Supplementary Material (ESI) for Chemical Communications

Supporting information

Motif-Programmed Artificial Proteins Mediated Nucleation of Octacalcium Phosphate on the Titanium Substrates

Toru Tsuji, Yuya Oaki, Masao Yoshinari, Takashi Kato, and Kiyotaka Shiba *

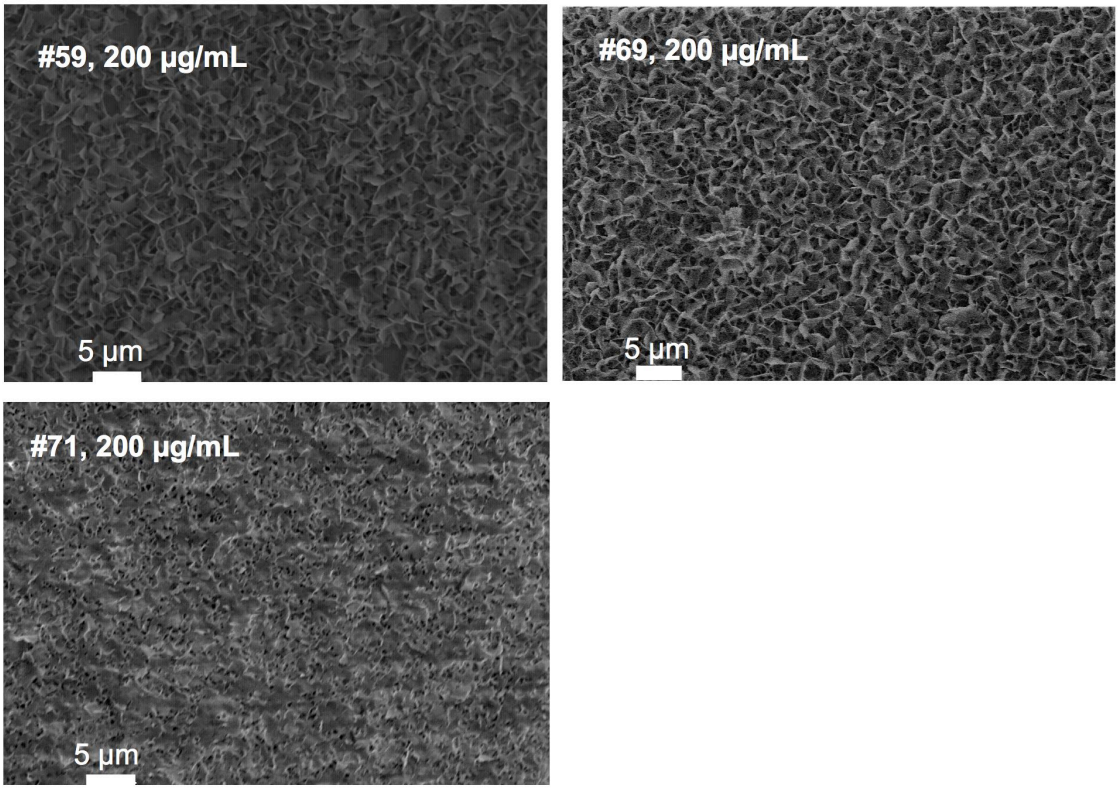

Fig. S1. SEM images of the surfaces of titanium plates incubated with calcium and phosphate ions in the presence of the indicated concentrations of protein.

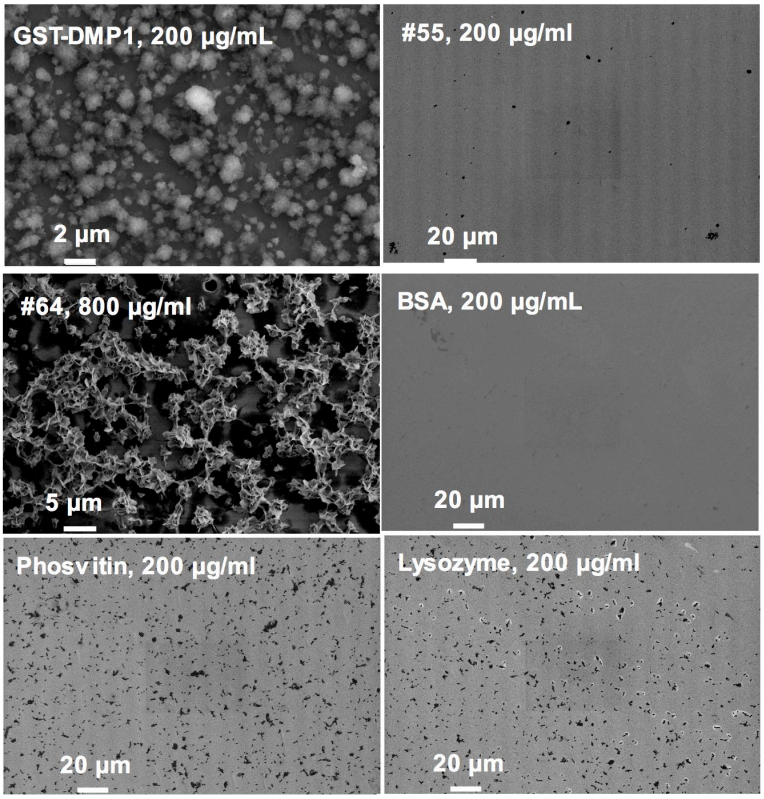

Fig. S2. SEM images of the surfaces of titanium plates incubated with calcium and phosphate ions in the presence of the indicated concentrations of protein.

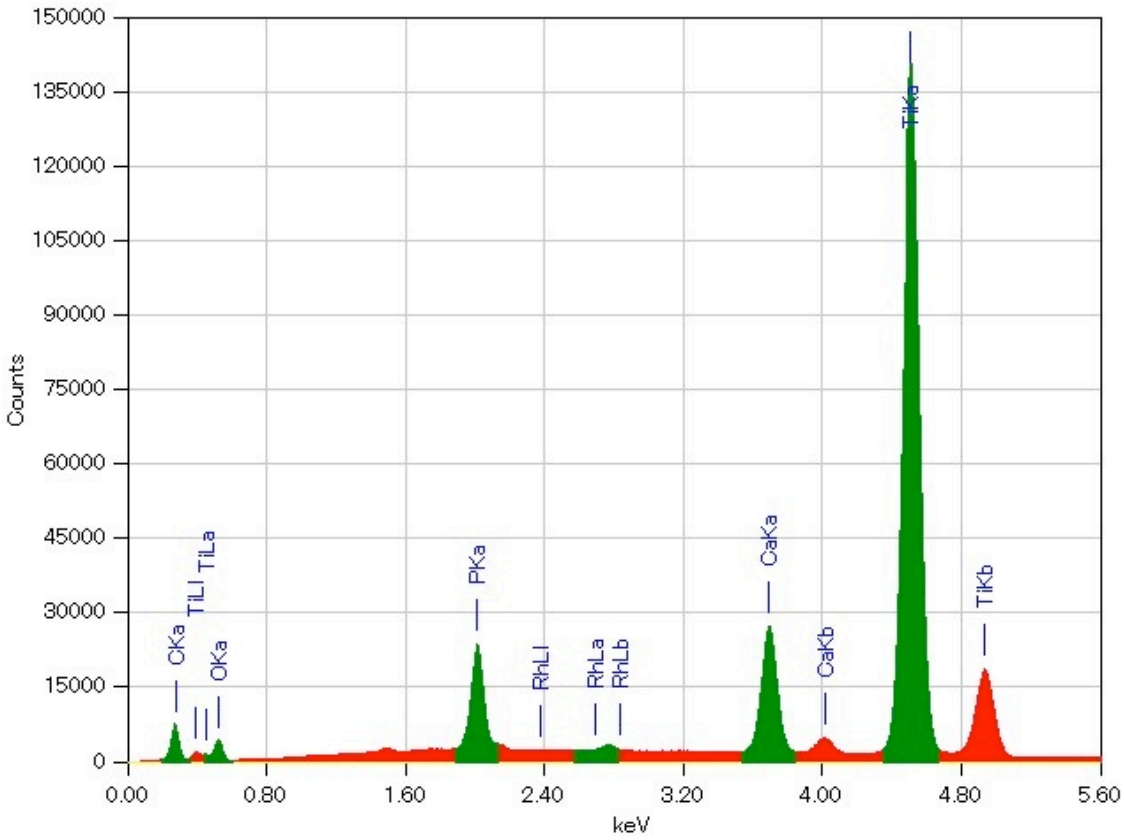

Fig. S3. Representative data of energy dispersive X-ray (EDX) analysis of the mineral phase. The atomic ratio of calcium to phosphorous (Ca/P) obtained from this spectrum was 1.318.

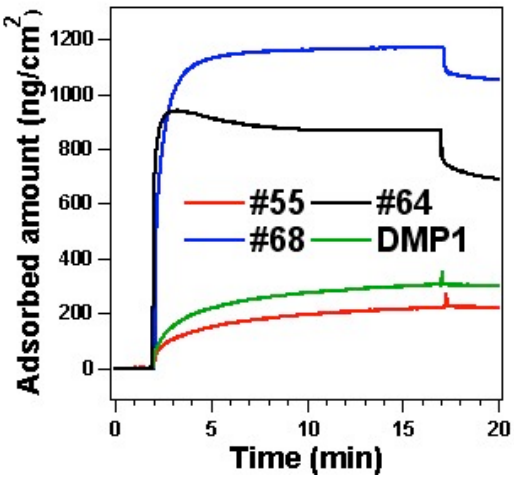

Fig. S4. Interactions between proteins and a titanium substrate analyzed by QCM measurements. Time-dependent changes in amounts of protein (#55, red; #64, black; #68, blue; and DMP1, green) adsorbed onto a titanium sensor. At time = 2 min, sample solutions were injected into the QCM measuring chamber. After 15 min, a buffer was injected.

EXPERIMENTAL PROCEDURES

Preparation of Proteins

Construction of a library of motif-programmed proteins has been previously reported[1]. The proteins were expressed in *Escherichia coli* and purified using TALON resin as described before[2], and then dialyzed against 0.001N-HCl and freeze-dried. Before use, the proteins were dissolved in solution containing 2.16 mM KH_2PO_4, and the pH was adjusted to 7.40 ± 0.2 (Horiba, model F22). BSA (albumin bovine Cohn Fraction V; Iwai Chemical), phosvitin (from egg yolk; Sigma) and lysozyme (from chicken egg white; Sigma) served as controls, and were dialyzed as describe above. Expression and purification of a glutathione-S-transferase (GST)-DMP fusion protein was described previously[3]. GST-DMP was dialyzed against 0.25 % of aqueous ammonium before use.

QCM measurements

Interactions between proteins and titanium were assessed based on QCM measurements (QCM-D300, Q-Sense AB, Gotenborg). QCM sensors sputter-coated with titanium and cleaned with UV/O_3 cleaner (ProCleaner, BioForce Nanosciences Inc., Iowa) for 10 min were used. Prior to measuring the interactions, the titanium sensor and the chamber were equilibrated with 1.1 mM KH_2PO_4 (pH 7.4) at 25˚C. The proteins were dissolved in 1.1 mM KH_2PO_4 (pH 7.4), and the protein concentration was 100 μg/ml. The injection volume was 1 ml. Sauerbrey's equation was applied to translate changes in resonance frequency into adsorbed mass of the protein on the titanium sensor[4].

Mineralization Experiments

Polished wrought titanium plates (10 mm x 10 mm x 1 mm, JIS H4600, 99.9 mass %, titanium) were purchased from Shinkinzoku Co., Ltd. Osaka. The plates were treated for 10 min with UV/O_3 cleaner (ProCleaner, BioForce Nanosciences Inc., Iowa) and rinsed with Milli-Q water before

mineralization experiments. Mineralization was carried out by incubating a titanium plate in 1 ml of the solution containing 1.1 mM KH_2PO_4 (pH 7.4), 2.0 mM $CaCl_2$ and various proteins. After incubation in calcium phosphate solutions for 6 days at 25˚C, the substrates were washed with MilliQ water and air-dried. The concentrations of proteins tested in the mineralization experiments were 800, 200 and 80 μg/ml for #64; 800, 200 and 40 μg/ml for #68; and 200 and 40 μg/ml for #55, DMP1, BSA, phosvitin and lysozyme.

Characterization of Mineral Phases

FESEM (Hitachi, S-4700 operated at 2.0 kV), SEM (KEYENCE, VE-8800 operated at 2.0 kV) and TEM (JEOL, JEM2010-HC operated at 200 kV) were used for the observations, and the images were obtained using a CCD digital imaging system (Gatan, MSC-794). The elemental mapping was performed on a SEM (JEOL, JSM-6390LV operated at 20 kV) equipped with an energy dispersive X-ray analyzer (EDX, JEOL, JSD-2300 with a silicon drift detector). The samples, including titanium substrates, were directly used for the SEM analyses with no conductive treatment. For the TEM samples, the calcium phosphate nanosheets were removed from the titanium substrate and dispersed on a copper grid supported by a collodion membrane. Sintered titanium mesh (Bekinit, Tokyo, Japan) with a fiber diameter of 20 μm, a pore size of 50-100 μm, and a volumetric porosity of 87%, was used. The prepared disks (3.5 mm in diameter and 1.0 mm thick) were treated for 60 min with UV/O_3 cleaner (PC440, Bioforce Nanosciences) and rinsed with Milli-Q water. Mineralization was carried out by incubating the disks in 1 ml of solution containing 1.1 mM KH_2PO_4 (pH7.4), 2.0 mM $CaCl_2$ and 800 mg/ml of #68. After incubation for 6 days at 25˚C, the disks were washed with Milli-Q water, air-dried and embedded in epoxy resin. After curing the resin, the specimens were cut through the middle using a cutting machine (Finecut, HS-100, Heiwa Tech, Japan) to observe interior regions of the titanium mesh. These specimens were ground down using 1,200 grit, polished using 0.3 μm alumina, and then ultrasonically cleaned with ethanol and distilled water. Thereafter, the specimens were coated with carbon before EPMA analysis

(JXA-8200, JEOL, Tokyo, Japan). The presence of calcium phosphate coating the interior regions of the titanium mesh was confirmed by elementary mapping of calcium, phosphorous, and titanium. EPMA analysis entailed detection of the X-ray intensities of Ca-Ka, P-Ka and Ti-Ka at an accelerating voltage of 25 kV.

References

1. (a) K. Shiba, Y. Takahashi and T. Noda, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 3805; (b) T. Tsuji, K. Onuma, A. Yamamoto, M. Iijima and K. Shiba, *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 16866.

2. K. Shiba and T. Minamisawa, *Biomacromolecules*, 2007, ***8***, 2659.

3. G. He, T. Dahl, A. Veis and A. George, A. Connect Tissue Res. 2003, *44*, 240.

4. F. Höök, B. Kasemo, T. Nylander, C. Fant, K. Sott and H. Elwing, *Anal. Chem*. 2001, ***73***, 5796.