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Using the synergism strategy for highly sensitive and specific electrochemical sensing of *Streptococcus pneumoniae Lyt-1* gene sequence

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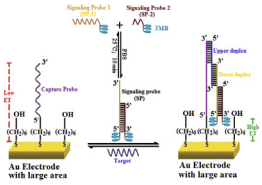
HIGHLIGHTS

- Introduction of an electrochemical method for Lyt-1 gene sequence of *Streptococcus pneumoniae*.
- This sensing method is highly versatile and is demonstrated for real application.
- A synergism strategy involving dual-hybridized signaling probe is used for improving the sensitivity.
- The sensor features a low detection limit of ~0.5 fM for the target.

GRAPHICAL ABSTRACT



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ABSTRACT

With the help of the interaction mode of capture probe-target-signal probe (CP-T-SP), an electrochemical sensing method based on the synergism strategy of dual-hybridized signaling probes modified with 6 MB (methylene blue), background suppression and large surface area Au electrode is developed for the detection of *Streptococcus pneumoniae* (*S. pneumoniae*) *Lyt-1* gene sequence. The proposed sensor features a very low detection limit (LOD) of ~0.5 fM for the target. This method also exhibits highly versatility and can apply to the construction of other sensors for the analysis of similar designated pathogenic bacteria gene sequence (PBGS).

1. Introduction

Rapid and accurate detection of pathogen DNA have received a great deal of attention in the field of biomolecular sensing owing to its important role in future disease diagnosis [1–5]. In order to achieve this aim, a lot of biological detection technologies, such as polymerase chain reaction (PCR), bioassay, SERS primers, Bio-MassCode mass spectrometry and so on, have been developed for the detection of pathogen DNA in recent years [6–9]. While most of these methods are suffering from the obvious drawbacks of time consuming, complicate processing steps and complex operation, as one of bio-sensing technologies, electrochemical DNA (E-DNA)

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biosensor exhibits many remarkable features of simple fabrication procedure, high sensitivity and selectivity, fast response, easy miniaturization and convenient operation [10–12]. All these features endow this technology a bright prospect for future real world application [13,14]. *Streptococcus pneumoniae* is the most common cause of community-acquired pneumonia, the second most common cause of meningitis, and a leading cause of otitis media in children [15]. The high sensitive DNA detection is an effective way to achieve the detection of its pathogen. The reported traditional detection methods of *S. pneumoniae* DNA are mainly PCR-based technology to improve the sensitivity of method [16–23], thereinto, the work worthy to be mentioned is S. Campuzano research group's detecting method of sensor, they constructed a disposable amperometric magneto-genosensor for the rapid, specific and sensitive detection of *S. pneumoniae* DNA, the developed method can detect as low as 1.1 nM for the target DNA with the help of asymmetric PCR amplicon [22]. They also made a clinical evaluation for this method used for the direct detection of pneumococci in clinical samples successfully [23]. Because of having above-mentioned drawbacks inevitably, such PCR-based technology is waiting for improving in detecting diagnosing pneumococcal infections in the majority of patients. E-DNA biosensor technology can be one of the best choices to solve this problem. Based on this, we design and fabricate an electrochemical DNA sensing method for the detection of *S. pneumoniae Lyt-1* gene sequence. The synergism strategy was used to improve the sensitivity of method for the target DNA. It is predicted that background suppression via surface or construction design may be an effective way but has not been rigorously tested in E-DNA sensors to improve the sensitivity theoretically [24]. The large surface area electrode can produce high detection current signal which also may be helpful to decrease detection limit of the sensor. Although the use of multi-redox labels is an understandable direct way to increase the detection current signal, it is still a big trouble awaiting for overcoming because of the difficulty in modifying more redox moieties on oligonucleotide probe. Up to now, not more than 3 redox moieties, such as Fc (ferrocene) and MB, have been achieved in practical synthesis. Dual-hybridized redox moiety-modified probes can be one of practicable means to achieve this aim. So using the synergism of these three aspects should be a feasible way to improve the sensitivity of sensor. Besides the high sensitivity, the proposed sensor also exhibits good mismatch discrimination ability, fast sensing speed, easy regeneration and satisfactory working capability in real complex matrixes, such as the serum and the whole blood.

2. Experiment

2.1. Reagents and materials

A 38-base thiolated DNA probe was used as the surface-immobilized target capture probe (**CP**). A long 28-base 3MB-modified DNA probe was used as the signaling probe 1 (**SP-1**) and a short 16-base 3MB-modified DNA probe was used as the signaling probe 2 (**SP-2**). The 16-base italicized portion of two probes can be hybridized to form the signaling probe (**SP**). A long thiolated 28-base 3MB-modified DNA probe (**SP-3**) was used to hybridize with **SP-2** to form **S-SP** which is used for comparing the blank MB current signal values obtained with **SP** free in the solution and restricted on the electrode surface through Au–S bond. 3**MB-CP** is used for calculating the probe coverage of **CP** which is attached on the gold electrode surface. 5 probes were obtained from TaKaRa Inc. (Dalian, China). 4 DNA targets were obtained from Beijing Xinlilai Biotechnology Inc. (Beijing, China) and used as received. The sequence information of all probes and targets is shown in the following. The double underlined portion of **CP** forms a 12-base duplex with the double underlined portion of *S. pneumoniae Lyt-1* gene sequence, **FY-0M**, whereas the single underlined portion of **FY-0M** hybridizes to the single underlined portion of **SP-1**. The mismatches in the targets are highlighted in bold.

CP : 5′SS $(CH_2)_6$ GCCCCAACCCCCTTCCCCCCCCCCTT ATATTCTACTCC 3′

3MB-CP:5′ SS $(CH_2)_6$ GCCCCAACCCCCTTCCCCCCCCCCTTA-TATTCTACTCC -T(MB)T(MB)T(MB) 3′

SP-1: 5′ T(MB)T(MB)T(MB)-*TGACCGGCTCGACCTG*ACATTAATTTCC 3′

SP-2: 5′ *CAGGTCGAGCCGGTCA*-T(MB)T(MB)T(MB) 3′

SP-3:5′T(MB)T(MB)T(MB)-*TGACCGGCTCGACCTG*ACATTAATTTCC$(CH_2)_6$SS3′

***Streptococcus pneumoniae Lyt-1* perfect match gene sequence target (FY-0M)**:

5′ GGAGTAGAATAT GGAAATTAATGT 3′

1C-base mismatch of 7 site in upper 12-base portion target (FY-7-C-1M):

5′ GGAGTA**C**AATATGGAAATTAATGT 3′

1A-base mismatch of 18 site in down 12-base portion target (FY-18-A-1M): 5′ GGAGTAGAATATGGAAA**A**TAATGT 3′

1C-1A-base mismatch of 7 and 18 site in two separate portions of target (FY-7-C-18-A-2M): 5′ GGAGTA**C**AATATGGAAA**A**-TAATGT 3′

The reagents 6-mercapto-1-hexanol (C_6–OH) and tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) were used as received (Sigma–Aldrich, St. Louis, MO). The bovine calf serum were from Sigma–Aldrich. The whole blood was provided by the university hospital. All other chemicals were of analytical grade. All the solutions were made with deionized (DI) water purified through a Millipore system (18.2 MΩ·cm, Millipore, Billerica, MA). The sensors were interrogated in a phosphate buffer solution (PBS) containing 10 mM sodium phosphate and 1.0 M sodium chloride (pH 7.4), 50% serum (1:1 serum/PBS) and 50% whole blood (1:1 whole blood/PBS).

2.2. Sensor preparation

Prior to sensor fabrication, 3 different size gold disk electrodes (Φ = 6, 2, 0.5 mm, Fig. 1) with geometric area of 0.2826, 0.0314 and 0.001963 cm^2 (CH Instruments, Austin, TX) were polished with a 0.5% 0.05 μm alumina powder (CH Instruments, Austin, TX) solution, rinsed with DI water and sonicated in a low power sonicator 5 min to remove bound particulates. They were then electrochemically cleaned by a series of oxidation and reduction cycles in

Fig. 1. The picture of 3 Au electrodes with different size (Φ = 6, 2, 0.5 mm).