Increase of Adhesion Force of Poly(carboxylate) Ligand on Calcium Phosphate Crystals by an NH···O (Oxyanion) Hydrogen Bond

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Ca–O bond is strengthened by carboxylate ligand with an intramolecular NH···O hydrogen bond. A novel NH···O hydrogen-bonded polymer, poly[1-carboxylato-2-(1-ethoxycarbonyl-3-methylsulfanylpropylcarbamoyl)-ethylene-*alt*-ethylene] exhibits higher adhesion force on the surface of calcium phosphate crystals than the non-NH···O one.

Calcium biominerals occurring in nature, such as those that make up mollusk shells, pearl, coral reef, bone, and tooth, are often mediated and regulated by a small amount of biopolymer ligands.[1] Through this process, a small amount of organic materials strongly binds to a specific crystal face and controls the crystal growth, and the resulting biominerals possess highly controlled shapes, sizes, and morphologies under ambient conditions.[2] There are many amino acid residues, such as Asp and phosphorylated Ser, that are present in protein-bound calcium phosphate biominerals.[3] We have suggested that the strong binding of these biopolymer ligands to inorganic crystals is due to formation of an NH···O hydrogen bond toward Ca-coordinated carboxylate oxygen from the neighboring amide group.

Recently, we have demonstrated that an NH···O hydrogen bond from a neighboring amide NH effectively strengthens Ca–O bond.[4] The NH···O hydrogen bond in carboxylic acid derivatives having the neighboring amide NH lowers the pK_a value.[5] Because of this change in the pK_a value, the carboxylate anion is hardly protonated by water under neutral conditions. Actually, the NH···O hydrogen-bonded benzoate derivative has a higher formation constant toward Ca^{2+} ion than the nonsubstituted benzoate without the NH···O hydrogen bond.[4] The NH···O hydrogen bond effectively affects to strength of Ca–O bond.

In the present study, we prepared a novel NH···O hydrogen-bonded poly(carboxylate) ligand, poly[1-carboxy-2-(1-ethoxycarbonyl-3-methylsulfanylpropylcarbamoyl)-ethylene-*alt*-ethylene] (**1H**), as well as its carboxylate anion state (**1**) (Chart 1), to be used as an artificial model in order to investigate the role and behavior of an acidic peptide in Ca biominerals; we also synthesized this polymer ligand–calcium phosphate composite to confirm the strong binding toward Ca ion on the crystal. This polymer ligand strongly binds to calcium phosphate crystals and is prevented from hydrolysis by the NH···O hydrogen bond. Furthermore, the methylsulfanylpropyl group has a high affinity for a dental material that is containing Au, Ag, Pt, and so on. We found that this polymer exhibits higher adhesion force than the non-NH···O hydrogen-bonded polymer.

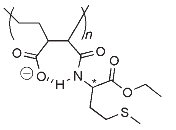

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Chart 1.

The carboxylic acid form of the polymer ligand, **1H**, was synthesized by the reaction of poly(ethylene-*alt*-maleic anhydride) with L-methionine ethyl ester (H-Met-OEt).[6] Tetramethylammonium salt of **1H** (**1NMe$_4$**) was prepared by the neutralization of the carboxylic acid with NMe$_4$OH. The polymer ligand was characterized by ^{1}H NMR spectra. In the ^{1}H NMR spectra in Me$_2$SO-d_6, the amide NH signal of **1H** was observed at 8.32 ppm, and that of the carboxyl group appeared at 12.23 ppm. Other ^{1}H NMR signals of the polymer were observed between 1.17 and 4.29 ppm. On the other hand, in **1NMe$_4$**, the ^{1}H NMR signal of the carboxyl group disappeared, and the amide NH signal found at 12.23 ppm. Significantly large downfield shift ($\Delta\delta = 3.91$ ppm) indicates that this polymer ligand forms a strong intramolecular NH···O hydrogen bond in the carboxylate anion state, as was demonstrated in our previous reports.[7] Furthermore, this NH···O hydrogen bond exhibits a thermostability.[7] Thus, near room temperature (223–323 K), the hydrogen bond is never dependent on the temperature from results of our model ligands (data not shown).

In order to estimate the value of the adhesion force, an experiment was carried out as shown below. We prepared cattle teeth (hydroxyapatite crystal) attached the sticker with a hole 5 mm in diameter (Figure 1a). The mixture of the polymer ligand [**1** or poly(acrylate)] and aluminosilicate was applied to the hole, and a hook (piano wire) was attached to it (Figure 1b). The hook was undergone by an externally applied force (Figure 1c). The adhesion force was assessed by measuring the level of stress (force/area) at which the point of the hook came off the tooth.

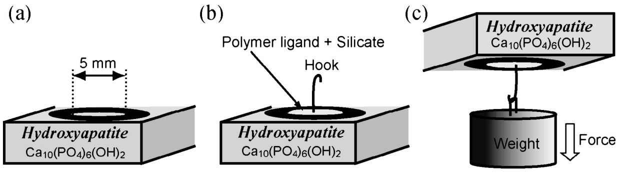

Figure 1. Schematic drawings of the experimental procedure for the measurement of the adhesion force (descriptions of these drawings were presented in the text).

Table 1. The average adhesion force of poly(carboxylate) ligands toward hydroxyapatite crystals (at room temperature)

Polymer	Dentine / MPa	Enamel / MPa
1	0.26	0.25
poly(acrylate)	0.05	0.04

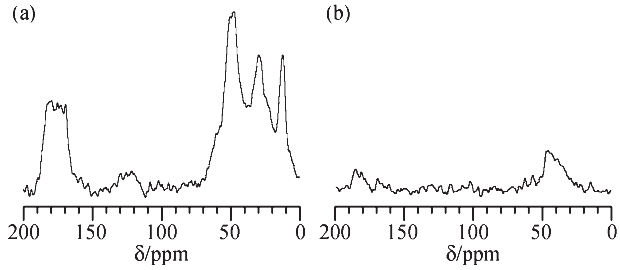

Figure 2. Solid-state ^{13}C CP/MAS NMR spectra of hydroxyapatite composites in the presence of (a) polymer ligand **1** and (b) poly(acrylate) (75 MHz, 7.5 mmϕ tunable probe, 4000-rps-spinning rate, at room temperature). Intensities of base-lines of these spectra are normalized by a noise level.

This experimental procedure was undertaken 5 times toward both the dentine and enamel layers of the cattle tooth individually, where the molecular weight of each polymer ligand was almost the same. Table 1 lists the average adhesion force of **1** compared with the results of the usage of poly(acrylate) without the NH···O hydrogen bond. All experimental results demonstrate that the adhesion force of the NH···O hydrogen-bonded polymer ligand **1** toward dentine or enamel is about 5 times stronger than poly(acrylate).

The strength of Ca–O bond affects the resistance to hydrolysis in polymer–hydroxyapatite composite, which is estimated by the amount of the remaining polymer after the washing process utilizing a solid-state ^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR spectroscopy.[7,8] When we synthesized hydroxyapatite composites in the presence of **1** and poly(acrylate), individually,[9] polymer ligand **1** is strongly bound to hydroxyapatite crystals, while poly(acrylate) weakly bound to hydroxyapatite crystals. Figure 2 shows the solid-state ^{13}C CP/MAS NMR spectra of **1**–hydroxyapatite composite and poly(acrylate)–hydroxyapatite composite after the washing process to remove free and dislodged polymer ligand. Comparing both spectra, the signal intensities of **1** are higher than those of poly(acrylate). The signal intensity of poly(acrylate)–hydroxyapatite composite was significantly low, which indicates most of ligand was dislodged from hydroxyapatite crystals because of weak Ca–O bindings in poly(acrylate)–hydroxyapatite composite.

In the present study, we demonstrated that the NH···O hydrogen bond strengthens Ca–O bond not only kinetically, but also mechanically. Actually, the adhesion force of the NH···O hydrogen-bonded polymer ligand **1** is 5 times higher than that of non-NH···O hydrogen-bonded poly(acrylate) ligand. In conclusion, formation of the NH···O hydrogen bond supports the stabilization of metal–oxygen bond, and the NH···O hydrogen-bonded polymer ligand strongly binds to inorganic crystals with the high adhesion force.

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6 **1H** was prepared as follows: poly(ethylene-*alt*-maleic anhydride) (220 mg, 1.8 mmol) and H-Met-OEt (310 mg, 1.8 mmol) in 10 mL of DMF were stirred for 12 h. To the mixed solution was added 3.5% HCl aq solution (100 mL) to give a white powder. The powder was collected by centrifugal separation and dried over P_2O_5 under reduced pressure. The obtained white powder was dissolved into EtOH (100 mL) and the solution was dropped into ether (400 mL) to obtain a white powder. Yield, 250 mg (47%). ^{1}H NMR (400 MHz, 303 K, in Me_2SO-d_6, δ): 12.23 (1H, br, COOH), 8.32 (1H, br, NH), 4.29 (2H, br, ethoxy CH_2), 4.09 (1H, br, Met H), 2.03 (3H, br, Met H), 1.89 (2H, br, Met H), 1.35 (6H, br, CH_2, CH), 1.17 (3H, br, ethoxy CH_3). Anal. Calcd. for $(C_{13}H_{21}O_5NS)_n(C_6H_8O_4)_{1.3n}(H_2O)_n$: C, 49.11; H, 6.62; N, 2.76; S, 6.44. Found: C, 49.00; H, 6.89; N, 2.76; S, 6.44%. Transducing ratio of amino acid group was 43% from the elemental analysis.

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9 Typical composing of hydroxyapatite crystals and a polymer ligand was undergone below. To ten-mM MeOH solution of a polymer ligand (10 mL) was added 50 mg/mL of hydroxyapatite aqueous suspension (20 mL) and the mixed suspension was stirred at 303 K. After 5 h, the precipitate was collected by filtration and washed with pure water and MeOH. The obtained white powder was dried over P_2O_5 under reduced pressure.