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Electrochemical Biosensor for Detection of *Staphylococcus aureus* Based on Nucleic Acid Aptamers

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Abstract

Staphylococcus aureus is a gram-staining positive cocci bacillus baterium and also one of the foodborne pathogens, which is a serious potential hazard to human health and food safety. We constructed an electrochemical biosensor for the detection of *S. aureus* based on nucleic acid aptamers to achieve highly specific detection of *S. aureus*. The detection of *S. aureus* was realized by using Aptamer (Apt) to capture *S. aureus*, which resulted in a change in the spatial conformation of Apt and a decrease in the electrochemical signal. Under the optimized experimental conditions, the detected electrochemical signals were positively correlated with the concentration of *S. aureus* with a linear range of 1×10^1 - 1×10^5 CFU/mL, a detection limit of 4.76 CFU/mL, and an experimental recovery of 97.43% - 99.37%. Therefore, we successfully constructed an electrochemical biosensor for the specific detection of *S. aureus*, which has the advantages of high specificity, sensitive detection and convenient operation.

Keywords

S. aureus, Nucleic Acid Aptamer, Electrochemistry

1. Introduction

Staphylococcus aureus (*S. aureus*) is a gram-staining positive coccus, and a purulent bacterium widely found in nature, on human as well as animal skin surfaces [1]. *S. aureus* is highly pathogenic, causing a wide range of purulent and toxigenic diseases, and is the most common causative agent of nosocomial infections [2] [3]. In addition, *S. aureus* is one of the most important foodborne pa-

thogens, and food poisoning caused by contaminated food poses a major threat to human life and health [4] [5]. Therefore, it is of great significance to realize the rapid, sensitive and specific detection of *S. aureus*.

The traditional bacterial culture method for the detection of *S. aureus*, although inexpensive and commonly used in resource-limited settings, the method is time-consuming and not able to meet the requirements of rapid detection [6]. PCR method, although highly sensitive, but the instrument is expensive and sophisticated, requires highly qualified personnel to operate, and is difficult to popularize [7]. The traditional ELISA method for the detection of *S. aureus* is characterized by low chemical stability, high cost, and difficulty in recycling [8]. Therefore, it is necessary to develop inexpensive, rapid and sensitive assays for the detection of *S. aureus*. Based on this, we constructed an electrochemical biosensor using nucleic acid aptamers for the detection of *S. aureus*.

A nucleic acid aptamer is essentially a segment of oligonucleotide sequence, which is screened by exponentially enriched ligand evolution technique [9]. Based on cDNA libraries, oligonucleotide sequences with high specificity are screened after dozens or tens of rounds of binding, isolation, and amplification in three steps [10]. Aptamers are more easy to prepare and have long preservation time as opposed to antibodies, which are widely used in disease diagnosis, and drug therapy [11] [12].

In summary, we used nucleic acid aptamers to construct an electrochemical sensor for the specific detection of *S. aureus* by generating the *S. aureus*-Apt complex, which changes the spatial conformation of Apt and reduces the electrochemical signal. This method has the advantages of high specificity, sensitivity, convenience and low cost in the detection of *S. aureus*. In Table 1, the recoveries were 95.76% - 101.20% and the detection limit was 4.29 CFU/mL.

2. Materials and Methods

2.1. Materials and Chemical Reagents

Potassium ferricyanide ($K_3Fe(CN)_6$), 6-MCH, were purchased from Aladdin (Shanghai, China); LB agar plates, nucleic acid sequences were purchased from Bioengineering (Shanghai, China); all other reagents were analytically pure and did not require further purification or treatment. All bacteria were cultured in the laboratory.

Table 1. Recovery results for the *S. aureus* in pure water and pure milk.

	Pure milk			
Sample	*S. aureus* Added (CFU/mL)	*S. aureus* Found (CFU/mL)	Recovery (%)	RSD (n = 3)
1	50	48.72	97.43	30.1
2	100	97.99	97.99	42.9
3	200	198.74	99.37	31.8
4	300	288.86	96.28	25.5

Apt: 5'-SH-GCAATGGTACGGTACTTCCTCGGCACGTTCTCAG TAGCGCTCGCTGGTCATCCCACAGCTACGTCAAAAGTGCACGCTACT TTGCTAA-Fc-3'

2.2. Instrument Parameters

The working electrode was a gold electrode (Au), the auxiliary electrode was a platinum wire electrode (Pt), the reference electrode was Ag/AgCl, and the electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) curves were carried out on an electrochemical workstation (CHI660e) in 0.5 mm of $[Fe(CN)_6]^{3-/4-}$. The 0.5 mm $[Fe(CN)_6]^{3-/4-}$ was analyzed by using a PBS solution configuration.

2.3. Preparation for Detection

Prior to the use of the electrochemical sensor for the detection of *S. aureus*, the gold electrode was polished with 0.3 μm and 0.05 μm alumina powder, ultrasonically cleaned, and blown dry with N_2. Then, the electrode was submerged in Apt solution for 10 h to fully modify Apt on the electrode surface by Au-S bonding. Finally, the membrane electrode was immersed in 10 μL of 0.3 mM MCH solution to prevent non-specific site binding. The construction of the electrochemical biosensor was completed.

Pre-treatment of the golden Portuguese was required before its detection. The *S. aureus* were inoculated from the medium into Luria-Bertani (LB) plates for activation, and the activation step was repeated three times to obtain a well-grown *S. aureus*. Then, the *S. aureus* were re-inoculated into the medium and incubated at 37˚C for 12 h. The concentration of *S. aureus* was expressed as CFU/mL in this experiment. The concentration of *S. aureus* was measured using a hemocytometer and light microscope and expressed as CFU/mL in this experiment. A solution of *S. aureus* with a concentration of 1×10^1 - 1×10^5 CFU/mL was prepared and the bacteria dissolved in *S. aureus* solution was diluted to different concentrations using 0.01 M PBS (pH 7.4) buffer. Other bacterial treatments were the same as for *S. aureus*.

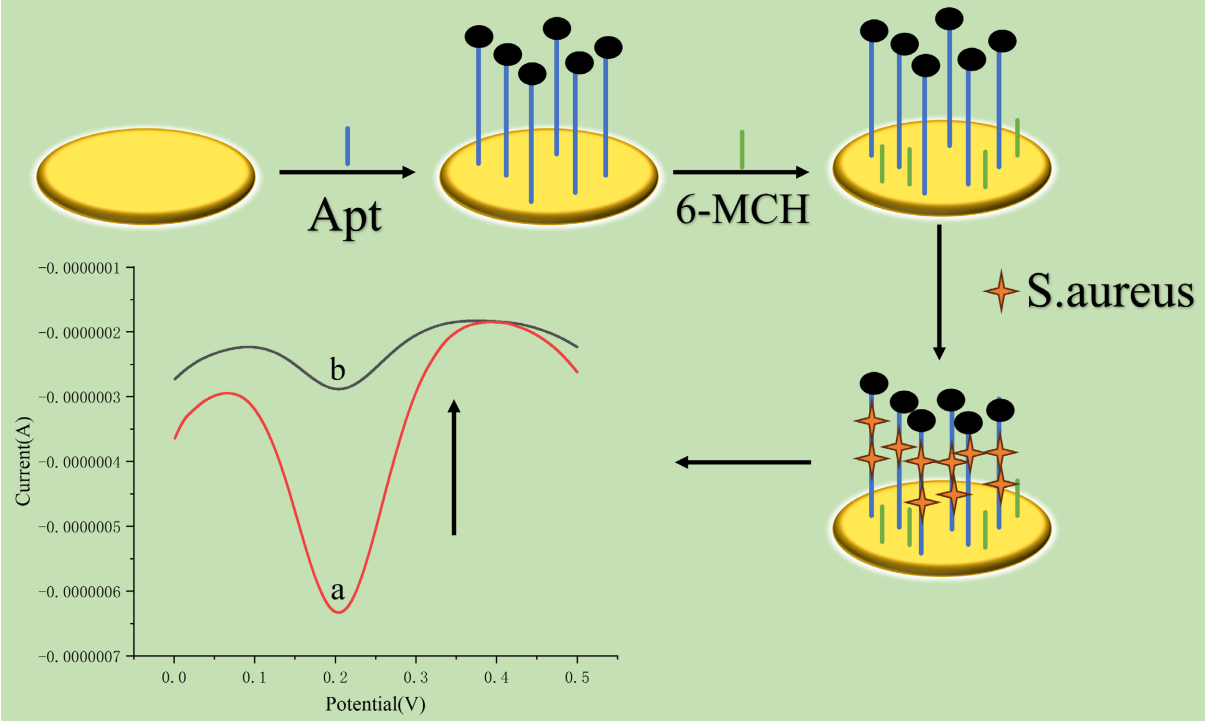
2.4. *S. aureus* Determination Procedures

The treated *S. aureus* bacterial solution was added dropwise to the surface of Apt/MCH/Au, respectively, and incubated at 37˚C for 30 min in order for *S. aureus* to fully bind to Apt, followed by electrochemical signal detection. Other bacteria were detected in the same way as *S. aureus*, and in this experiment, Square Wave Voltammetry (SWV) was used as the electrochemical signal output for observing the signal changes.

3. Results and Discussion

3.1. Principle of the Proposed Sensing Strategy

As shown in Scheme 1, in order to realize rapid and sensitive detection of *S. aureus*,



Scheme 1. Principle of the proposed sensing strategy.

we constructed an electrochemical biosensor based on nucleic acid aptamer and three-electrode system. We sequentially modified the nucleic acid aptamer and 6-MCH on the gold electrode, and 6-MCH prevented nonspecific adsorption and increased the stability of the sensor. When the *S. aureus* was not present in the solution to be tested, the aptamer was in an irregularly curled state, which facilitated electron transfer between the redox probe $[Fe(CN)_6]^{3-/4-}$ and the electrode in the electrolyte solution, and the electrochemical signal value was high. However, when the nucleic acid aptamer successfully captured the *S. aureus*, the spatial conformation of the nucleic acid aptamer was changed, and the electron transfer was blocked, and the electrochemical signal value decreased. We used SWV as the detection signal, and the SWV current signal decreased significantly after the capture of *S. aureus*, and the measured SWV current intensity was closely related to the concentration of *S. aureus*. We successfully constructed an electrochemical biosensor based on a nucleic acid aptamer for the specific detection of *S. aureus*.

3.2. Characterizations of Sensor Fabrications

We used SWV curves to characterize the process of biosensor construction changes. From Figure 1, it can be seen that the bare gold electrode has the fastest rate of electron transfer and has the largest current response (curve a), followed by a gradual decrease in the current response value when the Apt signaling probe (curve b) and 6-MCH (curve c) are sequentially modified on the electrode. When Apt captures the *S. aureus*, it leads to the change of Apt spatial conformation, which effectively hinders the rate of electron transfer, and the electrochem-

ical signal decreases (curve d), which can indicate the success of our electrochemical sensor construction.

3.3. Optimization of Analytical Conditions

In order to obtain the best experimental conditions, we choose to optimize the concentration of Apt. We performed SWV assay for different concentrations of Apt loaded onto the gold electrode separately. We established groups with different concentrations of Apt, 1 μM for group a, 2 μM for group b, 3 μM for group c, 4 μM for group d, and 5 μM for group e. As shown in Figure 2(A), Figure 2(B), the electrochemical signals were relatively stable when Apt was at 4 μM (curve d), and by adding more Apt concentration, the electrochemical signals became flat and the increase in the difference of the SWV currents tended to be stabilized, which indicated that the higher concentration of the aptamer didn't make the electrode This indicates that higher aptamer concentration did not increase the amount of aptamer binding on the electrode surface. Therefore, the sensor was assembled using 4 μM aptamers to capture a large amount of *S. aureus*.

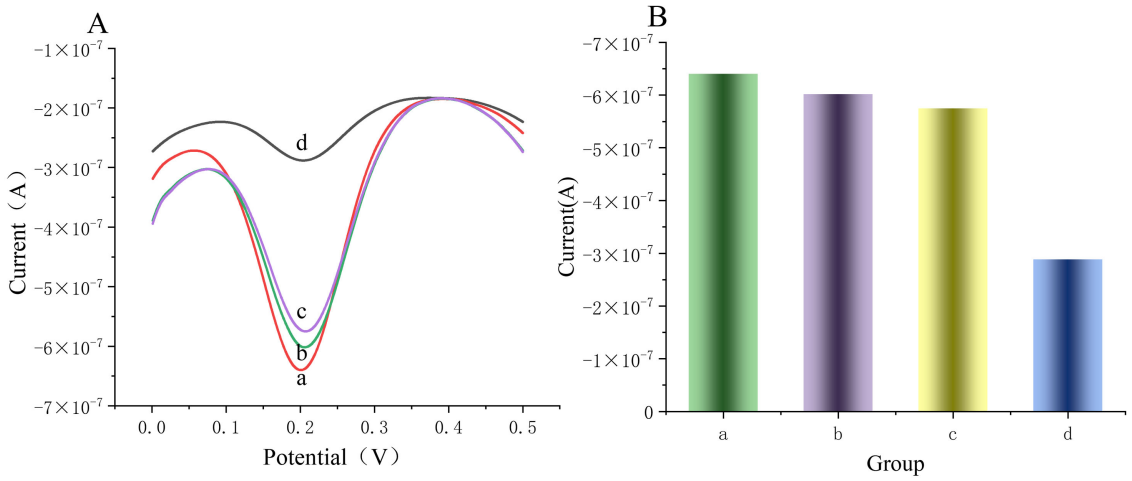


Figure 1. The current of SWV of sensor fabrications ((A)-(B)).

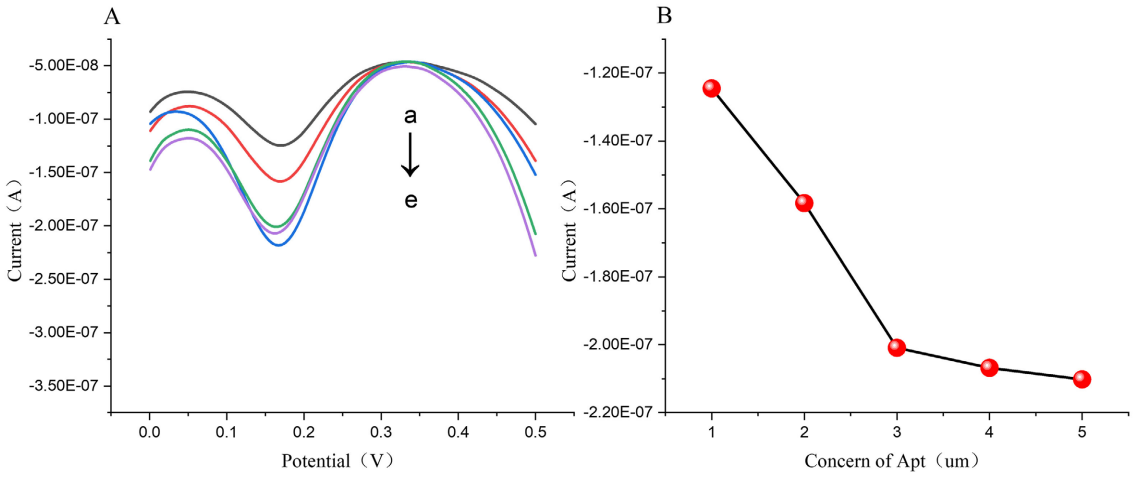


Figure 2. Optimization of the experimental conditions. ((A)-(B)) SWV the current of the concern of Apt.

3.4. Assay Performances of Proposed Method

The electrochemical sensor platform was used to detect different concentrations of *S. aureus*, and the electrochemical signal values were analyzed to evaluate the sensitivity of the developed electrochemical biosensing platform for detecting target genes. Figure 3(A) and Figure 3(B) show how different concentrations of *S. aureus* affect the responsiveness of the electrochemical signal.

In this experiment, *S. aureus* was cultured to an initial concentration of 1×10^1 CFU/mL As the concentration of the target gene continued to increase, the electrochemical signal signals were linearly related to the logarithm of the concentration of *S. aureus* (C, C represents the concentration of Lg *S. aureus*). In this experiment, a linear regression equation was developed which showed a linear relationship between the electrochemical signal level and the target gene concentration, $Y = -1.98 \times 10^{-7}$ LgC + 1.437×10^{-8}, $R^2 = 0.99236$. The limit of detection was determined to be 4.76 CFU/mL based on the generalized equation LOD = $3\sigma/k$, where k is the slope of the linear regression equation and σ is the standard deviation of the blank signal.

3.5. Specificity of the Electrochemical Biosensor and Detection of *S. aureus* in the Environment Sample

In order to verify that the biosensor is specific for the detection of *S. aureus*, under the optimal experimental conditions, we selected different species of bacteria for testing the specificity of the sensor. Five groups were set up, namely group a blank control, group b 100 CFU/mL *S. mutans*, group c 100 CFU/mL Salmonella, group d 100 CFU/mL *S. aureus*, and group e 100 CFU/mL *S. aureus*. As can be seen from Figure 4: the electrochemical signal of the sensor in response to *S. aureus* is much lower than that of other bacterial groups, which is due to the change of spatial conformation after the recognition of *S. aureus* by Apt, which hinders the electron transfer and finally leads to the decrease of electrochemical

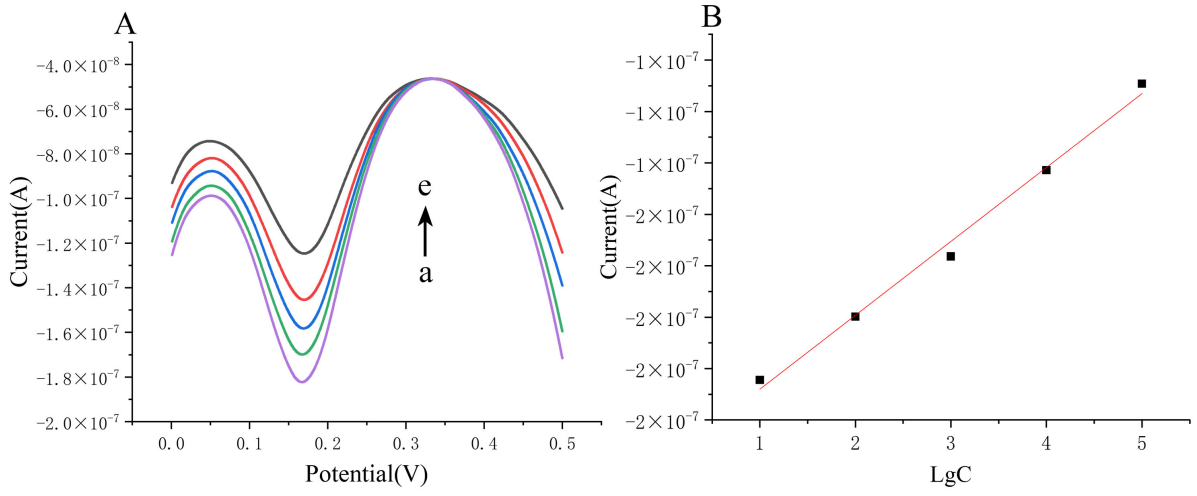


Figure 3. (A) SWV current after treatment of the sensor with different concentrations of *S. aureus*: a) 1×10^1 CFU/mL, b) 1×10^2 CFU/mL, c) 1×10^3 CFU/mL, d) 1×10^4 CFU/mL, e) 1×10^5 CFU/mL, respectively. (B) Plots of current intensity versus the concentration of *S. aureus*.

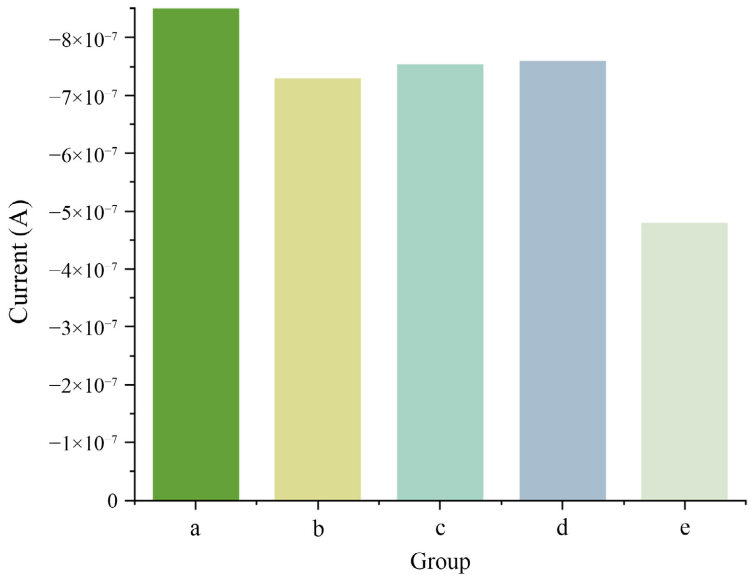


Figure 4. Specificity of the biosensor.

signal. Thus, the sensor has a large difference in electrochemical signal for *S. aureus* relative to the particularly small signal changes caused by non-specific detection of other bacteria. This demonstrates the good selectivity of the experimental method for *S. aureus* in complex environments. This demonstrates the excellent specificity of the electrochemical biosensing platform we developed for the detection of *S. aureus*.

In order to test the practicality of the method, we used pure milk as the actual detection samples, and added different concentrations of *S. aureus* into the actual samples to obtain 100 - 400 CFU/mL of the actual detection samples, respectively. As shown in **Table 1**, the recoveries of the method were in the range of 97.43% - 99.37%, which indicates that our constructed electrochemical biosensor has good practicality for the detection of *S. aureus* in real samples.

4. Conclusion

We constructed an electrochemical biosensor for the detection of *Staphylococcus aureus* using Fc as an electrochemical signaling molecule. When the solution to be tested contains *S. aureus*, the nucleic acid aptamer undergoes a spatial conformation change to capture *S. aureus*, and with successful capture, the rate of electron transfer on the electrode surface is effectively hindered, resulting in a decrease in the electrochemical signal response value. This method can be used to monitor the onset and progression of *S. aureus*-induced diseases, as well as to observe efficacy in the prognostic stage of the disease. In addition, the sensor also provides a research direction for the detection of other disease markers or pathogenic bacteria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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