Secure Binding of Alternately Amidated Poly(acrylate) to Crystalline Calcium Carbonate by NH···O Hydrogen Bond

Norikazu Ueyama,* Hidekazu Kozuki, Mototsugu Doi, Yusuke Yamada, Kazuyuki Takahashi, Akira Onoda, Taka-aki Okamura, and Hitoshi Yamamoto

Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Received October 12, 2000

ABSTRACT: Alternately amidated poly(1-carboxylate-3-*N*-alkylamidotetramethylene)s (alkyl = *t*-Bu, *n*-Bu) were synthesized from poly(acrylic anhydride) and alkylamines as ligands for Ca(II) complexes. The anion form of the alternately amidated polycarboxylates possesses an NH···O hydrogen bond between the carboxylate oxygen and the adjacent amide NH. The reinforcement of Ca−O bond strength by the NH···O hydrogen bond, upon the mineralization of calcium carbonate, is maintained at the interface between crystalline $CaCO_3$ and the polymer ligands because it prevents the removal of the alternately amidated poly(1-carboxylate-3-*N*-alkylamidotetramethylene) ligand from the crystalline $CaCO_3$ under neutral conditions. Under the same conditions, a commercial poly(acrylate) ligand readily releases Ca(II) ion to separate from the surface of the crystal by hydrolysis with water. The binding carboxylate ligand ^{13}C on calcium carbonate is detectable using solid-state ^{13}C CP/MAS spectroscopy.

Introduction

In the initial stage of biomineralization of shells, oriented $CaCO_3$ crystals grow on a nucleating protein sheet.[1−4] The proteins and inorganic ions regulate the phase of the deposited mineral.[3,5] Belcher has proposed that the incorporation of one linear polypeptide is essential for controlling the polymorph of a single $CaCO_3$ crystal.[3] These proteins have been believed to exist along the edge of the crystal as a polyanion ligand to bind Ca(II).

A soluble organic matrix protein has been isolated from the nacreous layer of oyster pearls. The protein consists of a repeated Gly-Xaa-Asn (Xaa = Asp, Asn, or Glu) fragment intervening between two Zn(II) binding peptide parts.[6] The carboxylate of the anionic Asp or Glu presumably binds Ca(II) ion without dissociation during the crystallization of $CaCO_3$.

Various artificial complexations have been investigated as models of biomineralization using synthetic peptides, e.g., poly(acrylate), poly(Glu) or poly(Asp),[7] stearic acid monolayer,[5] glycoproteins,[8] chitin fibers,[9] porphyrin-template monolayer,[10] and mercaptophenol-protected gold colloids.[10] These ligands have also been thought to control the polymorph, and the polymorph of the calcium carbonate clusters was identified by IR or microscopy.[11] However, the characterization of these native and synthetic polymers on the crystal surface is still unclear because the large contents of these polymers in crystalline $CaCO_3$ clusters disturb the analysis of these polymers by spectroscopic methods.

A number of Cu(II), Ca(II), Zn(II), and Mn(II) complexes of novel carboxylate ligands having an NH···O hydrogen bond between carboxylate oxygen and neighboring amide NH were synthesized to reveal the functions of this type of hydrogen bonds.[12,13] The NH···O hydrogen bond protects the metal−carboxylate bond from dissociation with water due to the shift in the pK_a of the conjugated carboxylic acid.[14] Previously, we synthesized novel poly(amide) ligands containing the NH···O hydrogen bond derived from 2,6-diaminobenzoic acid and dimethylmalonyl dichloride, isophthalyl dichloride, or *trans*-fumaryl dichloride.[15] The ease of the dissociation of an anion ligand from the Ca binding by water results in separation from the edge of the $CaCO_3$ crystals during crystallization.

However, conjugation between the carboxylate ($-CO_2^-$) and the benzene ring in benzoate leads to a lower shift of the pK_a accompanied by that from the NH···O hydrogen bond.[15] The conjugation also decreases the nucleophilicity of the carboxylate. Thus, it is necessary to examine an alkane carboxylate having an NH···O hydrogen bond for the mineralization of $CaCO_3$ as models of Asp-containing proteins.

This paper presents the synthesis of partially amidated poly(acrylic acid), poly(1-carboxylate-3-*N*-alkylamidotetramethylene), with the neighboring amide NH to form an NH···O hydrogen bond, and the incorporation of the polymer ligand into $CaCO_3$ crystals in comparison with that of poly(acrylic acid) itself.

Experimental Section

Materials. All solvents were purified by distillation before use. The synthesis of poly(acrylic anhydride) was carried out under argon atmosphere. Poly(acrylic acid) (M_v = 450 000) was obtained from Sigma-Aldrich. 2,4-Pentanedicarboxylic anhydride was purchased from Sigma-Aldrich. $N_3P_3Cl_6$ was of commercial grade.

Synthesis of Poly(acrylic anhydride). Poly(acrylic anhydride)-450000 was prepared by the literature method.[16] Tri(*n*-butyl)amine (1.4 mL, 5.9 mmol) was added to a solution of poly(acrylic acid) (M_v 450 000, 0.21 g, 3.0 mmol as its monomer unit) in methanol. The reaction mixture was stirred overnight and evaporated to dryness. The residue was washed with CH_2Cl_2 and dried over anhydrous Na_2SO_4. The residue was dissolved in fresh CH_2Cl_2, and $N_3P_3Cl_6$ (0.52 g, 1.5 mmol) was added to the solution with stirring. The mixture was allowed to stand overnight, and diethyl ether was added to the solution. A white precipitate was obtained, collected by filtration, washed with CH_2Cl_2, and then dried in vacuo. White solid was obtained in 0.16 g (81%) yield. IR (in KBr); *n* 1804 cm^{-1} (C═O), 1760 cm^{-1} (C═O), 1035 cm^{-1} (CO−O−CO).

Poly(1-carboxyl-3-*N*-*t*-BuNHCOC$_4$H$_6$) (1). A large excess of dried *t*-$BuNH_2$ (13 mL, 120 mmol) was added to **1** (0.64 g, 3.2 mmol for the monomer unit). The mixture was stirred overnight and evaporated to dryness before the residue was

10.1021/ma001771q CCC: $20.00
Published on Web 03/09/2001

dissolved in water. The dropwise addition of concentrated HCl aqueous solution at a controlled pH of 2−3 results in precipitation of a white solid. The product was collected by filtration and washed with water and diethyl ether, successively, and then dried over P_2O_5. A white polymer was obtained in 41% yield. Calcd for $C_{10}H_{17}NO_3$: C, 60.28; N, 7.03; H, 8.60. Found: C, 60.52; N, 7.12; H, 7.82. ^{1}H NMR (Me_2SO-d_6): 12.0 ppm (br, COO*H*), 6.9 (br, CON*H*-*t*-Bu), 1.17 ppm (s, *t*-Bu). The ratio of the integral intensity between −COO*H* and −CON*H* was 1.00:0.90.

Poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ tetraethylammonium complex) (1NEt$_4$). The titled ammonium complex was synthesized by a reaction between **1** (29 mg, 0.15 mmol) and (NEt_4)($OCOCH_3$) (44 mg, 0.17 mmol) in 10 mL of MeOH/H_2O (1:1) at room temperature. The solution was concentrated in vacuo and gave a white solid which was washed with water and dried. ^{1}H NMR (Me_2SO-d_6): 8.5 ppm (br, CON*H*-*n*Bu), 1.2 ppm (br, CH_3).

Poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ sodium complex) (1Na). The sodium complex was synthesized by a reaction between **1** (56 mg, 0.28 mmol) in 10 mL of MeOH and sodium bicarbonate (24 mg, 0.28 mmol) in 50 mL of H_2O. The solution was concentrated under reduced pressure. The residue was washed with 20 mL of diethyl ether and dried over P_2O_5. A slightly yellowish material was obtained in 76% yield (47 mg). ^{13}C CP/MAS NMR: 184.4 ppm (COO^-), 177.0 ppm (C═O), 51.7 ppm (CH_2), 43.8 ppm (CH), 29.7 ppm (CH_3).

Poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ calcium complex) (1Ca). A methanol (40 mL) solution of **1** (20 mg, 0.10 mmol) was added to a 10 mL aqueous solution of $Ca(OAc)_2H_2O$ (9 mg, 0.5 mmol) at room temperature. The solution was concentrated under reduced pressure and washed with diethyl ether (20 mL) to obtain a white solid. ^{13}C CP/MAS NMR: 183.6 ppm (COO^-), 177.7 ppm (C═O), 51.0 ppm (CH_2), 44.5 ppm (CH), 29.0 ppm (*t*-Bu).

Poly(1-carboxyl-3-*N*-*n*-BuNHCOC$_4$H$_6$) (2). The titled polymer was synthesized by the same method described above. The yield was 34%. Calcd for $C_{10}H_7NO_3H_2O$: C, 55.28; N, 6.45; H, 8.81. Found: C, 55.18; N, 6.88; H, 8.53. ^{1}H NMR (Me_2SO-d_6): 12.8 ppm (br, COO*H*), 7.6 ppm (br, CON*H*-*n*Bu), 1.27 ppm (t, −CH_3). The ratio of the integral intensity between −COO*H* and −CON*H* was 1.00:0.88.

Poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ tetraethylammonium complex) (2NEt$_4$). The ammonium complex was synthesized by a method similar to that described above for **1NEt$_4$**. ^{1}H NMR (Me_2SO-d_6): 8.1 ppm (br, CON*H*-*n*Bu), 1.2 ppm (br, CH_3).

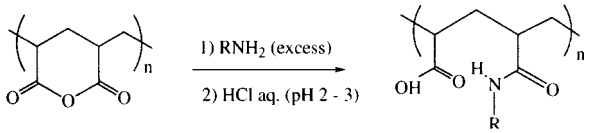
Poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ sodium complex) (2Na). This complex was synthesized by the same method as described for **1Na**. A yellow solid was obtained in 52% (27 mg) yield. ^{13}C CP/MAS NMR: 184.6 ppm (COO^-), 178.2 ppm (C═O), 41.3 and 32.7 ppm (CH_2), 21.4 ppm (CH), 13.9 ppm (CH_3).

Poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ calcium complex) (2Ca). The titled complex was synthesized by the same method as described for **1Ca**. ^{13}C CP/MAS NMR: 184.9 ppm (COO^-), 177.2 ppm (C═O), 43.8 and 32.4 ppm (CH_2), 21.0 ppm (CH), 14.4 ppm (CH_3).

Poly(acrylate sodium complex) (4Na) and Poly(acrylate calcium complex) (4Ca). 4Na was prepared by a reaction of poly(acrylic acid) with sodium bicarbonate at room temperature as described for the synthesis of **1Na**. **4Ca** was synthesized by a method similar to that described for the synthesis of **1Ca**.

5-(*t*-Butylamino)-2,4-dimethyl-5-oxopentanoic Acid (3). 5-(*tert*-Butylamino)-2,4-dimethyl-5-oxopentanoic anhydride was synthesized according to the literature.[17] The anhydride (60 mg, 0.42 mmol) was suspended in distilled *t*-$BuNH_2$ (30 mL, 290 mmol) at room temperature. A large excess of *t*-$BuNH_2$ was removed by evaporation, and the residue was dissolved in distilled water. The aqueous solution was filtered, and concentrated HCl aqueous solution was added to precipitate a white solid which was collected by filtration. The material was washed with water and dried in vacuo. The crude material was recrystallized from a THF−hexane medium. Yield: 23 mg (25%). Calcd for $C_{11}H_{21}N_1O_3$·0.12 H_2O: C, 60.76; H, 9.85; N, 6.43. Found: C, 60.75; H, 9.77; N, 6.61. ^{1}H NMR (Me_2SO-d_6): 11.97 ppm (br, COO*H*), 7.35 ppm (br, CON*H*-*t*-Bu), 2.29 ppm (m, C*H*), 2.21 ppm (m, C*H*), 1.80 ppm (m, CH_2), 1.23 ppm (s, *t*-Bu), 1.06 ppm, 0.95 ppm (d, CH_3). ^{13}C NMR (Me_2SO-d_6): 177.5 ppm (*C*OOH), 174.8 ppm (*C*ONH), 49.9 ppm (*C*Me$_3$), 37.5 ppm, 37.4 ppm (*C*H), 36.7 ppm (*C*H$_2$), 28.7 ppm (*t*-Bu), 18.6 ppm, 16.8 ppm (*C*H$_3$).

Scheme 1. Synthesis of Alternately Amidated Poly(1-carboxylate-3-*N*-alkylamidotetramethylene)



(NMe$_4$){5-(*t*-Butylamino)-2,4-dimethyl-5-oxopentanate} (3NMe$_4$). A methanol solution of (NMe_4)($OCOCH_3$) was added to **3** (23 mg, 0.11 mmol) in methanol at room temperature. The solution was concentrated under reduced pressure. The residue was washed with ethyl ether. The crude material was recrystallized from methanol−ethyl ether. ^{1}H NMR (Me_2SO-d_6): 7.88 ppm (br, CON*H*-*t*-Bu), 2.15 ppm (m, C*H*), 1.93 ppm (m, C*H*), 1.72 ppm (m, CH_2), 1.22 ppm (s, *t*-Bu), 1.09 ppm (t, C*H*), 0.97 ppm (m, CH_2), 0.89 ppm (q, CH_3).

Crystalline $CaCO_3$ Containing Poly(1-carboxylate-3-*N*−*t*-BuNHCOC$_4$H$_6$) (1CaCO$_3$). Poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ sodium complex) (**1Na**) (0.10 mmol per monomer unit) was suspended in a 40 mL aqueous solution of $CaCl_2$ (1.29 g, 10 mmol). An aqueous solution (20 mL) of ammonium carbonate (0.96 g, 10 mmol) was dropwise added to the mixture at acidic conditions (pH 3.8−4.0) to give a white $CaCO_3$ precipitate. The precipitate was collected by filtration and washed with water. The precipitated $CaCO_3$ was suspended in methanol to dissolve as a free polymer ligand. Crystalline $CaCO_3$ was collected by filtration, washed with methanol again, and then dried in vacuo.

The synthesis of crystalline $CaCO_3$ was performed under basic conditions by the successive addition of $CaCl_2$ and ammonium carbonate. Control experimental was carried out without polymer ligand to obtain crystalline $CaCO_3$ under acidic conditions (pH 3.8−4.0) and basic conditions (pH 8.2−8.4). The obtained crystals were washed with water and methanol successively.

Crystalline $CaCO_3$ Containing Poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$) (2CaCO$_3$). This $CaCO_3$-crystalline composite containing poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$) was synthesized from **2Na** by a method similar to that for **1CaCO$_3$**.

Physical Measurements. IR spectrum measurements were taken on a Jasco A-102 spectrometer and a Jasco DS-402G spectrometer. Samples were prepared as KBr pellets. ^{1}H NMR spectra were obtained with a JEOL EX-270 or a JEOL GSX-400 in dimethyl-d_6 sulfoxide at 30 °C. NOESY spectra of 5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid and its carboxylate anion were measured using a Varian Unity 600. ^{13}C CP/MAS NMR spectra was obtained on a CMX-300 spectrometer. SEM pictures were obtained from a JEOL JSM-5800.

Energy minimization calculations for (NMe_4){DL-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate} and (NMe_4){*meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate} were carried out using BIOGRAF (Dreiding force field) software (MSI, Ltd.).

Results and Discussion

Synthesis of Altenately Amidated Poly(1-carboxylate-3-*N*-alkylamidotetramethylene). Partially amidated poly(acrylic acid), poly(1-carboxylate-3-*N*-alkylamidotetramethylene), was synthesized as shown in Scheme 1.

Poly(acrylic anhydride) has been considered to possess a linear structure forming a six-membered anhydride ring with the neighboring two carboxylic acid residues because of its good solubility in DMF.[16] Poly(acrylic

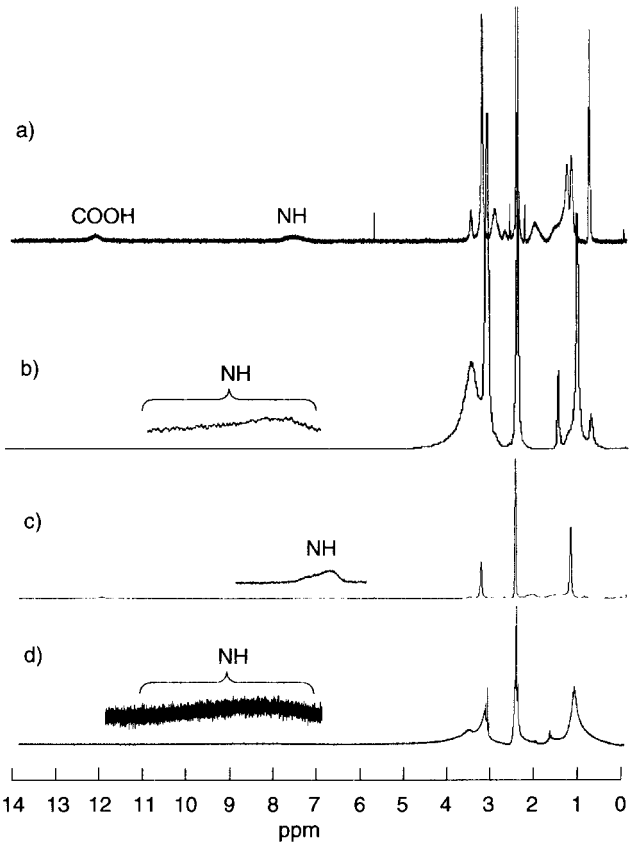


Figure 1. ^{1}H NMR spectra of (a) poly(1-carboxyl-3-*N*-*t*-BuNHCOC$_4$H$_6$) (**1**), (b) poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ tetraethylammonium complex) (**1NEt$_4$**), (c) poly(1-carboxyl-3-*N*-*n*-BuNHCOC$_4$H$_6$) (**2**), and (d) poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ tetraethylammonium complex) (**2NEt$_4$**) in Me$_2$SO-d_6 at 303 K.

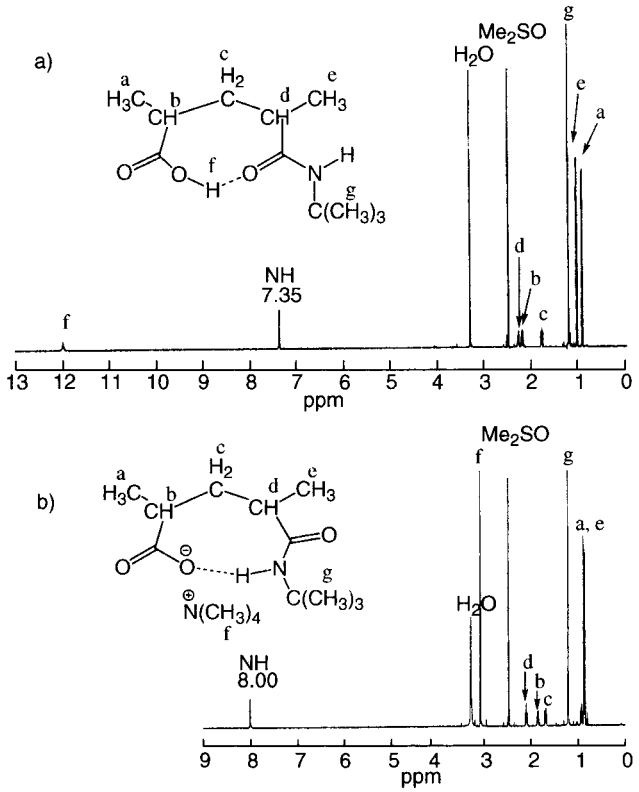


Figure 2. ^{1}H NMR spectra of (a) *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid (**3**) and (b) tetramethylammonium *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate (**3NMe$_4$**) in Me$_2$SO-d_6 at 303 K. Each assignment was carried out using 2D techniques.

anhydride) exhibits characteristic IR bands at 1804 and 1760 cm^{-1} due to a six-membered anhydride skeleton similar to those (1802 and 1761 cm^{-1}) reported for glutaric anhydride.[18] In the case of open chain anhydrides, the higher frequency is always observed with the more intense of the two bands, e.g., 1825 and 1760 cm^{-1} for capric anhydride.[19]

Formation of NH···O Hydrogen Bond in Alternately Amidated Poly(1-carboxylate-3-*N*-alkylamidotetramethylene) Anion. Figure 1 shows the ^{1}H NMR spectra of poly(1-carboxyl-3-*N*-*t*-BuNHCOtetramethylene) (**1**) and its carboxylate anion (**1NEt$_4$**) in Me$_2$SO-d_6 at 303 K. The observed integral ratio of 1.00:0.90 for COOH and CONH-*t*-Bu indicates the almost quantitative formation of an alternating copolymer consisting of *tert*-butylamidoethylene and acrylic acid units. An amide NH ^{1}H NMR signal at 6.8 ppm in the carboxylic acid state **1** shifts to a broad one in the range 7.7−8.3 ppm in the carboxylate anion state **1NEt$_4$**. The average shift over 0.9 ppm is ascribed to the existence of the NH···O hydrogen bond even in a hydrogen-bond-disrupting solvent such as Me$_2$SO. The broadness of the amide NH signals comes from an atactic structure of the starting commercial poly(acrylic acid). When the polymer ligand **1** is a block copolymer consisting of poly(amide) and poly(acrylic acid) parts, the amide NH also exhibits a broad signal due to the coexistence of both hydrogen-bonded and non-hydrogen-bonded parts.

Similarly, the ^{1}H NMR spectra of poly(1-carboxyl-3-*N*-*n*-BuNHCOtetramethylene) (**2**) and its carboxylate anion (**2NEt$_4$**) are also shown in Figure 1. The observed shift from 7.6 ppm for **2** to 8.0−8.5 ppm for **2NEt$_4$** also supports the presence of the NH···O hydrogen bond in the carboxylate anion state even in Me$_2$SO. These combined data strongly support the belief that the partially amidated polymer consists of alternately amidated poly(1-carboxylate-3-*N*-BuCOtetramethylene) units between amidated ethylene and acrylic acid units as shown in Scheme 1. Although the head-to-tail structure seems to be predominantly involved with the steric congestion, the accurate structure cannot be discussed because of the heterotacticity of the commercial poly(acrylic acid). The broadness of the amide NH signal in **1NEt$_4$** and **2NEt$_4$** is due to the presence of the mixture of head-to-tail or head-to-head and isotactic, syndiotactic or heterotactic structures.

Formation of Eight-Membered NH···O Hydrogen Bond in a Model Carboxylate. To examine the effect of the polymer tacticity in the starting poly(acrylic acid) on the Ca(II) binding, a simple model compound, *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid, was prepared. The reaction of 2,4-pentanecarboxlic anhydride with *t*-BuNH$_2$ produces only a *meso*-isomer, as reported in the literature.[17] Figure 2 shows the ^{1}H NMR spectrum of *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid (**3**) and its tetramethylammonium carboxylate complex (**3NMe$_4$**) in Me$_2$SO-d_6 at 303 K. **3** exhibts an NH signal at 7.35 ppm in Me$_2$SO-d_6 at 303 K, whereas **3NMe$_4$** shows an NH signal at 8.00 ppm. A relatively large shift (0.65 ppm) between **3** and **3NMe$_4$** is due to the formation of an NH···O hydrogen bond between the carboxylate and neighboring amide NH in the complex, as demonstrated for a dinuclear calcium complex, [Ca$_2${(2-OCO-3-CH$_3$C$_6$H$_3$NHCO)$_2$C(CH$_3$)$_2$}$_2$-(CH$_3$OH)$_6$].[14]

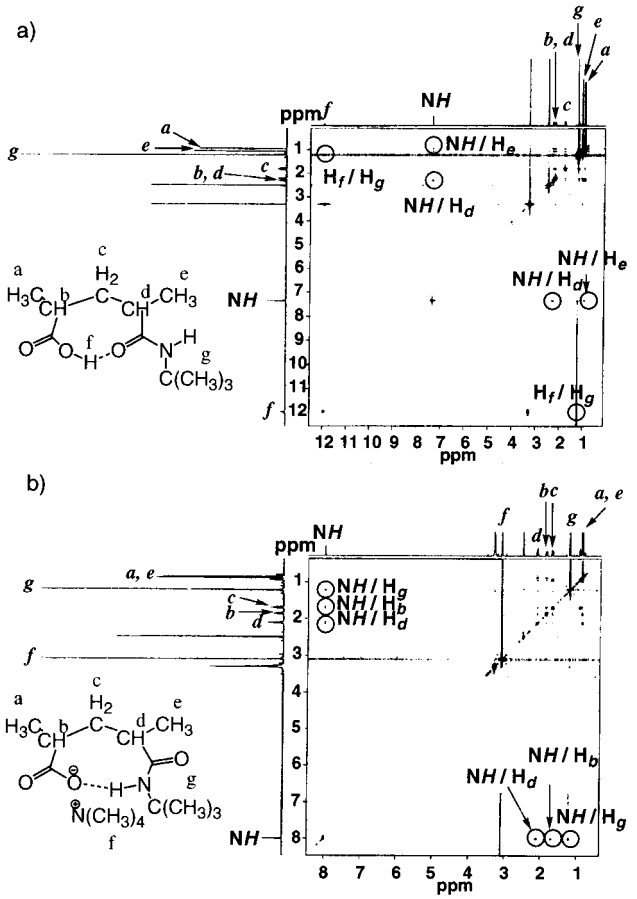


Figure 3. NOESY spectra of (a) *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid (**3**) and (b) tetramethylammonium *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate (**3NMe$_4$**) in Me_2SO-d_6 at 303 K.

The NOESY spectra (Figure 3) of carboxylic acid **3** in Me_2SO-d_6 indicate that the amide NH does not correlate with COOH, but the COOH interacts with *t*-Bu protons. The results supports the belief that carboxylic acid **3** has a *meso*-structure. In the NOESY spectrum of the anion state **3NMe$_4$**, the amide NH exhibits NOE with methine CH_b without interaction with CH_3. The amide NH locates near the carboxylate group, as supported by the structure for the anion state in Figure 3.

The structure of the *meso*-isomer in **3NMe$_4$** corresponds to the unit of isotactic poly(1-carboxylate-3-*N*-*t*-BuNHCOtetramethylene), as shown in Figure 4, while the DL-isomer structure resembles that of the syndiotactic unit. The formation of the NH···O hydrogen bond in *meso*- and DL-isomers was theoretically examined using molecular mechanics calculations (Dreiding force field[20]) at $\epsilon = 38$. The most stable conformation for each isomer was obtained using a Monte Carlo simulation. Obtained energy-minimized structures having the NH···O hydrogen bond between a carboxylate oxygen and an amide NH correspond to the polymer ligand structure as shown in Figure 4. Thus, the total energies obtained for the *meso*-isomer and the DL-isomer are −9.77 and −9.39 kcal/mol, respectively, whereas the calculated NH···O hydrogen bond energies are −8.20 and −8.10 cal/mol. Thus, the *meso*-isomer corresponding to the isotactic polymer ligand is more stable than the DL-isomer and provides a stronger NH···O hydrogen bond.

Previously, we reported the synthesis of a novel Cd(II) complex, [Cd_2{(3*S*,5*S*)-3,5-bis[(*n*-butylamino)carbonyl]-1,3,5-trimethylcyclohexanecarboxylate}$_2$(MeOH)$_2$(H$_2$O)$_2$]·2MeOH, which has an *eq*−*eq* conformation between *t*-Bu-amidated carbonyl and carboxylate, similar to the *meso*-configuration of 5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid. The crystal structure of the Cd(II) complex exhibits the formation of an eight-membered NH···O hydrogen bond, as shown in Figure 5.[21] The wide-line skeleton in the Kemp's acid ring in the *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanoic acid corresponds to a *trans*-zigzag backbone in the isotactic poly(1-carboxylate-3-*N*-alkylamidotetramethylene). Thus, the *meso*-configuration between carboxylate and *N*-alkylamidocarbonyl groups prefers to preferentially form an eight-membered NH···O hydrogen bond. This is coincident with the results of the molecular mechanics calculations.

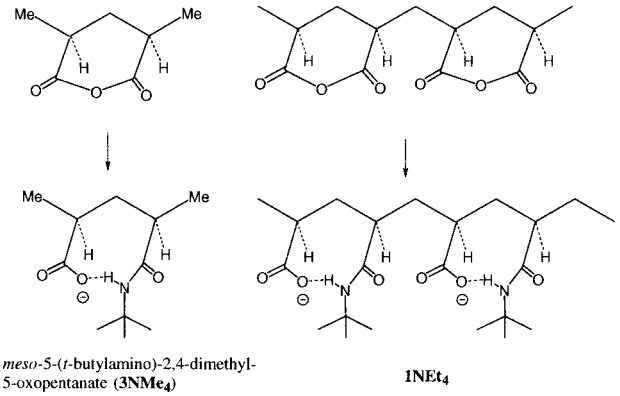


Figure 4. One of the possible configurations of alternately amidated poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$) prepared from atactic poly(acrylic acid). Tetramethylammonium *meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate (**3NMe$_4$**) corresponds to these polymer configurations.

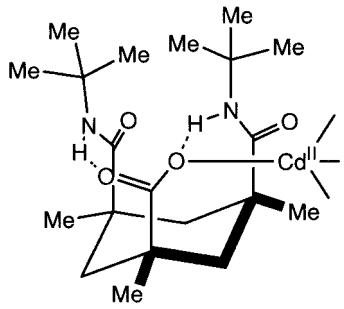


Figure 5. Eight-membered NH···O hydrogen bond in [Cd_2-{3,5-bis[(*n*-butylamino)carbonyl]-1,3,5-trimethylcyclohexanecarboxylate}$_2$(MeOH)$_2$(H$_2$O)$_2$]·2MeOH. The wide line structure corresponds to a *trans*-zigzag unit in the polymer ligands.

Scheme 2. Synthesis of Polymer Ca(II) Complexes

$$2\ \text{poly(COOH)}_n + n\ \text{Ca(OCOCH}_3)_2 \longrightarrow \text{Ca[poly(COO)}_n]_2 + 2n\ \text{CH}_3\text{COOH}$$

Ca(II) Complexes of Alternately Amidated Poly(acrylic acid). Calcium complexes (**1Ca**, **2Ca**, and **4Ca**) were synthesized by a reaction of $Ca(OCOCH_3)_2$ with alternatively amidated poly(1-carboxylic acid-3-*N*-alkylNHCOtetramethylene)s (**1** and **2**) and poly(acrylic acid) (**4**). This synthetic method has an advantage in obtaining a calcium complex having a ratio of (1:2) in calcium/carboxylic acid as shown in Scheme 2. Sodium complexes (**1Na**, **2Na**, and **4Na**) were also synthesized by a similar method using $Na(OCOCH_3)$ as a starting material.

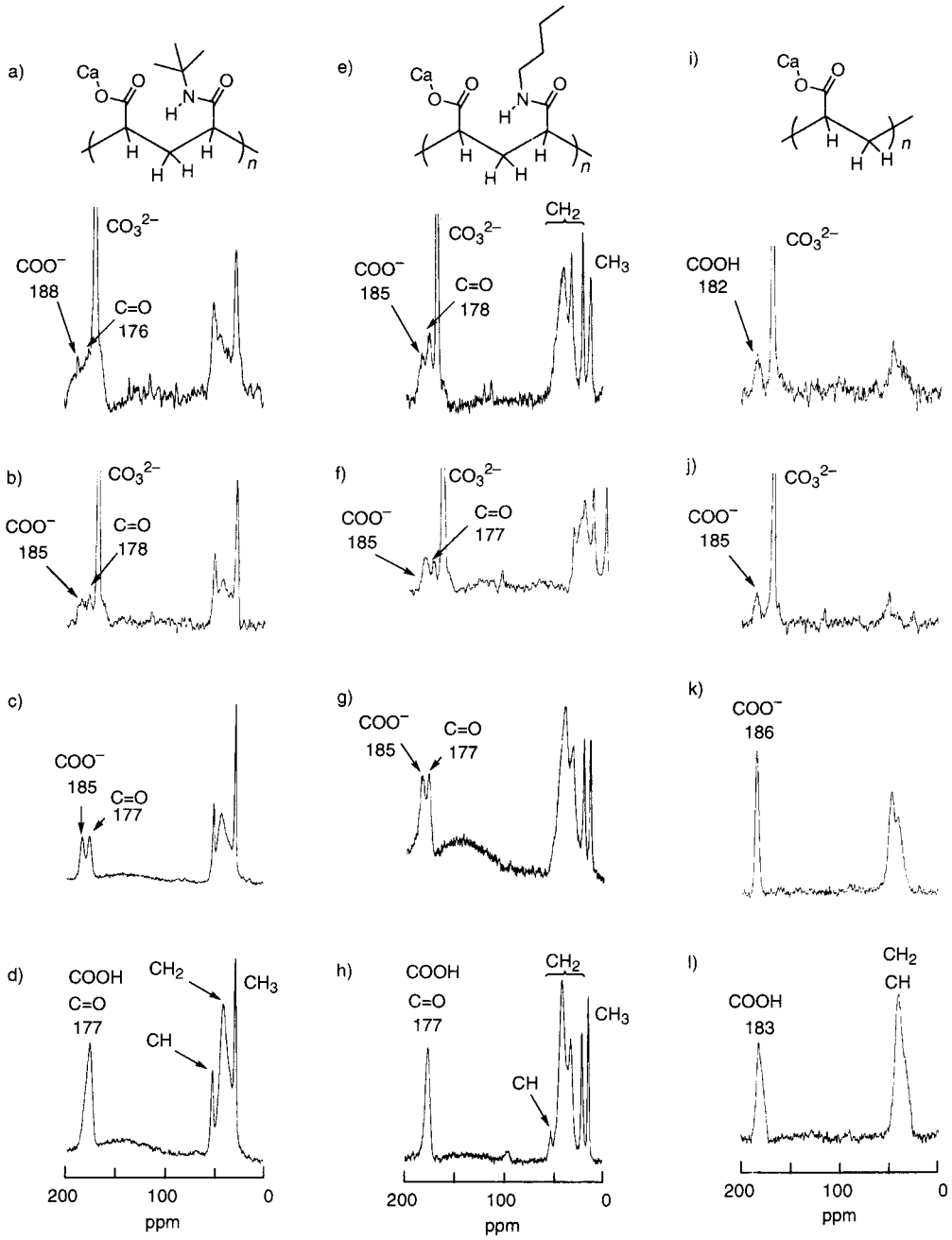


Figure 6. ^{13}C CP/MAS spectra of (a) crystalline $CaCO_3$ containing poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$) (**1CaCO$_3$**) obtained under acidic conditions, (b) **1CaCO$_3$** obtained under basic conditions, (c) poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$ sodium complex) (**1Na**), (d) poly(1-carboxyl-3-*N*-*t*-BuNHCOC$_4$H$_6$) (**1**), (e) crystalline $CaCO_3$ containing poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$) (**2CaCO$_3$**) obtained under acidic conditions, (f) **2CaCO$_3$** obtained under basic conditions, (g) poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ sodium complex) (**2Na**), (h) poly(1-carboxyl-3-*N*-*n*-BuNHCOC$_4$H$_6$) (**2**), (i) crystalline $CaCO_3$ containing poly(acrylate) (**4CaCO$_3$**) obtained under acidic conditions, (j) **4CaCO$_3$** obtained under basic conditions, (k) poly(acrylate sodium complex) (**4Na**), and (l) poly(acrylic acid) (**4**) in the solid state at 303 K.

Figure 6 shows the ^{13}C CP/MAS spectra of poly(1-carboxylate-3-*N*-*t*-BuNHCOtetramethylene sodium complex) (**1Na**) and the starting poly(1-carboxylic acid-3-*N*-*t*-BuNHCOtetramethylene) (**1**). **1Na** exhibits a carboxylate signal at 177 ppm and an amide CO signal at 185 ppm for **1Na** in the solid state, whereas the carboxylic acid **1** shows a signal at 177 ppm overlapped with those of amide and COOH carbons. Similarly, **1Ca** shows these signals at 178 and 185 ppm, respectively.

The ^{13}C CP/MAS spectra of poly(1-carboxylate-3-*N*-*n*-BuNHCOtetramethylene sodium complex) (**2Na**) compared with the ^{13}C spectra of the carboxylic acid (**2**) and its anion state (**2NEt$_4$**) in the solid state are also shown in Figure 6. **2Na** exhibits the ^{13}C signals of carboxylate CO and amide CO at 177 and 185 ppm (Figure 6c), while **2Ca** shows these at 178 and 184 ppm, respectively, in the solid state. Thus, the carboxylate form binding to solid $CaCO_3$ can be detected using a solid-state ^{13}C CP/MAS spectroscopic method. It is noted that the carboxylate form exists as a binding ligand for Ca ion in the solid state. The ^{13}C CP/MAS spectra of poly(acrylate sodium complex) (**4Na**) indicate a carboxylate CO signal at 186 ppm in the solid state, whereas the corresponding carboxylic acid CO signal appears at 183 ppm. The change from COOH to COO$^-$ is detectable using a ^{13}C NMR spectroscopic method although the difference is small (3 ppm).

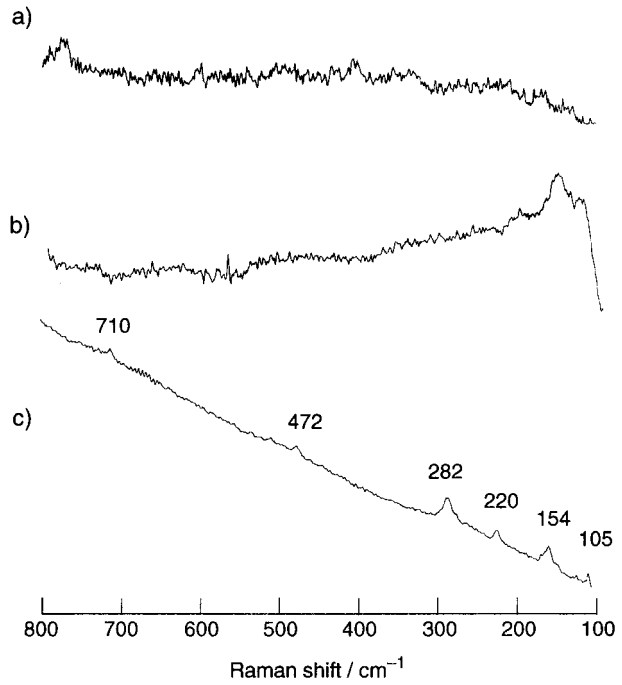


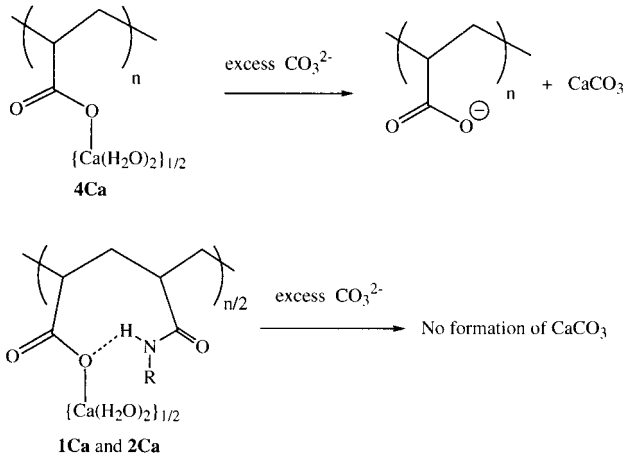
Figure 7. Raman spectra of reaction mixtures obtained by bubbling CO_2 into polymer Ca(II) complex water/methanol solutions: (a) poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$) Ca complex (**1Ca**), (b) poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$) Ca complex (**2Ca**), and (c) poly(acrylate Ca complex) (**4Ca**) in the solid state at 303 K.

Tight Binding of Calcium Ion in Poly(1-carboxylate-3-*N*-amidated tetramethylene calcium complex)s. The formation of crystalline calcium carbonate from poly(1-carboxylate-3-*N*-alkylNHCOtetramethylene calcium complex)s (**1Ca** and **2Ca**) or poly(acrylate calcium complex) (**4Ca**) was examined by the addition of CO_2 gas or CO_3^{2-} in MeOH/water mixed solvent under neutral conditions. The Raman analysis of **1Ca** and **2Ca**, which are obtained by the concentration of the reaction products, does not exhibit the formation of crystalline $CaCO_3$ as shown in Figure 7. The CO_2-bubbled or CO_3^{2-}-added Ca complexes show two intense Raman bands at 2927 and 1454 cm^{-1} for the obtained polymer from **1Ca** and at 2926 and 1450 cm^{-1} for that obtained from **2Ca**, which are assignable to carboxylate stretchings due to the preferable formation of an RCOOCa complex without the formation of crystalline $CaCO_3$.

On the contrary, poly(acrylate calcium complex) (**4a**) gave a crystalline $CaCO_3$ under the neutral conditions after the addition of ammonium carbonate, as shown in Scheme 3. The Raman spectrum (Figure 7) of the obtained polymer mixture indicates the formation of crystalline $CaCO_3$ which provides three characteristic Raman bands at 710, 472, and 282 cm^{-1} for unknown complex, different from those of vaterite (755, 304, and 215 cm^{-1}), calcite (714, 283, and 156 cm^{-1}), and aragonite (704, 208, and 182 cm^{-1}).[22]

Previously, we found that a dinuclear calcium complex, $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2\}_2(CH_3OH)_6]$, can be produced by a direct reaction between calcium carbonate and the dicarboxylic acid ligand which forms one NH···O hydrogen bond at each carboxylate.[14] Thus, **1Ca** and **2Ca** possess a stronger Ca−O bond compared with that of poly(acrylate calcium complex). The NH···O hydrogen bond in the poly(1-carboxylate-3-*N*-alkylNHCOtetramethylene calcium complexes) protects the Ca−O bond presumably due to the stabilization of the carboxylate anion as discussed in the previous paper.[15]

Scheme 3. Formation of Crystalline $CaCO_3$ from Polymer Ca(II) Complexes



Scheme 4. Synthesis of Crystalline $CaCO_3$ in the Presence of Polymer Na(I) Complexes

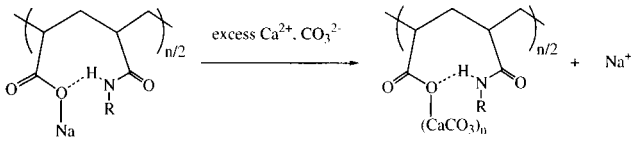


Table 1. Polymorphs of Crystalline $CaCO_3$ Forming in the Presence of Polymer Ligands, Poly(1-carboxylate-3-*N*-alkylamidotetramethylene) (1 and 2) and Poly(acrylic acid) (4) Obtained under Acidic Conditions

	acidic conditions (pH 3.8−4.0)		
polymer	binding	calcite/%[a]	vaterite/%[a]
poly(AA-*alt*-*t*-Bu amide) (**1**)	O	2	98
1Na	O	5	95
1Ca	O	63	37
poly(AA-*alt*-*n*-Bu amide) (**2**)	O	23	77
2Na	O	19	81
2Ca	O	83	17
poly(acrylic acid) (**4**)	X	14	86
4Ca	O	75	25

[a] The polymorphs were determined by IR spectroscopy.

Crystalline Calcium Carbonate Formation from Calcium Ion and Carbonate Anion in the Presence of Poly(1-carboxylate-3-*N*-amidated tetramethylene)s under Acidic Conditions. Crystalline $CaCO_3$ was obtained by the reaction of excess Ca ion and CO_3^{2-} anion in the presence of alternately amidated polycarboxylates, the sodium complexes, or their Ca complexes, according to Scheme 4. The crystalline $CaCO_3$ was carefully washed with water, methanol, and Me_2SO. On the other hand, crystalline $CaCO_3$, which was obtained by the control experimental without polymer ligand under acidic conditions (pH 3.8−4.0), consists of calcite. The formation of calcite is coincident with the results reported by Mann et al.[23]

Table 1 lists the polymorph of crystalline calcium carbonate obtained by the addition of ammonium carbonates to calcium chloride under acidic conditions in the presence of poly(acid), its poly(sodium carboxylate) complex, or poly(calcium carboxylate) complex in MeOH/water mixed solvents. The polymorphs were detected by the characteristic IR bands of $CaCO_3$. In all cases,

poly(acid)s (**1**, **2**, and **4**) and poly(sodium carboxylate) (**1Na**, **2Na,** and **4Na**) can control the polymorph to give vaterite. Thus, the anion state of these poly(1-carboxylate-3-*N*-alkylNHCOtetramethylene)s can control the polymorph at the nucleation stage. The protection from protonation of carboxylate and the chelate effect can dislodge Ca(II) ion by decomposition of crystalline calcium carbonate.

On the contrary, crystalline $CaCO_3$ obtained from **1Ca** and **2Ca** exhibits a mainly calcite structure without the control of morphology, as listed in Table 1. Specifically, the Ca(II) polymer inhibits the formation of vaterite with the control of calcium carbonate crystals by the starting polymer carboxylate ligands under acidic conditions. The addition of CO_2 gas or CO_3^{2-} to poly(1-carboxylate-3-*N*-amidated tetramethylene calcium complex)s does not form crystalline $CaCO_3$, as described above. Therefore, nucleation for the crystallization of $CaCO_3$ in the presence of **1Ca** and **2Ca** proceeds without involvement of the polymer ligands and results in formation of calcite, although the polymer ligand ultimately binds to $CaCO_3$ crystals.

Crystalline calcium carbonates washed with water, methanol, and Me_2SO were analyzed by the ^{13}C CP/MAS spectra in the solid state, as shown in Figure 6, as well as those of their poly(carboxylate sodium)s and poly(carboxylic acid)s described before. The observation of ^{13}C CP/MAS signal of the polymer ligand and carbonate carbon in well-washed crystalline $CaCO_3$ indicates the presence of polymer ligand as carboxylate tightly bound to the crystal edges or the attached amorphous Ca cluster. Calcium carbonate containing poly(1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$) (**1CaCO$_3$**) exhibits a carboxylate CO signal at 186 ppm and an amide CO signal at 178 ppm accompanied by a strong CO_3^{2-} signal at 169 ppm, as shown in Figure 6. The significant intensity loss of the CO_3^{2-} carbon signal is due to sufficient cross-polarization, as discussed in the previous paper.[15] Similar ^{13}C CP/MAS spectra were observed for crystalline $CaCO_3$ containing poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$) (**2CaCO$_3$**), compared with the spectra of poly(1-carboxylate-3-*N*-*n*-BuNHCOC$_4$H$_6$ sodium salt) (**2Na**) and poly(1-carboxyl-3-*N*-*t*-BuNHCOC$_4$H$_6$) (**2**) in the solid state at 303 K. Crystalline $CaCO_3$ shows the carboxylate CO signal at 185 ppm and the amide CO signal at 178 ppm indicative of the binding of the polymer ligand to the crystals.

The ^{13}C CP/MAS spectra of crystalline $CaCO_3$ (**4CaCO$_3$**) containing poly(acrylate) obtained from acidic conditions do not show two separate carboxylate CO signals but a COOH signal at 182 ppm, as shown in Figure 6. A clear carboxylate COO^- signal for $CaCO_3$ obtained under basic conditions appears at 185 ppm as well as that at 186 ppm for poly(acrylate sodium complex), whereas a carboxylic acid signal appears at 183 ppm in a poly(acrylic acid) in the solid state at 303 K. The crystalline $CaCO_3$ obtained even under basic conditions gradually provides the COOH signal, when the composite was washed with solvents under neutral conditions. Thus, in the case of poly(acrylic acid), a part of the polymer ligand separates from crystalline $CaCO_3$ to give a free poly(carboxylic acid) under basic conditions. These data are consistent with the reported results that poly(acrylic acid) is involved in the nucleation of the $CaCO_3$ crystallization but then separates from the crystals under neutral conditions.[7] The surface of crystalline $CaCO_3$ is thought to be covered with

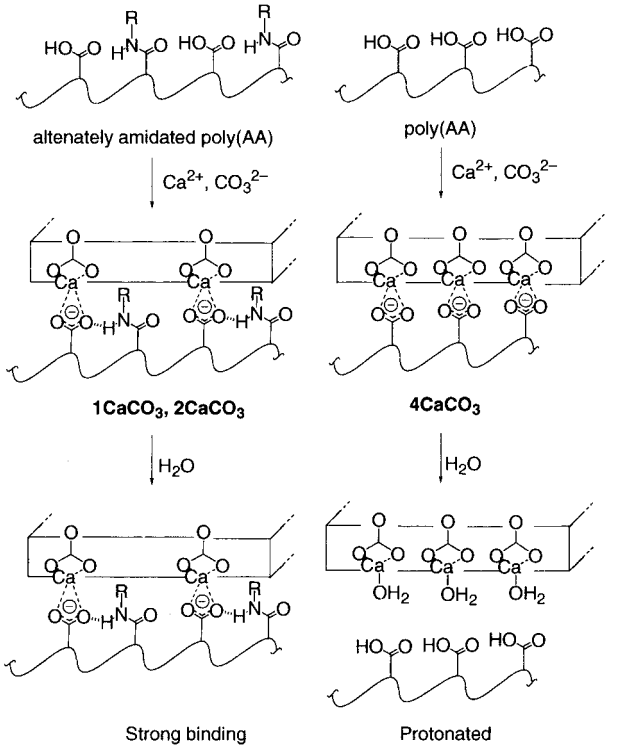


Figure 8. Protection of alternately amidated poly(carboxylate) from protonation by water in a $CaCO_3$ composite.

coordinated water molecules, and the presence of their protons on surface provides hydrophobic environments.[24] Carboxylate having a high pK_a then readily converts to carboxylic acid detached from the crystals. The removal of the carboxylate from nucleation environments to the crystal surface leads to easy removal of low-pK_a carboxylate on the **4CaCO$_3$** crystals (Figure 8).

Molecular dynamics calculations for an 8-mer model, (1-carboxylate-3-*N*-*t*-BuNHCOC$_4$H$_6$)$_8$, having a head-to-tail unit indicate 6−7 Å as an average distance between two carboxylates with an almost trans-zigzag main-chain structure. Because the Ca···Ca distance is ca. 5 Å in aragonite, vaterite, and calcite, the carboxylate−carboxylate distance presumably affects the nucleation in crystalline $CaCO_3$ formation. The results also support the belief that an eight-membered NH···O hydrogen bond between carboxylate and neighboring amide NH is preferentially formed in the 8-mer model as well as the data for (NMe$_4$){*meso*-5-(*tert*-butylamino)-2,4-dimethyl-5-oxopentanate} (**3NMe$_4$**) by the molecular mechanic calculations.

SEM Images of Crystalline $CaCO_3$−Poly(carboxylate) Composites. Figure 9 shows the scanning electron micrographs (SEM) of **1CaCO$_3$** from **1** or **1Na** under acidic conditions. The **1CaCO$_3$** exhibits a SEM image of the microcrystals in vaterite which is presumably produced by the control of the polymer ligands upon nucleation. A mixture of calcium chloride and ammonium carbonate without the polymer ligand results in formation of the most stabilized calcite at room temperature as mentioned before. In the presence of the synthetic polymer, metastable vaterite is formed. Formation of similar vaterite occurs with the addition of divalent cations as reported in the literature.[25−27] These polymer ligands with the NH···O hydrogen bond do not produce aragonite.

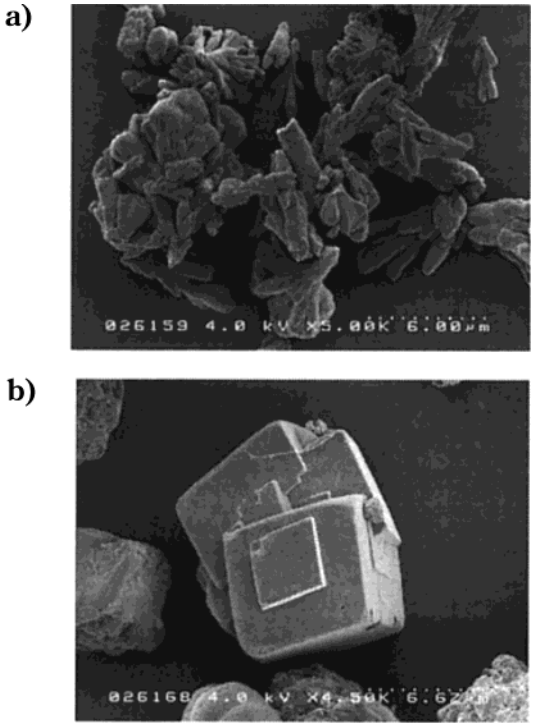


Figure 9. SEM image of (a) **1CaCO$_3$** produced from **1Na** under neutral conditions and (b) **4CaCO$_3$** obtained from **4Na** under neutral conditions.

Conclusions

The anion form of alternately amidated poly(carboxylate), poly(1-carboxylate-3-*N*-RNHCOtetramethylene) (R = *t*-Bu, *n*-Bu), gives a stronger eight-membered NH···O (carboxylate) hydrogen bond between COO^- and neighboring amide NH in Me_2SO, compared with the COOH···O=C(amide) hydrogen bond. The shift (0.9 ppm) of the amide NH ^{1}H NMR signal shows the existence of the NH···O hydrogen bond even in hydrogen-bond-disrupting solvents such as Me_2SO. The Ca(II) complex of the alternately amidated poly(carboxylate) strongly binds Ca(II) even in the presence of excess CO_3^{2-}. The strong Ca−O (carboxylate) bond is protected from hydrolysis with water.

A crystalline calcium carbonate containing poly(1-carboxylate-3-*N*-BuNHCOC$_4$H$_6$)s shows a carboxylate CO signal at 185−186 ppm and an amide CO signal at 178 ppm accompanied by a relatively weak CO_3^{2-} signal at 169 ppm. The shift of the COO^- is indicative of the polymer ligand bound to the crystals.

On the other hand, the ^{13}C CP/MAS broad signal at 182−184 ppm of crystalline $CaCO_3$ containing poly(acrylate) indicates a mixture of COO^- and COOH in the polymer chain. A clear carboxylate COO^- signal for poly(acrylate sodium salt) is observed at 186 ppm, and a carboxylic acid signal appears at 182 ppm in poly(acrylic acid) in the solid state at 303 K. In the case of poly(acrylic acid), a part of the polymer ligand separates from the crystalline $CaCO_3$ to give a partially free poly(acid). Poly(acrylic acid) is involved in the nucleation of $CaCO_3$ crystallization but is then dislodged from the crystals.

In a soluble organic matrix protein from nacreous layer of oyster pearls, a repeated Gly-Xaa-Asn (Xaa = Asp, Asn, Glu, or Tyr) fragment was found.[6] It is likely that, when the Ca binding site is carboxylate in Asp or Glu, the carboxylate forms an NH···O hydrogen bond with a neighboring amide NH of Asn, especially in a folded form of the peptide chain.

References and Notes

(1) Aizenberg, J.; Hanson, J.; Koetzle, T. F.; Weiner, S.; Addadi, L. *J. Am. Chem. Soc.* **1997**, *119*, 881−886.
(2) Addadi, L.; Weiner, S. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 153−169.
(3) Belcher, A. M.; Wu, X. H.; Christensen, R. J.; Hansma, P. K.; Stuky, G. D.; Morse, D. E. *Nature* **1996**, *381*, 56−58.
(4) Frankel, R. B.; Mann, S. *Encyclopedia of Inorganic Chemistry*; John Wiley & Sons Ltd.: Chichester, 1994; Vol. 1.
(5) Mann, S.; Heywood, B. R.; Rajam, S.; Birchall, J. D. *Nature* **1988**, *334*, 692−695.
(6) Miyamoto, H.; Miyashita, T.; Okushima, M.; Nakano, S.; Morita, T.; Matushiro, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9657−9660.
(7) Addadi, L.; Moradian, J.; Shay, E.; Maroudas, N. G.; Weiner, S. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2732−2736.
(8) Albeck, S.; Weiner, S.; Addadi, L. *Chem. Eur. J.* **1996**, *2*, 278−284.
(9) Xu, G.-F.; Yao, N.; Aksay, I. A.; Groves, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 11977−11985.
(10) Kuther, J.; Seshadri, R.; Nelles, G.; Assenmacher, W.; Butt, H.-J.; Mader, W.; Tremel, W. *Chem. Mater.* **1999**, *11*, 1317−1325.
(11) White, W. B. In *Infrared Spectra of Minerals*; Farmer, V. C., Ed.; Mineralogical Society: London, 1974; pp 227−284.
(12) Ueyama, N.; Yamada, Y.; Takeda, J.; Okamura, T.; Mori, W.; Nakamura, A. *J. Chem. Soc., Chem. Commun.* **1996**, 1377−1378.
(13) Yamada, Y.; Ueyama, N.; Okamura, T.; Nakamura, A. To be submitted.
(14) Ueyama, N.; Yamada, Y.; Takeda, J.; Okamura, T.; Nakamura, A. *Inorg. Chem.* **1998**, *38*, 475−478.
(15) Ueyama, N.; Hosoi, T.; Yamada, Y.; Doi, M.; Okamura, T.; Nakamura, A. *Macromolecules* **1998**, *31*, 7119−7126.
(16) Gregorio, F.; Marconi, F. *J. Org. Chem.* **1981**, *46*, 4569−4570.
(17) Allinger, N. L. *J. Am. Chem. Soc.* **1959**, *81*, 232−236.
(18) Wasserman, H. H.; Zimmerman, H. E. *J. Am. Chem. Soc.* **1950**, *72*, 5787.
(19) Bellamy, L. J. *The Infrared Spectra of Complex Molecules*; Chapman and Hall: London, 1975.
(20) Mayo, S. L.; Olafson, B. D.; GoddardIII, W. A. *J. Phys. Chem.* **1990**, *94*, 8897−8909.
(21) Kozuki, H.; Onoda, A.; Ueyama, N. *Acta Crystallogr.*, to be submitted.
(22) Bhagavantam, S.; Venkatarayudu, T. *Proc. Indian Acad. Sci.* **1939**, *9A*, 224.
(23) Mann, S.; Didymus, J. M.; Sanderson, N. P.; Heywood, B. R.; Samper, E. J. A. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 1873−1880.
(24) Wheeler, A. P.; Rusenko, K. W.; George, J. W.; Sykes, C. S. *Comp. Biochem. Physiol.* **1987**, *87*, 953−960.
(25) Brecevic, L.; Nothig-Laslo, V.; Kralj, D.; Popovic, S. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 1017−1022.
(26) Noethig-Laslo, V.; Brecevic, L. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2005−2009.
(27) Nassrallah-Aboukais, N.; Boughriet, A.; Fischer, J. C.; Wartel, M.; Langelin, H. R.; Aboukais, A. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 3211−3216.

MA001771Q