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An enhanced chemiluminescence resonance energy transfer aptasensor based on rolling circle amplification and WS_2 nanosheet for *Staphylococcus aureus* detection

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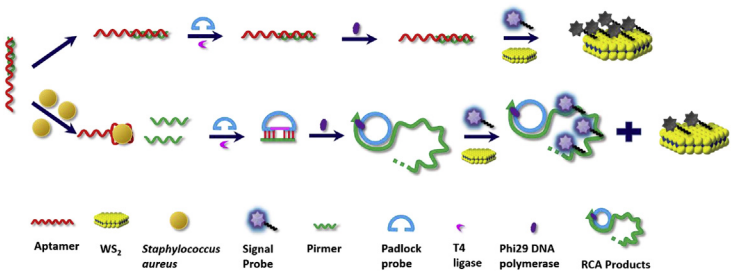
HIGHLIGHTS

- Co^{2+} enhanced ABEI functional flowerlike gold nanoparticles (Co^{2+}/ABEI-AuNFs) was first used as chemiluminescent donor.
- Rolling circle amplification (RCA) technique was also applied in the WS_2 nanosheet based CRET system for the first time.
- The improved WS_2 nanosheet based CRET platform can overcome the limitation that only DNA or RNA can be detected and expand the detection scope.
- A steady-state CRET aptasensor based on P-Iodophenol (PIP) and RCA was fabricated to detect *S. aureus* with high sensitivity and specificity.

GRAPHICAL ABSTRACT



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ABSTRACT

A chemiluminescence resonance energy transfer aptasensor was fabricated for the detection of *Staphylococcus aureus* (*S. aureus*) with Co^{2+} enhanced *N*-(aminobutyl)-*N*-(ethylisoluminol) (ABEI) functional flowerlike gold nanoparticles (Co^{2+}/ABEI-AuNFs) as donor and WS_2 nanosheet as acceptor. In the presence of *S. aureus*, rolling circle amplification (RCA) can be started. Partially complementary sequence of RCA product functional ABEI-AuNFs (cDNA-ABEI-AuNFs) were then annealed to multiple sites of the RCA product to form duplex complex. This complex is less adsorbed onto the WS_2 nanosheet, thus attenuating the quenching of ABEI-AuNFs chemiluminescence by WS_2 nanosheet. In the absence of target *S. aureus* (and hence the absence of RCA and duplex formation), the free cDNA-ABEI-AuNFs is completely adsorbed onto the WS_2 nanosheet and chemiluminescence quenching ensues. Under optimal conditions, the logarithmic correlation between the concentration of *S. aureus* and the CL signal was found to be linear within the range of 50 cfu/mL to 1.5 × 10^5 cfu/mL (R^2 = 0.9913). The limits of detection of the developed method were found to be 15 cfu/mL for *S. aureus*. The selectivity and the capability of the

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biosensor in meat samples were also studied. Therefore, this simple and easy operation method can be used to detect *S. aureus* with high sensitivity and specificity.

1. Introduction

Staphylococcus aureus (*S. aureus*) is gram-positive bacterium with a wide distribution. As one of the most common food borne pathogens for humans and animals, *S. aureus* can be able to produce heat-resistant toxins in food [1]. And even trace concentrations of bacteria in food can cause septicemia, osteomyelitis, pneumonia, toxic shock syndrome, and endocarditis [2,3]. Especially when the infection occurs among certain high risk people such as infants, elderly and chronically ill individuals, the symptoms are more serious [4,5]. Thus it is critical to develop rapid, sensitive and selective methods to detect *S. aureus* in food.

There are many kinds of methods to detect *S. aureus*, such as bacteria isolation-culture based conventional methods [6], polymerase chain reaction (PCR) based assays [7,8] and biosensor based detection methods [9,10] and so on. According to the detection signal, there are colorimetric [11,12], fluorescence [13,14], chemiluminescence(CL) [15,16], Raman scattering and surface plasmon resonance (SPR) biosensor [17,18]. Among these optical biosensors, CL biosensors is one kind of promising methods in food safety detection, because in CL reaction, the energy is produced by chemical reactions, and excitation is not needed for sample radiation. So the interference of light scattering, source instability and high backgrounds can be avoided [19].

Among the established CL-based detection methods, CL resonance energy transfer (CRET) is a promising optical detection mode that mainly benefits from the separation-free detection mode and robustness against matrix interferences. CRET involves non-radiative dipole-dipole transfer of energy from a chemiluminescent donor to a suitable acceptor molecule [20]. And the introduction of quantum dots (QDs), grapheme, amorphous carbon nanoparticles [21,22] and two-dimensional transition metal dichalcogenide (2D-TMDC) nanosheets [23–25] as energy acceptors have made significant progress in the CRET system. Recently, extensive attention has been focused on the 2D-TMDC (e.g., WS_2, etc.) due to their 2D layer structure analogous to graphene. Being an ultrathin direct bandgap semiconductor, WS_2 nanosheet has found widespread applications in catalysis, lithium ion batteries and electrochemical biosensing [26–28].

According to the reports, WS_2 has been applied in fluorescence resonance energy transfer (FRET) assays [29], but the application in CRET was not very common. Jingjin Zhao and Shulin Zhao [25] reported a CRET assay to detect MicroRNA with luminol as the donor and WS_2 nanosheet as acceptor. However, only MicroRNAs or DNA sequences can be detected in that CRET mode. Therefore, in this work, to expand the detection scope, CL reagents functionalized gold nanoparticles (AuNPs) and aptamer were first used in WS_2 nanosheet based CRET assay. Thus, other targets such as antibiotics, food borne pathogens and so on can be detected by the universal CRET assay. Compared with luminol, CL reagents functionalized AuNPs is one of the most attractive improvements because it can construct the nanosized platform in CL reactions. The nanosized CL platform can combine CL properties of the CL reagents with the unique optical properties, excellent catalytic activity, easy modification as well as good biocompatibility and stability of nanoparticles [30,31]. And their application can also improve the analytical sensitivity, simplicity, speed, and cost of bioassays. To further improve the sensitivity of the methods, the CL enhancer Co^{2+} and the signal amplification technique rolling circle amplification (RCA) were introduced to the CL system.

There has been some reports that metal irons and CL reagents were co-modified on the surface of gold nanoparticles to enhance the CL intensity [32–34]. However, the reported methods were either tedious and time-consuming for the synthesis or hard for immobilizing recognized molecules such as aptamer on the surface. Therefore, a simpler, easier and more universal method was needed. In our previous study [35], *N*-(aminobutyl)-*N*-(ethyl-isoluminol) (ABEI) and chitosan were used to prepare ABEI functionalized flowerlike gold nanostructures (ABEI-AuNFs) and both of them coexisted on the surface of the nanoparticles. Also, chemically active functional groups of chitosan can strongly interact with divalent metal ions (for example Co^{2+} and Cu^{2+}) through primary amine groups [36]. Thus, CL enhanced Co^{2+}/ABEI-AuNFs can be prepared through the interaction of Co^{2+} and chitosan. In addition, RCA is a simple and efficient molecular amplification technique especially when it combines with aptamer. During the process, long ssDNA with tens to hundreds of tandem repeats can be produced [37–40]. And signal molecules such as fluorescent dye, quantum dots and ABEI-AuNFs and so on can assembled onto the repetitive sequences so that the detection signal can be amplified.

Herein, we report an improved RCA based CRET aptasensor for detecting *S. aureus* by the CRET process between the chemiluminescent donor Co^{2+}/ABEI-AuNFs and chemiluminescent acceptor WS_2 nanosheet. By virtue of CL functional nanoparticles as well as the specific recognition of aptamer, the improved CRET assay are more universal in food safety detection. Co^{2+}/ABEI-AuNFs was first used as CRET donor and first applied in 2D-TMDC based CRET system. In addition, RCA technique was also applied to improve the CL signal in the CRET system for the first time.

2. Materials and methods

2.1. Materials and instruments

A 0.1 M stock solution of ABEI was prepared by dissolving ABEI (TCI (Shanghai) Development Co., Ltd, Shanghai, China) in 0.1 M NaOH solution without further purification and stored at 4 °C. A $HAuCl_4$ stock solution (0.2% $HAuCl_4$, w/v) was prepared by dissolving $HAuCl_4 \cdot 4H_2O$ (Shanghai Reagent, Shanghai, China) in purified water and stored at 4 °C. P-Iodophenol (PIP) (purity >98.0%) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (purity >98.0%) were purchased from TCI (Shanghai) Development Co., Ltd (Shanghai, China). Chitosan was purchased from Sigma-Aldrich Shanghai Trading Co., Ltd, (Shanghai, China). The tungsten disulfide (WS_2) nanosheet was purchased from Nanjing XFNano Material Tech Co., Ltd. (Nanjing, China). H_2O_2 used in detecting buffer were freshly prepared daily from 30% (v/v) H_2O_2 (Xinke Electrochemical Reagent Factory, Bengbu, China). All other reagents used in this study were of analytical grade. The oligonucleotides used in this work (synthesized by Shanghai Sangon Biological Science & Technology Company, Shanghai, China) were shown in Table 1. The meat tested in this work was bought form local market. Ultrapure water was prepared with a Millipore Milli-Q system and was used throughout the study.