Identification and Design of Peptides for the Rapid, High-Yield Formation of Nanoparticulate TiO_2 from Aqueous Solutions at Room Temperature

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Titania (TiO_2) nanoparticles are widely used, or are under active development, for a range of applications in (photo)catalysis, photovoltaics, enzyme support, energy storage, and photonics. The peptide-directed room-temperature formation of titania nanoparticles can be an attractive alternative to higher-temperature synthetic methods. However, the influence of the peptide primary structure on the titania precipitation activity at room temperature is not well understood. Through the selective binding of phage-displayed 12-mer peptides to TiO_2 substrates, we have identified 20 peptides with an affinity for titania. The average numbers of arginine, lysine, and histidine residues present in these 20 peptides were distinctly higher than for the overall peptide-bearing phage library. Synthetic 16-mer versions of four of these peptides (i.e., 12-mer peptides with C-terminal tetrapeptide tags for quantitative spectrophotometry) induced the formation of 8.1–38.7 mol TiO_2/mol peptide after exposure for only 10 min to an otherwise water-stable Ti(IV) complex at room temperature and a pH of 6.3. X-ray diffraction analyses, electron diffraction analyses, and high-resolution transmission electron microscopy revealed that the peptide-induced titania contained fine (<10 nm) anatase and monoclinic β-TiO_2 nanocrystals, along with an amorphous phase. The titania yield increased with the number of positive charges carried by these peptides. On the basis of these results, a peptide was designed that exhibited the highest titania formation activity reported to date for a peptide (82.9 mol TiO_2/mol peptide), as well as a reduced pH dependence for such titania formation.

1. Introduction

The biomimetic or biomolecule-enabled formation of inorganic materials is a field of growing prominence in materials science.[1–6] The inspiration for this emerging field is provided by certain organisms that produce minerals under physiological conditions (biomineralization) through the use of specific biomolecules.[2] Examples of such biomolecules include the silicatein and silaffin proteins that have been isolated from the silica structures generated by sponges and diatoms, respectively.[2,7,8] These proteins have also been utilized to induce the *in vitro* formation of silica, and of nonbiological inorganic materials, from aqueous precursor solutions at room temperature.[2,9–13] Examples of the latter include TiO_2, Ga_2O_3, ZrO_2, and $BaTiOF_4$ formed with the aid of native and recombinant silicateins, and rutile TiO_2 formed with the aid of a recombinant silaffin.[10–13] The formation of titania from precursor solutions exposed to lysozyme (a bactericidal enzyme), peptide R5 (a synthetic analog of silaffin natSil1), poly-L-lysine, polyallylamine, spermidine, and spermine have also been recently reported.[14–18]

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Such room-temperature biomolecule-enabled and biomimetic titania syntheses can be attractive alternatives to higher-temperature processes for forming titania (e.g., flame aerosol synthesis via oxidation of $TiCl_4$ vapor at ≥ 1700 °C,[19] combustion synthesis of an aqueous solution of titanyl nitrate and glycine at ≥ 650 °C,[20] and sol–gel processing and firing at ≥ 500 °C[21]), particularly for applications involving the integration of temperature-sensitive materials with titania (e.g., for the encapsulation of enzymes or dyes within a titania matrix[14,15]).

While these recent observations demonstrate that proteins and biomolecule analogs can be used to induce the formation of titania under mild conditions, the influence of protein primary structure on the titania yield or morphology at room temperature is not well understood. Through the selective binding of phage-displayed 12-mer peptides to TiO_2 crystals, we have identified 20 peptides with an affinity for titania. 16-mer versions of these peptides, each consisting of a phage display-identified 12-mer peptide with a C-terminal tetrapeptide tag for spectrophotometric identification, were then synthesized. The titania precipitation activities of such peptides upon exposure to a water stable Ti(IV) complex have been quantified. Correlations between the primary structures of these peptides and the associated titania formation activities have been utilized to design a new peptide exhibiting an enhanced titania precipitation activity with a reduced pH dependence for such titania precipitation.

2. Experimental Procedure

2.1. Peptide Library Screening ("Biopanning"). TiO_2-binding peptides were identified using the Ph.D.-12 phage display library (New England Biolabs; Beverly, MA). The target binding, elution, and phage amplification steps were conducted according to the manufacturer's instructions.[22] Rutile TiO_2 single crystals of (100), (110), or (001) orientation (10 × 10 × 0.5 mm, double-side polished, MTI Corporation, Richmond, CA) were used as targets. A given substrate was washed 3 times with a 150 mM NaCl solution containing 50 mM tris-(hydroxymethyl)aminomethane-HCl (pH 7.5) and 0.2 vol % of the detergent Tween-20 (referred to herein as 0.2% TBST). The phage library was then incubated with a TiO_2 substrate for 1 h in a 0.2% TBST solution with rotation at 30 rpm. The TiO_2 substrate was then washed at least 10 times with 0.2% TBST to remove any nonbound phage particles, after which the bound phages were eluted by incubation for 10 min with a solution containing 0.2 M glycine-HCl, pH 2.2. The supernatant was removed from the TiO_2 target and then neutralized with 1 M Tris-HCl, pH 9.1. The eluted phages were amplified through infection of *Escherichia coli* strain ER2738 grown in Luria Broth (LB). The phage population was further enriched for titania-binding clones by three subsequent attachment/elution ("biopanning") cycles utilizing 0.2% TBST. The fifth round of biopanning was conducted with 0.5% TBST. To select for high-affinity TiO_2 binding clones, additional biopanning rounds were conducted using more stringent washing conditions (i.e., with 0.5% or 0.8% TBST solutions). All other aspects of the biopanning procedure remained unchanged for these additional rounds. After the final biopanning round, *E. coli* ER2738 cells were infected with the eluted phage and plated on LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-glactoside (X-gal, Invitrogen, Carlsbad, Ca) and isopropyl β-D-thioglactoside (IPTG, Invitrogen, Carlsbad, Ca). DNA was isolated from 225 independent blue plaques and sequenced using a 3100 Avant (Applied Biosystems, Ca) automated sequencer.

2.2. Titania Precipitation. The peptides used in this study were purchased from EZBiolab, Inc. (Westfield, IN) and received as desalted grade. All peptides contained the C-terminal tetrapeptide Gly−Gly−Gly−Trp in order to enable quantification by spectrophotometry at 280 nm. Peptide stock solutions were prepared with 0.2 μm filtered 18.2 MΩ H_2O (Nano-Pure Diamond Ultrapure Water System, Barnstead International, Dubuque, IA). Select peptides possessing high titania precipitation activities were chemically synthesized and purified to >70% by EZBiolab, Inc. (Westfield, IN). These peptides were further purified by gel-filtration chromatography utilizing a Superdex Peptide 10/300 GL column (GE Healthcare, Piscataway, NJ) with a Dionex FPLC system (Sunnyvale, CA; see Figure S1, Supporting Information). In a typical precipitation experiment, 50–250 μL of 110 mM titanium(IV)-bis-ammonium-lactato-dihydroxide (TiBALDH; Alfa Aesar, Ward Hill, MA) was added to an equal volume of a 200 mM phosphate-citrate buffer solution that contained the peptide at a concentration of 4 mg/mL. This solution was mixed by vortexing and incubated for 10 min. The precipitates were recovered by centrifugation at 13 200 rpm and washed 5 times with 1 mL of H_2O and and once with 1 mL of methanol (Fisher Scientific, Waltham, MA). The precipitates were then dried for 30 min in a vacuum centrifuge (Eppendorf North America, Inc., Westbury, NY) at 30 °C. Where indicated, the phosphate-citrate buffer was replaced with an equal concentration of 4-morpholineethanesulfonic acid (MES), N-(2-acetamido)iminodiacetic acid (ADA), or bis(2-hydroxyethyl)amino-tris(hydroxyl methyl)methane (Bis-Tris) obtained from Amresco, Inc. (Solon, OH), or with sodium citrate or sodium phosphate buffers (Sigma-Aldrich, St. Louis, MO).

2.3. Colorimetric Assay for Titanium Dioxide. The amount of titania induced to form in the presence of a given 16-mer peptide was evaluated with a quantitative colorimetric assay.[23] Briefly, the precipitates were dissolved in a solution of concentrated sulfuric acid and ammonium sulfate by heating to 90 °C for 1 h. A portion of this solution was combined with an ethanolic solution of $NaClO_4$ (Sigma, Milwaukee, WI) and 5-chlorosalicylic acid (Fluka, Seelze, Germany). Ammonium hydroxide was used to adjust the solution pH to 3, and the absorbance was measured at 355 nm utilizing a Thermo Scientific GENESYS UV/vis spectrometer (Waltham, MA). Reported values represent the average of at least three independently prepared samples.

2.4. Materials Characterization. Scanning electron microscopy was conducted with a field emission gun microscope (Leo 1530 FEG SEM, Carl Zeiss SMT Ltd., Cambridge, UK) equipped with an energy dispersive X-ray spectrometer (INCA EDS, Oxford Instruments, Bucks, UK). Transmission electron microscopy was conducted with a JEOL 4000 EX instrument. X-ray diffraction analyses were conducted with Cu Kα radiation at a scan rate of 0.3°/min using an X-Pert ProAlpha 1 diffractometer equipped with an incident beam Johannsen monochromator, 1/28 divergence slits, 0.048 soller slits, and an Xcelerator linear detector (PANalytical, Almelo, The Netherlands). Quantitative evaluation of the amount of pyrolyzable material contained in the titania precipitates was obtained by thermogravimetric analysis (Netzsch 449C Simulta-

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Table 1. Summary of the Properties and Amino Acid Residue Compositions of the Peptides Identified through Additional High-Stringency Biopanning Rounds (Modified Biopanning Methods 1 and 2) Using a Ph.D.-12 Peptide Library and a Single Crystal (001)-Oriented Rutile TiO_2 Target[a]

	no. of unique sequences identified	average pI	average number of (K + R)/peptide	average number of H/peptide	average number of hydrophobic residues/ peptide
starting library	3×10^9	n.a.	0.9	0.8	5.5
initial screening	19	8.3	1.2	0.4	6.0
first additional 0.5% TBST round[b]	6	8.6	1.0	1.7	5.0
second additional 0.5% TBST round[b]	5	9.6	1.6	1.8	4.6
third additional 0.5% TBST round[b]	2	11.3	2.0	2.5	2.5
first additional 0.8% TBST round[c]	10	8.3	0.8	1.1	5.3
second additional 0.8% TBST round[c]	3	10.0	1.7	2.3	3.3

[a] Starting library peptide sequence enrichment information is as reported by manufacturer.[22] [b] Modified biopanning method 1. [c] Modified biopanning method 2.

neous Thermal Analyzer, Selb, Germany) at a heating rate of 5 °C/min to 600 °C in air.

3. Results and Discussion

3.1. Phage-Displayed Peptide Library Screening. To identify TiO_2-binding peptides, a commercial combinatorial phage-displayed peptide library (M13 phage) was screened against rutile TiO_2 single crystal substrates. The surface of each phage displayed five copies of the minor coat protein pIII fused to a 12-mer peptide characteristic for the phage clone. The library contained approximately 3×10^9 different phage clones representing the same number of different 12-mer peptides.[22] Given that some peptides have been reported to exhibit specific binding to certain inorganic crystal faces,[24,25] single crystals with three different orientations were used as targets in this work: (100)-, (110)-, and (001)-oriented rutile crystals. Four rounds of screening were conducted for each of the (100)-, (110)-, and (001)-oriented TiO_2 substrates. Each round involved the steps of phage binding, removal of low-affinity phage by washing, and elution of bound phages by a rapid pH decrease. A 0.2% TBST wash solution was used to remove phages with low binding affinity to titania during the first four rounds, whereas a more stringent 0.5% TBST wash solution was used for the fifth round. DNA was isolated and sequenced from 55 phage clones selected after the final round of biopanning against the three distinct TiO_2 substrates, which led to the identification of 53 unique 12-mer peptide sequences. A summary of the characteristics of peptides identified from biopanning against the (001)-oriented TiO_2 substrate is provided in Table 1. The peptides identified from the initial screening possessed calculated isoelectric points (pI) ranging from strongly acidic (3.1) to highly basic (12.0), with a modest average enrichment of lysine (K) and arginine (R) residues relative to the starting phage-displayed library. Additional rounds of biopanning were then conducted using washing conditions of higher stringency. In one set of experiments (modified biopanning method 1), three additional rounds of screening were conducted with a 0.5% TBST wash solution (for a total of eight rounds of biopanning). In another set of experiments (modified biopanning method 2), two additional rounds (seven rounds total) were conducted using a 0.8% TBST wash solution. The results of such modified screening against the (001)-oriented TiO_2 crystal are summarized in Table 1, with sequence details provided in Tables S1 and S2 of the Supporting Information. Through successive rounds of selection with the more stringent washes in modified biopanning method 1, the number of unique peptides detected decreased from 19 down to 2, while the average isoelectric points increased significantly (from 8.3 to 11.3) due to the enrichment of the basic amino acid residues, K and R (Table 1). A distinct enrichment in the histidine (H) content was also noted. These same trends were also observed with modified biopanning method 2, which resulted in a final population of three unique sequences with an average pI of 10.0 (Table 1). The increase in K, R, and H residues in the peptides identified with the modified biopanning methods occurred largely at the expense of hydrophobic residues, which decreased from the population average of 50% of the 12-mer residues after the initial (less stringent) five rounds of biopanning to averages of 21% and 28% of the 12-mer residues for the final pool of unique sequences isolated through the higher stringency modified screening procedures. The enrichment of K, R, and H residues was also observed in peptides identified via the increased stringency biopanning methods conducted with the (110)- and (100)-oriented TiO_2 crystal substrates (see Table S3, Supporting Information). These screening results are consistent with the recent report of Chen et al., who identified two anatase TiO_2 binding heptapeptides enriched in basic amino acid residues via screening with a constrained phage-displayed heptapeptide library.[26]

Of the total of 20 unique 12-mer peptides (sequences provided in Table S3, Supporting Information) identified through the higher stringency biopanning methods, one peptide sequence (Ti-4) was identified after screening against all three rutile TiO_2 substrates (i.e., against each of the (100)-, (110)-, and (001)-oriented rutile crystals; Figure 1). Peptide Ti-3 was also repeatedly identified during high stringency

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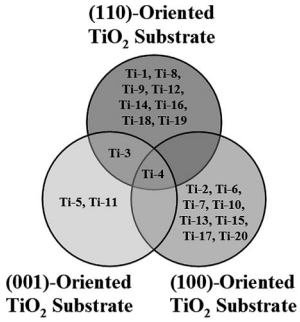

Figure 1. Venn diagram indicating the unique and common peptide sequences identified from the higher-stringency (i.e., with 0.5% or 0.8% TBST wash solutions) panning experiment against three TiO_2 substrates.

biopanning against both the (001)- and (110)-oriented TiO_2 crystal substrates. This Ti-3 peptide appears to have a high affinity for several inorganic and organic surfaces, as it has also been identified through the Ph.D.-12 library screening of FePt and GaN, as well as several acrylate-based polymers.[27,28] The remaining 18 peptides were each identified via the higher-stringency biopanning against only one of the three different rutile TiO_2 substrates (i.e., against only one of the (001)-, (110)-, or (001)-oriented rutile crystals; Figure 1).

3.2. Peptide-Induced Titania Formation. On the basis of the pattern of K, R, and H enrichment observed during the biopanning process, peptides enriched in these residues were chosen to evaluate their capabilities of inducing the formation of titania from an aqueous solution of TiBALDH. Peptides possessing a range of pI values (6.0–12.4) were also selected for evaluation of titania precipitation activity. These selected peptides were (commercially) synthesized with an extra C-terminal glycine spacer and tryptophan residue (i.e., a Gly−Gly−Gly−Trp tag) to enable evaluation of the peptide concentration by spectrophotometry at 280 nm. The peptides were added to the TiBALDH solution buffered to pH 6.3 by a sodium phosphate/citrate buffer and incubated for 10 min at room temperature. Precipitates induced by the addition of peptides were collected by centrifugation and washed at least 3 times with H_2O. The buffered TiBALDH precursor solutions were stable in excess of 24 h at room temperature in the absence of the selected peptides. The amount of titanium present in the peptide-induced precipitates was determined by a quantitative colorimetric assay.[23] The purified phage display-derived peptides with the highest titania precipitation activities, Ti-1, Ti-2, Ti-3, and Ti-4, induced the formation of 8.1–38.7 mol of TiO_2 per mole of peptide (see Table 2). The titania precipitation activities of the peptides increased with the number of calculated positive charges carried by the peptides at the assay pH of 6.3. The titania precipitation activity of peptide Ti-1 (6.3 side-group positive charges) exceeded the activities of peptides Ti-2 and Ti-3 (4 and 3.7 side-group positive charges, respectively) that, in turn, exceeded the activity of peptide Ti-4 (2.7 side-group positive charges). Thus, it appeared that both the binding of peptides to TiO_2 surfaces and the induction of TiO_2 formation from aqueous TiBALDH solutions were promoted by the presence of multiple positive charges within the peptide. These results are in accordance with previous observations that strongly polycationic molecules (natural and synthetic polyamines, highly basic recombinant silaffins rSilC and rSil1L) are capable of precipitating titania from aqueous Ti(IV) complexes at near-neutral pH.[12–18]

The results of these peptide-titania precipitation analyses were then used to design peptides with enhanced titania precipitation activity. On the basis of the sequence of the phage display-derived peptide with the highest titania precipitation activity, Ti-1, two modified peptides were designed: dTi-1(H/R) and dTi-1(RKK) (see Table 2). For the designed peptide dTi-1(H/R), the single histidine residue of peptide Ti-1 was replaced by an arginine residue. This modification was expected to decrease the pH dependence of the titania precipitation activity in the range $4 < \text{pH} \leq 8$, since the arginine side chain should remain positively charged over this range, whereas the positive charge of the histidine side chain should become progressively reduced with increasing pH values (i.e., the histidine side group $pK_a = 6$). Indeed, as shown in Figure 2, the titania formation activity of Ti-1 was substantially lower at pH 8 than at pH 4. A comparison of the curves for the peptides Ti-1 and dTi-1(H/R) in Figure 2 reveals that the pH dependence of the precipitation activity of the Ti-1 peptide in the pH range 7–8 was reduced by replacing the histidine with arginine. The influence of histidine residues on the pH dependence of titania precipitation activity was further confirmed by the observation that the histidine-rich peptide Ti-3 failed to induce the formation of any titania at pH 8 (Figure 2). The second designed peptide, dTi-1(RKK), contained a tetrameric repeat of the first three amino acid residues (RKK) of peptide Ti-1 (Table 2). The titania formation activity of dTi-1(RKK) was observed to be pH-independent from pH 4–8, as the number of positive charges carried by this designed peptide remained unchanged over this range (Figure 2).

Consistent with the observed correlation between titania precipitation activities and positive peptide charges, the dTi-1(RKK) peptide (which possessed the highest number of positive charges) exhibited the highest titania precipitation activity, 46.7 mol TiO_2/mol peptide, which corresponded to 83.9% conversion of the available Ti(IV) in TiBALDH to TiO_2 (Table 2). This titanium depletion from the starting TiBALDH solution upon exposure to a 2 mg/mL (0.99 mM) concentration of the dTi-1(RKK) peptide was on the order of the titanium depletion reported by Cole and Valentine (>92%) for TiBALDH solutions exposed to much higher concentrations of spermidine and spermine (13.3 mM) and well in excess of the titanium depletion induced by a higher concentration of the R5 peptide (about 20% depletion at R5 concentrations of > 3 mM) reported by Sewell and Wright.[17,18] The significant depletion of soluble Ti(IV) upon exposure to 1 mM dTi-1(RKK) peptide raised the concern that the titania formation activity of this peptide may have been limited by the availability of TiBALDH. Consequently, the activity of the dTi-1(RKK) peptide was examined over

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Table 2. Titania Precipitation Activities of Peptides[a]

peptide (purified)	pI	sequence	H	K + R	number of positive charges	mol TiO_2/ mol peptide	yield (%)
dTi-1(RKK)	12.8	**R K K R K K R K K R K K**G G G W	0	12	12	46.7 ± 2.0	83.9 ± 4.4
Ti-1	12.4	**R K K R** T **K** N P T **H K** L G G G W	1	6	6.3	38.7 ± 1.6	75.4 ± 3.3
dTi-1(H/R)	12.6	**R K K R** T **K** N P T **R K** L G G G W	0	7	7	34.9 ± 0.7	67.3 ± 6.1
Ti-3	10.1	**K** S L S **R H** D **H** I **H H H** G G G W	5	2	3.7	29.1 ± 1.1	61.5 ± 5.7
Ti-2	12.3	M **R** M I **R R** F P S S L **K** G G G W	0	4	4	21.2 ± 1.0	40.9 ± 1.8
Ti-4	10.0	T Q **H** L S **H** P **R** Y A T **K** G G G W	2	2	2.7	8.1 ± 1.1	16.4 ± 2.3

peptide (desalted)	pI	sequence	H	K + R	number of Positive charges	mol TiO_2/ mol peptide	yield (%)
Ti-1	12.4	**R K K R** T **K** N P T **H K** L G G G W	1	6	6.3	42.0 ± 2.8	81.8 ± 5.5
Ti-3	10.1	**K** S L S **R H** D **H** I **H H H** G G G W	5	2	3.7	24.2 ± 0.9	47.3 ± 1.8
Ti-2	12.3	M **R** M I **R R** F P S S L **K** G G G W	0	4	4	20.0 ± 2.1	38.6 ± 4.1
Ti-4	10.0	T Q **H** L S **H** P **R** Y A T **K** G G G W	2	2	2.7	5.9 ± 0.2	11.8 ± 0.0
Ti-9	11.0	N **H H H** Q P L A **R** N Q S G G G W	3	1	2	2.5 ± 0.2	0.5 ± 0.0
Ti-8	7.1	A S I E E L **R** V P **R** Q A G G G W	0	2	2	0.6 ± 0.0	<0.1
Ti-6	10.5	L **K** M N P S I S S S L **K** G G G W	0	2	2	0.4 ± 0.0	<0.1
Ti-20	6.0	L A T P F T A T S A T G G G G W	0	0	0	0.4 ± 0.0	<0.1
Ti-5	12.0	S **R** P S **R** Q P S A S P T G G G W	0	2	2	0.2 ± 0.0	<0.1
Ti-11	9.6	L L A D T T **H H R** P W T G G G W	2	1	1.7	0.0 ± 0.0	0.0 ± 0.0
Ti-18	7.8	S P G L S L V S **H** M Q T G G G W	1	0	0.3	0.0 ± 0.0	0.0 ± 0.0

[a] Peptide theoretical isoelectric point (pI) was calculated with software available at the Web site http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/compo-p.html. Titania precipitation activities for chromatographically purified and desalted grade peptides were determined (differences in activity of the same peptide reflect the differences in purity). pI = isoelectric point. H = number of histidine residues. K + R = combined number of lysine and arginine residues. The yield (%) refers to the percentage of soluble Ti(IV) present in the starting precursor solution that was precipitated as titania in the presence of the peptide. Reported values represent the average of at least three independently prepared samples. The ± ranges indicated for the titania precipitation activities and yields refer to ± one standard deviation.

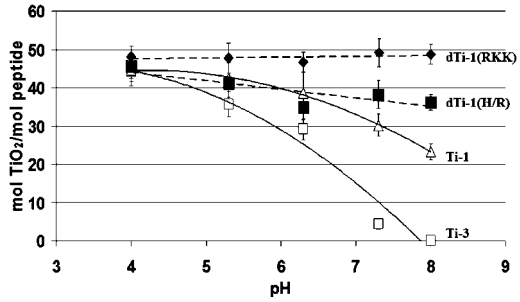

Figure 2. pH dependence of titania precipitation activity. The titania precipitation activities of peptides possessing histidine residues (solid lines) were more affected (reduced) by increasing pH than those lacking histidine (dashed lines). The precipitation activities of peptides Ti-1 and Ti-3 are marked with open triangles and squares, respectively, and are indicated with solid lines. The precipitation activities of peptides dTi-1(H/R) and dTi-1(RKK) are marked with filled squares and diamonds, respectively, and are indicated with dashed lines.

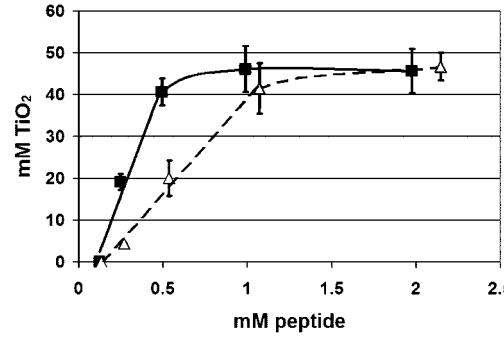

Figure 3. Dependence of the quantity of titania formed (at pH 6.3) on the peptide concentration. The values obtained for peptides dTi-1(RKK) and Ti-1 are marked by filled squares with a solid line and open triangles with a dashed line, respectively.

a range of peptide concentrations. A linear increase in the amount of precipitated titania with increasing peptide concentration was observed for dTi-1(RKK) concentrations below 0.5 mM (1 mg/ml), whereas the titania precipitation activity for this peptide reached a plateau at higher peptide concentrations (Figure 3). Hence, at a 1 mM concentration of the dTi-1(RKK) peptide, the precipitation activity was indeed limited by the TiBALDH availability. In the linear regime of Figure 3, the titania formation activity of dTi-1(RKK) was almost twice as high (82.9 mol TiO_2/mol peptide, as determined from least-squares fitting of the precipitation yields up to a dTi-1(RKK) concentration of 0.5 mM) as under Ti-limiting conditions (46.7 mol TiO_2/mol peptide; Table 2). For peptide Ti-1, which exhibited the highest titania precipitation activity (38.7 mol TiO_2/mol peptide; Table 2) of the phage display-derived peptides, a good fit to linear behavior could be obtained in Figure 3 for precipitation yields up to a peptide concentration of 1 mM; that is, the Ti-limiting condition for peptide Ti-1 was reached at peptide concentrations beyond 1 mM.

3.3. Characterization of the Peptide-Induced Titania. The structures of the precipitates generated by the peptides Ti-1, Ti-2, Ti-3, Ti-4, dTi-1(H/R), and dTi-1(RKK) were evaluated by scanning electron microscopy (Figure 4 and Figure S2 of the Supporting Information). Secondary electron micrographs revealed that the precipitation products generated by peptides Ti-1, Ti-2, Ti-3, and dTi-1(RKK) consisted of particles approximately 50–100 nm in diameter that were necked together to form a nanoparticulate network (Figure 4A and Figure S2 of the Supporting Information). The Ti-4 and dTi-1(H/R) peptides also induced the formation of nanoparticulate networks, but the apparent particles possessed larger diameters (Figure 4B,C).

Energy dispersive X-ray spectroscopy (EDXS) indicated that the peptide-induced precipitates contained titanium, oxygen, and phosphorus (see Figure 4D, E). The presence of phosphorus indicated the incorporation of phosphate ions from the buffer solution in the precipitate. The presence of

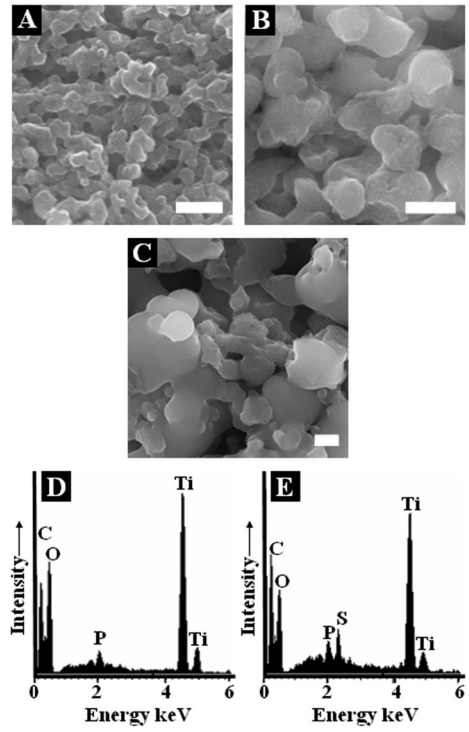

Figure 4. Structures and elemental compositions of peptide-induced titania precipitates. (A−C) Secondary electron images of titania particles generated in the presence of (A) Ti-1 peptide, (B) Ti-4 peptide, or (C) dTi-1(H/R) peptide. Elemental analyses by EDXS of the titania particles formed in the presence of the (D) Ti-1 peptide or (E) Ti-2 peptide. Scale bars: (A and B) 200 nm, (C) 400 nm.

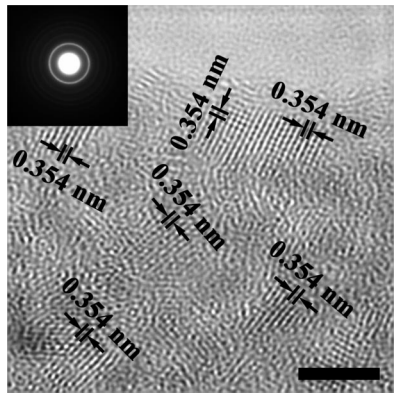

Figure 5. High-resolution transmission electron image and electron diffraction pattern (inset) of a cross-section of a dTi-1(H/R)-induced titania particle. The lattice fringe spacings indicated by arrows were consistent with both the (101) plane spacing of anatase and the (110) plane spacing of monoclinic β-TiO_2. Similar results were obtained with the titania induced by the other peptides. The scale bar corresponds to 5 nm.

sulfur in the Ti-2-induced precipitate was due to incorporation of the peptide, which contained two residues of the sulfur-bearing amino acid, methionine (Figure 4E). These observations were consistent with TGA analyses, which indicated the presence of 45.8 + 2.5% pyrolyzable material in the precipitates formed by the Ti-1, Ti-2, Ti-3, and Ti-4 peptides.

High-resolution transmission electron microscopy (TEM) analyses (Figure 5 and Figure S3 of the Supporting Information) revealed that the peptide-induced titania particles consisted of a mixture of fine nanocrystals (<10 nm in size) along with an amorphous phase. In their investigation of

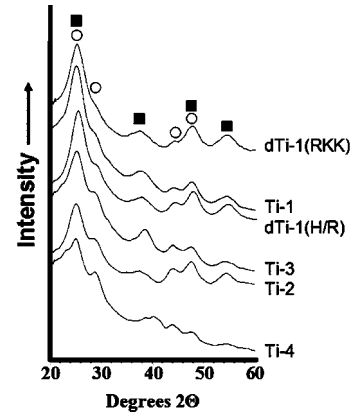

Figure 6. X-ray diffraction analyses of peptide-induced titania. The peptide used for titania precipitation is indicated next to the X-ray diffraction pattern. Open circles and filled squares represent diffraction peaks attributable to the monoclinic (β-TiO_2) and anatase polymorphs of TiO_2, respectively.

silicatein-induced TiO_2 formation, Sumerel et al. reported the formation of a mixture of anatase and amorphous oxide.[12] In the present work, electron and X-ray diffraction analyses (Figures 5 and 6) revealed the presence of both anatase and monoclinic β-TiO_2 crystals within the peptide-induced nanoparticles. Indexing of several electron diffraction patterns yielded *d* spacings of 3.5, 2.1, 1.73, and 1.22 Å, which were consistent with the *d* spacings associated with the (101) plane of anatase and the (110) plane of β-TiO_2, the ($\bar{6}01$) plane of β-TiO_2, the (105) plane of anatase, and the (910) plane of β-TiO_2, respectively. The relatively broad electron diffraction rings (Figure 5 inset) and X-ray diffraction peaks (Figure 6) were consistent with the presence of very fine titania crystallites of the type observed in Figure 5. The relatively high background intensities observed at low 2θ values in the X-ray diffraction analyses were also consistent with the presence of an amorphous phase within the precipitates.

The titania-forming peptides identified through extensive biopanning against three rutile TiO_2 single-crystal substrates yielded precipitates containing nanocrystals of only the anatase and monoclinic (β) polymorphs of titania. While nanocrystalline mixtures of anatase and monoclinic titania can exhibit attractive photocatalytic properties,[29] the inability of any of the titania-forming peptides to induce rutile titania formation was striking. Recent work has shown that a 17 kDa recombinant silaffin, rSilC, possessing a particularly highly repetitive arrangement of arginine and lysine residues can induce the formation of rutile TiO_2 from an aqueous TiBALDH solution under ambient conditions and neutral pH.[13] Those results coupled with the present work suggest that 16-mer titania-forming peptides lack the molecular complexity (e.g., three-dimensional structure and cooperative effects between domains) that may be required for rutile TiO_2 formation under ambient conditions.

3.4. Influence of Reaction Conditions on Precipitate Structure. In several recent studies, solution conditions (i.e., pH, buffer chemistry, and buffer concentration) have been found to influence both the ability of biomolecules to induce precipitation and the morphology of the resulting pre-

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cipitates.[15,18,30–32] In order to assess the possible influence of these factors on the peptide-mediated formation of titania, precipitation trials were conducted utilizing pH 3–8 phosphate-citrate buffers (i.e., constant chemistry and variable pH) and several pH 6 buffers (i.e., variable chemistry, constant pH). Peptide Ti-1 was observed to generate a white precipitate under all conditions examined. SEM analyses indicated that the Ti-1-induced TiO_2 nanoparticulate networks also possessed similar morphologies under all of the conditions tested (see Figures S4 and S5, Supporting Information). The X-ray diffraction patterns (see Figures S6 and S7, Supporting Information) obtained from the Ti-1-induced precipitates under varied conditions were nearly identical, which indicated that the sizes and phases of the titania nanocrystals were not appreciably affected by the different solution conditions.

4. Summary

Phage-displayed, 12-mer peptides that were identified on the basis of their binding affinity to titania single crystals exhibited a strong enrichment in arginine, lysine, and histidine residues. Synthetic 16-mer versions of these peptides (containing a tryptophan-bearing tetramer tag for spectrophotometric identification) that possessed > 2 net positive charges under the precipitation assay conditions induced the rapid formation (≤ 10 min) of nanoparticulate titania from an otherwise stable aqueous TiBALDH solution at pH 6.3. The amount of titania formed by a given peptide was found to increase with the number of positive charges carried by the peptide. X-ray diffraction analyses, electron diffraction analyses, and high-resolution transmission electron microscopy revealed that the peptide-induced TiO_2 nanoparticles (≥ 50 nm in size) contained very fine (< 10 nm) anatase and monoclinic β-TiO_2 crystal domains. These observations were used to design a peptide that possessed a titania precipitation activity of 82.9 mol TiO_2/mol peptide over a relatively wide pH range of 4–8.

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Supporting Information Available: Peptide sequences identified during additional steps of the modified biopanning methods 1 and 2, MALDI-MS analyses of purified peptides, SEM and TEM images of peptide-induced TiO_2, and SEM images and XRD characterization of the TiO_2 induced by peptide Ti-1 under varied conditions of pH or with varied buffers at fixed pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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