Electronic Supplementary Information

Effect of Peptide Conformation on TiO_2 Biomineralization

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Experimental section

Materials

Oligo(L-lysine) (OLL) and lysine-rich peptides containing glycines were purchased from Sigma-Aldrich and Peptron Co., respectively. An aqueous solution of titanium(IV) bis(ammonium lactato) dihydroxide (TBALDH, 50 wt% in H_2O, Aldrich) was chosen as a precursor for TiO_2 mineralization. 5-chlosalicylic acid (98%, Fluka), anhydrous sodium perchlorate (95%, Wako), sulfuric acid (95%, Duksan), ammonia water (25~30%, Duksan), and ethyl alcohol (99.9%, Duksan) were used for activity measurement by chlorosalicyclic acid assay (CSA).[1] All aqueous solutions were prepared using deionized (DI) water (R=18.2 MΩ).

Biomineralization of titanium precursor

Simple mixing of a peptide solution with aqueous titanium precursor solution induced mineralization to form solid TiO_2. Details on the reaction protocols are described elsewhere.[2] Briefly, 1 ml of lysine solution (75 μM) was mixed with 150 μL of TBALDH solution (340 mM) at room temperature, and then DI water was added to make a total volume of 1.5 ml. The final concentrations of peptide and TBALDH were 50 μM and 340 μM, respectively. The pH of the mixture was adjusted to be 6.5±0.3 at the beginning of the reaction for each sample. Mineralization was performed by stirring the solution at room temperature.

Specific activity measurement

The specific activity is defined as the moles of TiO_2 produced per mole of peptide per minute. To determine the specific activity, the isolated solid TiO_2 precipitates were analyzed by CSA assay. In detail, the solid TiO_2 precipitates were mixed with 5-chlorosalicylic acid, sodium perchlorate, ethanol, and deionized water. The chemically treated solution was colored light yellow at pH 4. The concentration of TiO_2 was determined from UV-vis spectroscopy of this yellowish solution at 355 nm (S-3100, Scinco). A standard curve was prepared for the quantification of the TiO_2 precipitate.

Molecular dynamics simulations

All simulations and analyses were performed using the GROMACS 4.5.5 simulation package.[3-5] The GROMOS96-45a3 force field (FF) was used,[6] since this version of the FF has successfully predicted the secondary structures of short α-helical and β-sheet peptides.[7-9] The coordinates of the tetralysines, pentalysines, and their derivatives were generated in an initially α-helical structure using Swiss-Pdb Viewer.[10] A single peptide was solvated with simple point charge water in a periodic box measuring 3.5 nm per side. Four to five counterions (Cl^-) were added to neutralize the system. A cutoff of 12 Å was applied for electrostatic forces, with the inclusion of particle mesh Ewald for long-range electrostatics.[11] The LJ forces were smoothly switched to zero between 10 and 12 Å. A temperature of 298 K and a pressure of 1 bar were maintained by applying a Berendsen thermostat in the NPT ensemble.[12] Simulations were performed over 20 ns with a time step of 2 fs, and the last 10 ns were used for analyses.

Simulations of peptides with the different extents of amine protonation

To understand the effect of the lysine charge on their interactions, peptides with the different extents of amine protonation (0%, 50%, and 100%) were simulated. Figure S1 shows the distributions of the distances between the N atoms of the protonated (net charge of +1) or unprotonated (neutral) amino groups in the lysine residues, where “K” and “k” respectively designate the protonated and unprotonated lysine. Tetralysines with 0%- and 50%-protonated amino groups show the decrease in distances between neighboring lysine residues, presumably because of neutral amines of lysines, which induce much less repulsive forces than do charged lysines. Since the distances between lysines decrease, the non-monotonic effects of the number of glycines are observed less significantly than those for charged lysines (100%-protonated amines), indicating the dependence on the lysine protonation.

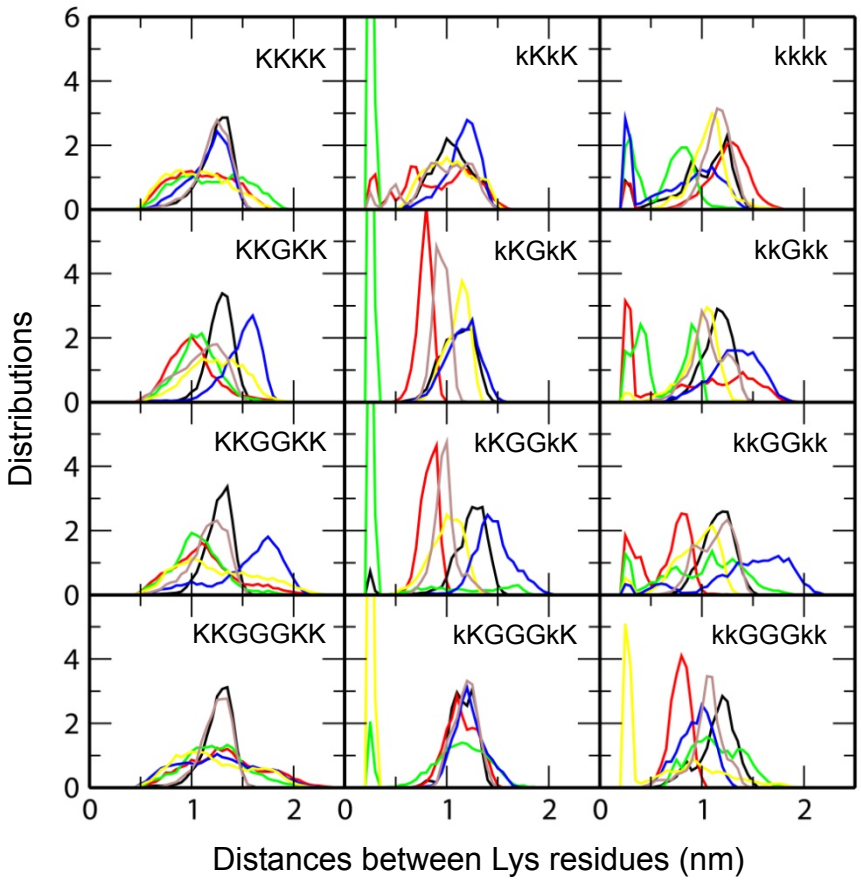

Figure S1. The distributions of the distances between the N atoms of the amino groups of the lysine residues for the tetralysine molecule. Protonated (+1) and unprotonated (neutral) lysines are named "K" and "k", respectively. Six distributions are differently colored for each system.

Estimation of TBALDH size

The size of TBALDH was estimated assuming that molecule was assumed to be fully protonated in the aqueous medium.

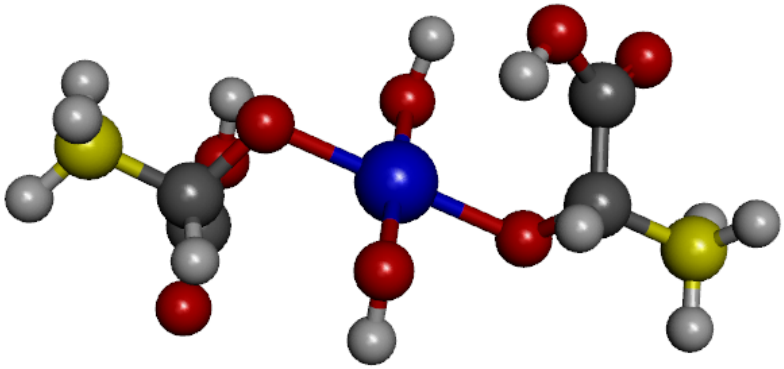

Figure S2. Structure of TBALDH. The distance between the carbons of the end methyl groups (colored yellow) is 8.365 Å. (Atom colors: blue (Ti), red (O), dark gray (C), light gray (H))

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