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Gold nanoprobe functionalized with specific fusion protein selection from phage display and its application in rapid, selective and sensitive colorimetric biosensing of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is one of the most ubiquitous pathogens in public healthcare worldwide. It holds great insterest in establishing robust analytical method for *S. aureus*. Herein, we report a *S. aureus*-specific recognition element, isolated from phage monoclone GQTTLTTS, which was selected from f8/8 landscape phage library against *S. aureus* in a high-throughput way. By functionalizing cysteamine (CS)-stabilized gold nanoparticles (CS-AuNPs) with *S. aureus*-specific pVIII fusion protein (fusion-pVIII), a bifunctional nanoprobe (CS-AuNPs@fusion-pVIII) for *S. aureus* was developed. In this strategy, the CS-AuNPs@fusion-pVIII could be induced to aggregate quickly in the presence of target *S. aureus*, resulting in a rapid colorimetric response of gold nanoparticles. More importantly, the as-designed probe exhibited excellent selectivity over other bacteria. Thus, the CS-AuNPs@fusion-pVIII could be used as the indicator of target *S. aureus*. This assay can detect as low as 19 CFU mL^{-1} *S. aureus* within 30 min. Further, this approach can be applicable to detect *S. aureus* in real water samples. Due to its sensitivity, specificity and rapidness, this proposed method is promising for on-site testing of *S. aureus* without using any costly instruments.

1. Introduction

In recent years, pathogens have become public threats worldwide, which have been found to affect humans' health, food safety and even national security with a considerable economic loss (Harris et al. 2010; Sartor 2008). The overuse of antibiotics, on the other hand, has further escalated the threat of pathogen contamination, resulting in an increasing incidence of antibiotic-resistant pathogenic bacteria (Fischbach and Walsh 2009). *Staphylococcus aureus* (*S. aureus*), as one of the most ubiquitous pathogens in public healthcare worldwide (Nawattanapaiboon et al. 2015; Wen et al. 2013), has been a major common cause of bloodstream infections, osteomyelitis, endocarditis and toxic shock syndrome (Paulsen et al. 2015; Rao et al. 2013). Such infections could be lethal with improper medical treatment, thus, *S. aureus* has been considered to be one of the most serious risks in the public health all around the world (Zhong et al. 2015). According to the U.S. Center for Disease Control and Prevention (CDC 2011), approximately 1 in 120 Americans annually suffered from food poisoning caused by *S. aureus* in the United States (Scallan et al. 2011). This situation is even more serious in developing countries. Therefore, it is critical for human safety to develop accurate and rapid methods to detect *S. aureus*.

To date, great efforts have been devoted to explore convenient and accurate approaches for identifying *S. aureus*, but each of them showed its own drawbacks. The conventional methods serve as the basis in pathogenic bacteria testing laboratories despite rather laborious and time-consuming protocols (Liebana et al. 2009). Real-time fluorescent quantitative PCR has been employed for detecting the fluorescence of specific nucleic acids in *S. aureus* (Thet et al. 2013). However, this method requires highly skilled operators and expensive equipments. This is clearly insufficient, and many researchers have recently geared their efforts towards the development of rapid and sensitive strategies (Brigati et al. 2004). These techniques include immunoassays and nanoparticle-based platforms with specific and selective diagnostic probes for pathogens (Bedi et al. 2013). Gold nanoparticles (AuNPs), which

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possess unique surface plasmon resonance (SPR) with colors of red or blue corresponding to their dispersion or aggregation state, have drawn considerable attention in the development of rapid and visual sensing scheme (Chai et al. 2010; Ndokoye et al. 2014). Over the past decade, AuNPs based colorimetric assays have become an important research topic and been applied to monitor (bio)chemical substances (Liu et al. 2011; Niu et al. 2014). The concentration changes of targets can be easily transformed into color changes, which can be observed by the naked eye alone; hence, no sophisticated instruments are required.

As for pathogenic bacteria detection, AuNPs is generally modified with probe molecules, which can recognize target bacteria and improve the selectivity of AuNPs sensors. Antibodies, especially monoclonal antibodies are the most used because of their high specificity toward target antigens on the surfaces of pathogens (Lesniewski et al. 2014). However, the preparation process of antibodies is complex and costly, and few antibodies for specific targets are available (Lai et al. 2015). Furthermore, antibodies are usually very large and only a few antibodies can be immobilized on the surface of a single AuNP, which limit their widespread applications in pathogenic bacteria detection (Lai et al. 2015). Apart from antibodies, some antibiotics (such as vancomycin, daptomycin, etc.), proteins (such as avidin, biotin, lectin, etc.), DNA/RNA aptamers and carbohydrates (such as glycan) have also been conjugated with nanoparticles and used as targeting ligands to probe target bacteria cells (Bohara and Pawar 2015). For example, Xu's group had successfully developed vancomycin-modified FePt NPs to capture bacteria with vancomycin-resistance at a concentration as low as ~10 CFU (colony-forming unit) mL^{-1} (Gu et al. 2003; Gao et al. 2006). Despite the wonderful sensitivity, vancomycin shows poor specificity as it can bind to different vancomycin-resistant bacteria, such as *S. aureus*, *Enterococcus faecalis* and *Streptococcus pneumonia* (Bohara and Pawar 2015). Recently, Burcu et al. immobilized gold-coated magnetic spherical nanoparticles with biotin-labeled anti-*E. coli* antibodies and used them in the separation and detection of *E. coli* cells, with a limit of the detection (LOD) of 8 CFU mL^{-1} in 70 min (Guven et al. 2011). He's group successfully combined positive dielectrophoresis driven on-line enrichment and DNA aptamer-fluorescent silica nanoparticle label to detect *S. aureus* with high sensitivity (Shangguan et al. 2015). Adak et al. conjugated bovine serum albumin (BSA) to glycan with high affinity for *S. aureus* and printed as dot-matrix arrays by thermal injection (Adak et al. 2013). After exposed the glycan-BSA microarrays to *S. aureus* and decoded by a fast Fourier transform (FFT)-based algorithm, they detected *S. aureus* with a LOD at 10^3 CFU mL^{-1}. Although avidin, biotin, lectin, aptamer and carbohydrate ligands provided some advantages over antibodies, such as high binding affinity, stability and versatile target binding (Lazcka et al. 2007), they are experienced some drawbacks.

Phage display, as a well-established powerful technology, provides a tool to express heterologous proteins or peptides of interest on phage surface for a wide range applications (Liu et al. 2009; Mao et al. 2009; Petrenko and Smith 2000; Qi et al. 2012; Smith 1985). The f8/8 landscape phage library, containing about 2×10^9 different clones, was constructed by one of our coauthors, by replacing the N-terminal Glu-Gly-Glu (E2-G3-D4) peptide on every copy of the pVIII protein (Scheme S1) with random octapeptides. As a result, 4000 copies of random octapeptides were displayed on the phage surface as the N-terminal portion of the major coat protein pVIII (Petrenko et al. 1996). F8/8 landscape library has been used to select specific ligands with high affinity for many different targets, such as proteins (Lang et al. 2014; Qi et al. 2014), cancer cells (Wang et al., 2014) and bacteria, such as *Bacillus anthracis* (Brigati et al. 2004) and *Salmonella typhimurium* (Sorokulova et al. 2005), as well as swine fever virus (Yin et al. 2014). The selected phages have many merits including high specificity, multivalency, resistance to heat, organic solvents and many other stresses (Brigati et al. 2004), as well as rapidly and inexpensively in large quantities (Petrenko and Smith 2000). Furthermore, the target-specific pVIII protein can be obtained by simple isolation without losing its activity and selectivity.

In the present study, selection from f8/8 landscape phage library against *S. aureus* in a high-throughput way, we identified *S. aureus*-specific phage monoclone GQTTLTTS, which was further isolated to obtain *S. aureus*-specific pVIII proteins (fusion-pVIII). We then immobilized them on the cysteamine (CS) modified AuNPs (CS-AuNPs) by covalent conjugation. Combining the recognition capability of *S. aureus*-specific fusion-pVIII and the unique SPR property of CS-AuNPs, we developed a novel bifunctional gold nanoprobe for label-free, rapid and sensitive colorimetric detection of *S. aureus*. At last, we used this colorimetric method to detect *S. aureus* in real water samples. This strategy established in this work is expected to provide a novel method for fast and convenient detection of other pathogens.

2. Experimental section

2.1. Materials and reagents

S. aureus was kindly provided by Prof. Lingyun Qu in the First Institute of Oceanography, State Oceanic Administration (Qingdao, China). *Vibrio parahemolyticus* (*V. parahemolyticus*), *Vibrio anguillarum* (*V. anguillarum*), *Edwardsiella tarda* (*E. tarda*), *Escherichia coli* (*E. coli*) Trans 5α were generously gifted by Prof. Jie Huang in Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (Qingdao, China). *Proteus vulgaris* (*P. vulgaris*) was provided by Prof. Xiaolu Jiang in College of Food Science and Engineering, Ocean University of China (Qingdao, China). *Bacillus cereus* (*B. cereus*) was kindly provided by Prof. Xiangzhao Mao in College of Food Science and Engineering, Ocean University of China (Qingdao, China). The Nunc-Immuno MicroWell 96-well plate used in biopanning was from Nunc (Roskilde, Denmark). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99%), cysteamine hydrochloride (2-aminoethanethiol, CS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, USA). Tert-butyl carbamate (BOC) was purchased from J&K Chemical Ltd. (Beijing, China). BSA was purchased from Salarbio (Beijing, China). Tween 20 and tris(hydroxymethyl)-aminomethane (Tris) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals were used without further purification. Ultrapure water (18.2 MΩcm) was prepared using a Millipore Milli-Q system and used throughout. All other reagents were of the highest grade, and all solutions were prepared with ultrapure water.

2.2. Instrumentation

The SDS-PAGE was run on the Biorad Mini-PROTEAN Tetra Electrophoresis System (Hercules, California, U.S.), and corresponding images were acquired on the ImageScanner III from GE Healthcare (Piscataway, New Jersey, U.S.). Transmission electron microscopic (TEM) images were obtained using an H-7650 transmission electron microscope (Hitachi, Japan) with an accelerating voltage of 80 kV. Samples for TEM examination were prepared by putting a droplet of the treated solution on a copper grid coated with a thin carbonfilm and evaporating in air at room temperature. The UV–vis–NIR absorption spectra were collected using UV-1800 scanning spectrophotometer (Shimadzu, Japan) and ultrapure water was used as reference. The surface charge (zeta