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Effects of polymer architecture and charge density on the pH-responsive Ca(II) release from brushite

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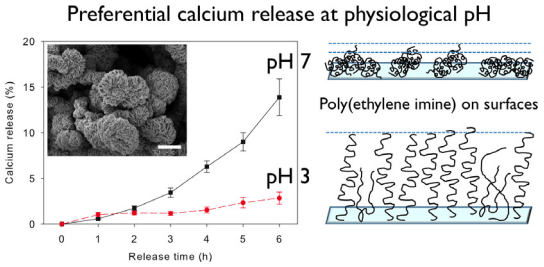
HIGHLIGHTS

- Kinetics of calcium release from brushite mineral.
- Poly(ethylene imine) to regulate the calcium release.
- Changes of conformation and adsorption behavior of poly(ethylene imine).
- Preferential calcium release at physiological pH.

GRAPHICAL ABSTRACT



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ABSTRACT

Calcium is one of the major components in the biomineralization of living species, where the transport and release of calcium as well as the consequent nucleation and crystallization are required to be controlled. In this report, the pH-responsive release of calcium from brushite, dicalcium phosphate dihydrate, was studied with addition of some structurally modulated poly(ethylene imine). Poly(ethylene imine) made the calcium delivery preferential at the physiological pH at the same time promoting the sustained behavior, while the release was intrinsically faster at acidic conditions without additive. The release at pH 7 was more than three times faster than at pH 3 for the best case among the systems studied in the present study. The observed phenomenon was attributed to the altered boundary layers of the calcium diffusion at the brushite surfaces when the pH changed, arising from the conformational change of poly(ethylene imine) combined with the charge reversal of brushite surfaces. The current results could be of critical implications in the fields of bioinspired and biomimetic mineralization as well as the controlled drug release from mineral carriers.

1. Introduction

Calcium is the vital component of diverse biominerals, mainly involved in the forms of calcium carbonate and calcium phosphate. For example, the shells of mollusks and the spicules of sea urchins/cucumbers contain calcium carbonate as the main inorganic constituent [1,2]. The bones and enamels of vertebrates possess calcium phosphate as apatite [3]. Also, the cuticles of crabs include both calcium carbonate and calcium phosphate [4].

The biomineral formation requires the elaborate management of calcium in the biological systems to induce intricately developed structures. It is executed through the combinations of calcium uptake, transportation, storage, deployment, and mineralization, which involve calcium in the forms of precursor minerals as well as ions [5]. The meticulous regulation on the calcium is not limited to

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the mineral formation. The plasma and cytosolic levels of calcium concentrations are also tightly maintained in the millimolar and micromolar ranges, respectively, since the ions act as physiological controllers during glycogen metabolism and muscle contraction [6,7].

Various biomimetic and bioinspired approaches have been made to selectively transport calcium in targeted environments. Extensive efforts have been made in the use of liposomes that release calcium ions, and the release was usually activated through the applications of heat and additives that altered the confined structures [8–10]. Similarly, polymer vesicles and gels have been used to regulate the delivery of calcium ions [11–13]. However, most approaches have been limited to the use of spatially confined calcium ions. Although the use of calcium minerals could be found in some endodontic filling materials, the calcium release was optimized mostly through trial and error [14,15]. The high solubility of calcium minerals at low pH has been utilized for the release of the drugs and proteins coexisting with the minerals [16–19], while the contrasting examples of the preferential release at physiological conditions are difficult to find. In addition, the protection against acidic degradation is essential for the diverse protein drugs, and calcium phosphate is becoming one of the key components of the delivery system [20].

In this work, calcium release was studied from the crystals of brushite, also known as dicalcium phosphate dihydrate (DCPD: $CaHPO_4{\cdot}2H_2O$). The release was controlled with poly(ethylene imine) (PEI) as an adsorbing species to form a protective layer on the crystal surfaces and enable the sustained release. The pH-responsive release was studied with PEIs of modulated molecular structures to ultimately achieve preferential release at physiological conditions. PEI was selected as a model compound because of its ability to adjust its conformation along the pH change [21,22]. The conformational change as well as the adsorption behavior was correlated to the profile of calcium release to reveal the key characteristics of PEI to enable the desired behavior of calcium release.

2. Materials and methods

2.1. Preparation of dicalcium phosphate dihydrate (DCPD) crystals

DCPD was prepared according to a known method, which is in brief as follows [23]. A gelatin matrix (Sigma, bovine skin, ~75 Bloom, 10 w/v%, 50 mL) containing $NaH_2PO_4{\cdot}2H_2O$ (Fluka, 99.0%, 1.1 M) was prepared. When the same volume of an aqueous solution of $Ca(NO_3)_2{\cdot}4H_2O$ (Sigma Aldrich, 99.0%, 2.7 M) was gently placed on the matrix, the formation of DCPD was initiated at the interface. The crystals were filtered after ca. 24 h (pore size 1 μm, cellulose acetate, Hyundae micro) and thoroughly washed with deionized water (resistivity 18.2 MOhm cm, Direct Q3, Millipore). Drying (>48 h) was done at room temperature before further use.

2.2. Calcium release from DCPD crystals

The calcium release from DCPD crystals into water was studied in the presence fluoride ion. This method made it possible to accelerate the release due to the driving force to form stable fluorapatite ($Ca_{10}(PO_4)_6F_2$), which acted as a sink of the released calcium. It also helped to make DCPD dissolution relatively constant in the solution in equilibrium with solid fluorapatite of the very low solubility. A typical release experiment utilized 90.6 mg DCPD in a 20-mL aqueous solution with 100-ppm fluoride (5.26 mM NaF, Sigma Aldrich, 99.0%). The Ca/F ratio in the system was set as 5:1 following the theoretical composition of fluorapatite. The pH of the fluoride solution was initially adjusted to 3 or 7 using a small amount of 1 N HNO_3 (aq). The release experiment proceeded at room temperature in a vial made of isotactic-polypropylene (diameter 27 mm, height 61 mm), and a rod-shaped magnetic stir bar (Teflon-coated: length 14 mm, diameter 5 mm) was used for vigorous stirring (ca. 120 rpm).

The percentage of calcium release from DCPD at a given time was calculated from the concentration of the remaining fluoride: [100 − (remaining ppm of fluoride)]%. The *in situ* measurement of the fluoride concentration was performed with a fluoride-selective electrode (Thermo Electron Corporation, an Orion 4-Star pH/ISE Benchtop meter equipped with an Orion 9609BNWP Combination fluoride electrode and an Orion 8102BNUWP ROSS Ultra glass combination pH electrode). Each release experiment was monitored until 24 h, after which the solid phases in the reaction system were filtered, washed with deionized water, and dried at room temperature. The reported value of calcium release was the average of five independently performed experiments.

2.3. Calcium release controlled by PEI adsorption

The control of the calcium release was attempted with addition of poly(ethylene imine) (PEI, Sigma Aldrich). Three different PEIs were used: a linear PEI (LP: M_w 1,300, M_n 1,200) and two branched PEIs (BP2: M_w 2,000, M_n 1,800; BP25: M_w 25,000, M_n 10,000). Polymer concentrations in the range 0.010–10 μM were investigated, and all the other experimental details were the same as without polymers.

The structures of PEIs were studied by titration measurements of the primary amines using phenolphthalein indicator (Fluka, 1 wt% in EtOH/water = 1:1) [24]. The phenolphthalein (0.1 mL) indicator was added to the aqueous solution of PEI (100 mL in a 250 mL round-bottom flask), and the PEI concentrations were 10 μM for LP and BP2, and 1.0 μM for BP25. The initial pH was about 10, and 0.10 N HCl (Daejung Chemical, Korea) was added dropwise at room temperature with stirring (a Teflon-coated stir bar, ca. 20 rpm) until the red solutions became clear. The final pH was about 7. For each PEI, the titration experiment was repeated five times using independently prepared solutions.

PEI adsorption on DCPD was monitored by zeta-potential measurements (Zetasizer Nano ZS90; Malvern instrument, UK). DCPD powder (90.6 mg) was dispersed in the 20 mL aqueous solutions of PEI at various concentrations (0–10 μM). The solution pH was adjusted to 3 or 7 with a small amount of 1 N HNO_3 (aq). The measurement was performed at room temperature after 1–2 min of shaking, and about 2 mL of the mixture was used. Three independently prepared mixtures were used for each condition, and the measurements were repeated five times for each mixture.

2.4. Crystal characterizations

Surface area of DCPD crystals was measured using an adsorptive molecular probe of methylene blue (MB) [25,26]. DCPD powder (100 mg) was placed in a 0.10 mM MB aqueous solution (20 mL) and equilibrated for 24 h. After filtration, the absorbance was measured at 665 nm (Jasco V-560 UV/Vis spectrophotometer, Japan), which was converted to the concentration of MB remaining in the solution using a pre-obtained calibration curve. Finally, the surface area of DCPD was obtained from the adsorbed amount of MB with an occupying area of 1.3 nm^2 per MB molecule [27]. The reported surface area was the average of three measurements.

Crystal morphology was observed before and after the calcium release using scanning electron microscopy (SEM). A JEOL JSM-6401F microscope was used after thin Au coating (Cressington Sputter Coater 108) to minimize surface charging.

The crystal phases were also studied before and after the calcium release by wide-angle X-ray diffraction (XRD) using a Bruker