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Array-based functional peptide screening and characterization of gold nanoparticle synthesis

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ABSTRACT

Based on inorganic material production through biomineralization in organisms, the use of biological molecules in nanomaterial production has received increasing attention as a vehicle to synthesize inorganic materials with selected properties in ambient conditions. Among various biological molecules that interact with metallic surfaces, short peptides are putative ligand molecules as they exhibit potential to control the synthesis of nanoscale materials with tailored functions. Herein, using a spot synthesis-based peptide array, the gold nanoparticle (AuNP) binding activities of approximately 1800 peptides were evaluated and revealed various activities ranging from positive (high-affinity binding peptides) to negative (weak- or null-affinity binding peptides). Among 50 peptides showing the highest AuNP binding activity, 46 sequences showed the presence of tryptophan-based motifs including W[X_n]W, H[X_n]W, and W[X_n]H (W: tryptophan, X: any amino acid, n: 1–8 amino acid residues), whereas none of these motifs was found in the WORST50 peptides. Notably, three peptides showing the highest binding affinities possessed bi-functionality in AuNP binding and Au(III) reduction in solution and on solid surfaces. In addition, the characterization of truncated peptide derivatives revealed unique peptide motifs for their function expressions that also supported the importance of tryptophan-based motifs for peptide-AuNP binding. These findings open the door for peptide-mediated precise regulation of AuNP synthesis in ambient condition and for site dependent controlled AuNP integration onto nanotechnological devices.

Statement of Significance

The development of a technique for functionally regulated nanosized material production in ambient condition is broadly required according to the expansion of nanomaterial based applications. Short peptides, which bind to metallic surfaces, have great potential for the technique development, but the realization remains a difficult challenge due to the lack of metal binding peptide varieties. Herein, approximately 1800 peptides with the gold nanoparticle (AuNP) binding activity are reported and characterized. Furthermore, by three highest binding peptides, the expression of bi-functionality in AuNP binding and Au(III) reduction was serendipitously discovered in solution and on solid surfaces. These findings will be attributed to new technique development of functional nanoparticle synthesis in mild condition, and for site-dependent AuNP integration in various nanotechnological devices.

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1. Introduction

Nanoparticles are of great interest to researchers in a wide range of fields, and remarkable progress has been made in the development of robust synthesis methods that allow precise control of inorganic nanoparticles [1,2]. For example, various ligands that bind to nanoparticle surfaces have demonstrated abilities to be modified in size and shape, to act as assembly scaffolds, to

Abbreviations: AuNP, gold nanoparticle; SH, sulfhydryl; AuP, AuNP binding peptides screened with a peptide array; GRAVY, grand average of hydropathy; SEM, scanning electron microscope; SPR, surface plasmon resonance; TEM, transmission electron microscopy.

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prevent particle oxidation, and to enhance and refine particle properties during synthesis [3].

Living organisms are well known to use material properties of crystalline minerals produced by biomineralization of organic–inorganic hybrid materials for a variety of purposes, such as mechanical support, photonics, protection, and navigation (e.g., hydroxyapatite in bones and teeth [4], silica in diatoms [5] and sponges [6], calcium carbonate in mollusk shells [7], and magnetite in bacteria [8–10]). The precise control of biominerals even at the nanometer level has garnered considerable attention owing to the potential to mimic the bioprocess of highly regulated inorganic nanomaterial production in ambient conditions [11–13]. In particular, peptides are putative ligand molecules for nanoparticle production and functionalization because variants are derived abundantly from combinations using 20 natural amino acids via chemical synthesis [14,15]. Various peptides with unique characteristics have been used as non-covalently bound linkers for nanomaterial surface modification, as mediators for controlled self-assembly of nanomaterials, and as templating/catalyzing reagents for nanomaterial production [16,17].

Various functional peptide screening approaches, including phage display [18], cell surface display [19], and peptide array [20–22] techniques, have been used to identify and characterize various metal binding peptides including gold nanoparticle (AuNP) binders [19,23–26]. Interactions between functional peptides and Au crystal surfaces have been investigated for development of a technique for AuNP synthesis in ambient conditions [25,27–30], however, detailed analyses are still required because the direct imaging of metal-biomolecule interfaces at a molecular level in aqueous condition and the design of peptide mediated-AuNP property are still highly challenging. One of the obstacles to this research is the number of known AuNP binding peptides. To evaluate the interaction mechanism, additional AuNP-binding peptides with various physicochemical properties are required. Among various peptide screening techniques, coherent membrane-supported peptide array libraries by spot-synthesis have several advantages, including ease of peptide sequence identification (DNA sequencing is not necessary), chemical synthesis without a biological organism, and the acquisition of binding activity data from positive (high-affinity binding peptide) to negative (weak- or null-binding affinity) with amino acid sequences [31–34]. Moreover, given the ease of peptide sequence design, this technique can be used to elucidate the functional expression of the peptide derivatives, including their truncations.

In this study, to investigate the interaction mechanism between peptide and metallic surfaces, we used peptide array technology to identify a list of metallic particle binding peptides with various physicochemical properties (hydrophobicity and isoelectric point (pI)) and characterized high-affinity binding peptides. Herein, as a model material, we had chosen Au because the material reveals highly stable metallic surface compared to other metals and is applicable for a wide range of bio-nanotechnology fields such as catalysis [35,36], biosensors [37,38], diagnostic tools [39,40], cell analysis [41–43]. The screening strategy was to repeatedly modify the peptide library based on amino acid appearance frequency in peptides with a high affinity for binding to AuNPs during previous rounds of the assay. The aim was to design a peptide library preferentially containing amino acid residues found in high affinity binding peptides (500 amino acids in the TOP50 10-mer peptides from 600 peptides evaluated). Triplicate repetitions of peptide library synthesis and binding assays were performed to screen strongly binding peptides. The effects of high affinity binding peptides on AuNP synthesis were investigated to clarify the motifs imparting unique functions to the peptides in solution and on solid support. The results presented herein could be useful for a wide range of bionanotechnological applications and for the understanding of the relationship between peptide sequence and synthesized AuNP that can be applicable to the design of AuNP synthesis in mild condition.

2. Materials and methods

2.1. AuNP synthesis

AuNPs were prepared by a previously reported method with slight modifications [44]. Briefly, AuNPs were formed by quickly injecting 1.20 mL of ice-cold, freshly prepared $NaBH_4$ (10 mM) into a rapidly stirred solution containing 0.50 mL of $HAuCl_4$ (10 mM) and 20.00 mL of N-hexadecyltrimethylammonium chloride (100 mM). The solution (pH 5.5) was stirred for 1 min and then left undisturbed at 25 °C for 2 h. Spectrometric analysis of the UV–Vis spectra was used to determine the absorbance (0.132) at 512 nm, and the AuNP concentration was estimated to be 4.20 nM [45].

2.2. Peptide array synthesis using spot technology

A cellulose membrane (grade 542; Whatman, Maidstone, UK) was activated using β-alanine as the N-terminal basal spacer. Activated Fmoc amino acids (0.5 M) were spotted on the membrane using a peptide auto-spotter (ResPepSL; Intavis AG, Köln, Germany), in accordance with the manufacturer's instructions. After addition of the first residue, the remaining amino groups were blocked by 4% acetic anhydride. With each elongation step, the membrane was deprotected with 20% piperidine in N,N′-dimethylformamide (DMF) and subsequently washed thoroughly with DMF and then ethanol. After the final deprotection, side-chain protecting groups were removed with a solution of m-cre sol:thioanisol:ethandithiol:trifluoroacetic acid (1:6:3:40) for 3 h. Finally, the membrane was thoroughly washed with diethyl ether, ethanol, and phosphate-buffered saline (PBS; pH 7.4), and dried without heat. Each peptide spot has an area density up to 10 μmol/cm^2 [46].

2.3. Screening of AuNP-binding peptides from peptide arrays

To screen AuNP binding peptides, a series of peptides were designed and synthesized onto a cellulose membrane (Fig. 1a). First, 9 previously reported representative Au binding peptides [19,23–26] (Fig. 1b) were synthesized onto the cellulose membrane. The peptide array was soaked directly in the solution containing synthesized AuNPs and maintained for 16 h with gentle shaking (termed the first round assay). Binding affinity between these peptides and the AuNP material was quantitatively evaluated based on the color intensity derived from AuNP binding onto peptide spots, which was measured using ImageQuant TL software (GE Healthcare, Tokyo, Japan) from the digitized images of the peptide array. To compile the library for the next round, peptide sequences were designed in the program R (https://cran.r-project.org) to preferentially include amino acids found in high binding affinity peptides within the previous library. Percentages of each amino acid comprising high affinity binding peptides were set as parameters. For example, if the TOP50 10-mer peptide sequences comprising 500 amino acids contained 50 alanine residues in their sequences, alanine was set to include at 10% in 600 peptide sequences for the next round. However, as cysteine possesses a sulfhydryl (SH) functional group that can assist to modify metal surfaces, this amino acid was excluded in the peptide library design. Moreover, when some amino acid species (asparagine, aspartic acid, and phenylalanine) were not found in the amino acid sequences of peptides with highest binding affinity identified in the previous assay, to maintain sequence diversity, the peptide array design for the following round of screening was modified to include 0.5% of each amino

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