Tight Binding of Poly(carboxylate) Ligand to Calcium Carbonate with Intramolecular NH···O Hydrogen Bond

Norizaki Ueyama, Kazuyuki Takahasi, Akira Onoda,*
Taka-aki Okamura, Hitoshi Yamamoto

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

Summary: Novel poly(carboxylates), partially amidated poly(acrylate), poly{[1-carboxylate-2-(*N*-*t*-butyl)carbamoyl]ethylene-*alt*-ethylene}, poly[1-(*N*-oxysuccinyl-aminomethenyl)ethylene], poly[1-(*N*-oxymalenyl-aminomethnyl)ethylene] and poly[1-(*N*-oxyphthalyl-aminomethnyl)ethylene] with intramolecular NH---O hydrogen bond between amide NH and coordinating oxygen were synthesized as model ligands for $CaCO_3$ biomineralllization. The FE/SEM and its backscatter of the $CaCO_3$ composite of gold colloid-conjugated poly{[1-carboxylate-2-(*N*-*p*-methylthiophenyl)carbamoyl]ethylene-*alt*-ethylene} indicate that the polymer ligand is located at the surface of vaterite crystals and the oriented carboxylate ligands control the $CaCO_3$ polymorph.

Introduction

Biominerals, e.g., shell and pearl, consist of oriented $CaCO_3$ crystals and soluble organic matrix proteins that contain anionic amino acid residues such as Asp and Glu. [1] These proteins are believed to exist along the edge of the crystal as a polyanion ligand to bind Ca(II). The carboxylate of the anionic Asp or Glu in the protein presumably binds the Ca(II) ion without dissociating during the crystallization of $CaCO_3$. Various biomineralization model complexes using synthetic peptides, e.g. poly(Glu) or poly(Asp), stearic acid monolayer,[2] glycoproteins[3] as welll as poly(acrylic acid). [1] These ligands also seem to control the polymorph at the nucleation stage.

Hydrogen bond networks were often found between the carboxylate oxygen atom and the main chain amide NH in the Ca center of Ca-binding proteins. The hydrogen-bonding networks have been considered to enable the fixation of carboxylate ligand orientation and maintain a suitable main chain conformation in the Ca-binding loop. Recently we found that the NH---O hydrogen bond protects the metal-carboxylate bond from dissociation with water due to the shift of pK_a of the conjugated carboxylic acid as well as those of thiol and thiolate as demonstrated in NH---S hydrogen bonded metal-thiolate complexes.[4]

CCC 1022-1360/00/$ 17.50+.50/0

Crystalline calcium carbonate with polyamide having a 6-membered ring intramolecular NH---O hydrogen bond

Three characteristic polyamides containing carboxylic acid, $\{NHC_6H_3(COOH)NHCOC(CH_3)_2CO\}_n$ (**1**), $\{NHC_6H_3(COOH)NHCO\text{-}m\text{-}C_6H_4CO\}_n$ (**2**) and $\{NHC_6H_3(COOH)NHCOCH{=}CHCO\}_n$ (**3**), are utilized as models of biomineralizing proteins, as shown in Fig. 1.[5] All of the polymer ligands, **1** ~ **3**, have intramolecularly hydrogen-bonded carboxylate groups to bind Ca ion strongly. Combined data with the ^{13}C CP/MAS NMR spectra of crystalline calcium carbonates (**1·$CaCO_3$**, **2·$CaCO_3$** and **3·$CaCO_3$**) containing the polymer ligands show that **1** and **2** bind to a $CaCO_3$ cluster but **3** is not involved in the $CaCO_3$ cluster. This fact indicates that the relative position of the carboxylate group in the polymer ligands primarily influences their ability to bind to the $CaCO_3$ cluster. Both **1** and **2** have carboxylate-parallel oriented moieties in the MD minimized structures. In the moieties, the distances between adjacent carboxylates are almost 10 Å for **1** and 9 Å for **2**, almost twice the distances (5.7 Å) of Ca ion separation in calcium carbonate. On the other hand, **3** cannot have such a parallel-oriented carboxylate 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. Thus, the parallel-oriented carboxylate groups are crucial for making inorganic-organic complexes.

$[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2\}_2(CH_3OH)_6]$, was synthesized as a structural model of 8-coordinated Ca(II).[6] The complex has four NH---O hydrogen bonds between the amide NH and the carboxylate oxygen anion. Two types of bridging coordination of the carboxylate ligand to Ca(II) were found in the Ca(II) dimer complex having a short Ca-Ca distance (3.86 Å). The amide NH forms a strong NH---O hydrogen bond with the anionic one of the two carboxylate oxygens. Ligand exchange reaction

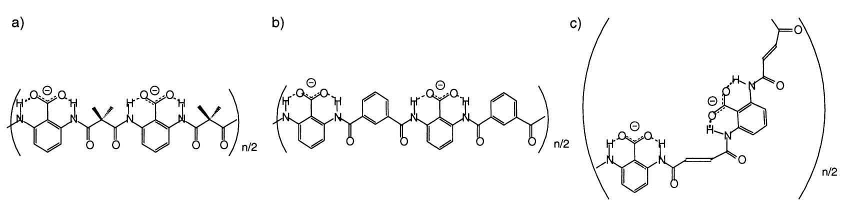
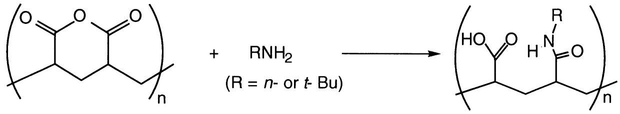


Figure 1. Synthetic polymer(carboxylate anion) ligands with 6-membered ring NH---O hydrogen bonds. a) $\{NHC_6H_3(COO)NHCOC(CH_3)_2CO\}_n$ (**1**), b) $\{NHC_6H_3(COO)NHCO\text{-}m\text{-}C_6H_4CO\}_n$ (**2**) and c) $\{NHC_6H_3(COO)NHCOCH{=}CHCO\}_n$ (**3**).

between the dinuclear calcium complex and 8 equimolar amounts of 2,4,6-trimethylbenzoic acid or 2-CH_3-6-*t*-$BuCONHC_6H_3COOH$ indicates that the NH---O hydrogen bond prevents the dissociation of the Ca-O bond.

Although the COO plane is twisted from the benzene ring, the amide NH is directed toward one of the carboxylate oxygen atoms to form an NH---O hydrogen bond. The combined data indicate that a strong NH---O hydrogen bond is formed between the amide NH and the C-O^- anion oxygen. The coordination of six methanol molecules outside the dinuclear site suggests a convergence of these water molecules in this type of Ca-binding complex by these bulky ligands.

Strong binding of partially amidated poly(acrylic acid) to crystalline calcium carbonate by 8-membered ring NH---O hydrogen bond



Partially amidated poly(acrylic acid) was synthesized by the above Scheme. An amide NH ^{1}H-NMR signal at 6.8 ppm in the carboxylic acid state **4** shifts to 7.7 - 8.3 ppm in the carboxylate anion state **4·NEt$_4$**.[7] The shift over 0.9 ppm is ascribed to the existence of a NH---O hydrogen bond even in hydrogen bond-disrupting solvents like Me_2SO. The broadness of the amide NH signals comes from the atactic structure of the starting poly(acrylic acid). On the other hand, when the polymer ligand **4** is a block copolymer consisting of poly(amide) and poly(acrylic acid) parts, the amide NH exhibits a broad signal due to the existence of both hydrogen bonded and non-hydrogen bonded structures.

A simple model compound, *meso*-5-(*t*-butylamino)-2,4-dimethyl-5-oxopentanoic acid, was prepared in order to investigate the solution structures of its acid and carboxylate states. The ^{1}H NMR spectrum of *meso*-5-(*t*-butylamino)-2,4-dimethyl-5-oxopentanoic acid (**3**) exhibits an NH signal at 7.35 ppm in Me_2SO-d_6 at 303 K, whereas its tetramethylammonium carboxylate complex (**3·NMe$_4$**) in Me_2SO-d_6 at 303 K gives an NH signal at 8.00 ppm. A relatively large shift (0.65 ppm) between **3** and **3·NMe$_4$** is due to the formation of an NH---O hydrogen bond between the carboxylate and neighboring amide. The NOESY spectra of the anion state **3·NMe$_4$**, the amide NH exhibits NOE with methine CH_b without interaction with CH_3, supporting that the amide NH locates near the carboxylate group, as shown in Fig. 2a. The structure of the *meso*-isomer in **3·NMe$_4$** corresponds to the unit of isotactic poly(1-carboxylate-3-*N*-*t*-BuNHCOtetramethylene), while the *dl*-isomer structure resembles that of the

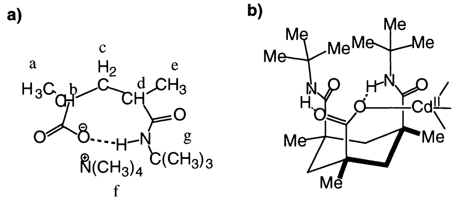


Figure 2. a) Proposed solution structure of tetramethylammonium*meso*-5-(*t*-butylamino)-2,4-dimethyl-5-oxopentanate (**3** · **N Me$_4$**) in Me$_2$SO-d_6 at 303 K. b) Molecular structure of Cd$^{II}_2$\{3,5-bis(*t*-butylamino-1,3,5-trimethyl-cyclohexanecarboxylate)$_2$.[7]

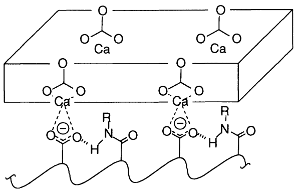


Figure 3. Amidated poly(acrylate) ligand incorporated into crystalline calcium carbonate.

syndiotactic unit.

A crystalline calcium carbonate composite (**4**·**CaCO$_3$**), of poly(1-carboxylate-3-*N*-*t*-BuNHCOtetramethylene) gives a carboxylate CO signal at 186 ppm and an amide CO signal at 178 ppm accompanied by a relatively weak CO_3^{2-} signal at 169 ppm. The remarkable intensity loss of the CO_3^{2-} ^{13}C signal is ascribed to sufficient cross-polarization. ^{13}C CP/MAS spectra of crystalline $CaCO_3$ (**5**·**CaCO$_3$**) containing poly(1-carboxylate-3-*N*-*n*-BuNHCOtetramethylene) were similar to the ^{13}C spectra of poly(1-carboxylate-3-*N*-*n*-BuNHCOtetramethylene sodium salt) (**5**·**Na**) and poly(1-carboxyl-3-*N*-*t*-BuNHCOtetramethylene) in the solid state at 303 K. **4**·**CaCO$_3$** shows a carboxylate CO signal at 185 ppm and an amide CO signal at 178 ppm indicative of the polymer ligand bound to the crystals.

On the other hand, the ^{13}C CP/MAS spectra of crystalline $CaCO_3$ containing poly(acrylate) does not show a clear carboxylate CO signal but a broad signal at 182 ~ 184 ppm. 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. The broad signal indicates a mixture of COO^- and COOH in the polymer chain. Thus, 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). These data are consistent with the reported fact that poly(acrylic acid) is involved in the nucleation of $CaCO_3$ crystallization but is then dislodged from the crystals.[1a] The surface of crystalline $CaCO_3$ is thought to be covered with coordinated water molecules and the presence of their protons on the surface provides a hydrophobic environment around the Ca(II) ion. Since the pK_a of the carboxylic acid becomes higher, the carboxylate readily converts to carboxylic acid to become dislodged from the crystals.

Poly(carboxylate) ligands with 7-membered ring NH---O hydrogen bond in the main chain

Poly[1-carboxylate-2-(*N*-*t*-BuNHCOCH$_2$)ethylene-*alt*-ethylene] was prepared from poly(ethylene-*alt*-maleic-anhydride) and *t*-BuNH$_2$. The polymer ligand has an intramolecular 7-membered ring NH---O hydrogen bond in the main chain, as shown in Fig. 4. The CaCO$_3$ composite of this polymer provides fine vaterite crystals under acidic and basic conditions.

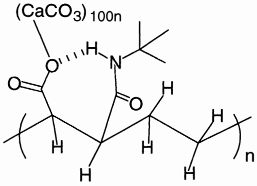


Figure 4. Proposed structure of poly[1-carboxylate-2-(*N*-*t*-BuNHCOCH$_2$)ethylene-*alt*-ethylene].

Poly(carboxylate) ligands with 7-membered ring NH---O hydrogen bond in the side chain

Novel three poly(carboxylate) ligands, $-\{CH_2CH(CH_2NHCO\text{-}R\text{-}COO^-)\}_n-$ (R: CH_2CH_2, CH=CH, *o*-Ph) were synthesized from poly[(1-aminomethynyl)ethylene] and the carboxylic anhydrides. The two polymer-CaCO$_3$ composites give the stable binding of carboxylate of the polymer ligands to vaterite CaCO$_3$ crystals (Fig. 5a and 5c). These ligands have an intramolecular NH---O hydrogen bond at the side chain. A structural change between the amide and phenyl planes converts from the NH···O hydrogen bonded form into a higher nucleophile form that is readily hydrolyzed with water due to the high pK_a.

One of the interesting points is that poly[1-(*N*-oxymalenyl-aminomethenyl)ethylene] forms calcite (Fig. 5b). The ligand has a thermodynamically unstable intramolecular NH---O hydrogen bond that is sensitive against temperature. This behavior is established by a simple model ligand, 4-*t*-BuNHCOCH$_2$=CH$_2$COO$^-$ having two conformers. One conformer form a strong NH---O hydrogen bond and the other is strong nucleophile without the hydrogen bond as shown in Fig. 6.

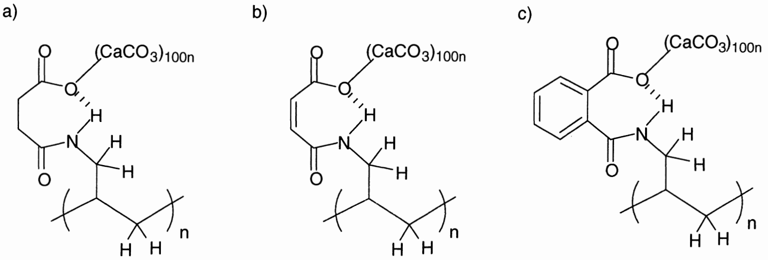


Figure 5. Poly(carboxylate) ligand having an intramolecular hydrogen bond at the side chain. a) Poly(*N*-oxysuccinyl-aminomethenyl)ethylene], b) poly[1-(*N*-oxymalenyl-aminomethnyl)ethylene] and c) poly[1-(*N*-oxyphthalyl-aminomethnyl)ethylene].

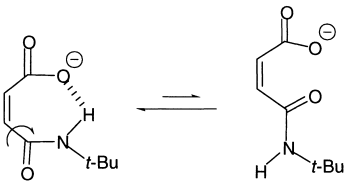


Figure 6. Conformational change in a model compound, (t-BuNHCOCH=CHCOO$^-$)(NEt$_4$).

Location of polymer ligand on the $CaCO_3$ crystal

Novel gold colloid-conjugated poly{[1-carboxylate-2-(*N*-*p*-methylthiophenyl) carbamoyl] ethylene-*alt*-ethylene}, as shown in Fig.7, was synthesized for the analysis of the inorganic crystals/organic ligand composite using a FE/SEM and its backscatter methods. The data indicate that the polymer ligand is located at the surface of vaterite crystals and the oriented carboxylate ligands control the $CaCO_3$ polymorph. It is likely that nucleation of $CaCO_3$ crystallization starts along the carboxylate matrix and the polymer ligand locates on the surface of the crystal.

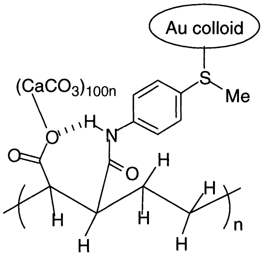


Figure 7. Gold colloid-conjugated poly{[1-carboxylate-2-(*N*-*p*-methylthiophenyl) carbamoyl]ethylene-*alt*-ethylene}.

[1] (a) Addadi, L.; Moradian, J.; Shay, E.; Maroudas, N. G.; Weiner, S. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 2732-2736. b) Flankel R. B. M.; Mann, S. In *Encyclopedia of Inorganic Chemistry*; John Wiley & Sons: Chichester, 1994; Vol. 1.

[2] Mann, S.; Heywood, B. R.; Rajam,S.; Birchall, J. D. *Nature*, **1988**, *334*, 692-695.

[3] Albeck, S. W.; Weiner, S.; Addadi, L. M. *Chem. Eur. J.*, **1996**, *2*, 278-284.

[4] (a) Okamura, T.; Takamizawa, S.; Ueyama, N.; Nakamura, A. *Inorg. Chem.*, **1998**, *37*, 18-28. (b) Ueno, T. ; Kousumi, Y.; Yoshizawa-Kumagaye, K.; Nakajima, K.; Ueyama, N.; Okamura, T.; Nakamura, A. *J. Am. Chem. Soc.*, **1998**, *120*, 12264-12273. (c) Ueyama, N.; Nishikawa, N.; Yamada, Y.; Okamura, T.; Nakamura, A. *J. Am. Chem. Soc.*, **1996**, *118*, 12826-12827.

[5] Ueyama, N.; Hosoi, T.; Yamada, Y.; Doi, M.; Okamura, T.; Nakamura, A. *Macromolecules* **1998**, *31*, 7119-7126.

[6] (a) Ueyama, N.; Takeda, J.; Yamada, Y.; Okamura, T.; Nakamura, A. *Inorg. Chem.* **1999**, *38*, 475-478.

[7] Ueyama, N.; Kozuki, H., Doi, M.; Yamada, Y.; Takahashi, K.; Onoda, A.; Okamura, T.; Yamamoto, H. *Macromolecules* **2001**, *34*, 2607-2614.