Journal of Colloid and Interface Science 454 (2015) 80–88

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

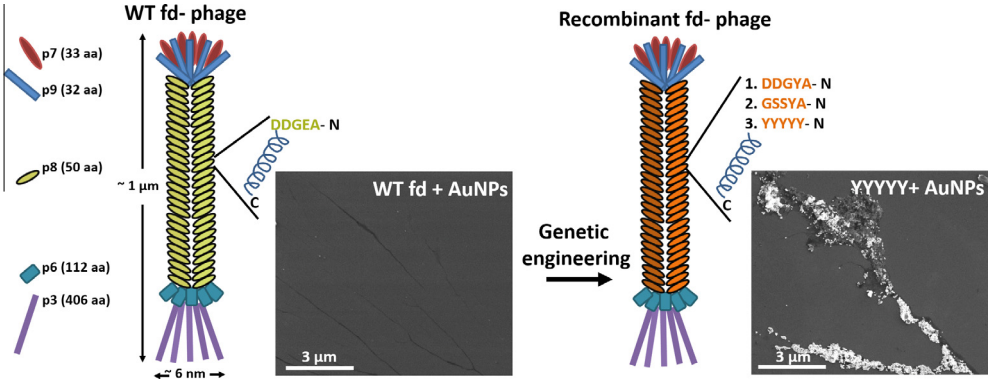
www.elsevier.com/locate/jcis

Engineering filamentous bacteriophages for enhanced gold binding and metallization properties

Nuriye Korkmaz Zirpel *, Taner Arslan, Hyeji Lee

Korea Institute of Science and Technology, Europe Forschungsgesellschaft mbH, Campus E 71, Saarbruecken D-66123, Germany

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 March 2015
Accepted 5 May 2015
Available online 13 May 2015

Keywords:
Bottom-up manufacturing
Self-assembly
Bacteriophage
Genetic engineering
Nanoparticle

ABSTRACT

Filamentous bacteriophages are nanowire-like virion molecules consisting of a single stranded DNA (ssDNA) as the genomic material packed in a protein cage. In this study, Tyr containing 5-mer peptides were displayed on phage filaments for enhanced Au binding and reduction properties. Wild type fd (AEGDD) and engineered YYYYY, AYSSG and AYGDD phages were investigated by Quartz crystal microbalance (QCM), Atomic force microscopy (AFM), Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX) analyses. Presence of only one Tyr unit on five aa flexible region of p8 coat proteins increased Au binding affinities of engineered phages. YYYYY phages were shown to have the strongest Au surface and AuNP binding affinities. Recombinant phages were shown to be coated with Au clusters after one-step metallization reaction. With further genetic modifications, phages can be programmed to function as site specific self-assembling biotemplates for bottom-up manufacturing in nanoelectronics and biosensor application studies.

1. Introduction

Bacteriophages are virus particles infecting only bacteria. F-specific filamentous (Ff) group bacteriophages include M13, fd and f1 phages with almost identical structures [1]. They amplify themselves in Gram negative bacteria carrying F-pili [2]. Wild type (WT) fd phages (~7 nm × 880 nm) are composed of a single stranded DNA (6408 bases) packed in a protein cage of coat proteins. The ssDNA is surrounded by 2750 copies of major coat protein p8 α-helical subunits composed of 50 amino acid (aa) residues. On head and tail parts of the phage, five copies of minor coat proteins p7, p9 and p3, p6 are found

* Corresponding author. Fax: +49 681 9382 240.
E-mail addresses: n.korkmaz@kist-europe.de (N. Korkmaz Zirpel), t.arslan@kist-europe.de (T. Arslan), cccoonu@naver.com (H. Lee).

http://dx.doi.org/10.1016/j.jcis.2015.05.006
0021-9797/

respectively [3–5]. Bacteriophages have been applied in various interdisciplinary research fields such as drug delivery, tissue engineering and biosensor development studies [6]. The most popular application area of filamentous phages is phage display technique where phages expressing a random peptide library on p3 coat proteins are applied in order to identify short specific peptide sequences having binding affinities to other peptides [7], proteins [8], solid materials [9] or cells [10]. For example, Lee et al. constructed bacteriophages which can specifically bind single-walled nanotubes (SWNTs) and $FePO_4$ which were then used to develop high-power lithium ion battery electrodes [11].

Nanodevice fabrication is a fast growing research field employing both bottom-up [12] and top-down [13] manufacturing techniques. Bottom-up fabrication is applied for the production of functional systems via controlled self-assembly of nanoscale building units. There is a tremendous effort to manufacture nanowires (NWs) via bottom-up approach employing self-assembling biotemplates. In nature, there are many examples of self-assembling building blocks. Until now various biomolecules such as DNA [14], viruses [15], microtubules [16], proteins [17] and actin filaments [18] have been applied as building blocks for bottom-up fabrication. Cung et al. have applied bacteriophages as templates for lead zirconate titanate (PZT) NW production [19]. Resulting NWs showed very high piezoelectric constants (132 pm/V). Other than bacteriophages, plant viruses such as tobacco mosaic virus (TMV) molecules have been also investigated for nanobiotechnological applications [20]. Atanasova et al. developed TMV/ZnO hybrid structures to be integrated into field-effect transistors for functional nano-device fabrication [21].

In order to obtain metal decorated biotemplates, either chemical or genetic modifications can be applied. Au–S linkage is one of the most commonly applied technique for obtaining Au–protein hybrid systems. It was previously shown that sulfur carrying amino acids like cysteine have strong binding affinities against Au [22]. Expression of three Met residues on p8 major coat protein subunits of filamentous fd bacteriophages resulted in enhanced Au binding and metallization properties [23]. Slocik et al. applied gold binding peptides (e.g. 12-mer A3 peptide: AYSSGAPPMPPF) identified by phage display technique in order to produce water stabilized AuNPs [24]. They reported that A3 peptides are not only able to bind Au templates but also able to reduce Au ions. The binding of A3 peptide to Au was explained by hydrophobic interactions or hydrogen bonding. After replacing the Tyr residue of A3 peptide with Ser, no reduction occurred showing that the presence of only one Tyr residue enables $HAuCl_4$ reduction. Similarly, Flg tag peptide which also contains one Tyr subunit was reported to be able to reduce Au ions as well. Being inspired by these results, in this study we expressed Tyr containing 5-mer peptides on N-terminal region of major coat protein p8 subunits and compared Au binding affinities and Au reduction potentials of engineered phages. We modified the original N-terminal AEGDD sequence of p8 coat proteins to AYGDD, AYSSG (first five aa of previously identified A3 peptide), YYYYY and tested the Au binding affinities by Quartz crystal microbalance (QCM), Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX) analyses. One-pot Au metallization reaction was successful without applying additional reducing agents. EDX analyses revealed that recombinant bacteriophages were coated with AuNPs after treatment of phages with different concentrations of AuNPs or with Au clusters after metallization experiments at different $HAuCl_4$ precursor concentrations. Genetically engineered filamentous phages with improved material binding properties may serve as self-assembling nanobiotemplates for bottom-up manufacturing.

2. Materials and methods

2.1. Cloning and phage amplification

Escherichia coli MC1061 (New England Biolabs) and *E. coli* K91BluKan (K91BK-kindly provided by Prof. Dr. Georg P Smith (University of Missouri, USA)) cells were utilized for plasmid and phage amplification correspondingly. Backbone fd-tet vector was delivered by Smith's laboratory. Flexible five aa N-terminal AEGDD region of the major coat protein p8 was substituted with AYGDD, AYSSG (first five aa of previously identified A3 peptide) and YYYYY by assembly polymerase chain reaction (APCR) with the primers listed in Table 1. The first PCR reaction was performed using FP1 & RP1 primers carrying the base pairs coding for desired inserts; and fd-tet vector as the template in order to amplify a 397 bp fragment. Second PCR reaction was conducted in order to amplify a 329 bp fragment having a 5′ overlapping complementary sequence to the first PCR product using primers FP2 & RP2 (Table 1) and the template fd-tet vector. Lastly, the APCR was performed using two PCR products as templates and the far primers FP1 and RP2. Gel extracted and purified APCR product was next treated with restriction enzymes *BsrG*I and *Not*I (NEB) and inserted into the digested fd-tet vector by overnight ligation at 16 °C using T4 DNA ligase (NEB). The ligated vector was transformed into *E. coli* MC1061 cells by electroporation using a micropulser (voltage: 2.5 kV; capacity: 25 μF; resistance: 200 Ω, BIORAD). Transformed cells were plated on Tetracycline (Roth) supplemented LB agar plates for colony selection. Following the sequencing analyses (Eurofins Genomics) *E. coli* K91BK cells were electroporated with vectors of interest and plated on Tetracycline and Kanamycin (Roth) supplemented LB agar plates. Phages were isolated by PEG/NaCl treatment. Purified phages were dialyzed against 1× PBS at 4 °C overnight and stored at 4 °C after quantification by UV–vis spectrometry as previously described [www.biosci.missouri.edu/smithgp/PhageDisplayWebsite/PhageDisplayWebsiteIndex.html].

2.2. Au binding measurements

Au surface binding tests were conducted by QCM measurements with Qsense E1 (Biolin Scientific, Sweden), according to manufacturer's instructions. Gold coated quartz crystals (LOT Quantum Design) were mounted to the instrument's probe arm and 1× PBS was injected to the system until the frequency signal became steady. After 300 μl of bacteriophages (10^{10} cfu/ml in 1× PBS) was given at a flow rate of 15 μl/min, the sensor was washed with autoclaved MilliQ water (18.2 MΩ cm) at the same flow rate. Five overtone resonance frequencies (25, 35, 45, 54 and 64 MHz) were applied for frequency change measurements at 23 °C. Data analysis was performed with QTools software program and experiments were repeated at least for three times.

Affinity of bacteriophages to AuNPs was analyzed by SEM and EDX measurements after incubating the bacteriophages (10^{10} vir/ml) with various dilutions (1:1, 1:3 and 1:10) of 20 nm and 5 nm citrate stabilized AuNPs (Sigma) in 100 μl of 1× PBS. Samples were incubated for 2 h at room temperature on a rolling drum (7 rpm). In order to get more dispersed phage filaments decorated with AuNPs, we decreased the final phage concentrations to 6×10^{10} vir/ml and 6×10^{9} vir/ml (in MilliQ) and conducted the AuNP treatment experiments by mixing 10 μl of phages with 50 μl, 150 μl, 250 μl and 500 μl of 20 nm AuNPs.

2.3. One-pot metallization

One-pot or one-step metallization of phages was performed in 100 mM HEPES buffer (PAN Biotech) without using any additional