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Calcium Complexes of Carboxylate-Containing Polyamide with Sterically Disposed NH···O Hydrogen Bond: Detection of the Polyamide in Calcium Carbonate by ^{13}C Cross-Polarization/Magic Angle Spinning Spectra

Norikazu Ueyama,* Tsutomu Hosoi, Yusuke Yamada, Mototsugu Doi, Taka-aki Okamura, and Akira Nakamura*

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

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ABSTRACT: Carboxylate-containing polyamides were synthesized from 2,6-di(amino)benzoic acid and dimethylmalonyl dichloride, isophthaloyl dichloride, and fumaryl dichloride. These polymers, $\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}_n$, $\{NHC_6H_3(COO)NHCO\text{-}m\text{-}C_6H_4CO\}_n$, and $\{NHC_6H_3(COO)NHCO\text{-}trans\text{-}CH{=}CHCO\}_n$, have seven to eight carboxylate units in a polymer chain, and their Ca(II) complexes possess NH···O hydrogen bonds, which prevents the Ca−O dissociation by hydrolysis. $CaCO_3$ was obtained from aqueous Ca^{2+} and CO_3^{2-} in the presence of the polyamide or the Na salt of these polyamides ([Ca^{2+}]/[monomer unit] = 100) to give calcite, vaterite, and/or their mixture. We were able to observe polymers in $CaCO_3$ utilizing the ^{13}C cross-polarization/magic angle spinning solid-state NMR. The binding of Ca(II) by carboxylate appears to be an important factor in these studies and may be relevant to biomineralization of $CaCO_3$.

Introduction

In the initial stage of the biomineralization of shells, the oriented calcium carbonate crystals grow on a nucleating protein sheet.[1−6] The protein and inorganic ions regulate the phase of the deposited mineral.[7−18] The protein is believed to exist along the edge of crystal as a polyanion ligand to bind Ca(II). Recently, Belcher et al.[18] proposed that a linear acidic polypeptide is sufficient to control the polymorph of calcium carbonate. In both cases, whether the form of polypeptide is sheet or linear, a small amount of acidic proteins as a polyanion ligand are thought to bind the Ca(II) ion.

Miyamoto et al.[19] isolated a soluble organic matrix protein in the nacreous layer of oyster pearls. The protein has a repeated Gly-Xaa-Asn (Xaa = Asp, Asn, Glu, or Tyr) fragment that intervenes between the two Zn(II)-binding peptide parts. The carboxylate of the anionic Asp or Glu presumably binds the Ca(II) ion without dissociation during the crystallization of $CaCO_3$.

Crystallization of $CaCO_3$ in the presence of various synthetic polymers has been investigated as a model of biomineralization using synthetic polypeptides, e.g. poly(Glu) or poly(Asp),[3,20] stearic acid monolayer, glycoproteins.[3,4,21,22] These macromolecular ligands are also thought to control the polymorph. The polymorph of the calcium carbonate clusters can be identified by IR or microscopy.[17,18,22−24] However, the characterization of these polymers is still difficult because the small contents of these organic polymers in $CaCO_3$ make the direct observation of them by spectroscopic methods difficult.

Previously we synthesized a novel carboxylate ligand having a sterically forced NH···O hydrogen bond and its Cu(II), Ca(II), Zn(II), and Mn(II) complexes.[25,26] The NH···O hydrogen bond protects the metal-carboxylate bond from hydrolysis due to the lowering of the pK_a of the corresponding carboxylic acid.[27] When the Ca−O bonds of polypeptide are easily dissociated by water, the polypeptide is thought not to be occluded in the $CaCO_3$ crystal. Strong Ca−O bonds are thus required to control the polymorph of $CaCO_3$.

We designed novel polyamide ligands containing the NH···O hydrogen bond. These polymers were synthe-

* To whom correspondence should be addressed.

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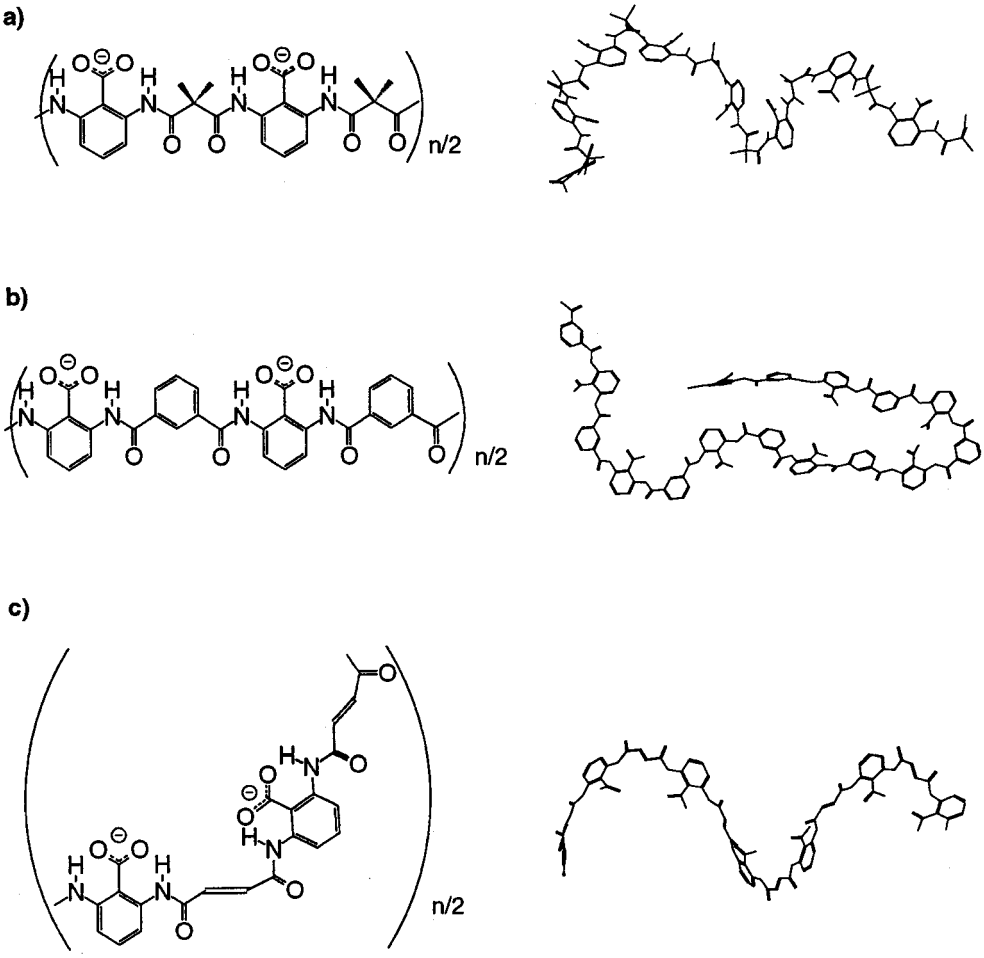
Scheme 1. MD Minimized Structures of Polyamides. a) {$NHC_6H_3(COOH)NHCOC(CH_3)_2CO$}$_n$ (1), (b) {$NHC_6H_3(COOH)NHCO$-*m*-C_6H_4CO}$_n$ (2), and (c) {$NHC_6H_3(COO)NHCOCH{=}CHCO$}$_n$ (3)



sized from 2,6-diaminobenzoic acid and dimethylmalonyl dichloride, isophthalyl dichloride, or fumaryl dichloride. $CaCO_3$ crystals were grown on these polyamides or sodium salt of the polyamides. Thus, we were successful in observing of a few of the polymers ([Ca^{2+}]/[monomer unit] = 100) in the $CaCO_3$ crystals by ^{13}C cross-polarization/magic angle spinning CP/MAS solid-state NMR.

Experimental Section

Materials. 2,6-Diaminobenzoic acid dihydrochloride was prepared by the hydrolysis of 2,6-di(acetylamino)benzoic acid.[28] Dimethylmalonyl dichloride, isophthaloyl dichloride, and fumaryl dichloride were purchased from Aldrich and used without other purification. Organic solvents were dried over ambient drying reagents and distilled before use.

{$NHC_6H_3(COOH)NHCOC(CH_3)_2CO$}$_n$ (1). Dimethylmalonyl dichloride (0.46 mL, 2.2 mmol) was added dropwise to a solution of 2,6-diaminobenzoic acid di(hydrochloride) salt (0.48 g, 2.2 mmol) in 1-methyl-2-pyrrolidone (20 mL) at 3−5 °C and warmed to room temperature. After 10 h the reaction mixture was poured into a 2% HCl aqueous solution to give a white precipitate which was collected by filtration. The white materials were washed with water and ether and dried over P_2O_5 under reduced pressure. The polymer was obtained in 61% (320 mg) yield. ^1H NMR (Me_2SO-d_6) δ 10.75 (*s*, 2H, NH), 7.91 (*d*, 2H, *m*-ArH), 7.45 (*t*, 1H, *p*-ArH), 1.51 (*s*, 6H, Me).

{NHC_6H_3 (COOH)NHCO-*m*-C_6H_4CO}$_n$ (2). 2,6-Diaminobenzoic acid dihydrochloride (0.29 g, 1.3 mmol) was dissolved in 20 mL of *N*-methyl-2-pyrrolidone (NMP) under argon. The solution was added solid isophthaloyl dichloride (0.257 g, 1.33 mmol) in one portion at −78 °C. The cooling bath was changed to an ice bath, and the mixture was stirred at 4−7 °C for 3 h and at room temperature for 7 h under argon. The polymer was precipitated by pouring the reaction solution into 300 mL of methanol. After thoroughly washing with water and methanol and drying, the polymer was obtained in 53% (190 mg) yield. ^1H NMR (Me_2SO-d_6) δ 11.58 (*s*, 2H, NH), 8.55 (*s*, 1H, ArH), 8.16 (*d*, 2H, ArH), 7.99 (*d*, 2H, ArH), 7.78 (*t*, 1H, ArH), 7.58 (*t*, 1H, ArH).

{$NHC_6H_3(COO)NHCO$-*trans*-$CH{=}CHCO$}$_n$ (3). 2,6-Diaminobenzoic acid dihydrochloride (1.3 g, 5.7 mmol) was dissolved in 40 mL of NMP under argon. The solution was cooled with an ice bath and 0.62 mL (5.7 mmol) of fumaryl dichloride was added dropwise while the solution was stirred at 0 °C. After 3 h under argon, the polymer was isolated by pouring the reaction solution into 700 mL of 2% HCl aqueous solution and washed with water, methanol, and ethanol, successively. Dry polymer was obtained in 65% (0.86 g) yield.^1H NMR (Me_2SO-d_6) δ 10.68 (*s*, 2H, NH), 7.60 (*d*, 2H, *m*-ArH), 7.50 (*t*, 1H, *p*-ArH), 7.13 (*d*, 2H, ethylenH).

[{$NHC_6H_3(COO)NHCOC(CH_3)_2CO$}Na]$_n$(1Na). [{$NHC_6H_3$-$(COO)NHCOC(CH_3)_2CO$ (56.0 mg, 0.23 mmol) was dissolved in 30 mL of tetrahydrofuran (THF). An aqueous solution (20 mL) of $NaHCO_3$ (19.0 mg, 0.23 mmol) was added to the solution with stirring in 1 h at room temperature. The reaction mixture was concentrated under reduced pressure to give brown powder in 97% (59 mg) yield. ^1H NMR (Me_2SO-d_6) δ 14.06 (*s*, 2H, NH), 8.18 (*d*, 2H, *m*-ArH), 7.13 (*t*, 1H, *p*-ArH), 1.53 (*s*, 6H, Me).

[{$NHC_6H_3(COO)NHCO$-*m*-C_6H_4CO}Na]$_n$ (2Na). A dispersion of {$NHC_6H_3(COOH)NHCO$-*m*-C_6H_4CO} (29.5 mg, 0.105 mmol) in 4 mL of *N*,*N*-dimethylformamide (DMF) was added an aqueous solution (4 mL) of $NaHCO_3$ (9.0 mg, 0.10 mmol) and the reaction mixture became a clear solution. After

Table 1. ^1H NMR Chemical Shift of Amide NHs for Carboxylic Acids, Their Sodium Salts, and Ca(II) Complexes in the Solid State

compounds	δ(NH), ppm
$\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}_n$ (**1**)	10.8
$[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Na]_n$ (**1Na**)	14.1
$[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Ca]_n$ (**1Ca**)	13.3
$\{NHC_6H_3(COOH)NHCO$-*m*-$C_6H_4CO\}_n$ (**2**)	11.6
$[\{NHC_6H_3(COO)NHCO$-*m*-$C_6H_4CO\}Na]_n$ (**2Na**)	15.6
$[\{NHC_6H_3(COO)NHCO$-*m*-$C_6H_4CO\}Ca]_n$ (**2Ca**)	13.4
$\{NHC_6H_3(COO)NHCOCH{=}CHCO\}_n$ (**3**)	10.7
$[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}Na]_n$ (**3Na**)	15.0
$[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}Ca]_n$ (**3Ca**)	14.7

stirring for 1 h, the solution was concentrated under reduced pressure. White powder was obtained in 88% (28 mg) yield. ^1H NMR (Me_2SO-d_6) δ 15.56 (*s*, 2H, NH), 8.67 (*s*, 1H, ArH), 8.54 (*d*, 2H, ArH), 8.22 (*d*, 2H, ArH), 7.76 (*t*, 1H, ArH), 7.39 (*t*, 1H, ArH).

$[\{NHC_6H_3(COO)NHCO$-*trans*-$CH{=}CHCO\}Na]_n$ (3Na). A dispersion of $\{NHC_6H_3(COOH)NHCO(CH)_2CO\}$ (121 mg, 0.52 mmol) was dissolved in 40 mL of Me_2SO and 5 mL of 1 M $NaHCO_3$ (aq). The solution was concentrated under reduced pressure to give brown powder which was dissolved in water and reprecipitated from acetonitrile. The obtained brown precipitate was collected with filtration and washed with ethanol and diethyl ether to give a Na salt in 62% (83 mg) yield. ^1H NMR (Me_2SO-d_6) δ14.96 (*s*, 2H, NH), 8.38 (*d*, 2H, *m*-ArH), 7.33 (*t*, 1H, *p*-ArH), 6.88 (*d*, 2H, ethylenH).

$[\{NHC_6H_3(COO)NHCO$-*m*-$C_6H_4CO\}_2Ca]_n$ (2Ca). $\{NHC_6H_3$-$(COOH)NHCO$-*m*-$C_6H_4CO\}$ (0.49 g, 0.17 mmol) was dissolved in 10 mL of Me_2SO. An aqueous solution (10 mL) of $CaCl_2$ (0.75 g, 10 mmol) was added, and the mixed solution gave insoluble materials. The obtained white precipitate was collected by filtration and washed by water, methanol, and ethanol to give white Ca(II) complex in 41% (42 mg) yield. ^1H NMR (Me_2SO-d_6) δ 13.34 (*s*, 2H, NH), 8.61 (*s*, 1H, ArH), 8.30 (*d*, 2H, ArH), 8.13 (*d*, 2H, ArH), 7.62 (*t*, 1H, ArH), 7.44 (*t*, 1H, ArH).

$[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Ca]_n$ (1Ca) and $[\{NHC_6H_3(COO)NHCO$-*trans*-$CH{=}CHCO\}Ca]_n$ (3Ca) were synthesized by analogous method of **2Ca**.

Synthesis of Crystalline $CaCO_3$. $\{NHC_6H_3(COOH)$-$NHCORCO\}$ (R = $C(CH_3)_2$, Ph, *trans*-CH=CH) (0.10 mmol per monomer unit) was suspended in 40 mL aqueous solution of $CaCl_2$ (0.75 g, 10 mmol). An aqueous solution (20 mL) of ammonium carbonate (0.96 g, 10 mmol) was dropped to give a white $CaCO_3$ precipitate. The precipitate was collected with filtration and washed with water. The precipitated $CaCO_3$ was suspended in THF or Me_2SO, which is a good solvent for $\{NHC_6H_3(COOH)NHCORCO\}$. The calcium carbonate was collected with filtration after being stirred for 1−2 days.

Physical Measurements. IR spectrum measurement was taken on a Jasco FT-IR 8300. Samples were prepared as KBr pellets. ^1H NMR spectra in solution state were obtained with a JEOL EX-270 or a JEOL GSX-400 in dimethyl sulfoxide-d_6 at 30 °C. Solid-state ^{13}C CP/MAS spectra were taken on a Chemagnetics CMX-300 spectrometer at room temperature. The rotating speed was ~8000 rps, and contact time was 5.00 ms in the CP/MAS measurements.

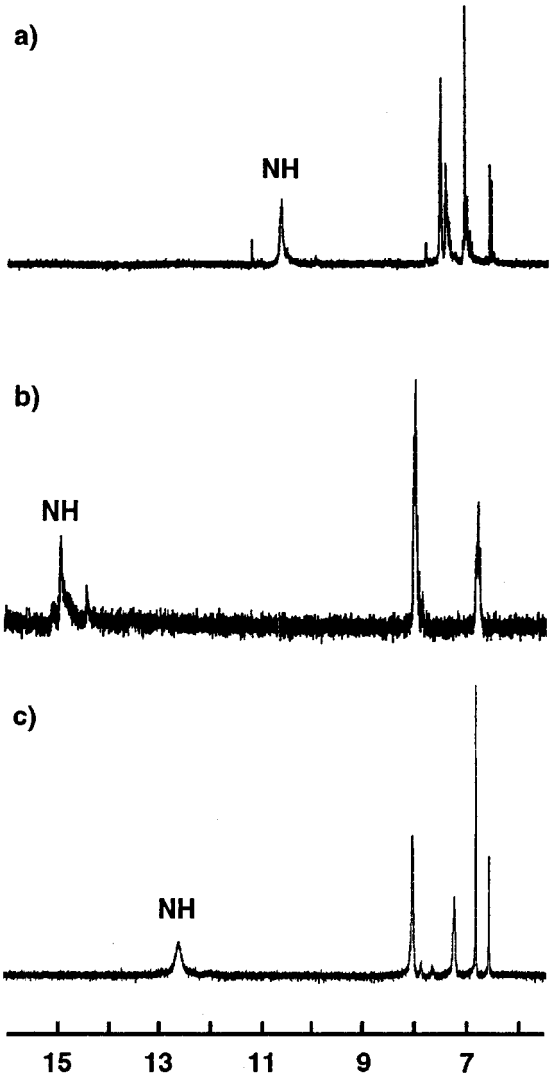


Figure 1. ^1H NMR spectra in the aromatic and amide region of (a) $\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}_n$ (**1**), (b) $[\{NHC_6H_3$-$(COO)NHCOC(CH_3)_2CO\}Na]_n$ (**1Na**), and (c) $[\{NHC_6H_3$-$(COO)NHCOC(CH_3)_2CO\}Ca]_n$ (**1Ca**) in Me_2SO-d_6 at room temperature.

Results and Discussion

Ligand Design. One of the features of our designed amide polymers is intramoleculer NH···O hydrogen bond to carboxylates. Recently, we reported that the intramolecular NH···O hydrogen bonds from amide NH to the binding oxygens protect the metal−oxygen bonds from dissociation. A small amount of protein is considered an impurity for the biominerals so that the proteins are excluded in the process of crystallization. The maintainance of the anion state of carboxylate ligands is necessary for biomineralizations to control the polymorph and morphology. The NH···O hydrogen bonds

Table 2. Selected IR Bands for Carboxylic Acids, Their Sodium Salts, and Ca(II) Complexes in the Solid State

Compounds	ν(NH), cm^{-1}	ν(CO), cm^{-1}
$\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}_n$ (**1**)	3329, 2978	1698
$[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Na]_n$ (**1Na**)	3432, 2976	1673
$[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Ca]_n$ (**1Ca**)	3423, 2962	1654
$\{NHC_6H_3$ $(COOH)NHCO$-*m*-$C_6H_4CO\}_n$ (**2**)	3376	1683
$[\{NHC_6H_3(COO)NHCO$-*m*-$C_6H_4CO\}Na]_n$ (**2Na**)	3426, 2929	1675
$[\{NHC_6H_3(COO)NHCO$-*m*-$C_6H_4CO\}Ca]_n$ (**2Ca**)	3385, 3005	1667
$\{NHC_6H_3(COO)NHCOCH{=}CHCO\}_n$ (**3**)	3361, 3066	1673
$[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}Na]_n$ (**3Na**)	3410, 2919	1660
$[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}Ca]_n$ (**3Ca**)	3395	1663

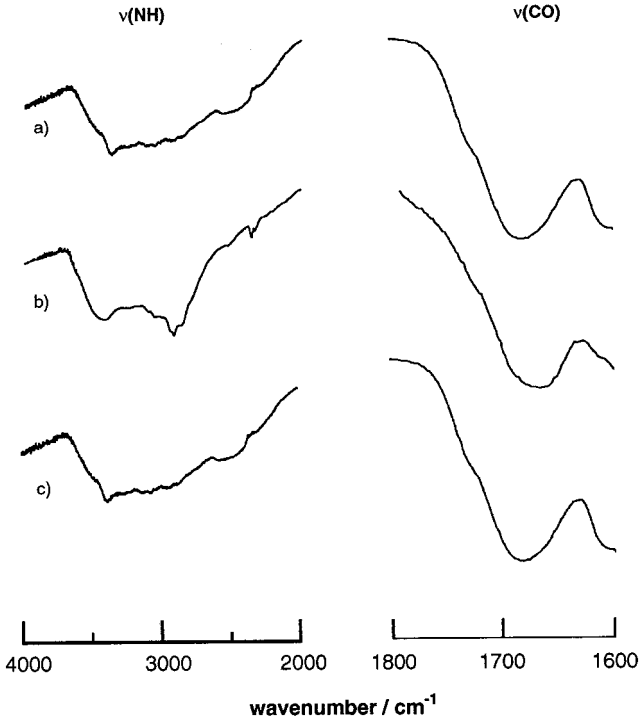


Figure 2. IR spectra of a) $\{NHC_6H_3(COOH)NHCO\text{-}m\text{-}C_6H_4CO\}_n$ (**2**), b) $[\{NHC_6H_3(COO)NHCO\text{-}m\text{-}C_6H_4CO\}Na]_n$ (**2Na**), and c) $[\{NHC_6H_3(COO)NHCO\text{-}m\text{-}C_6H_4CO\}Ca]_n$ (**2Ca**) in the solid state.

are now thought to be a main factor in protecting the calcium-carboxylate bond in both native protein and our polymers.

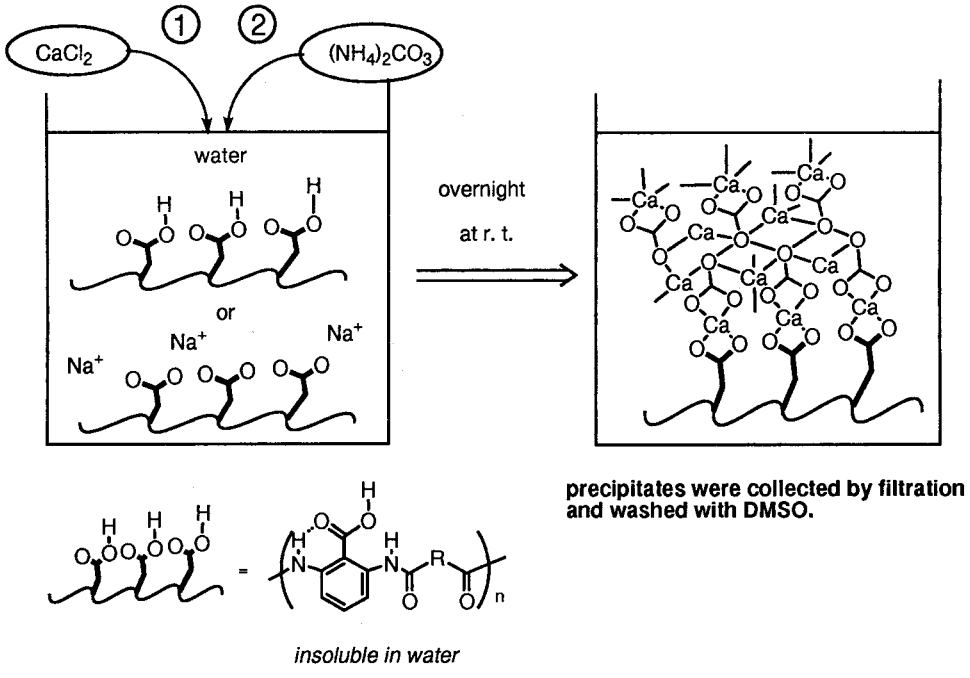
Our polymers consist of 2,6-di(amide)benzoic acid and flexible or rigid spacers. The energy-minimized octamer structures of each polyamide are illustrated in Scheme 1. The energy-minimized calculation by molecular dynamics was performed by Biograf software using Dreiding II force field. Three kinds of different spacers are used: the flexible dimethylmalonic group, the rigid *m*-substituted phenyl group, and the rigid *trans*-fumaric groups. These specifically configurated carboxylate ligands with three different spacers are expected to interact with calcium ions in different ways and to result in control of their polymorph or morphologies.

Detection of Intramolecular NH···O hydrogen bonds by ^{1}H NMR and IR Spectra. Figure 1 shows the ^{1}H NMR spectra of **1**, **1Na**, and **1Ca** in $Me_2SO\text{-}d_6$ at 30 °C. The carboxylic acid form of ligand **1** exhibits an amide NH signal at 10.8 ppm which is not involved in NH···O hydrogen bond. The carboxylate form of complex **1Na** gives a shifted NH signal at 14.1 ppm due to the increasing acidity of hydrogen-bonded amide NH. Such a large shift of amide NH is observed for a simple carboxylate having double NH···O hydrogen bonds, for example, $(NEt_4)\{2,6\text{-}(t\text{-}BuCONH)_2C_6H_3CO_2\}$, in $CDCl_3$. On the other hand, **1Ca** shows a NH signal with a relatively small shift at 13.3 ppm due to the influx of carboxlate oxygen electron into Ca(II) with the partially covalent Ca−O bond formation. Actually, a neutral Ca(II) complex, $[Ca\{OCO\text{-}2,6\text{-}(t\text{-}BuCONH)_2C_6H_3\}_2(H_2O)_2]$, having the NH···O hydrogen bond exhibits a shorter Ca−O bond (2.278 Å) than that (2.35 Å) in octahedral calcium complexes with six coordinating O-atoms from the Cambridge Structural Data Base.[29]

Table 1 lists the ^{1}H NMR spectra of **2**, **2Na**, and **2Ca** in $Me_2SO\text{-}d_6$ at 30 °C. Ligand **2** exhibits an amide NH signal at 11.6 ppm which is not involved in the NH- - - O hydrogen bond. **2Na** gives a strongly hydrogen-bonded amide NH signal at 15.6 ppm. A weakly hydrogen-bonded amide NH signal is observed at 13.4 ppm for **2Ca**. A similar trend is found for the ^{1}H NMR spectra of **3**, **3Na**, and **3Ca** which show each signal at 10.7, 15.0, and 14.7 ppm in $Me_2SO\text{-}d_6$. The amide NH signals for these compounds shift to upfield presumably due to decrease in the acidity by forming a completely conjugated fumaryl diamide plane.

In the IR analysis, the ν(NH) bands for carboxylic acid **1** in the solid state are observed at 3339 and 2978 cm^{-1} as listed in Table 2. The shifted ν(NH) bands for carboxylate **1Na** and **1Ca** appear at 3432, 2976 cm^{-1} and 3423, 2962 cm^{-1}, respectively.

Scheme 2. Method for Preparing Crystalline Calcium Carbonate



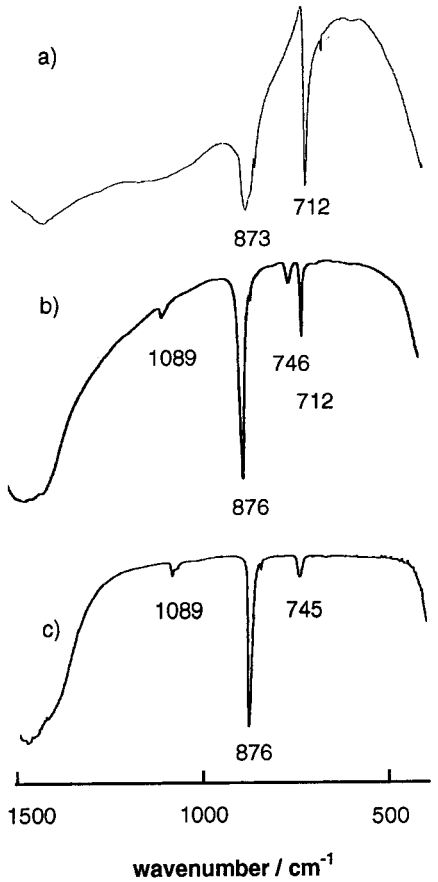


Figure 3. IR spectra of crystalline $CaCO_3$ complexes grown on a) $\{NHC_6H_3(COO^-)NHCOC(CH_3)_2CO\}_n$ (**1**), b) $\{NHC_6H_3(COO^-)NHCO\text{-}m\text{-}C_6H_4CO\}_n$ (**2**), and c) $\{NHC_6H_3(COO^-)NHCOCH{=}CHCO\}_n$ (**3**) in the KBr solid state.

The IR spectra of **2**, **2Na**, and **2Ca** are shown in Figure 2 in the solid state. Carboxylic acid **2** exhibits the ν(NH) band at 3376 cm^{-1} and the ν(CO) band at 1683 cm^{-1}, whereas **2Na** gives two ν(NH) bands at 3426 cm^{-1} assignable to a noninteracting amide NH and 2929 cm^{-1} due to the formation of the NH···O hydrogen bond. Table 2 indicates the IR spectra of **3**, **3Na**, and **3Ca** in the solid state. **3Na** exhibits two amide NH bands at 3410 and 2919 cm^{-1}. Such two separated different ν(NH) values were also observed for **2Na** as described above.

Formation of Crystalline $CaCO_3$ in the Presence of Poly(amide)s or Their Na Salts. Crystalline $CaCO_3$ was synthesized by adding $(NH_4)_2CO_3$ to an $CaCl_2$ solution in the presence of poly(amide) or Na salt of these polymers as shown in Scheme 2. The molar ratio of Ca ion to carboxylic acid group is 100. The crystalline $CaCO_3$ was washed thoroughly with Me_2SO or THF to remove any contaminating polymer ligand that is not involved in the crystals. The crystals then were dried under reduced pressure to thoroughly remove water and Me_2SO solvents. The crystal phase of $CaCO_3$ obtained from aqueous solution was determined by the IR analysis. Figure 3 shows the IR spectra of crystalline $CaCO_3$ in the presence of **1**, **2**, and **3**. Both $CaCO_3$ crystals of **1** and **2** exhibit crystalline bands at 876 and 712 cm^{-1} assignable to calcite.[20] A small band at 746 cm^{-1} is due to the contamination by vaterite.[20] Other crystalline $CaCO_3$ polymorphs are listed in Table 3.

Characterization of Poly(amide) Ligands in $CaCO_3$ by ^{13}C CP/MAS Spectroscopy. A ^{13}C-{^1H} CP/MAS method was used to obtain the high-resolution ^{13}C NMR spectra of solid $CaCO_3$-containing polyamide carboxylate ligands. Figure 4a shows the solid-state CP/MAS ^{13}C spectrum of commercial grade $CaCO_3$ (calcite). An observed sharp signal at 168.1 ppm is attributed to

Table 3. Polymorphs of Crystalline $CaCO_3$ Grown on Polyamide Determined by IR and Powder X-ray Diffraction

polyamide ligands coexisting with crystalline $CaCO_3$	polymorph of calcium carbonate
$[\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}]_n$ (**1**)	calcite
$[\{NHC_6H_3(COO^-)NHCOC(CH_3)_2CO\}Na]_n$ (**1Na**)	calcite
$[\{NHC_6H_3(COOH)NHCO\text{-}m\text{-}C_6H_4CO\}]_n$ (**2**)	calcite + vaterite
$[\{NHC_6H_3(COO^-)NHCO\text{-}m\text{-}C_6H_4CO\}Na]_n$ (**2Na**)	calcite + vaterite
$[\{NHC_6H_3(COOH)NHCOCH{=}CHCO\}]_n$ (**3**)	vaterite
$[\{NHC_6H_3(COO^-)NHCOCH{=}CHCO\}Na]_n$ (**3Na**)	calcite

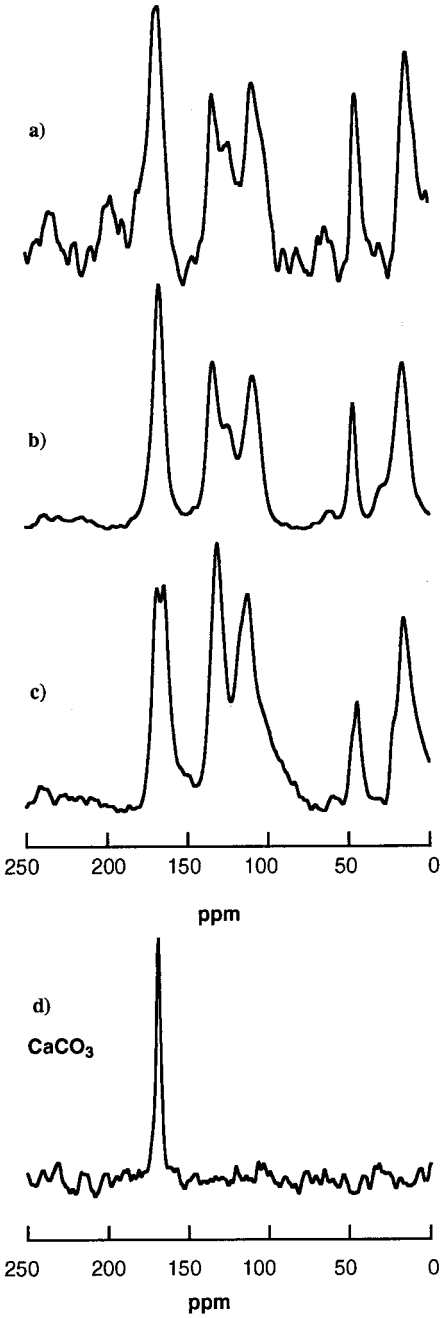


Figure 4. ^{13}C NMR spectra of a) $\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}_n$ (**1**), b) $[\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}Na]_n$ (**1Na**), c) crystalline $CaCO_3$ containing **1**, and d) crystalline $CaCO_3$ without **1** in the solid state.

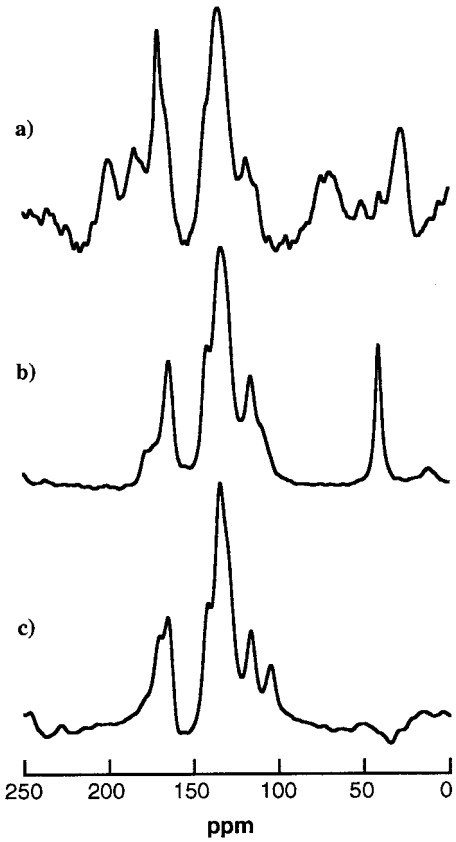


Figure 5. ^{13}C NMR spectra of a) $\{NHC_6H_3(COOH)NHCO\text{-}m\text{-}C_6H_4CO\}_n$ (**2**), b) $[\{NHC_6H_3(COO)NHCO\text{-}m\text{-}C_6H_4CO\}Na]_n$ (**2Na**), and c) crystalline $CaCO_3$ containing **2** in the solid state.

the CO_3^{2-} anion. The signal gives a significant intensity loss by the difficult condition to have sufficient cross-polarization.

Figure 4a–c illustrates the solid-state CP/MAS ^{13}C spectra of malonic **1**, **1Na**, and **1CaCO$_3$**. The methyl signal of the dimethylmalonic group for each of these compounds appears at 15.3, 15.9, and 17.7 ppm, respectively. The downfield shift of the methyl signal corresponds to a similar shift of the tertiary carbon signal of malonic residue from 42.7, 46.7 to 47.1 ppm, due to the conformational change around the flexible malonic chain. A clear difference was found between carboxylic acid and carboxylate at 162.4–167.4 ppm just as at the 109.5–134.2 ppm region of the aromatic ^{13}C signal between benzoic acid and its anionic conjugate base. **1CaCO$_3$** gives ^{13}C signals around 168 ppm of the polyamide ligand without any CO_3^{2-} anion ^{13}C signal as shown in Figure 4d. Such a successful characterization of the organic ligand in **1CaCO$_3$** indicates that the CP/MAS is useful in the detection of organic materials in crystalline $CaCO_3$.

Five ^{13}C signals for the benzoyl and benzoic acid aromatic carbons in **2** appear at 104.4, 116.2, 130.0, 134.0, and 141.5 ppm and two such peaks at 164.0 ppm for isophthaloyl carbonyl and at 169.9 ppm for carboxylic acid carbonyl as shown in Figure 5a. **2Na** and **2CaCO$_3$** exhibit shifted carboxylate ^{13}C signals at 178.5 and 176.7 ppm, respectively. In the anionic benzoate state, the benzoate aromatic ^{13}C signal also shifts to 109.3 ppm from 104.4 ppm of benzoic acid.

Figure 6 shows the solid-state CP/MAS ^{13}C spectra of **3**, **3Na**, and **3CaCO$_3$**. The carboxylic acid ^{13}C signal at 168.4 ppm for **3** shifts to 173.5 ppm upon conversion

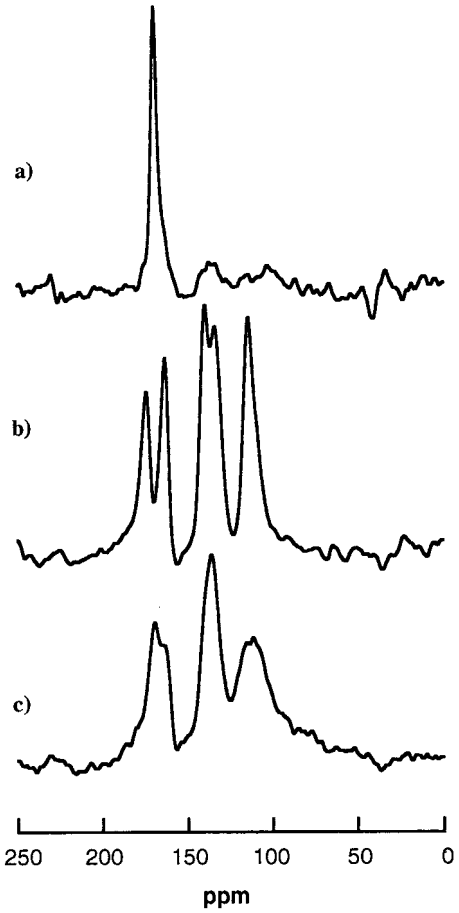


Figure 6. ^{13}C NMR spectra of a) $\{NHC_6H_3(COO)NHCOCH{=}CHCO\}_n$ (**3**), b) $[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}Na]_n$ (**3Na**), and c) crystalline $CaCO_3$ containing **3** in the solid state.

to carboxylate of **3Na**, whereas the aromatic ^{13}C signal of **3Na** also shifts from 108 and 136–138 ppm of **3** to approximate 114 and 140 ppm of **3Na**. However, **3CaCO$_3$** does not give any polyamide ligand ^{13}C signal. The result obtained for **3CaCO$_3$** reveals that polyamide carboxylate is not involved in the crystalline $CaCO_3$. Presumably distal carboxylate ligands do not cooperate with strongly binding Ca ion because of *E*-geometry of each carboxylate group as shown in Scheme 1.

Morphologies of the Calcium Carbonates. Figure 7 shows scanning electron micrographs of **1CaCO$_3$**, 2$CaCO_3$, and **3CaCO$_3$**, respectively. **3CaCO$_3$** shows the same morphology of calcite crystals without additives. This result is reasonable, because the ^{13}C NMR spectrum of **3CaCO$_3$** indicated that it includes no carboxylate ligands. The morphology of **1CaCO$_3$** resembles that of **3CaCO$_3$**. Although the ^{13}C NMR spectrum of **1CaCO$_3$** indicates that it includes ligand **1**, the ligand is not able to control the morphology of calcium carbonate crystals. **2CaCO$_3$** has an unique morphology compared with the morphology of the other two crystals. The shape of the crystals indicates that the crystal growth is inhibited at the {401} plane. The separation of calcium ions is 9.88 Å, which is almost the same separation as the adjacent carboxylate ligands of **2**. Considered with the ^{13}C NMR spectrum, these results indicate that **2** controls the morphology of calcium carbonate.

Conclusion

Three characteristic polyamides are utilized as the models of biomineralizing proteins. The ^{13}C CP/MAS

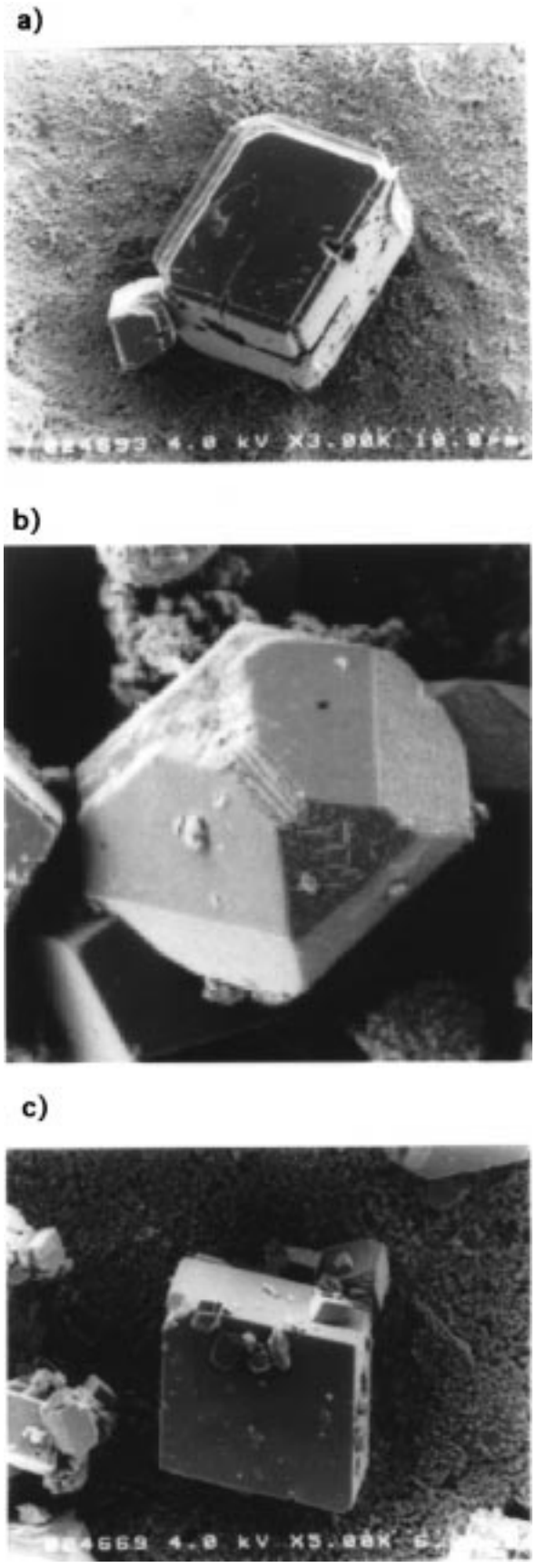


Figure 7. Scanning electron micrographs of calcium carbonates a) **1CaCO$_3$**, b) **2CaCO$_3$**, and c) **3CaCO$_3$**.

NMR was a good tool to detect the participation of the polyamide ligand in $CaCO_3$ microcrystal. Combined data with ^{13}C CP/MAS NMR spectra of **1CaCO$_3$**, **2CaCO$_3$**, and **3CaCO$_3$** show that **1** and **2** bind to the $CaCO_3$ cluster. **3** is not involved in the $CaCO_3$ cluster, however, although all of the polymers, **1**−**3**, have intramolecularly hydrogen-bonded carboxylate groups to bind the calcium ion strongly. This fact suggests that the relative position of the carboxylate group in the polymers primarily influences their ability to bind the $CaCO_3$ cluster. Both **1** and **2** have carboxylate-parallel−oriented moieties in the MD minimized structures. In the moieties, the distances of adjacent carboxylate distances are almost 10 Å for **1** and 9 Å for **2** which are almost twice the distances of calcium ion separation in calcium carbonate as shown in Figure 8a. On the other hand, **3** cannot have a parallel-oriented carboxylates group because of the *trans*-geometry of the fumaryl spacer. The results indicate difficulty for **3** to interact with calcium carbonate at more than two points as depicted in Figure 8b. Thus, the parallel-oriented carboxylate groups are very important for making inorganic−organic complexes. This idea had been recognized intuitively, these data support the idea experimentally utilizing ^{13}C CP/MAS NMR spectroscopy.

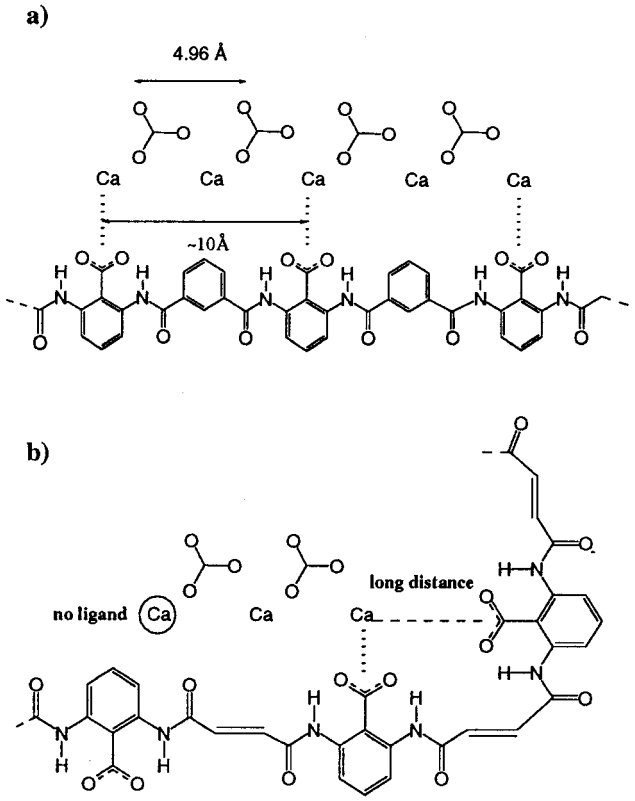


Figure 8. The reason for the difficulties in interaction between the poly(amide) ligands and Ca^{2+} ions in calcium carbonate. a) $[\{NHC_6H_3(COO^-)NHCO\text{-}m\text{-}C_6H_4CO\}]_n$ (**2**) and Ca^{2+} ions and b) $[\{NHC_6H_3(COO)NHCOCH{=}CHCO\}]_n$ (**3**) and Ca^{2+} ions.

Although the parallel-oriented carboxylates are important for the binding to $CaCO_3$ clusters, which also have been implied in some literature,[30,31] the main factor for controlling the polymorph is still unclear. The structure of the polymorph-controlling protein proposed by Miyamoto et al.[19] has a collagenous structure which can be thought to tightly array carboxylate groups in one direction. It is necessary to investigate linear polyamides having various combinations of rigidity of the main chain and separation of carboxylate groups. The combination of ^{13}C CP/MAS and IR spectroscopy will be powerful tools for understanding the correlation

between the structures of polymers and polymorphs of $CaCO_3$ deposited on them.

Supporting Information Available: X-ray diffraction data of calcium carbonates (2 pages). Ordering information is given in masthead page

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