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Bacteriophages as biorecognition elements in capacitive biosensors: Phage and host bacteria detection

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ABSTRACT

Herein, we introduced a molecular imprinting based capacitive biosensor for real-time and highly sensitive bacteriophage detection. The sensing mechanism was based on the binding of target phage into the specific cavities on the electrode surface which resulted in a measurable change in the total capacitance of the system. Phage detection was investigated in the concentration range of 1.0×10^1–1.0×10^5 plaque forming units (pfu)/mL and the limit of detection (LOD) was measured as 10 pfu/mL which shows the high sensitivity of the system compared to results reported for previous studies. The system also allowed the detection of phages in river water samples which is very important for the usability of the system as in-field analysis for different applications e.g. investigating the contamination of drinking water via wastewater or reservoir water in the future. Recently, due to their high specificity towards their host bacteria, being cost-effective and also stable in harsh environments, bacteriophages have been used as biorecognition elements in many studies. Due to this reason, the applicability of the phage imprinted biosensor was also investigated for host bacteria detection. *E. coli* detection has been performed in the concentration range of 1.0×10^2–1.0×10^7 colony forming units (cfu)/mL with a LOD value of 100 cfu/mL. This system offers direct, real-time, very sensitive and rapid detection of bacteriophage and its host bacteria.

1. Introduction

Bacteriophages (phages) are viruses which infect host bacteria with either a narrow or broad specificity [1]. They are ubiquitous in almost all environments including soil, food, ground and surface water [1]. They have robust stability under suboptimal environmental conditions. Quick and sensitive detection of bacteriophages is important during biotechnological and biopharmaceutical processes in order to determine the infections of bacterial cultures with bacteriophages [2]. Bacteriophages also play an important role in spreading antibiotic resistance via transduction even though the impact has partly been neglected so far [3,4]. Metagenome studies of bacteriophages have revealed that there are antibiotic resistant genes present in the phage population [5]. Therefore, targeting single phage species and studying the phageome of these specific phages for resistance genes in complex systems is important.

The most commonly used biological assay for bacteriophage detection is the double agar overlay plaque assay which is laborious and time consuming [6]. There are a lot of efforts to develop novel diagnostic tools for viruses including bacteriophages, such as atomic force microscopy (AFM) [7], interferometry [8], electrochemistry [9] and biosensor systems [10–13]. A lot of work has been focused on biosensors as detection methods owing to their advantages as being easy to operate, highly sensitive and capable of real time measurement [14,15]. Further, no labelling is required in real time measurement which makes the whole process cost- and time-effective [2].

Many of the biosensor studies are based on the use of antibodies as biorecognition elements due to the high specificity between an antibody and its antigen. Antibodies possess high affinity towards their target, they can be easily implemented into reliable and portable biosensor systems [10]. However they have some disadvantages when they are used in the detection systems. They are expensive and unstable in harsh conditions resulting in limited reusability. An alternative to circumvent these drawbacks is the use of molecularly imprinted polymers (MIPs). Contrary to natural antibodies, MIPs are robust and stable [16,17]. They can be reused for a long period which also makes them cost-effective [18].

Recently bacteriophages have also been used as biorecognition elements in biosensors for bacterial cell detection [19–22]. Application of phages as a detection probe has many advantages in rapid diagnosis [23]. As a biorecognition element, they offer very high selectivity towards their host bacteria, they are inexpensive and resistant to harsh conditions including organic solvents and high

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temperatures, they can discriminate between viable and non-viable bacterial cells and working with them involves no health concerns [10,24]. Due to different functional groups on the surface of bacteriophages, their immobilization on an electrode surface is highly feasible [24].

In the present study a molecular imprinting method was used to develop a capacitive biosensor for real-time, highly sensitive, label-free and fast detection of a bacteriophage. Due to the high abundance and relevance for biotechnology and bioprocesses, an *E. coli* phage was used as a model bacteriophage.

The developed system enables highly sensitive and selective detection without requiring any additional signal amplification. Moreover, the system can also be used for detection of host bacteria owing to the specific interactions between the bacteriophage and its host bacteria. We demonstrate this applicability of the sensor for the detection of the phage host bacterium in the last part of the study.

2. Materials and methods

2.1. Materials

N-hydroxymethyl acrylamide (cat.no.697931), the initiator 2,2′-azobis (2-methylpropionitrile) (cat no. 441090), acryloyl-chloride and 1-dodecanethiol were obtained from Aldrich (Deisenhofen, Germany). Polyethylene glycol 400-dimethacrylate (PEG 400 DMA) (cat.no.15179-100) was purchased from Polysciences, Inc, USA. Glutaraldehyde (50%, w/v), trimethylamine, 3-aminopropyltriethoxysilane (APTES) were purchased from Fluka (Buchs, Switzerland). Tyramine (99%) was supplied from Sigma Chemical Co. (St. Louis, USA). Glass microscope cover slips (24 × 50 mm) (Menzel-Glaser) were used as the phage stamp in molecular imprinting. All other chemicals used were of analytical grade. All buffers were prepared with water processed using a reverse osmosis step with a Milli-Q system from Millipore (Bedford, MA, USA). Prior to use, all buffers were filtered through a Millipore filter (pore size: 0.22 μm) and degassed for 1 h.

2.2. Bacteriophage sample preparation

The bacteriophage used in the study was isolated from a batch of *E. coli* used in a bioprocessor unit, contaminated with a lytic phage. The phage was propagated against TOP10 *E. coli* cells by using a standard double agar overlay assay, as described elsewhere [6]. In this technique, different dilutions of the phage samples are mixed with the host bacterium. Then, these samples are dispersed onto a solid medium. On incubation, host bacteria forms a lawn on the solid medium. However where the phage particles lyse or inhibit the growth of the cells, they form a localized clear or translucent zone. These zones are termed as plaques and the infectious phage unit is thus termed as a 'plaque forming unit (pfu)'. Phages were extracted from the agar by the addition of SM buffer (100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl pH 7.5, 0.01% gelatin) to the plates, incubating for 30 min at room temperature, carefully rotating the plates. The SM-buffer was collected, and NaCl (0.5 M) was added to the solution to release phages bound to bacterial debris (30 min, room temperature), after which the solution was centrifuged (8000*g*, 20 min) and sterile filtered (0.22 μm). The phages were precipitated by addition of 10% (w/v) PEG 6000, collected by centrifugation (10,000*g*, 30 min), and resuspended in SM-buffer.

2.3. Preparation of bacteriophage imprinted capacitive biosensor electrodes

The bacteriophage imprinting was done in 3 steps:

In the first step, glass slides serving as phage stamps were prepared and cleaned as previously described [15,25,26]. Following cleaning, glass slides were modified by using 10% APTES in ethanol (v/v) at room temperature for 1 h in order to introduce amino groups on the surface. Then, they were rinsed with ethanol and water, respectively and dried with nitrogen gas. Following APTES modification, glass slides were immersed in 5% glutaraldehyde solution (v/v) in 10 mM phosphate buffer (pH 7.4) at room temperature for 2 h in order to obtain aldehyde groups on the surface. Then, the glass slides were rinsed again with water and dried with nitrogen gas. In order to immobilize the bacteriophages on the surface of the slides, 200 μL of bacteriophage suspension (in 10 mM phosphate buffer, pH 7.4, 10^4 pfu/mL) was dropped on the glass slides and incubated at room temperature overnight. Then, they were washed with deionized water to remove the unbound phages from the surface and dried with nitrogen gas.

In the second step, modification of the gold electrodes was carried out. Gold electrodes were cleaned as previously described [26] and then electropolymerization of tyramine was performed by using cyclic voltammetry (CV) in an ethanolic solution of 10 mM tyramine. Cyclic voltammetric scans (15 cycles) were performed using an IviumStat (Netherlands) covering a potential range of 0–1.5 V (Ag/AgCl) and a scan rate of 50 mV s^{-1}. By this way, free primary amino groups were introduced via the deposition of polytyramine on the surface of the electrode. In the next step, electrodes were immersed in a solution containing 30 mM acryloyl chloride and 30 mM trietyhlamine in toluene overnight at room temperature. The reaction of acryloyl chloride with the free primary amino groups on the surface generated amide groups and left free vinyl groups that would be involved in the subsequent polymerization step. In the last step, electrodes were rinsed with distilled water and dried with nitrogen gas.

For the imprinting of the bacteriophages on the capacitive gold electrode, a monomer solution containing N-hydroxymethyl acrylamide and PEG-400 DMA were mixed in 1 mL of 10 mM phosphate buffer (pH 7.4). The concentration of the monomers were 1.8×10^{-1} M and 3.0×10^{-1} M for N-hydroxymethyl acrylamide and PEG-400 DMA, respectively. Then, the photo initiator [2,2′-Azobis (2-methylpropionitrile]) (1 mg) was added into this solution. The monomer solution (1.5 μL) was pipetted onto the electrode surface and the bacteriophage stamp was brought into contact with this surface. UV polymerization (365 nm, 400 W) was continued for 15 min. After polymerization, the phage stamp was removed from the surface. The phage-imprinted electrode was rinsed with distilled water and immersed in 1-dodecanethiol (10 mM in ethanol) for 20 min in order to cover any pinholes on the surface of the electrode.

Preparation of the phage imprinted capacitive gold electrodes are shown schematically in Scheme 1B.

2.4. Surface characterization of the capacitive electrodes with scanning electron microscopy (SEM)

For scanning electron microscopy, specimens were mounted on aluminium holders with adhesive carbon tape and sputtered with 10 nm palladium/gold. The samples were examined in a DELPHI correlative light and electron microscope (Phenom-World) at the IQ Biotechnology Platform, Lund University.

2.5. Real time bacteriophage analysis with capacitive biosensors

Capacitive measurements were performed in a continuous flow system with an automated capacitance measuring device, CapSenze Biosystems (CapSenze AB, Lund, Sweden). The continuous flow system was developed by CapSenze Biosystems (Lund, Sweden). The system was 85 × 65 × 30 cm in size and operated