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Recognition of *Salmonella typhimurium* by immobilized phage P22 monolayers

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Abstract

Phages are promising alternatives to antibodies as the biorecognition element in a variety of biosensing applications. In this study, a monolayer of bacteriophage P22 whose tailspike proteins specifically recognize *Salmonella* serotypes was covalently bound to glass substrates through a bifunctional cross linker 3-aminopropyltrimethoxysilane. The specific binding of *Salmonella typhimurium* to the phage monolayer was studied by enzyme-linked immunosorbent assay and atomic force microscopy. *Escherichia coli* and a Gram-positive bacterium *Listeria monocytogenes* were also studied as control bacteria. The P22 particles show strong binding affinity to *S. typhimurium*. In addition, the dried P22 monolayer maintained 50% binding capacity to *S. typhimurium* after a one-week storage time. This is a promising method to prepare phage monolayer coatings on surface plasmon resonance and acoustic biosensor substrates in order to utilize the nascent phage display technology.
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Keywords: Bacteriophage; *Salmonella typhimurium*; Lipopolysaccharide membrane

1. Introduction

The development of accurate and rapid detection methods of microorganisms, particularly those infectious pathogens in soil, food, and water, remains a technical challenge. It becomes more urgent due to the growing threat of bioterrorism. Different types of chemicals, toxins, and biological molecules are either being used or have the potential to be used as warfare agents [1–3]. Biorecognition strategies are being developed to utilize biomolecules such as enzymes, DNA, bacteriophages (or phages), and antibodies to detect complementary molecules through bio-specific interactions. Phages are virus particles that carry their genetic information in the form of DNA or RNA and can attach to specific receptors that are present on a limited range of host bacterial cells [4–7]. Since their discovery a century ago, phages have found new applications including phage therapy [8,9], water treatment [10], high-throughput screening [11,12], and biosensing [4,5,13–15]. The specific targeting phage probes can be directly attached to the sensor surface in surface plasmon resonance (SPR) and acoustic biosensors [16]. This paper presents a method to chemically immobilize phage particles on glass substrates while maintaining their binding affinity and specificity to bacterial surface receptors. The selectivity and durability of phage are key attributes to its ultimate use as a biosensing element.

Currently, antibodies are the most prevalent biosensing element for bacteria in immunosensors [17–20]. Phage display for bio-detection is a nascent technology suitable for real-time and inexpensive field detection [5]. Antibodies can be immobilized on solid substrates via their F_c fraction

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leaving the F_{ab} portion free for detection using physical adsorption [21], Langmuir–Blodgett (LB) technique [22,23], and covalent coupling [24,25]. Immunosensors have serious limitations including the non-antigenic nature of the analyte, incompatibility with the sample matrix or extraction process, and the time- and labor-intensive process of making the antibodies. In addition to the high cost, antibodies are highly fragile and sensitive to environmental conditions.

Compared to antibodies, phages are less fragile and less sensitive to environmental stress such as pH and temperature fluctuation [26–28], which give them longer field life for detecting toxins, bacteria, and spores. In addition, the new phage display technology offers billion clone libraries of recombinant phages for high-throughput detection. This paper explores the possibility of using a covalently linked phage monolayer for specific bacterial detection by combining the traditional enzyme-linked immunosorbent assay (ELISA) and molecular-level atomic force microscopy (AFM) characterization techniques. The well-documented P22 phage and its interactions with *S. enterica serovar typhimurium* (*S. typhimurium*) [29–34] was chosen as a model system. P22 is known to bind to the repetitive O-antigen part present in the lipopolysaccharides (LPS) of *Salmonella* outer membrane. P22 consists of double-stranded DNA packaged in an icosahedral capsid head and the O-antigen recognizing tailspike protein (TSP). A total of 6 TSP gp9 (6×215.4 kDa) copies are non-covalently attached to the capsid head by the N-terminal domain of the gp9 while the C-terminal domain binds to their cellular LPS receptor. Gp9 exhibits endoglycosidase activity by hydrolyzing the repeating sections of the O-antigen portion of LPS specifically at the Rha–Galα(1$\rightarrow$3)-glycosidic linkage.

Here we report a method to chemically immobilize P22 in a monolayer and the study of the interactions of P22 with various bacteria including Gram-negative *S. typhimurium* (Fig. 1) and *Escherichia coli*, and the Gram-positive *Listeria monocytogenes*. Previous reports on phage immobilization include physical adsorption [13–15] and Langmuir–Blodgett deposition [5]. Chemical adsorption based immobilization utilizing the versatile silane chemistry may improve the durability of the phage coating. We employed a chemical vapor deposition (CVD) method developed in our group to make a smooth aminosilane monolayer on silicon oxide surface of the glass substrate [35]. The phage P22 was bound to the aminosilane monolayer using the well-established sulfo-NHS and EDC chemistry [36–41]. To the best of our knowledge, our results are among the first to demonstrate successful immobilization of phage by EDC/NHS activation. Other covalent attachment schemes are also available in literature for phage immobilization [42,43]. Through ELISA and AFM characterization, we showed that the phage-coated substrates are capable of differentiating among different bacterial types in an aqueous environment. Such phage coatings promise to achieve the same degree of sensitivity and selectivity as current immunosensors but with a fraction of the cost and significantly improved durability.

2. Experimental

2.1. Materials

3-Aminopropyltrimethoxysilane (APTMS) and *o*-phenylenediamine dihydrochloride (OPD) were purchased from Sigma–Aldrich. *N*-Hydroxysulfosuccinimide (sulfo-NHS) and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) were purchased from Pierce. HRP-labeled anti *Salmonella* IgG and anti *E. coli* IgG were purchased from US biological (s0060-20) and HRP-labeled anti *Listeria* IgG was purchased from Abcam (ab20357). Phage P22 (*S. enterica* subsp. *Enterica serovar typhimurium* bacteriophage) ATCC (19585-B1), *S. typhimurium* ATCC (19585), and *E. coli* strain ATCC (33780) were used. Cellulose acetate filters (0.22 μm) were purchased from Corning.

2.2. Bacteria culture

Three millilitres Luria–Bertani (LB Broth) was added to a 15 ml centrifuge tube. The media was inoculated with *S. typhimurium*. The tube was shaken in water bath at 37 °C

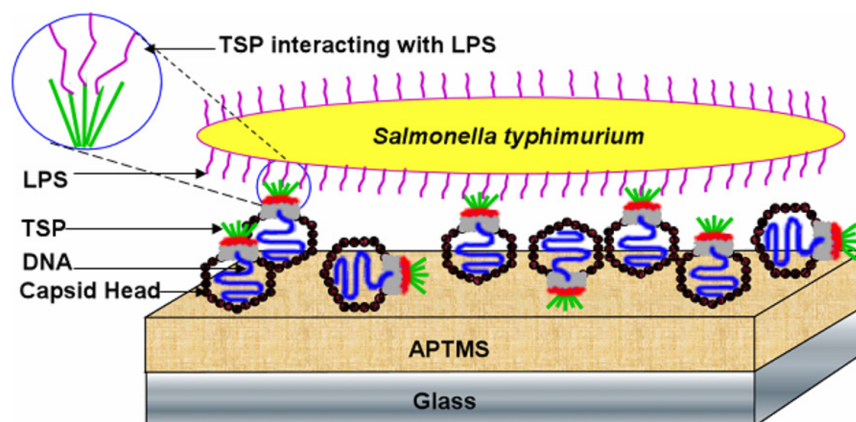

Fig. 1. Scheme of phage P22 immobilization for the detection of *S. typhimurium*.

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