Colloids and Surfaces B: Biointerfaces 159 (2017) 770–780

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Original Research Article

Construction of genetically engineered M13K07 helper phage for simultaneous phage display of gold binding peptide 1 and nuclear matrix protein 22 ScFv antibody

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ARTICLE INFO

Article history:
Received 22 February 2017
Received in revised form 27 July 2017
Accepted 17 August 2017
Available online 24 August 2017

Keywords:
Bacteriophage M13
Gold nanoparticle
Single-chain antibodies
Electron microscopy
Genetic engineering

ABSTRACT

The most common techniques of antibody phage display are based on the use of M13 filamentous bacteriophages. This study introduces a new genetically engineered M13K07 helper phage displaying multiple copies of a known gold binding peptide on p8 coat proteins. The recombinant helper phages were used to rescue a phagemid vector encoding the p3 coat protein fused to the nuclear matrix protein 22 (NMP22) ScFv antibody. Transmission electron microscopy (TEM), UV–vis absorbance spectroscopy, and field emission scanning electron microscopy (FE-SEM) with energy dispersive X-ray spectroscopy (EDX) analysis revealed that the expression of gold binding peptide 1 (GBP1) on major coat protein p8 significantly enhances the gold-binding affinity of M13 phages. The recombinant bacteriophages at concentrations above 5×10^4 pfu/ml red-shifted the UV–vis absorbance spectra of gold nanoparticles (AuNPs); however, the surface plasmon resonance of gold nanoparticles was not changed by the wild type bacteriophages at concentrations up to 10^{12} pfu/ml. The phage ELISA assay demonstrated the high affinity binding of bifunctional bacteriophages to NMP22 antigen at concentrations of 10^5 and 10^6 pfu/ml. Thus, the p3 end of the bifunctional bacteriophages would be able to bind to specific target antigen, while the AuNPs were assembled along the coat of virus for signal generation. Our results indicated that the complex of antigen-bacteriophages lead to UV–vis spectral changes of AuNPs and NMP22 antigen in concentration range of 10–80 μg/ml can be detected by bifunctional bacteriophages at concentration of 10^4 pfu/ml. The ability of bifunctional bacteriophages to bind to antigen and generate signal at the same time, makes this approach applicable for identifying different antigens in immunoassay techniques.

1. Introduction

During the last decade, there has been a great interest for the discovery of genetically engineered peptides for inorganics (GEPI). GEPI are peptides with specific binding affinity toward inorganic materials such as metals, metal oxides, and semiconductors [1,2]. Various biocombinatorial peptide libraries including phage display [3,4], bacterial [5,6], and yeast display [7] have been utilized to identify peptides that interact with inorganic surfaces. Among all available display techniques, phage display (specifically filamentous phage strain M13) has become the dominant combinatorial method to isolate material binding peptides [8].

One of the first inorganic material binding peptide sequences was generated toward gold, and is termed gold binding peptide 1 or GBP1. The GBP1 is a 14-mer binding motif, MHGKTQATSGTIQS, that was expressed on the outer surface of *E.coli* as part of *LamB* protein [9]. This peptide sequence in spite of being cysteine-free, exhibits strong binding affinity to gold surfaces. The interaction

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http://dx.doi.org/10.1016/j.colsurfb.2017.08.034
0927-7765/

of GBP1 with gold is carried out by a mechanism unlike thiol-Au covalent bonds. The binding affinity of GBP1 may be directed by physisorption of polar (serine, glutamine, and threonine) and cationic (lysine and hystidine) amino acids onto gold surfaces [10].Willett and co-workers showed that Arg, Asp, Ser, Thr, and Pro amino acids strongly adsorbed to gold surfaces as homopolypeptides [11]. Whereas, Ala is a weak binder and Ala substitutions will decrease the affinity of gold binding peptides for Au interfaces [12]. However, the amino acids adsorption preferences alone cannot guarantee the peptide binding preferences. The composition of amino acids, peptide sequence, and peptide conformation act synergistically to mediate the binding affinity of GBP1 [13].

In this study, the GBP1 sequence was selected to display on p8 coat protein of M13K07 helper phages. Molecular dynamics simulations confirmed that the above sequence exhibits high affinity binding to gold surface when triply repeated in tandem [14]. Tamerler *et al.,* reported the specificity of GBP1 for gold compared to platinum by using quartz-crystal microbalance (QCM) [15]. Kanata *et al.,* showed that the conformational structure of GBP1 contributes to its gold-binding property. Fourier transform infrared spectroscopy (FTIR) and scanning tunneling microscopy (STM) revealed that the GBP1 adopted the helical structure on the gold surface and polar amino acids (i.e. threonine and serine) responsible for Au binding aligned on the same side of the helix. The role of these amino acids in the gold binding activity of GBP1 was investigated by replacing all threonine residues with alanine residues. As a result, the gold binding affinity of the peptide reduced by alanine substitution [12].

The nuclear matrix protein 22 (NMP22) is a part of nucleus scaffold that provides structutal integrity of the cell and regulates mitosis, DNA replication, and gene expression. The urinary NMP22 values are significantly higher in patients with bladder carcinoma compare to normal controls. Therefore, NMP22 is known as a tumor marker with high sensitivity and specificity for diagnosis of bladder cancer in early stage [16]. Since the tumor markers can be measured in the immunoassay tests, the generation of anti-tumor marker antibodies is highly regarded in the field of cancer research. Single-chain variable fragments (ScFvs) are one of the most popular recombinant antibodies that can easily be produced by phage display technology. Because of their small size, specific antigen recognition, and attachment ability to different labels, ScFv antibodies can be used in immunologic assays for detection of tumor-associated antigens [17–19].

Recently, a novel T7 phage was constructed to display the GBP1 and a prostate cancer cell-binding peptide on the major coat 10 B protein in a tandem arrangement. The genetically modified T7 phages can induce the self-assembly of AuNPs into nanoclusters for prostate cancer-targeted photothermal therapy. The AuNP clusters selectively targeted the prostate cancer cells and killed the targeted cells under a mild visible-light irradiation without significant damages to other cells [20].

Phage display has provided a reliable method for isolation of target-specific antibodies among large antibody repertoire consisting up to 10^{12} members. The most used bacteriophage in the phage display technique is M13 filamentous phage. The M13 bacteriophage is a rod-shaped virus that only infects *E.coli* cells carrying F-pili. The phage particles consist of a protein capsid (~900 nm long, ~7 nm thick) packing a single-stranded circular DNA genome of about 6400 nucleotides. The phage capsid is composed of approximately 2700 copies of α-helical major coat protein p8 aligned along the SS-DNA. Five copies of minor coat proteins p7, p9 and p3, p6 are found on head and tail parts of the phage filament, respectively. The most commonly used phage coat proteins for displaying peptides of interest are p3 and p8 [21].

In a ScFv phage display library, the genes encoding the variable antibody domains are fused to the p3 phage coat protein gene on a separate plasmid called phagemid. The phagemid-based system needs a helper phage for the production of phage particles displaying antibody fragments on p3 coat proteins. Helper phage provides all the necessary proteins that are required to assemble functional phage particles [22,23]. The released phage particles can simultaneously display different foreign proteins on their coat proteins.Thus, a bifunctional phage display system can be designed that the coding sequence of a gold binding peptide and a ScFv antibody are fused to p8 and p3 genes, respectively. The resultant recombinant phage particles display the antibody on p3 coat proteins and several copies of gold binding peptide on p8 coat proteins, that could specifically bind antigen and gold nanoparticles [24].

Previously, the filamentous fd-bacteriophages were genetically engineered to enhance their gold binding properties. Expression of three methionine amino acids on the N-terminal of p8 major coat proteins improved the attachment of recombinant bacteriophages to Au surfaces and AuNPs via sulfur-gold interaction [25]. Similarly, the modification of first five amino acids of the N-terminal region of p8 coat proteins to tyrosine containing sequences enhanced the Au binding affinity and Au reduction potential of modified bacteriophages. It has been reported that, the presence of one tyrosine in the N-terminal of p8 coat proteins increased the Au binding affinity of modified bacteriophages and the highest affinity was achieved by substituting the first five amino acids of p8 coat proteins with five tyrosine residues [26].

In this study, the M13K07 helper phage is genetically modified and the coding sequence of GBP1 is inserted into the phage genome fused to p8 coat protein gene. The recombinant and wild type M13K07 helper phages are investigated in terms of their binding affinities to gold nanoparticles by ultraviolet-visible (UV–vis) absorption spectroscopy, transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM) with energy dispersive X-ray spectroscopy (EDX) analysis. Then, the phagemid clones encoding the variable domains of NMP22 antibody are rescued by both wild type and recombinant M13K07 helper phage. The susceptibility of NMP22 single chain antibody to detect its target antigen is evaluated by phage ELISA assay, and the optimal amount of NMP22 phage antibody that interact with specific antigen is determined in phage titration experiment. Finally, the simultaneous binding of recombinant bifunctional bacteriophage to NMP22 antigen and gold nanoparticles is inquired by UV–vis spectroscopy, and compared with the results obtained by phage ELISA assay.

2. Materials and Methods

2.1. Recombinant M13K07 helper phage vector construction

The recombinant M13K07 (R-M13K07) vector was generated through genetically modification of M13K07 helper phage (Amersham-Pharmacia-Biotech, Vienna, Austria). The R-M13K07 phage vector was constructed by inserting a synthetic DNA fragment into the M13K07 genome (Fig. S1a). The construction procedure is described in Supplementary Information, S1. The resulting recombinant M13K07 vector contains two copies of p8 gene: a wild type p8 gene and a GBP1-p8 fusion gene (Fig. S1b).

2.2. Helper phage amplification

The recombinant and wild type M13K07 vectors were transformed into *E.coli* TG1 cells, and streaked on LB-agar plates with 70 μg/ml kanamycin. Phage-infected colonies were formed after an overnight incubation at 37 °C. A single colony was picked and inoculated into 10 ml Super Broth medium (SB; 30 g of tryptone, 20 g of yeast extract, 10 g of MOPS per liter, pH 7) with 70 μg/ml