Published in final edited form as:

ACS Nano. 2010 July 27; 4(7): 3883–3888. doi:10.1021/nn100630v.

Unusual Reactivity of a Silver Mineralizing Peptide

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Abstract

The ability of peptides selected via phage display to mediate the formation of inorganic nanoparticles is now well established. The atomic-level interactions between the selected peptides and the metal ion precursors are in most instances, however, largely obscure. We identified a new peptide sequence that is capable of mediating the formation of Ag nanoparticles. Surprisingly, nanoparticle formation requires the presence of peptide, HEPES buffer, and light; the absence of any one of these compromises nanoparticle formation. Electrochemical experiments revealed that the peptide binds Ag⁺ in a 3 Ag⁺:1 peptide ratio and significantly alters the Ag⁺ reduction potential. Alanine replacement studies yielded insight into the sequence-function relationships of Ag nanoparticle formation, including the Ag⁺ coordination sites and the residues necessary for Ag synthesis. In addition, the peptide was found to function when immobilized onto surfaces, and the specific immobilizing concentration could be adjusted to yield either spherical Ag nanoparticles or high aspect ratio nanowires. These studies further illustrate the range of interesting new solid-state chemistries possible using biomolecules.

Keywords

Silver nanoparticles; biomineralization; peptide; amino acids

In vitro selection experiments involving RNA, phagemids, or whole cells can yield biomolecules that bind tightly to or mediate the formation of inorganic materials.^{1–10} Many of these biomolecules enable the synthesis of materials under more environmentally benign conditions (e.g., aqueous solutions, low temperatures, and neutral pH) and can be used to assemble nanomaterials on surfaces^{4, 11} and into thin films¹². The ability to perform spatially resolved nanoparticle synthesis using biological systems has been demonstrated to be a powerful tool in the fabrication of functional devices.⁵

One of our primary research objectives is to exploit biomineralization in the assembly of two or more chemically distinct nanoparticle materials in predetermined locations either on a surface or inside a living cell for applications in nanoscale patterning and intracellular imaging. In pursuing these goals it is important for us to characterize the specificity of biomolecule mineralization agents toward a range of metal precursors. During the course of our studies, we have discovered a peptide (SLKMPHWPHELLP) with an unusual reactivity in the conversion of Ag⁺ to Ag nanoparticles. The reaction required the presence of Ag⁺, HEPES buffer, light, *and* peptide to occur. The absence of any one of these ingredients compromised particle formation. These results are discussed within the context of changes in the Ag⁺ reduction potential upon coordination to the peptide and the photodecomposition of HEPES. Furthermore, as one of our primary objectives is to isolate peptides that mediate

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solid-state reactions when immobilized, the peptide was deposited onto TEM grids and assayed for activity. Incubation of the peptide-coated TEM grids in a solution of Ag^+ and HEPES showed that the immobilized peptide was active toward Ag particle formation. Surprisingly, depending upon the immobilization concentration, spherical particles or high aspect ratio wires that appeared to grow out from the points of triangular Ag particles were observed to form. The unusual reactivity of this peptide further illustrates the range of solid-state chemical processes that biomolecules can influence.

Results and Discussion

The peptide of interest in this work was SLKMPHWPHLLP, which was originally isolated from a phage library by Sandhage and coworkers⁸ based upon its ability to bind tightly to germania. The peptide, named Ge8, was also reported to catalyze the formation of germania networks when incubated with tetramethoxygermanium in methanol. Given the presence of a strong reducing agent (tryptophan), metal ion coordination and general acid/base catalysis site (histidine), and common metal nanoparticle capping agent (amine of lysine), we decided to investigate the reactivity and selectivity of Ge8 in the formation of a variety of metal and metal oxide nanoparticles. The catalytic activity of Ge8 toward metal oxide nanoparticle formation was investigated first with the metal precursors Zn^{2+} , Zr^{2+} , Ga^{2+} , Ti^{4+} , and Ge^{2+} . All metals were incubated in concentrations ranging from 10 μM – 10 mM with 690 μM Ge8 at room temperature. The experiments were repeated in acetone, methanol, and water. A range of buffers were explored for experiments in which water was the solvent: pH 7.3 HEPES buffer, pH 7.1 1X PBS buffer, pH 7.5 TBS buffer, and pH 7.5 Tris buffer. Only Ge^{2+} in methanol was observed to yield a precipitate in solution and solid network structures by TEM (identical in appearance to the material reported by Sandhage).

The reactivity of Ge8 with Au and Ag salts was then investigated. Incubation of 690 μM Ge8 with AgNO_3 or HAuCl_4 (at concentrations between 10 μM – 500 mM) in milliQ 18 M Ω H_2O at room temperature for 48 hrs did not result in Ag or Au nanoparticle formation as observed by visible spectroscopy and transmission electron microscopy (TEM). HEPES was then included in the reaction solution. HEPES was chosen because it is known to act as a reducing agent in the formation of peptide-capped Au nanoparticles.¹³ However, incubation of 100 mM HAuCl_4 with 690 mM Ge8 and 500 mM HEPES at room temperature resulted in the immediate formation of a massive black precipitate. When investigated by TEM, polydisperse micron-sized pieces of material with irregular morphologies were observed.

In contrast to the behavior of Ge8 and Au, solutions containing Ge8, HEPES, and Ag^+ turned from clear and colorless to a faint orange color over the course of 24 hrs. Visible spectroscopy revealed a broad absorbance centered at 410 nm, which we attribute to the Ag nanoparticle dipolar plasmon resonance (Figure 1). TEM showed that the solutions contained spherical particles $4.1 \text{ nm} \pm 0.9 \text{ nm}$ in diameter (Figure 1).

The role of light in the production of Ag nanoparticles with HEPES and Ge8 was then explored. Light is known to cause the spontaneous reduction of Ag^+ in solution even in the absence of buffers and peptides. Solutions containing 10 mM AgNO_3 and 100 mM HEPES, and exposed to light for 24 hrs did not yield the characteristic plasmon band of Ag nanoparticles (e.g., that shown in Figure 1) or show any evidence of bulk Ag formation. Moreover, when solutions containing Ge8, AgNO_3 , and HEPES were kept in the dark, nanoparticle formation was not observed over the course of 120 hrs. Thus, in this unusual reaction, the presence of light, HEPES, and Ge8 are required to convert Ag^+ to Ag nanoparticles.

Electrochemical studies were performed to determine the effect of the peptide on the Ag^+ reduction potential. Belcher has suggested that a glutamate hexamer (E_6) lowers the reduction potential of Ag^+ , enabling the rapid and photoinduced production of Ag nanoparticles.¹⁴ Cyclic voltammetry of HEPES solutions containing E_6 and Ag^+ revealed no difference in the Ag^+/Ag^0 redox potential vs. solutions not containing E_6 . Solutions containing Ag^+ and Ge8, however, showed that Ag^+ cannot be reduced in a potential window of +0.8 V to -0.4 V (vs. Ag^+/AgCl) for $\text{Ag}^+:\text{Ge8}$ ratios less than 3:1 (Figure 2). For ratios greater than 3:1 (4:1, 5:1....10:1), Ag^+/Ag^0 redox was observed to occur at a potential identical to that of the Ag^+/Ag^0 redox couple in HEPES buffer prepared without Ge8. This suggests that Ge8 binds to Ag^+ in a 3:1 ratio and renders Ag^+ reduction energetically more difficult.

In an attempt to elucidate the role of specific amino acids of Ge8 on its function, four alanine replacement experiments were performed. When either one of the histidines in Ge8 were replaced with an alanine, the Ag^+/Ag^0 redox couple was observed at any $\text{Ag}^+:\text{peptide}$ ratio. This suggests that the binding interaction between Ag^+ and Ge8 is compromised by the loss of a single histidine. Despite the loss of the $\text{Ag}^+/\text{Ge8}$ interaction, however, Ag nanoparticle formation was still observed (5.3 nm $\pm$ 1.4 nm diameter; Figure 3). When either the methionine or tryptophan residues were replaced with alanine, the Ag^+/Ag^0 redox couple was again only observed at $\text{Ag}^+:\text{peptide}$ ratios $>3:1$. This peptide, however, was unable to mediate the formation of nanoparticles, and Ag^+ reduction was not evident by eye, visible spectroscopy or TEM. It thus appears that the 3 $\text{Ag}^+:\text{1 Ge8}$ binding interaction requires the histidine residues, while the formation of Ag nanoparticles is dependent upon the presence of both methionine and tryptophan but not histidine.

In addition to systematically replacing select amino acids of Ge8 with alanine, two peptides containing more substantial changes were investigated. One peptide contained both methionine and tryptophan (AYSSGAWPMPPF), but was otherwise selected to be a largely random sequence compared to Ge8. This peptide was found to be active toward bulk Ag precipitation as observed by visible spectroscopy, but did not mediate the formation of stable Ag nanoparticles. The presence of methionine and tryptophan, although necessary for Ge8 function, is not sufficient in general for Ag nanoparticle formation by peptides.

The second peptide contained all of the residues of Ge8, but was randomly scrambled (MSLPHKPPHWLL) to determine the dependence of amino acid position on Ge8 function. Nanoparticle formation was observed with the scrambled Ge8 peptide, but with a larger size and size dispersity than particles synthesized with Ge8 (7 nm $\pm$ 3 nm). These data suggest that both the specific amino acids and their primary sequence influence Ag^+ reduction and Ag nanoparticle growth.

Our interest in using biomolecules to perform surface-bound, spatially resolved materials synthesis¹⁵ prompted us to investigate the ability of Ge8 to mediate the formation of Ag nanoparticles when immobilized onto a surface. TEM grids were glow discharged to provide a negatively charged surface, allowing electrostatic adsorption of the peptide onto the surface. Drops (20 μL) of solutions containing various concentrations of Ge8 were placed onto grids for two minutes and then the solution was wicked away with filter paper, leaving Ge8 adsorbed to the grid. The peptide-coated grid was then inverted onto a droplet containing 10 mM AgNO_3 and 100 mM HEPES and the grids examined by TEM. Solutions containing 8.7 mM and 4.4 mM Ge8 led to the formation of spherical nanoparticles, ca. 20 nm and 10 nm in diameter, respectively, following 24 hrs of grid exposure to the Ag^+/HEPES droplet. Ge8 transferred to the TEM grid from a solution containing 690 μM Ge8, however, yielded high-aspect ratio, curled wires, that appear to grow from the points of

triangular nanoparticles (Figures 4A and 4B). Electron diffraction of the wires revealed six rings in total that could be indexed closely to fcc Ag (Figure 4C).

Finally, the photochemistry involved in the synthesis of Ag nanoparticles was considered. It has been reported that fluorescent light causes the photodecomposition of HEPES in aqueous solution with the concomitant production of H_2O_2 .¹⁶ To determine if H_2O_2 might play a role in Ag nanoparticle formation, a solution containing 100 mM H_2O_2 and 10 mM AgNO_3 was prepared and kept in the dark. After 24 hrs a black solid was observed to form. These results are consistent with those reported by Eychmuller et al., who have employed H_2O_2 as a reducing agent in the synthesis of Ag microstructures.¹⁷ It is thus plausible that H_2O_2 produced from the photodecomposition of HEPES may be acting as the reducing agent in the production of the Ag nanoparticles and nanowires by Ge8. We note, however, that Ge8 must be serving as more than a simple nanoparticle capping agent as the production of solid Ag from HEPES and light did not occur in the absence of Ge8.

Peptides isolated via phage display to mediate the formation of inorganic nanoparticles are yielding interesting insights into the range of chemistries possible by biomolecules and are providing new opportunities to synthesize materials under more environmentally benign conditions (e.g. neutral pH, aqueous solutions). Of interest in this work was the selectivity of peptides for certain metal precursors and whether changes in reaction conditions will lead to changes in peptide function. For instance, how does an immobilized peptide behave compared to a freely diffusing peptide? Does the buffer system affect materials synthesis? Will a peptide selected to interact with one metal precursor accept another precursor?

The Ge8 peptide was originally selected via phage display to bind to germania. Ge8 was also discovered to mediate the formation of germania network structures from solutions of tetramethoxygermanium in methanol. The work presented here has shown that Ge8 is selective and does not by itself mediate the formation of inorganic nanoparticles when challenged with a number of other metal and metal oxide precursors. This may be contrasted to the behavior of the protein silicatein, which tolerates a number of metal precursors.^{18–20} Surprisingly, however, when Ge8 was incubated with Ag^+ and HEPES and kept in ambient light for ca. 24 hrs, nanoparticles were observed to form. The absence of any one of these ingredients completely compromised nanoparticle formation.

While the mechanism of Ag nanoparticle formation in the presence of Ge8 remains to be elucidated, some information was obtained concerning sequence-function relationships and the role of light in the reaction (Figure 5). It has been posited by others that peptides such as E6 may serve to lower the Ag^+ reduction potential. However, our studies showed that E6 does not alter the energy required to reduce Ag^+ . Ge8, in contrast, does bind to Ag^+ in a 3 Ag^+ :1 Ge8 ratio, but this appeared to prevent rather than promote Ag^+ reduction. The key residues involved in Ag^+ -Ge8 binding were determined to be the histidines, although replacing the histidines for alanine revealed that Ag^+ coordination was not necessary for nanoparticle formation. The most important amino acids for Ag nanoparticle formation were methionine and tryptophan. Alanine replacement showed an absolute dependence on these residues for Ag particle formation. Despite this, methionine and tryptophan were not found to be the exclusive requirement for Ag synthesis as another peptide containing these amino acids was not able to form stable nanoparticle sols under identical synthesis conditions. The arrangement of amino acids in Ge8 proved to be only important to the resulting particle size and size dispersity; a scrambled Ge8 peptide produced nanoparticles that were on average nearly 2× larger and 3× more polydisperse in diameter.

The possibility that the relevant photochemical process involved the decomposition of HEPES to produce H_2O_2 was considered and tested by incubating Ag^+ and H_2O_2 in the

dark. Solid black microparticles were observed to form from this solution, which were similar in appearance in the electron microscope to the Ag particles obtained by Eychmüller et al. We propose that the photodecomposition of HEPES, along with its inherent reducing power¹³, should be considered when choosing a buffer system for the biological synthesis of metal and metal oxide nanoparticles.

The Ge8 peptide was also observed to function when immobilized on surfaces. When adsorbed onto a surface from solutions containing 690 μM Ge8, Ag wires were observed to grow from the points of triangular Ag particles. While a detailed mechanism for the behavior remains to be elucidated, we speculate that certain densities of peptide on a surface are capable of stabilizing {111} facets of Ag, causing the growth of thin triangles bound by top and bottom {111} facets. When the triangles reach a certain size, it is possible that further growth becomes diffusion controlled, with the fastest rates of mass transport occurring at the tips of the triangles.

Conclusions

The synthesis of metal and metal oxide nanoparticles in the presence of a peptide isolated via phage display has been examined. The peptide of interest in this work, Ge8, was originally isolated from a phage library by Sandhage and coworkers⁸ based upon its ability to bind tightly to germania. The most surprising result to emerge from this study is that this peptide also mediates the formation of Ag nanoparticles, and product formation required the presence of the peptide, HEPES, and light. In addition, it was determined that H_2O_2 produced via the photoinduced decomposition of HEPES is likely a key reactant in Ag^+ reduction. In contrast to a prior hypothesis suggesting that the role of Ag-forming peptides is to lower the energy of Ag^+ reduction, the peptides studied here (e.g., Ge8, E₆) either did not lower the formal potential of the Ag^+/Ag^0 redox couple or made the redox couple completely inaccessible within the potential window explored (0.8 V to -0.4 V vs. Ag/AgCl). Finally, we have observed a dramatic change in particle growth upon immobilizing Ge8 on a solid support. The Ge8 peptide, which formed exclusively spherical nanoparticles in solution, produced Ag nanowires when adsorbed onto a TEM sample grid. The wires appeared to grow from the points of triangular nanoparticles. These studies illustrate that Ag nanoparticle formation reactions mediated by peptides do not require a lowering of Ag^+ reduction potentials and that decomposition reactions involving buffers such as HEPES can produce active byproducts that influence the production of inorganic nanoparticles.

Methods

Materials

All reagents were used as received from the manufacturer. All solutions were made using high purity water (18.2 M Ω , Millipore) and adjusted to the appropriate pH using NaOH, HCl or HNO_3 . All peptides were purchased from Genscript. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and AgNO_3 (Certified ACS) were purchased from Fisher (BioReagent, 1 M, pH 7.3).

Ag particle synthesis with Ge8

Peptide Ge8 (SLKMPHWPHLLP), the scrambled Ge8 (MSLPHKPPHWLL) or mutant peptides (SLKAPHWPHLLP, SLKMPAWPALLP, SLKMPHAPHLLP) (690 μM) were combined with 10 μM -10 mM AgNO_3 in the presence of 100 μM -1 mM HEPES at room temperature, in light, in a sealed eppendorf tube. The random peptide AYSSGAWPMPPF was incubated as stated above.

Transmission Electron Microscopy

Electron Microscopy was performed using a Phillips CM100 with and accelerating voltage of 100 kV. The images were obtained on an AMT CCD ($2k \times 2k$). Typically a 20 μ L drop of the nanoparticle solution was placed onto a glow discharged TEM grid (300 mesh carbon coated copper, EM science) for 30 seconds and the solution was wicked away. Peptide immobilization was achieved by placing a 20 μ L drop of 690 μ M Ge8 onto a glow-discharged grid for 2 minutes and the solution was wicked away. The grid was then inverted onto a droplet containing the appropriate concentration of AgNO₃ and HEPES. The inverted reactions were kept in a humid environment for 24h to avoid evaporation. The grid was then removed from the droplet and the solution wicked away.

Electron Diffraction

Diffraction data was collected on a FEI F20 transmission electron microscope with a 200 kV accelerating voltage equipped with a $4k \times 4k$ Gatan CCD (15 μ m pixel size). The camera length was determined by obtaining a diffraction pattern from the Cu grid bar. The grid bar diffraction was indexed to elemental copper, allowing the assignment of a camera constant to the experimental data, which were collected with identical objective and C2 lens settings.

UV-Visible spectroscopy

UV-visible spectra were obtained using either a Genesys 10UV Scanning spectrophotometer (Thermo) or a FLUOstar Omega plate reader (BMG Labtech).

Electrochemistry

Electrochemical measurements were performed using a 3 mm glassy carbon electrode with 100 mM KNO₃ as supporting electrolyte. A Ag/AgCl electrode was used as a reference, while a Pt wire was used as the counter electrode. All cyclic voltammograms were performed using a BASi potentiostat.

Acknowledgments

DLF thanks the Charles and Jane Butcher Foundation, the NIH (1R01GM086200-01), and the University of Colorado for support. This work was performed in part at The NIH Boulder Laboratory for 3D Electron Microscopy of Cells.

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Figure 1.

UV-visible spectrum and TEM image of Ag particles formed in a solution containing 690 μM Ge8, 10 mM AgNO_3 and 100 mM HEPES, exposed to ambient light and room temperature for 24 hrs.

Figure 2. Cyclic voltammograms of solutions containing 690 μM Ge8, 100 mM HEPES and A) 1, 2 and 3 molar equivalents of $\text{AgNO}_3\text{:Ge8}$ and B) 4 molar equivalents of AgNO_3 . Working, auxiliary, and counter electrodes were glassy carbon, Pt, and Ag/AgCl, respectively.

Figure 3.
TEM image of particles formed using Ge8 containing alanine instead of histidine.

Figure 4.

A) and B) TEM images of curled wires formed via immobilized Ge8 incubated in 10 mM AgNO_3 and 100 mM HEPES. C) Diffraction pattern of curled wires with d spacings corresponding to 1 (111), 2 (220), 3 (420) labeled.

Figure 5.

Summary of the peptides examined in this study and their ability to mediate the formation of Ag nanoparticles.