Small Au Nanoparticles Synthesized by Peptide-based Biomineralization for Catalytic Applications

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ABSTRACT Biomineralization processes have garnered considerable attention owing to their potential for synthesizing functionally and technologically important nanomaterials in an environment-friendly manner. In this study, we synthesized small gold nanoparticles (AuNPs) ranging in size from 2.5 to 2.9 nm using three biomineralization peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL) without other supplements such reducing agents or triggers. Dynamic light scattering and thermogravimetric analyses confirmed the presence of peptide molecules on the synthesized AuNPs. We quantified the catalytic activity of

the synthesized AuNPs by analyzing the reduction of 4-nitrophenol and methyl orange by borohydride. Overall, the AuNPs synthesized by peptide biomineralization exhibit catalytic properties and have potential for bioremediation of wastewater through environment-friendly processes.

INTRODUCTION

Nitroaromatic compounds (e.g., 4-nitrophenol) and organic dyes (e.g., methyl orange) have received attention owing to their potential hazardous side effects in animals and humans, including skin irritation, liver damage, and mutagenic effects. These chemicals are released into the water environment (lakes, rivers, and oceans) by various industries, posing risk to aquatic life, the ecosystem, and even human health. The importance of addressing the reduction of these target molecules and employing catalytic processes for environmental remediation cannot be overstated. Reduction of nitroaromatic compounds and organic dyes is crucial for mitigating their adverse effects on both environmental and human health.[1,2] Catalytic reactions offer promising solutions for efficient and selective reduction of these pollutants, leading to their detoxification and elimination from water supplies. Regarding the state of the art in water decontamination, various technologies have been investigated [3–5], including activated carbon filtration, microorganisms, chemical precipitation, membrane filtration, UV/Ozone treatment, photocatalysis, Fenton processes, and catalytic reduction.[6–8] In the development of these technologies, including catalytic reactions, efforts are underway to create effective, safer, and more environmentally-friendly decontamination methods.

Metallic nanoparticles possess large surface areas and greater edge step ratios per volume or mass unit compared to bulk metals, making them excellent candidates for reduction catalysis in

the removal of water contaminants.[9] The size of metal nanoparticles plays a crucial role in their catalytic abilities as chemical reactions occur primarily on the catalyst surface. Firstly, they the reactivity scales with surface area and secondly nanoparticles can have more edge sites that have higher potency. For example, gold nanoparticles (AuNPs) with diameters less than 10 nm are reported to be highly active catalysts; however, this catalytic activity diminishes considerably with increasing diameter.[10]

Many types of metallic nanocatalysts have been synthesized using various ligands, such as cetrimonium bromide, typically in combination with a reducing agent. However, these methods typically require toxic surfactants, high temperature and pressure conditions, strong reducing reagents, or organic solvents.[11,12] Materials prepared using these methods also require post-treatment processing, such as ligand exchange and cleaning to eliminate the potential of undesirable health and environmental effects of the particles.

Several biomineralization peptides capable of reducing Au(III) to Au(0) and forming AuNPs have been isolated from a series of Au-binding peptides identified using various peptide screening techniques (e.g., phage and cell surface display libraries).[13–17] GBP1 (MHGKTQATSGTIQS), a well-investigated Au-binding peptide,[18,19] was originally screened from cell surface displays using *Escherichia coli* and biomineralized 1–3 μm AuNPs.[17,20] A3 (AYSSGAPPMPPF), screened from phage display systems, has been reported to form spherical 10 nm AuNPs.[14] Midas-2 (TGTSVLIATPYV), also obtained from phage display, produced nanosized spherical particles and trigonal and hexagonal platelets, depending on the pH conditions.[13] In addition to these reports, several peptides as well as cellular extracts from various biological resources have been documented to contribute to AuNP synthesis through environment-friendly methods. However, all of these studies either report larger-sized

nanoparticles, or their nanoparticle synthesis process required the addition of reagents (e.g., reducing agents) and/or additional triggers (e.g., heat, light, or pH) (see Supplementary Table 1).[21–24]

Our research group has previously developed a peptide array technique to screen AuNP-binding peptides.[25] Subsequently, among these AuNP-binding peptide libraries, more than 150 peptides that are also suitable for AuNP biomineralization were isolated by adapting the peptide array technique.[26] The color and intensity of the biomineralized AuNPs (e.g., red, green, orange, and purple) were obtained at each spot on the array after the mineralization reaction. Because the optical properties of AuNPs are highly related to their particle size and morphology based on their localized surface plasmon resonance and light scattering properties, the color of the array spot allows rapid identification of a set of peptides suitable for the formation of AuNPs of defined size and morphology. For example, the B3 peptide (ASHQWAWKWE) creates a blue spot on the peptide array due to biomineralized AuNPs with decahedral and triangular nanoplate structures in solution.[27] Our aim was to find peptides capable of synthesizing small Au nanoparticles which would be predicted to have higher catalytic activity. Just three peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL) produced green-colored AuNPs on the peptide array – indicating that these peptides could synthesize small AuNPs. In fact, detailed analysis of these peptides showed that they synthesized small AuNPs (< 3 nm), not only on the peptide array, but also when free in solution (Scheme 1). These AuNPs are synthesized using a method involving simple mixing of chloroauric acid and a peptide without any other supplements, such as reducing agents or triggers. There is no report about peptide-based one-pot AuNP biomineralization ranging in this small size (< 3 nm) without other supplements such reducing agents or additional triggers (Supplementary Table 1). As their

surface area to volume plays a crucial role in catalytic activity, this study aims to further characterize and to compare these small AuNPs synthesized using G1–G3 peptides with the strongest mineralization activity and investigate their catalytic reduction activities on organic dyes and nitroaromatic compounds, leveraging their small size properties. In general, model nitroaromatic compound (4-nitrophenol: 4-NP) and organic dye (methyl orange: MO) are shown to be reduced by the electron transferred from the coadsorbed BH_4^- on the AuNP surface. Because AuNP synthesis is a one-pot process occurring under mild aqueous conditions, exploring their catalytic properties can provide an eco-friendly and simple method to synthesize small AuNPs for environmental applications.

MATERIALS AND METHODS

Peptide preparation for small AuNP synthesis

The peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL) were synthesized using the standard Fmoc-based solid-phase protocol with a ResPep SL automatic peptide synthesizer (Intavis Bioanalytical Instruments AG, Köln, Germany), as previously described.[28,29]

Table 1 A list of peptides used in this study[a]

Peptide name	Mineralization peptide	pI	GRAVY	Net charge
G1	ETGHHIWEWM	5.06	-0.99	-2
G2	TQWHEWHWYQ	6.05	-2.16	-1
G3	GMWHIEHIWL	6.05	0.26	-1

[a] pI and GRAVY were calculated from ProtParam tool (http://web.expasy.org/protparam/)[30]

Peptide-based small AuNP mineralization and AuNP characterization

Purified peptides were dissolved in Tris-buffered saline (pH 7.4) to obtain a peptide concentration of 0.25 mM, while 10% DMSO (final conc.) was supplemented for G3 peptide to dissolve in this solution. The biomineralization reaction was then initiated by adding $HAuCl_4$ at a final concentration of 0.5 mM. This reaction proceeded according to the following equation:

$$\text{Peptide} + \text{HAu(III)Cl}_4 \rightarrow \text{Au(0)NPs}$$

The reaction was conducted at 25 °C for 24 hours. To prevent evaporation during the reaction, mineral oil was overlaid on the reaction mixture. To monitor the mineralization reaction with peptide oxidation, a fluorescence microplate reader (SpectraMax ID5; Molecular Devices, San Jose, CA, USA) was used to measure the fluorescence spectrum of a single peptide molecule, as previously reported,[31,32] with an excitation wavelength of 350 nm. Transmission electron microscopy (TEM) analysis was performed using a Hitachi H7650 microscope (Hitachi, Tokyo, Japan) at a working voltage of 100 kV. To prepare the specimens, 1.5 µl of the sample dispersion was drop-cast onto a formvar-coated 200-mesh Cu grid. The average particle sizes (± standard deviation [SD]) were obtained by manually counting more than 200 randomly selected particles from the TEM images. Field-emission scanning electron microscopy (FE-SEM) was conducted using an S5500 microscope (Hitachi, Tokyo, Japan) at 15 kV, also using the same Cu grid. The yield of AuNPs was measured using inductively coupled plasma mass spectrometry (Agilent 5100 ICP-OES, Tokyo, Japan). The absorbance of the AuNPs in the Ultraviolet (UV)-visible range was measured using a microtiter plate reader (PowerScan 4; DS Pharma Biomedical Co., Ltd., Osaka, Japan) and a UV-Vis- Near-infrared (NIR) scanning spectrophotometer (UV-3100PC; Shimadzu Co., Ltd., Kyoto, Japan). The hydrodynamic mean diameter and Z-potential

of the synthesized AuNPs were determined by using the nanoPartica SZ-100 analyzer (HORIBA Scientific, Kyoto, Japan). The thermogravimetry analysis (TGA) was performed using a DTG-60 thermobalance (Shimadzu, model DTG-60, Kyoto, Japan).

Catalytic evaluation of small AuNPs synthesized by G1-G3 peptides in 4-Nitrophenol (4-NP) reduction

In a 96-well plate, the 4-nitrophenol (4-NP) (2 µl, 15 mM) and AuNP solutions (2 µl, 24 µg ml^{-1}) were added to $NaBH_4$ solution (196 µl, 20 mM). The degradation kinetics of 4-NP were monitored by measuring the absorbance at 400 nm, using a UV-Vis spectrometer. The absorbance was recorded at 30 s intervals for 240 s. To compare catalyst-specific activities, similar experiments were performed with varying gold concentrations (0–32 µg ml^{-1}). The reaction kinetics can be represented using the equation $K_{app} \times t = \ln(C_0/C_t)$, where K_{app} is the apparent rate constant, t is the reaction time, C_0 and C_t are the concentrations of 4-NP at t=0 and t, respectively, and C_0/C_t is equal to A_0/A_t, where A_0 and A_t are the absorbances at 400 nm at t=0 and t, respectively. In the subsequent methyl orange reduction reaction, absorption at 465 nm was measured. In these reactions, the concentration of $NaBH_4$ was much higher than that of 4-NP. Therefore, it could be approximated as constant, thus allowing the application of a pseudo-first-order rate equation.

Catalytic evaluation of small AuNPs synthesized by G1-G3 peptides in Methyl Orange (MO) reduction

In a 96-well plate, the methyl orange (MO) aqueous solution (2 µl, 5 mM) and AuNP solution (2 µl, 12 µg ml^{-1}) were added to an $NaBH_4$ aqueous solution (196 µl, 0.1 M). The kinetics of MO degradation were monitored by measuring the absorbance at 465 nm using a UV-Vis spectrometer. Absorbance was recorded at 30 s intervals for 240 s. Similar experiments were performed with varying gold concentrations (0–16 µg ml^{-1}) to compare catalyst-specific activities. In this reaction, the concentration of $NaBH_4$ was considerably higher than that of MO and could be deemed constant; therefore, a pseudo-first-order rate equation could be applied.

RESULTS

Characterization of biomineralized small AuNPs using G1-G3 peptides

In a previous study, we identified more than 150 peptides with AuNP biomineralization properties.[26,33] Among these gold biomineralizing peptides, three peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL) that biomineralized gold ions to AuNPs showed a green color on the peptide array.[26] The well-dispersed AuNPs biomineralized by G1, G2, and G3 in an aqueous solution were analyzed by High Resolution (HR)-TEM (Figure 1). Through the ICP-MS analysis, the yield of AuNPs was confirmed to be approximately 17.0, 12.1, and 20.2% of Au ions in each reaction condition. Through the ICP-MS analysis, the AuNPs yield (i.e., $100 \times [Au^0]_{t=24}/[Au^{3+}]_{t=0}$) was confirmed to be 17.0, 12.1, and 20.2% for G1, G2, and G3, respectively. The particles exhibited irregular shapes with a single crystallinity, and well-defined lattice fringes were distinguished. These AuNPs nanoparticles exhibited a similar size range, but with the following order: G2< G1< G3 (Figure 1). The amino acid, tryptophan, is fluorescent (em: 450 nm) when oxidized and this signal could be used to monitor the reduction of Au (III) by the peptide (Figure 2a and Supplementary Movie

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1).[34] This was also confirmed by the emission peak at 450 nm in the single-molecule fluorescence spectrum analysis (ex.: 350 nm) (Figure 2b). While this fluorescence was also observed in the supernatant from which AuNPs were removed (through ultra-centrifugation (100,000 × g, 1h)), fluorescence was not observed before the AuNP synthesis reaction. Based on these observations, we confirmed that part of the role of the peptide was to act as a reducing agent to biomineralize the AuNPs. The reaction proceeded for approximately 10 h (Figure 2a).

Dynamic light scattering (DLS) measurements were conducted to determine the size distribution of these AuNPs in solution. These data indicated mean particle sizes of 126.6 ± 0.8 nm, 95.8 ± 0.9 nm, and 88.0 ± 0.3 nm with a poly dispersity index (PDI) of 0.36, 0.17, and 0.20, respectively, indicating a moderately narrow size distribution. As the size range measured by DLS is significantly larger than TEM analysis, the particle seems to be surrounded by peptide molecules. The Z-potential of these AuNPs was found to be 5.6 ± 1.6 mV, 7.0 ± 1.7 mV, and 10.8 ± 2.0 mV. To further evaluate the AuNPs mineralized by peptides, thermogravimetric analysis (TGA) was performed (Figure 3). TGA of all these AuNPs revealed the presence of at least three different decomposition processes. The gradual reduction in weight observed between 25°C and 250°C suggested that the water content within these AuNPs had been removed. The rapid decrease in weight observed up to 400°C was attributed to the peptide decomposition. The remaining approximately 20% weight was attributed to the AuNP.

Catalytic evaluation of small AuNPs synthesized by G1-G3 peptides in 4-Nitrophenol (4-NP) reduction

4-NP can be reduced in the presence of a catalyst (Figure 4a). The yellow-colored 4-NP solution had a strong absorbance band with a maximum at 400 nm. The reduction reaction of the 4-NP

resulted in the solution becoming colorless (Figure 4b), and a decrease in the area of the absorbance band was observed (Figure 4c). The plot of $\ln(C_0/C_t)$ versus reaction time in Figure 4d shows a linear relationship, confirming the pseudo-first-order reaction. The rate constant K_{app} of G1_AuNPs, G2_AuNPs, and G3_AuNPs at the concentration of 24 µg ml^{-1} was 1.00×10^{-2} s^{-1}, 6.13×10^{-3} s^{-1}, and 1.05×10^{-2} s^{-1}, respectively. For For comparing the catalytic efficiency of different nanoparticles (G1-AuNPs, G2-AuNPs, and G3-AuNPs), the catalytic activity factor (K_1: K_{app}/m, m is the total mass of the catalyst) representing their relative catalytic activities was also calculated. The K_1 of G1-AuNPs, G2-AuNPs, and G3-AuNPs calculated from the slope of the linear plot was 8.12×10^{-6} s^{-1} mg^{-1}, 4.44×10^{-6} s^{-1} mg^{-1}, and 8.58×10^{-6} s^{-1} mg^{-1}, respectively (Supplementary Figure S1). The change of AuNP size and morphology before and after both catalytic reaction of 4-NP reduction was observed by TEM analysis and found the difference to be small. The AuNPs in this study maintain their size and morphology after the catalytic reaction.

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Catalytic evaluation of small AuNPs synthesized by G1-G3 peptides in methyl orange (MO) reduction

The orange-red solution containing MO showed the maximum absorbance at 465 nm. The degradation reaction of MO resulted in a colorless solution and a reduction in the peak maxima (Figure 5a and b). The UV-Vis spectrum versus reaction time is shown in Figure 5c. The absorbance at 465 nm decreased significantly within 120 s from the start of the reaction. The degradation of MO commenced immediately after the addition of AuNPs as catalysts. Figure 5d shows a plot of $\ln(C_0/C_t)$ versus reaction time. The rate constant K_{app} of G1-AuNPs, G2-AuNPs, and G3-AuNPs at the concentration of 12 µg ml^{-1}, calculated from the slope of this linear plot,

was 1.25×10^{-2} s^{-1}, 1.26×10^{-2} s^{-1}, and 2.58×10^{-2} s^{-1}, respectively. Moreover, K_{app} at a range of Au concentrations was observed in Supplementary Figure S2. The K_{app} is directly proportional to the concentration of AuNPs. The K_1 of G1-AuNPs, G2-AuNPs, and G3-AuNPs, calculated from the slope of the linear plot, was 2.11×10^{-5} s^{-1} mg^{-1}, 2.10×10^{-5} s^{-1} mg^{-1}, and 4.89×10^{-5} s^{-1} mg^{-1}, respectively (Supplementary Figure S2).

DISCUSSION

The three peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL) directly reduced gold ions to synthesize small AuNPs (G1:2.7 ± 1.9 nm, G2:2.9 ± 1.7 nm, G3:2.5 ± 1.5 nm) (Figure 1). Although some biomineralization peptides were reported to synthesize larger AuNPs, the size range below 3 nm obtained using this simple one pot protocol without any supplementation such as with $NaBH_4$ has not been previously reported. Generally, in the chemical synthesis of nanoparticles, the ligand concentration is increased to produce small Au NPs. Recent studies using plasma-induced reduction have shown that the resultant diameter is inversely proportional to the ligand solution concentration.[35] Moreover, the strength of the affinity of the ligand to the Au surface determines increases the strength of the effect. The peptides in this study, synthesized small AuNPs at relatively low concentration, without any reducing agent, suggesting that the peptide potency was high compared to that of traditional ligands like citrate.

Interestingly, these G1-G3 sequences contain multiple tryptophan and histidine residues. Mechanistic and kinetic studies on tryptophan-based peptides for AuNP synthesis have been previously investigated by Stayabrata Si *et al*. They reported the synthesis AuNPs with a size of 13.39 ± 2.7 nm using a peptide containing tryptophan at pH 11.0. UV-visible and fluorescence

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spectroscopic studies showed that the tryptophan residue of the peptide was converted to highly fluorescent forms such as ditryptophan/kynurenine. Additionally, Catherine J. Munro and Marc R. Knecht *et al*. reported AuNP synthesis with a size range of approximately 20 nm using the AuBP1 peptide (WAGAKRLVLRRE) under various aqueous conditions (pH, ionic strength, ion composition, peptide:Au^{3+} ratio). In these studies, the reduction activity was attributed to the tryptophan residue, where reactivity is sensitive to the local environment surrounding the residue. Furthermore, by substituting tryptophan with the oxidation product kynurenine in the AuBP1 peptide to create AuBP1 kynurenine (kyn) (kynAGAKRLVLRRE), the effect of the oxidized biomolecule on the reduction of Au^{3+} was examined. By comparing native AuBP1 and AuBP1 kynurenine, substantially diminished K_{app} values for the reduction reaction with increasing amounts of AuBP1 kynurenine exposed to a constant amount of Au^{3+}. These results highlighted the importance of the tryptophan residue for Au^{3+} reduction, where the tryptophan moiety donates an electron to the nearby metal ion and forms the tryptophyl radical, which is subsequently transferred to its oxidized form.

To confirm the importance of tryptophan in the small AuNP synthesis by the G1 peptide, we observed fluorescence from the amino acid tryptophan (emission at 450 nm) upon oxidation. This allowed us to monitor the reduction of Au(III) by the peptides (Figure 2a and Sup. Movie 1). Further confirmation was obtained through the emission peak at 450 nm in single-molecule fluorescence spectrum analysis (ex.: 350 nm) (Figure 2b). While this fluorescence was also observed in the supernatant from which AuNPs were removed through ultra-centrifugation (100,000 x g, 1h), it was not observed before the AuNP synthesis reaction. Additionally, we evaluated AuNP synthesis using three G1 derivatives: G1_1-6 (ETGHHI), G1_3-8 (GHHIWE), and G1_5-10 (HIWEWM). Among these sequences, only G1_1-6 (ETGHHI), which lacks a

tryptophan residue and showed no AuNP mineralization activity, while the other two sequences possessing tryptophan demonstrated AuNP synthesis properties. Based on these observations, we believe that the role of the tryptophan residue is to act as a reducing agent even for the biomineralization of small AuNPs, as reported in previous literature.

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In our previous work, the presence of histidine in a mineralization peptide (AuP1) was found to reduce AuNP mineralization activity. As the histidine residue is generally known to chelate metallic ions, the binding between $AuCl^{4-}$ and histidine might restrict Au biomineralization to form large particles due to steric hindrance, electrostatic interactions, or both. Therefore, the large number of histidine residues in G1-G3 peptides may be important in regulating the continuous biomineralization in the synthesis of smaller AuNPs.

The fluorescence emission observed during AuNP mineralization suggests that some amino acids, including tryptophan, act as reducing agents (Supplementary Movie 1). This is advantageous for developing a simple and highly reproducible green synthesis method for AuNPs. However, we wanted to address whether these peptides would interfere with the catalytic properties because various studies have reported that the size,[36,37] shape,[38] and capping ligands[39] of AuNPs all play key roles in the catalytic activity and in determining the reaction rate.

The sizes of G1-G3 AuNPs were all very small (2–3 nm), but no specific morphology was observed in HR-TEM (Figure 1). To further determine the elemental composition and the presence of specific facets on the AuNP surface, X-ray photoelectron spectroscopy analysis should be performed. The high dispersibility of the synthesized AuNPs in aqueous solution suggests that some peptide molecules bind to the particle surface as ligands. The presence of peptide molecules on AuNPs was supported by the results of TGA and DLS analysis (Figure 3).

Given that previous reports have shown the significance of Π-metal interactions, such as those involving tryptophan, in the binding of amino acids and peptides to the surface of AuNPs, all tryptophan-containing peptides (G1-G3) used in this study can be hypothesized to also interact with the synthesized gold surface through a similar mechanism.[40–43] In an attempt to obtain more detailed information about the peptides on the AuNP surface, we conducted Raman spectroscopy and Fourier transform infrared spectrometer analyses. However, we were unable to obtain signals that could be confidently attributed to peptides. This may possibly be attributed to the heterogeneity of peptides on the particle surface. In addition, the presence of large amounts of peptide molecules on AuNPs seems to contribute to the synthesis of small AuNPs by the peptides. The peptide molecules could be inhibiting crystal growth. In our previous study, B3 peptide (ASHQWAWKWE) synthesized triangular Au nanoplates and decahedral AuNPs. These particles are dominant in the (111) crystal facet. Therefore, this B3 peptide was considered to have a high affinity for this (111) crystal facet. The reason for synthesizing completely different-sized and shaped AuNPs by G1-G3 peptides and B3 peptide may lie not only in their crystal surface recognition ability but also in the differences in their self-assembly capability on the particle surface.

In this study, the catalytic properties of three AuNPs synthesized using different peptides were investigated. Interestingly, in these two different reactions, a different tendency was observed in each sample (Supplementary Tables 2 and 3). In 4-nitrophenol (4-NP) reduction, higher values of K_{app} and K_1 were obtained by G1-AuNP and G3-AuNP than by G2-AuNP. This was mainly because of the size of AuNPs. G2-AuNP was considered to exhibit superior catalytic activity because of its smaller particle size compared to that of G1 and G3, leading to a higher surface area. Further, in MO reduction, G3-AuNP exhibited superior catalytic activity than that of the

others. G3-AuNP also revealed higher Z-potential and smaller cumulant diameter. For the MO reduction reaction, these parameters may contribute to the catalytic property. Overall, the catalytic activity is proportional to the available Au surface area, which is dependent on the peptide density, Au NP size, and even the size, charge, and shape of the molecules reacting at the surface. Therefore, while the activities are similar for all three AuNP samples, subtle differences in the size and peptide will result in different catalytic activity values. When comparing the values of AuNPs presented in other studies, the K_{app} of G1-G3 AuNPs appeared to be similar to that of other AuNPs, but K_1 was higher when compared to that of other AuNPs. This is probably because of the relatively smaller size of G1-G3 AuNPs compared to that of the other AuNPs, as well as the absence of a support material. A significantly superior K_1 has been reported only for ascorbic acid-modified AuNPs with a hollow flower-like structure in the MO reduction reaction. [44] This is attributed to its unique shape, and with regard to the smaller particles obtained in this study, their activity may be enhanced by tuning their and shape. Various biological materials, such as bacteria, fungi, algae, plants, and pure proteins, have been utilized for the biomineralization of AuNPs. [45–50] However, tuning and designing the biomineralization events further is relatively challenging owing to sample complexity and large molecular sizes. However, peptides are relatively simple. Their physicochemical properties can be easily controlled by sequence substitution via the chemical synthesis process. In addition to utilizing this characteristic, it is expected that by optimizing each reaction condition (pH, temperature, concentration, etc.), catalysts with enhanced reactivity can be obtained in the future.

CONCLUSION

This study reports the formation of small AuNPs via biomineralization using peptides (G1:ETGHHIWEWM, G2:TQWHEWHWYQ, and G3:GMWHIEHIWL). AuNPs exhibit both catalytic and enzymatic properties. These AuNPs, with an average size of 2.7 ± 1.9 nm (G1), 2.9 ± 1.7 nm (G2), and 2.5 ± 1.2 nm (G3), were generated using a simple, green, one-pot synthesis process. The mineralization reaction proceeded under ambient conditions by mixing only the peptide and Au (III) ions in a neutral pH solution at 25 °C. This is the first study reporting the catalytic properties of small AuNPs (less than 3 nm) synthesized via an eco-friendly route using biomineralized peptides without any supplementation. The activity factor K_1 of G1-G3 AuNPs for 4-NP and MO reduction reactions was higher when compared to that of other previously reported AuNPs except in one report using a hollow flower-like unique structure.[44] As this study has shown the potential of AuNPs synthesized via mineralization peptides without other supplements such as reducing agents or additional triggers in catalytic reactions, these environmentally friendly AuNPs can be widely utilized as active components in biomimetic catalysis, theragnostics, sensing, and other fields.

ASSOCIATED CONTENT

Supporting Information

Supplementary Movie 1 Observation of the biomineralization process for small AuNP synthesis using the G1 peptide.

The reaction vial was irradiated using a UV lamp and monitored using a conventional digital camera. AuNP was synthesized by mixing 0.5 mM $HAuCl_4$ and 0.25 mM peptide in Tris-buffer saline (pH 7.4).

Supplementary Figure Legends

Figure S1. Evaluation of the activity factor (K_1) of 4-NP reduction using AuNPs synthesized from biomineralization peptides (G1, G2, and G3).

Figure S2. Evaluation of the activity factor (K_1) of MO reduction using AuNPs synthesized from biomineralization peptides (G1, G2, and G3).

Supplementary Tables

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Conflicts of interest

There are no conflicts to declare.

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ABBREVIATIONS

AuNP: Gold nanoparticle, HR-TEM: High Resolution Transmission Electron Microscopy, TGA: Thermogravimetry analysis, 4-NP: 4-nitrophenol, MO: methyl orange, PDI: poly dispersity index, DLS: Dynamic light scattering.

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