on the polymorphism of $CaCO_3$ minerals.

Adsorption of organic acids

Adsorption experiments of organic acids on the surfaces of calcite and aragonite showed significantly different results between calcite and aragonite and among organic acid species. Note that these organic acids exhibited very low adsorption amounts of less than 0.1 $\mu M/m^2$ on the calcite surface in the range of pH 8 to 10 (Fig. 6a), whereas greater adsorption of the organic acids on the aragonite surface was clearly ob-
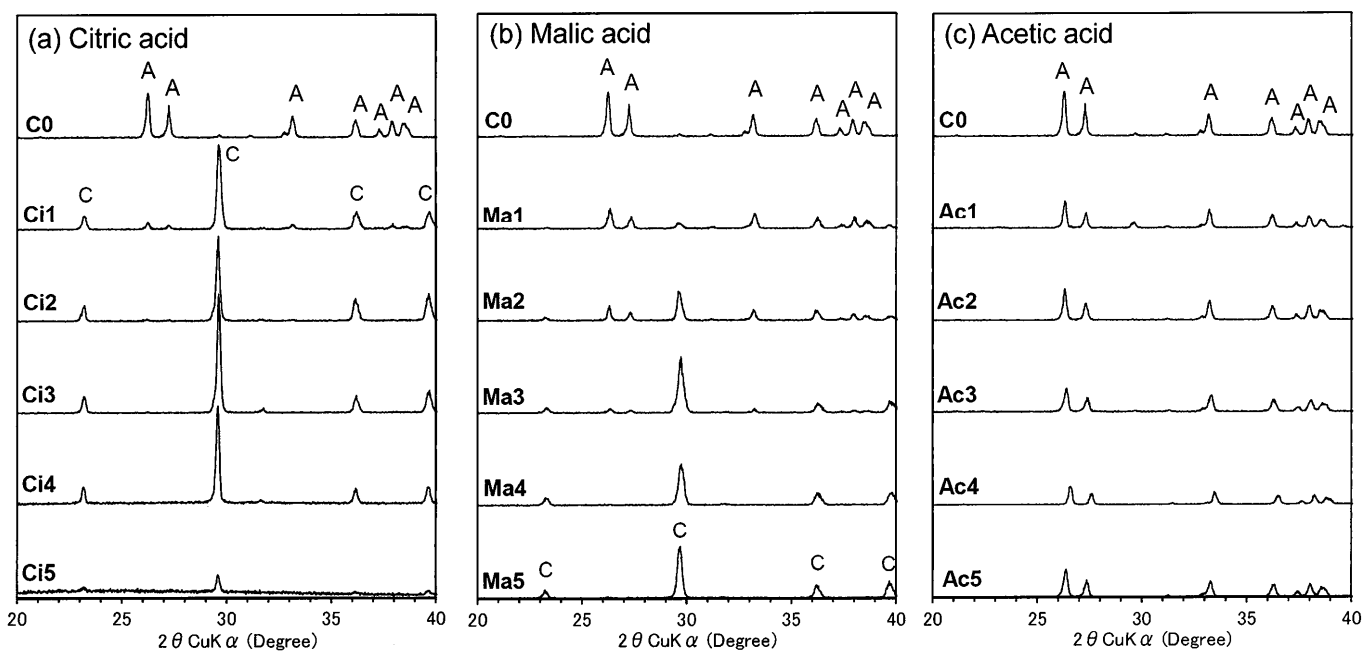

FIG. 5. XRD profiles of $CaCO_3$ minerals precipitated after 10 days incubation in solutions containing 0.01, 0.05, 0.1, 0.5, and 1.0 mM of citric acid (Ci1 to Ci5), and the same concentrations of malic acid (Ma1 to Ma5) and acetic acid (Ac1 to Ac5). The label C0 stands for the XRD profile of precipitate in the control system containing no organic acids. Labels A and C indicate aragonite and calcite, respectively.
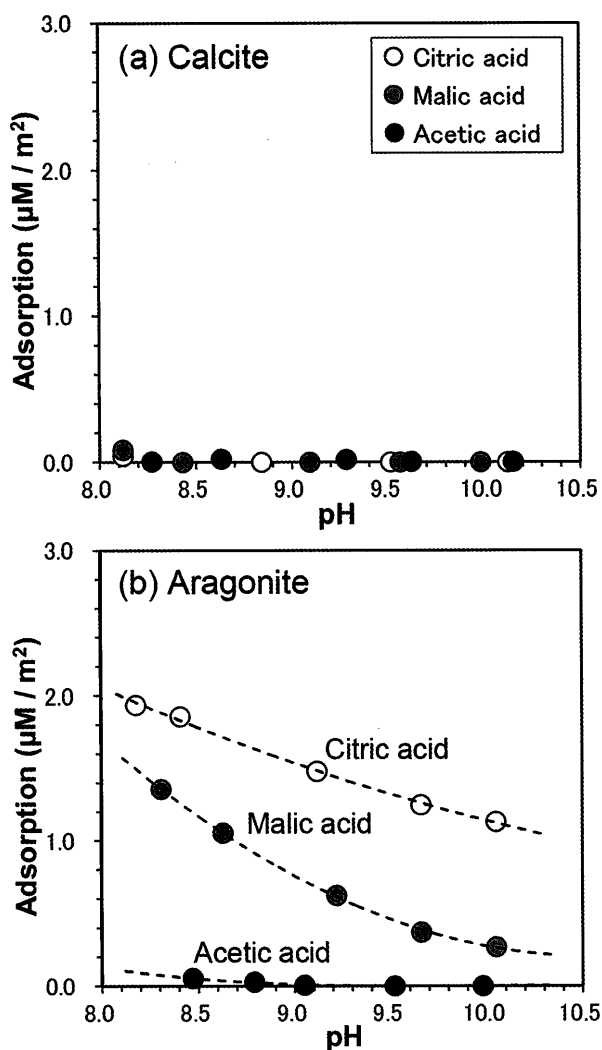
FIG. 6.   Variations in amounts of adsorption of citric acid, malic acid, and acetic acid on the surfaces of calcite (a) and aragonite (b) in solution with pH range of approximately 8 to 10. The amounts of adsorption were normalized by the specific surface area of each minerals.

served (Fig. 6b). In particular, citric acid showed significant greater adsorption on the aragonite surface up to approximately 2.0 $\mu M/m^2$ at pH 8, which decreased as the solution pH increased. Malic acid also exhibited greater adsorption on the aragonite surface with lesser amounts than that for citric acid, but greater than that for acetic acid. Thus, the adsorption affinity of the organic acids for the aragonite surface appears to be higher than that for the calcite surface. In addition, the adsorption ability of organic acids was greater in the following order: citric acid > malic acid > acetic acid. These adsorption characteristics of organic acids are consistent with the degrees of effect on the precipitation rate and polymorphism of $CaCO_3$ minerals.

DISCUSSION

The precipitation experiments on $CaCO_3$ minerals in the control system demonstrated that only aragonite was precipitated due to the effect of Mg^{2+} ions. The experiments

in solutions containing various concentrations up to 1.0 mM of organic acids confirmed progressive decrease in the precipitation rates of $CaCO_3$ minerals and favorable the formation of calcite as a dominant polymorph depending on the organic acid species and their concentrations. Mg^{2+} ions are widely accepted as having a strong inhibition effect on calcite formation, resulting in the enhancement of aragonite formation as the dominant polymorph of $CaCO_3$ minerals (Mucci and Morse, 1983; Morse and Mackenzie, 1990; Falini et al., 1994; Gutjahr et al., 1996; Zhang and Dawe, 2000; Park et al., 2008; Lin and Singer, 2009). The effect of Mg^{2+} ions on the polymorphism of $CaCO_3$ minerals has been explained by the following two molecular-scale reaction mechanisms: the step-pinning model and the impurity incorporation model. The former model is the inhibition mechanism of calcite growth through the step-pinning or blocking by hydrated Mg^{2+} ions adsorbed to step-edges or on terraces ahead of migrating steps and subsequent dehydration during crystal growth processes in supersaturated solutions (Reddy and Wang, 1980; Mucci and Morse, 1983; Gutjahr et al., 1996). The latter model explains the inhibition of calcite growth by incorporation of Mg^{2+} ions into the surface lattice of calcite structures (Berner, 1975; Davis et al., 2000). This incorporation induces a strain in the solid lattice, thereby increasing the internal free energy of the crystal. Therefore, the resultant increase in crystal solubility reduces the reaction affinity of crystal growth by decreasing the effective supersaturation. Although the molecular scale reaction mechanisms remain under debate (Morse et al., 2007; Astilleros et al., 2010), the preferential precipitation of aragonite in the control system appears to be attributed to the higher affinity of Mg^{2+} ions for the surface of calcite than for aragonite (De Groot and Duyvis, 1966; Berner, 1975; Mucci and Morse, 1985; Deleuze and Brantley, 1997).

In the systems with organic acids, it was determined that citric acid significantly decreased the precipitation rates of $CaCO_3$ minerals and inhibited the formation of aragonite by increasing its concentration. Malic acid also showed a decrease in the precipitation rate and inhibition of aragonite formation to a lesser extent than that with citric acid. However, acetic acid exhibited no clear effect on both the precipitation rate and the polymorphism of $CaCO_3$ minerals. These observations indicate that organic acids have a significant effect on both precipitation rate and polymorphism of $CaCO_3$ minerals in the following order: citric acid > malic acid > acetic acid. A similar effect on $CaCO_3$ precipitation by the interaction of organic compounds, including monomeric to polymeric carboxylic acids, has been previously reported by many researchers (Kitano and Hood, 1965; Berner et al., 1978; Amjad et al., 1998; Hoch et al., 2000; Klepetsanis et al., 2000; Wada et al., 1999, 2001; Lin et al., 2005; etc). At present, two fundamental processes have been accepted to explain the mechanisms of the effect of organic molecules. First is the formation of chelate complexes of the organic molecules with dissolved Ca^{2+} ions, which results in the reduction of effective supersaturation with respect to $CaCO_3$ minerals, thereby decreasing the rates of nucleation and crystal growth depending on the saturation states. The second is adsorption of the organic compounds on the specific surface of $CaCO_3$ minerals. The active growth sites on the $CaCO_3$ surface may be blocked by
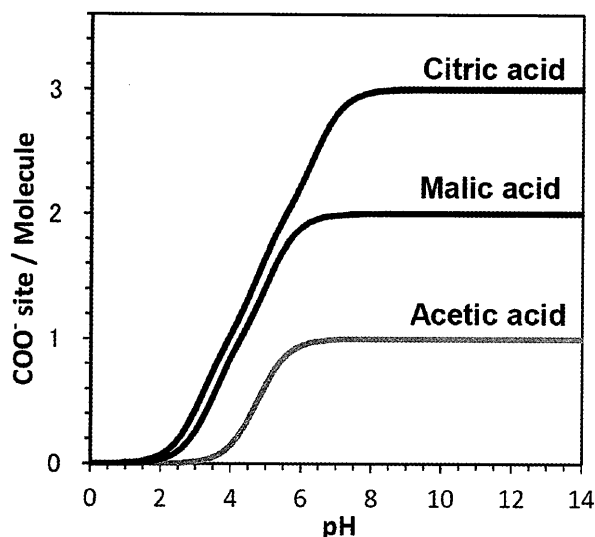
FIG. 7.   Calculated numbers of deprotonated COO⁻ sites per molecule of citric acid, malic acid, and acetic acid by geochemical code of ChemEQL.
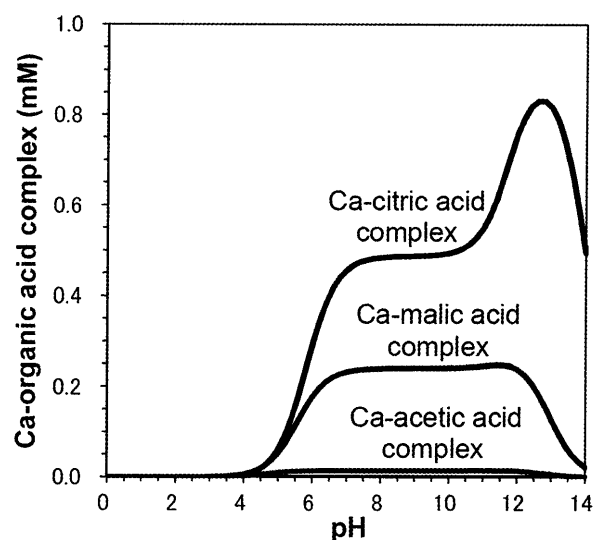
FIG. 8.   Calculated concentrations of Ca-complex in solutions of 5.0 mM Ca^{2+} and Mg^{2+} ions with 1.0 mM citric acid, malic acid, and acetic acid by geochemical code of ChemEQL. The log K values of organic complexes used in this calculation are as follows (Dawson et al., 1986): Ca-citric acid; log K = 3.6, Mg-citric acid; log K = 3.6, Ca-malic acid; log K = 1.9, Mg-malic acid; log K = 1.6, Ca-acetic acid; log K = 0.5, and Mg-acetic acid; log K = 0.5.

the adsorption reaction, thus preventing further growth of the $CaCO_3$ crystal.

As shown in Fig. 1, the organic acids used in this study are monomeric molecules with three, two, and one functional carboxyl group, respectively. These carboxyl groups tend to deprotonate and produce negatively charged COO⁻ sites with an increase in solution pH. The numbers of COO⁻ sites per molecule of each organic acid were calculated with the geochemical code of ChemEQL (Müller, 2004) by using their pK values as shown in Fig. 7. This calculation indicated that the numbers of COO⁻ sites increased with an increase in solution pH and remained constant for fully deprotonated forms above pH 7 to 8. The solution pH of the present experiments also remained above pH 7 to 8 throughout the 10 days of reaction (Fig. 2a, d, g). Thus, the citric, malic, and acetic acids in solutions of the experiments would be expected to present as fully deprotonated trivalent, divalent, and monovalent anionic forms, respectively. When these organic acids are present in solutions with alkaline earth metals such as Ca^{2+} and Mg^{2+} ions, complexation of the metals and organic acids tend to occur depending on the stability of the complexes. The ChemEQL calculation confirmed that the concentrations of Ca-complex in solutions of 5.0 mM Ca^{2+} and Mg^{2+} ions with 1.0 mM citric, malic, and acetic acids were approximately 0.49, 0.24, and 0.01 mM, respectively, in the range of pH 7 to 10 (Fig. 8). This result suggests that the effective Ca^{2+} activity on $CaCO_3$ formation in the present reaction systems decreased slightly by the formation of a Ca-complex, however, this complexation appears to be less effective on the precipitation rate and polymorphism because its concentrations were very low relative to the free Ca^{2+} ions.

The other factor is adsorption of organic acids on the surface of $CaCO_3$ minerals. The present adsorption experiments revealed that the adsorption ability of organic acids for the surface of aragonite was significantly greater than that for the calcite surface, and showed adsorption amounts in the follow-

ing order: citric acid > malic acid > acetic acid (Fig. 6). These adsorption behaviors were consistent with the effectiveness of these organic acids on the precipitation rate and polymorphism of the $CaCO_3$ minerals. Alternatively, a higher adsorption of organic acids caused a larger decrease in the precipitation rate of aragonite, resulting in favorable formation of calcite possibly due to the lower adsorption ability of organic acids for the calcite surface. The detailed adsorption mechanism of these organic acids on the surface of $CaCO_3$ minerals is not well understood; however, it is widely accepted that the aragonite surface exhibits higher adsorption affinity for anionic compounds including monomer to polymer than does the calcite surface (Mucci and Morse, 1985; Westin and Rasmuson, 2005). Additionally, the adsorption amounts increase with increasing degree of ionization, charge density, molecular size, stereochemical geometry, and flexibility of the molecules (Geffroy et al., 1999; Klepetsanis et al., 2000). In the present study, it would be reasonable to assume that the negatively charged functional groups of organic acids may have bound to the positively charged Ca sites on both aragonite and calcite surfaces, which may have inhibited further crystal growth depending on the degree of binding. In this process, substantially greater inhibition may have occurred on the aragonite surface because it is more significantly dominated by terminated Ca ions relative to the calcite surface (De Leeuw and Parker, 1998).

CONCLUSIONS

The precipitation experiments in this study confirmed that the organic acids significantly inhibited precipitation of $CaCO_3$ minerals and the formation of aragonite in the following order: citric acid > malic acid > acetic acid. Citric

acid was significantly more effective in both reducing the precipitation rate and preventing aragonite formation, resulting in the formation of calcite as the dominant polymorph by increasing its concentrations. Malic acid also exhibited similar inhibition, which was less effective than that of citric acid, and no significant inhibition was observed for acetic acid. The adsorption experiment revealed higher adsorption affinity of organic acids for the aragonite surface than that for the calcite surface, and showed a very strong correlation between adsorbed amounts and strength of the inhibition effect. This higher adsorption affinity of the organic acids contributed to the greater adsorption on the $CaCO_3$ surface and a decrease in the precipitation rate of the minerals. The higher adsorption affinity for aragonite caused a significantly stronger inhibition of precipitation, which favored the formation of calcite rather than aragonite.

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REFERENCES

ADDADI, L. and WEINER, S. (1985) Interaction between acidic proteins and crystals: stereochemical requirements in biomineralization. *Proceedings of the National Academy of Sciences of the United States of America*, **82**, 4110–4114.

AMJAD, Z., PUGH, J., and REDDY, M. (1998) Kinetic inhibition of calcium carbonate crystal growth in the presence of natural and synthetic organic inhibitors. Pp. 131–147 in: *Water Soluble Polymers, Solution Properties and Applications* (Z. Amjad, editor). Plenum Press, New York.

ARP, G., THIEL, V., REIMER, A., MICHAELIS, W., and REITNER, J. (1999) Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. *Sedimentary Geology*, **126**, 159–176.

ASTILLEROS, J.M., FERNÁNDEZ-DÍAZ, L., and PUTNIS, A. (2010) The role of magnesium in the growth of calcite: An AFM study. *Chemical Geology*, **271**, 52–58.

BAUMGARTNER, L.K., REID, R.P., DUPRAZ, C., DECHO, A.W., BUCKLEY, D.H., SPEAR, J.R., PRZEKOP, K.M., and VISSCHER, P.T. (2006) Sulfate reducing bacteria in microbial mats: Changing paradigms, new discoveries. *Sedimentary Geology*, **185**, 131–145.

BERNER, R.A. (1975) The role of magnesium in the crystal growth of calcite and aragonite from sea water. *Geochimica et Cosmochimica Acta*, **39**, 489–504.

BERNER, R.A., WESTRICH, J.T., GRABER, R., SMITH, J., and MARTENS, C.S. (1978) Inhibition of aragonite precipitation from supersaturated seawater: A laboratory and field study. *American Journal of Science*, **278**, 816–837.

DAVIS, K.J., DOVE, P.M., and DE YOREO, J.J. (2000) The role of Mg^{2+} as an impurity in calcite growth. *Science*, **290**, 1134–1137.

DAWSON, R.C., ELLIOTT, D.C., ELLIOTT, W.H., and JONES, K.M. (1986) *Data for Biochemical Research, 3rd edition.* Clarendon Press, Oxford, UK, 580 pp.

DECHO, A.W. (2010) Overview of biopolymer-induced mineralization: What goes on in biofilms? *Ecological Engineering*, **36**, 137–144.

DE GROOT, K. and DUYVIS, E.M. (1966) Crystal form of precipitated calcium carbonate as influenced by adsorbed magnesium ion. *Nature*, **212**, 183–184.

DE LEEUW, N.H. and PARKER, S.C. (1998) Surface structure and morphology of calcium carbonate polymorphs calcite, aragonite, and vaterite: An atomic approach. *Journal of Physical Chemistry B*, **102**, 2914–2922.

DE VRIND-DE JONG, E.W., and DE VRIND, J.P.M. (1997) Algal deposition

of carbonates and silicates. Pp.35–79 in: *Geomicrobiology: Interactions between Microbes and Minerals* (J.F. Banfield and K.H. Nealson, editors). Reviews in Mineralogy, 35, Mineralogical Society of America, Washington DC.

DELEUZE, M. and BRANTLEY, S.L. (1997) Inhibition of calcite crystal growth by Mg^{2+} at 100°C and 100 bars: Influence of growth regime. *Geochimica et Cosmochimica Acta*, **61**, 1475–1485.

FALINI, G., GAZZANO, M., and RIPAMONTI, A. (1994) Crystallization of calcium carbonate in presence of magnesium and polyelectrolytes. *Journal of Crystal Growth*, **137**, 577–584.

GEFFROY, C., FOISSY, A., PERSELLO, J., and CABANE, B. (1999) Surface complexation of calcite by carboxylates in water. *Journal of Colloid and Interface Science*, **211**, 45–53.

GUTJAHR, A., DABRINGHAUS, H., and LACMANN, R. (1996) Studies of the growth and dissolution kinetics of the $CaCO_3$ polymorphs calcite and aragonite II. The influence of divalent cation additives on the growth and dissolution rates. *Journal of Crystal Growth*, **158**, 310–315.

HOCH, A.R., REDDY, M.M., and AIKEN, G.R. (2000) Calcite crystal growth inhibition by humic substances with emphasis on hydrophobic acids from the Florida Everglades. *Geochimica et Cosmochimica Acta*, **64**, 61–72.

KAWAGUCHI, T. and DECHO, A.W. (2002) A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing $CaCO_3$ polymorphism. *Journal of Crystal Growth*, **240**, 230–235.

KAWANO, M. and HWANG, J. (2010) Influence of gram-negative bacterium Pseudomonas fluorescens on the precipitation rates and polymorphism of calcium carbonate minerals. *Clay Science*, **14**, 177–185.

KAWANO, M. and HWANG, J. (2011) Roles of microbial acidic polysaccharides in precipitation rate and polymorph of calcium carbonate minerals. *Applied Clay Science*, **51**, 484–490.

KITANO, Y. and HOOD, D.W. (1965) The influence of organic material on the polymorphic crystallization of calcium carbonate. *Geochimica et Cosmochimica Acta*, **29**, 29–41.

KLEPETSANIS, P.G., KLADI, A., OSTVOLD, T., KONTIYIANNIS, C.G., KOUTSOUKOS, P.G., AMJAD, Z., and REDDY, M. (2000) The inhibition of calcium carbonate formations in aqueous supersaturated solutions. Spontaneous precipitation and seeded crystal growth. Pp. 123–137 in: *Advances in Crystal Growth Inhibition Technology* (Z. Amjad, editor). Kluwer Academic/Plenum Publisher, New York.

LIN, Y.P., SINGER, P.C., and AIKEN, G.R. (2005) Inhibition of calcite precipitation by natural organic material: kinetics, mechanism, and thermodynamics. *Environmental Science & Technology*, **39**, 6420–6428.

LIN, Y.P. and SINGER, P.C. (2009) Effect of Mg^{2+} on the kinetics of calcite crystal growth. *Journal of Crystal Growth*, **312**, 136–140.

LOWENSTAM, H.A. and WEINER, S. (1989) *On Biomineralization.* Oxford University Press, New York, 336 p.

MANN, K., SIEDLER, F., TRECCANI, L., HEINEMANN, F., and FRITZ, M. (2007) Perlinhibin, a cysteine-, histidine-, and arginine-rich miniprotein from abalone (Haliotis laevigata) nacre, inhibits in vitro calcium carbonate crystallization. *Biophysical Journal*, **93**, 1246–1254.

MORSE, J.W. and MACKENZIE, F.T. (1990) *Geochemistry of Sedimentary Carbonates.* Elsevier, Amsterdam, 706 p.

MORSE, J.W., ARVIDSON, R.S., and LÜTTGE, A. (2007) Calcium carbonate formation and dissolution. *Chemical Reviews*, **107**, 342–381.

MUCCI, A. and MORSE, J.W. (1983) The incorporation of Mg^{2+} and Sr^{2+} into calcite overgrowths: influences of growth rate and solution composition. *Geochimica et Cosmochimica Acta*, **47**, 217–233.

MUCCI, A. and MORSE, J.W. (1985) Auger spectroscopy determination of the surface-most adsorbed layer composition on aragonite, calcite, dolomite, and magnesite in synthetic seawater. *American Journal of Science*, **285**, 306–317.

MÜLLER, B. (2004) *CHEMEQL V3.0, A program to calculate chemical speciation equilibria, titrations, dissolution, precipitation, adsorption, kinetics, pX-pY diagrams, solubility diagrams.* Limnological Research Center EAWAG/ETH, Kastanienbaum, Switzerland, 95 pp.

NOVITSKY, J.A. (1981) Calcium carbonate precipitation by marine bacteria. *Geomicrobiological Journal*, **2**, 375–388.

PARK, W.K., KO, S.J., LEE, S.W., CHO, K.H., AHN, J.W., and HAN, C. (2008) Effects of magnesium chloride and organic additives on the synthesis of aragonite precipitated calcium carbonate. *Journal of*

Crystal Growth, **310**, 2593–2601.

PARKHURST, D.L. and APPELO, C.A.J. (1999) *User's guide to PHREEQC (Version 2) – A computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations.* Water-Resources Investigations Report 99-4259, U.S. Geological Survey, Denver, Colorado.

PENTECOST, A. and RIDING, R.P. (1986) Calcification in cyanobacteria. Pp. 73–90 in: *Biomineralization in Lower Plants and Animals* (B.S.C. Leadbeater and R.P. Riding, editors). Clarendon Press, Oxford.

PIPICH, V., BALZ, M., WOLF, S.E., TREMEL, W., and SCHWAHN, D. (2008) Nucleation and growth of $CaCO_3$ mediated by the egg-white protein ovalbumin: a time-resolved in situ study using small-angle neutron scattering. *Journal of the American Chemical Society*, **130**, 6879–6892.

REDDY, M.M. and WANG, K.K. (1980) Crystallization of calcium carbonate in the presence of metal ions. I. Inhibition by magnesium ion at pH 8.8 and 25°C. *Journal of Crystal Growth*, **50**, 470–480.

RIDING, R. (1982) Cyanophyte calcification and changes in ocean chemistry. *Nature*, **299**, 814–815.

ROBERTS, J.A., BENNETT, P.C., GONZÁLEZ, L.A., MACPHERSON, G.L., and MILLIKEN, K.L. (2004) Microbial precipitation of dolomite in metha-nogenic groundwater. *Geology*, **32**, 277–280.

TOURNEY, J. and NGWENYA, B.T. (2009) Bacterial extracellular polymeric substances (EPS) mediate $CaCO_3$ morphology and polymorphism. *Chemical Geology*, **262**, 138–146.

WADA, N., YAMASHITA, K., and UMEGAKI, T. (1999) Effects of carboxylic acids on calcite formation in the presence of Mg^{2+} ions. *Journal of Colloid and Interface Science*, **212**, 357–364.

WADA, N., KANAMURA, K., and UMEGAKI, T. (2001) Effects of carboxylic acids on the crystallization of calcium carbonate. *Journal of Colloid and Interface Science*, **233**, 65–72.

WESTIN, K.J. and RASMUSON, Å.C. (2005) Crystal growth of aragonite and calcite in presence of citric acid, DTPA, EDTA and pyromellitic acid. *Journal of Colloid and Interface Science*, **282**, 359–369.

YANG, M., STIPP, S.L.S., and HARDING, J. (2008) Biological control on calcite crystallization by polysaccharides. *Crystal Growth & Design*, **8**, 4066–4074.

ZHANG, Y. and DAWE, R.A. (2000) Influence of Mg^{2+} on the kinetics of calcite precipitation and calcite crystal morphology. *Chemical Geology*, **163**, 19–138.

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