Direct Observation of Polymer-Binding Site on Calcite Crystal by FE/SEM: Regulation of Binding Abilities by a Rotation of Amide Group in Poly(carboxylate) to $CaCO_3$ Crystals

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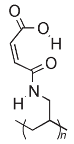
Poly{1-[(*Z*)-3-carboxyl-2-propenylaminomethyl]ethylene} binding on the edge of calcite crystals was observed directly by ^{13}C CP/MAS NMR and FE/SEM measurements. Comparing the results taken both before and after the washing process, we were able to make these observation because the polymer ligand weakly bound to the $CaCO_3$ crystals.

In natural $CaCO_3$ architectures, a small amount of biopolymer (acidic peptide, polysaccharide, etc.) strongly binds to a specific face of the $CaCO_3$ crystal, thereby providing various shapes and morphologies.[1] Various synthetic carboxylate or poly(carboxylate) ligands have been used as model acidic peptides in $CaCO_3$ architectures.[2–4] These ligands have highly designed structures and seem to bind to a specific face of the $CaCO_3$ crystal.[2,4] For example, Addadi et al. have been suggested that the sulfonated poly(styrene) and the poly(aspartate) cooperate to induce (001)-oriented calcite nucleation.[2] Their discussion, however, dealt mainly with the change of crystal face, and a binding site of ligand in the $CaCO_3$ crystal has not yet been found directly.

Here, we directly determined a location of a polymer ligand on $CaCO_3$ calcite crystals by utilizing novel poly{1-[(*Z*)-3-carboxyl-2-propenylaminomethyl]ethylene} (**1**). In crystalline **1**–$CaCO_3$ composite (**1CaCO₃**), **1** was completely dislodged from the $CaCO_3$ crystal by hydrolysis, which occurred during washing with water-containing solvents. Comparing the composite before and after the washing process, we found that **1** mainly binds to the edge of $CaCO_3$ crystals as detected by a ^{13}C cross polarization/magic angle spinning (CP/MAS) NMR spectrum and a field emission/scanning electron microscopy (FE/SEM) analysis.



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Scheme 1.

The polymer **1** was synthesized through the reaction of poly(allylamine hydrochloride) with maleic anhydride. Tetraethylammonium poly(carboxylate) salt (**1NEt₄**) was obtained by neutralization of **1** with NEt_4OH. At the initial stage of crystallization for $CaCO_3$ composite, the pH of the mixed solution of polymer MeOH solution and $CaCl_2$ aq reached a level between 3.8 and 4.0, and the pH of the cocktail was finally turned to ≈7.2 to 7.4 by the addition of $(NH_4)_2CO_3$ aq. At low pH, polymer ligands assume the COOH form. After the crystallization, the obtained composites were washed carefully with MeOH and distilled H_2O to completely remove the unbound polymer ligand. The $CaCO_3$ crystal obtained by this process was the most stable polymorph, calcite, as determined by a powder X-ray diffraction analysis.

To detect the binding of the polymer ligand to $CaCO_3$ crystals, ^{13}C CP/MAS measurements were performed. The ^{13}C CP/MAS is an effective technique for observing a polymer ligand in $CaCO_3$ composites.[5] Figure 1 shows the obtained ^{13}C CP/MAS spectra of **1CaCO₃** both before and after the washing process. The ^{13}C CP/MAS spectra of **1** and **1NEt₄** were also obtained in order to characterize each signal of **1CaCO₃**. On the ^{13}C CP/MAS analysis, ^{13}C signals of **1** were found before the washing process (Figure 1a), although each ^{13}C signal of **1** completely disappeared after the washing process except for the $^{13}CO_3{}^{2-}$ signal (Figure 1b). Carbonyl carbon signals were overlapping to the $^{13}CO_3{}^{2-}$ signal in the spectrum before the washing process. This

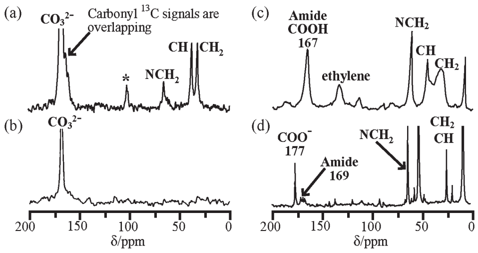

Figure 1. ^{13}C CP/MAS spectra of (a) **1CaCO₃** before the washing process, (b) **1CaCO₃** after the washing process, (c) **1** and (d) **1NEt₄** (in solid-state).

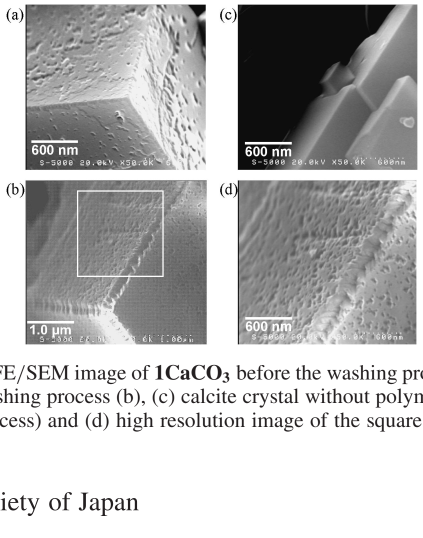

Figure 2. FE/SEM image of **1CaCO₃** before the washing process (a) and after the washing process (b), (c) calcite crystal without polymer (after the washing process) and (d) high resolution image of the square are of (b).

result indicates that the polymer ligand is easily dislodged from $CaCO_3$ crystals by washing with solvents.

FE/SEM images clearly show a polymer ligand binding site. Many hollows clearly appear on the edge and surface of $CaCO_3$ crystals after the washing process (Figures 2b and 2d); however, swells are found on the same place before the washing process (Figure 2a). In contrast, the calcite crystal without polymer shows a smooth surface after the washing process (Figure 2c). The ^{13}C CP/MAS spectrum also supports the supposition that **1** binds only to the edge and surface of the $CaCO_3$ crystal. Thus, it is clear that the binding polymers exist in swells before the washing process.

The proposed structures of polymer ligand–$CaCO_3$ composite, as suggested by our results, are shown in Figure 3. Before the washing process, **1** mainly binds to the edge of $CaCO_3$ crystal as COO^- form (Figure 3, left). Also, the crystal growth occurs on the opposite side from the polymer binding site, supported by the fact that the polymer ligand does not bind to inside the crystal from both ^{13}C CP/MAS and FE/SEM measurements. During the washing process, the polymer ligand is hydrolyzed to affect the COOH form, and finally, **1** is dissociated from the $CaCO_3$ crystal (Figure 3, right). The obtained hollow area had a diameter of 50 nm and clearly corresponds to the site where the polymer ligands bind before the washing process (Figure 2d). If **1** forms a *trans*-zigzag extended structure with a volume approximately 3.5 nm^3, the hollow would be filled with approximately 20 polymer ligands. In fact, an etching effect probably exists during such polymer dissociation. Therefore, there is a maximum of 20 polymers contained in each hollow. In contrast, the strong NH···O hydrogen-bonded polymer ligand, which has a rigid amide group localizing to the carboxylate oxyanion, hardly dislodged form $CaCO_3$ crystal during the washing process.[5] The reason that such a difference exists is described later.

During these processes, a conformational change occurs for **1** (Figure 4). Based on results of its model maleate derivateives,[6] (*Z*)-4-(*tert*-butylamino)-4-oxo-2-butenoate, **1** has two possible conformers that are an NH···O hydrogen-bonded form (conformer **A**) and a non-NH···O hydrogen-bonded form (conformer **B**), as shown in Figure 4. The formation of NH···O hydrogen bond between a carboxylate oxyanion and the neighboring amide NH group lowers the basicity of carboxylate oxyanion, and the carboxylate oxyanion hardly protonates by hydrolysis that occurs due to the pK_a shift of the carboxylic acid. This ligand must be dislodged without conformational change.

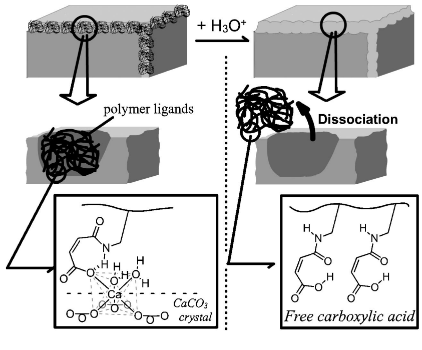

Figure 3. Proposed structures of **$1CaCO_3$** during washing process. The crystal edge and surface before washing process (left), and the edge and surface after the washing process (right).

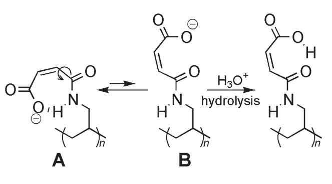

Figure 4. Possible conformational change of **1**.

However, during the washing process, **1** was protonated by hydrolysis. This hydrolysis occurs for the conformer **B**. The amide carbonyl of this conformer is directed to the carboxylate oxyanion. At this point, the basicity of the carboxylate oxyanion is increased. The conformer **B** forms by a rotation of the amide plane from the conformer **A**. The energy barrier of the rotation of amide plane between the hydrogen-bonded form and non-hydrogen-bonded form is relatively high (about 8 kcal/mol).[6] The conformer **B** has a relatively long lifetime because of this high energy barrier. Therefore, this conformer is readily hydrolyzed during the washing process and assumess the COOH form (Figure 4, right). Thus, **1** easily dislodged from $CaCO_3$ crystals during the washing process.

On the contrary, the poly(*N*-allylsuccinamate) ligand, because non-hydrogen-bonded conformer has a short lifetime, is hardly hydrolyzed during the washing process.[6,7] This ligand strongly binds to $CaCO_3$ crystal to obtain the metastable polymorph, vaterite.[7] Strong binding polymers to $CaCO_3$ crystals make vaterite crystals, although weakly binding polymers, such as **1**, give calcite crystals.

In this work, we directly detected a polymer ligand binding site on $CaCO_3$ calcite crystal by utilizing a novel polymer ligand **1**. Before washing, **1** mainly binds to an edge of calcite crystal; however, **1** easily dislodges from the $CaCO_3$ crystal after washing. Utilizing this polymer ligand, we successfully observed binding polymers on the $CaCO_3$ calcite crystal, by comparing FE/SEM images of the crystal surface of the composite before and after the washing process. These binding abilities of **1** are probably dependent on the reactivity of the carboxylate oxyanion, which will be described elsewhere in detail.[6]

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