Electrochemistry Communications 11 (2009) 816–818

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

DNA aptamer-based detection of lysozyme by an electrochemiluminescence assay coupled to quantum dots

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ARTICLE INFO

Article history:
Received 10 December 2008
Received in revised form 21 December 2008
Accepted 9 January 2009
Available online 13 January 2009

Keywords:
DNA
Electrochemiluminescence
Quantum dots
Lysozyme
Biosensors

ABSTRACT

A novel quantum dots (QDs) ECL biosensor for the detection of lysozyme was developed. Lysozyme was first incubated with probes immobilized at Au electrode in order to form the aptamer-lysozyme bioaffinity complexes. And the free probes were hybridized with the 5′-biotin modified cDNA oligonucleotides to form double-stranded DNA (ds-DNA) oligonucleotides. Avidin-QDs were bound to these hybridized cDNA through the biotin-avidin-system. The ECL signal of the biosensor was responsive to the amount of QDs bonded to the cDNA oligonucleotides, which was indirectly inverse proportional to the combined target protein.

1. Introduction

Semiconductor nanocrystals (NCs), or quantum dots (QDs), have been extensively studied because of their unique size-dependent electronic, magnetic, optical, and electrochemical properties [1]. In particular, Bard's group found that QDs could electrogenerated light emission during the potential cycling or pulsing [2]. This light from electrochemically generated reagents is called electrogenerated chemiluminescence, abbreviated ECL. An alternative approach to induce QDs ECL is the use of a coreactant. In the reaction with the coreactants, the efficient and stable ECL in aqueous solution can be obtained by applying a cathodic potential to the QDs [3]. Afterwards, highly luminescent semiconductor NCs gained increasing attention for the application in bioconjugates and optical biosensor [4]. The fabrication of QDs-based ECL immunosensors was reported in our group [5].

As a new class of single-stranded DNA/RNA molecules, aptamers are selected from synthetic nucleic acid libraries. Aptamers could be stored stable for a long-term, be transported at ambient temperature and undergo reversible denaturation. With these advantages, aptamers were regarded as the ideal recognition probes for a broad range of analyte detection [6]. By using some special methods such as optical, HPLC and electrochemical assay, many proteins like ATP, peptide and thrombin can be detected using high-affinitive DNA aptamers recognition [7]. However, to the authors' knowledge, there is no paper issued about detecting protein using DNA aptamer recognition via QDs ECL assay till now. Herein, based on our previous work, we developed a novel QDs ECL biosensor for lysozyme detection via the aptamer-lysozyme binding and aptamer-complementary DNA (cDNA) oligonucleotides match principle.

2. Experimental

2.1. Reagent and apparatus

Labeled DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these two oligomers employed are given below:

The anti-lysozyme aptamer:

5′-HS-$(CH_2)_6$-ATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3′

The complement DNA:5′-biotin-CTAAGTAACTCTGCACTCTTTAGCCCTGATGAATTCGTAGAT-3′

6-mercapto-1-hexanol (MCH), bovine serum albumin (BSA), lysozyme (from hen egg white) and thrombin (from human plasme) were purchased from Sigma–Aldrich. HIgG was obtained from Ningbo Xinzhi Biochemical Reagents (Ningbo, China). Avidin-QDs (with a CdSe/ZnS core–shell structure about 10 nm) were obtained from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). All other reagents were of analytical reagent grade and used without further purification. The buffer solution for oligonucleotides contained 100 mmol/L $Na_2HPO_4 + NaH_2PO_4$, 5 mmol/L $MgCl_2$, pH 7.4. The electrolyte for ECL measurement was 5 mL PBS solution containing 0.1 mol/L $K_2S_2O_8$ and 0.1 mol/L KCl.

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doi:10.1016/j.elecom.2009.01.009

Solution contained 0.1 mol/L KCl and 2 mmol/L $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ was used for electrochemical impedance spectroscopy (EIS) characterization. Millipore ultrapure water (resistivity $\geqslant$ 18.2 MΩ) was used throughout the experiment.

The ECL emission was detected using a model MPI-A electrochemiluminescence analyzer (Xi'an Remex Analysis Instrument Co., Ltd., Xi'An, China) with 600 V photomultiplier tube voltage. The EIS analyzes were performed on an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) with the frequency range of $0.1–1.0 \times 10^5$ Hz. In all electrochemical experiments, the conventional three-electrode system was employed with a modified gold electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a Pt wire as the counter electrode.

Prior to use, the gold electrode was cleaned with freshly made piranha solution (98% H_2SO_4:30% H_2O_2 = 7:3, v/v) for 10 min, all the oligonucleotides solutions were heat-treated in 90 °C for 3 min and then cooled in ice for 10 min.

2.2. The preparation of ECL biosensor

About 20 μL 5 μmol/L aptamer solution was first spread on the pre-cleaned gold electrode surface for 12 h at 37 °C in the 100% humidity. Next, this electrode was immersed in 1 mmol/L MCH for 1 h to remove the nonspecific DNA adsorption, and another 1 h in lysozyme solution. After being washed with wash buffer (Tween-20 in PBS) to remove the nonspecifically bound lysozyme, the electrode was covered with a 20 μL droplet 5′-biotin modified cDNA oligonucleotides solution (5 μmol/L in PBS) for another 1 h incubation in the 100% humidity. The whole fabrication process of this ECL biosensor is outlined in Fig. 1. Prior to ECL measurement, the biosensor was washed with wash buffer to remove the physical adsorption of QDs.

3. Results and discussion

Electrochemical impedance technique was employed to characterize the fabrication in whole process. Fig. 2 shows the EIS changes for surface-modified process. Compared with the bare Au electrode (Fig. 2a), the probe modified Au electrode shows a larger eT resistance (Fig. 2b), mainly due to the electrostatic repulsion between negative charges of the DNA aptamer backbone and the $Fe(CN)_6^{3-/-4}$ probe. After the probe modified electrode was immersed into the lysozyme solution, the eT resistance (Fig. 2c) decreased to a large extent, suggesting the formation of aptamer-lysozyme bioaffinity complex. This resistance decrease could be attributed to the fact that lysozyme (PI = 11) [8] is positive charged in PBS solution of pH 7.4. When cDNA solution was dispread onto the above electrode, a larger eT resistance (Fig. 2d) was observed. When the cDNA oligonucleotides were directly combined with the probe in the absence of lysozyme, the largest eT resistance (Fig. 2f) was obtained. It may be that the formation of ds-DNA structure essentially increased the negative charge at the electrode

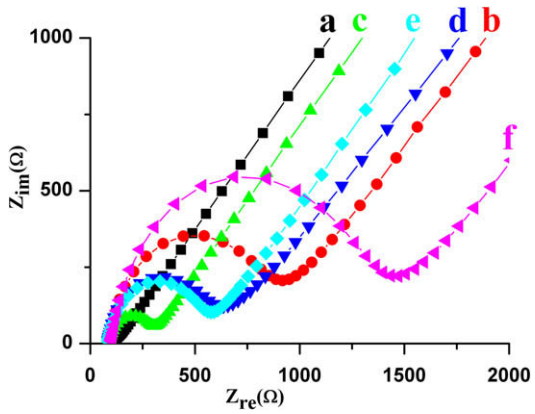

Fig. 2. EIS of the electrode at different stages in 0.1 mol/L KCl and 2 mmol/L $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. (a) Bare Au electrode, (b) probe/Au electrode, (c) lysozyme/probe/Au electrode, (d) cDNA/lysozyme/probe/Au electrode, (e) QDs/cDNA/lysozyme/probe/Au electrode, and (f) cDNA/probe/Au electrode.

surface. The eT resistance decreased after incubated in QDs solution for 60 min (Fig. 2e), which indicated that the avidin-QDs were successfully bond to the 5′-biotin modified cDNA oligonucleotides via the biotin-avidin-system.

QDs could be electrochemically reduced during potential scan and react with the coreactant $S_2O_8^{2-}$ to generate strong ECL signal in aqueous solution [5a]. Here, the ECL signal is responsive to the amount of QDs. In the absence of lysozyme, the strongest ECL signal was observed. We presume that all the probes immobilized on the gold electrode were hybridized to their cDNA oligonucleotides, so the maximum amount of QDs could be bound to cDNA through the biotin-avidin-system. This is useful to achieve the biggest ECL value. In order to testify that the amount of QDs was sufficient for binding, diluted QDs prime solution with 100 or 20 folds were used and the ECL signals kept unchanged. As the electrode was immersed into lysozyme solution, a portion of the probes were combined with lysozyme to form the aptamer-lysozyme bioaffinity complexes. This induced conformational change of these combined probes. When 5′-biotin modified cDNA oligonucleotides were added, only those free probes which were not combined with lysozyme could be hybridized with their cDNA oligonucleotides to form the ds-DNA oligonucleotides. In biotin-avidin-system, avidin-QDs could only bind to the biotin-modified cDNA oligonucleotides. So, when more amount of lysozyme was combined to the probes, fewer cDNA oligonucleotides could be hybridized with the free probes. Ultimately, fewer QDs were bond to the modified electrode, and this directly led to the poor ECL signal. Shown in Fig. 3 are the ECL signals that are responsive to the changing concentration of lysozyme. When the concentration of lysozyme exceeds 100 μg/mL, the ECL signal almost remains the same. This illuminates the bioaffinity saturation between the probes and lysozyme.

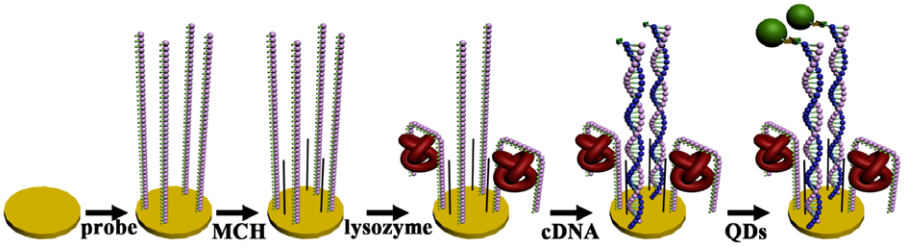

Fig. 1. Schematic diagram for the biosensor fabrication.

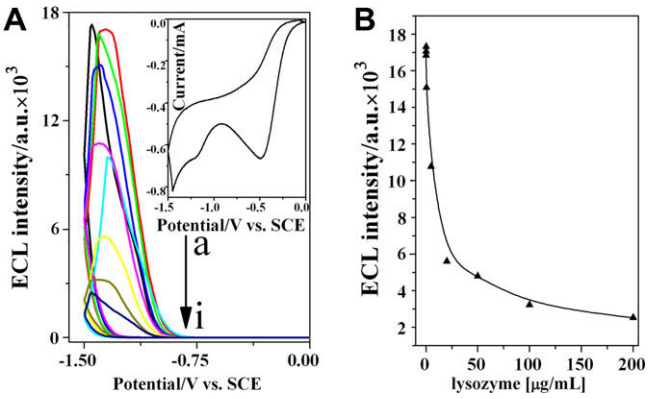

Fig. 3. (A) The ECL-potential curves of the lysozyme biosensor with different protein concentrations (μg/mL): (a) 0, (b) 0.1, (c) 0.2, (d) 0.5, (e) 2, (f) 5, (g) 20, (h) 100, (i) 200. The insert shows the cyclic voltammetry response of curve a during the ECL measurement. (B) The ECL-concentration curve draws from (A).

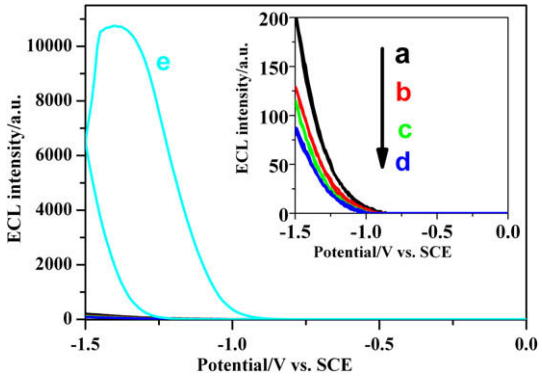

Fig. 4. Electrochemiluminescence intensity versus potential. (a) Bare Au electrode, (b) probe/Au electrode, (c) lysozyme/probe/Au electrode, (d) cDNA/lysozyme/probe/Au electrode, (e) QDs/cDNA/lysozyme/probe/Au electrode. The inset is the amplification form of curve (a), (b), (c) and (d).

In order to gain a better understanding for the ECL signal generation, the ECL signals of the modified electrode after each step were examined. The results showed these signals were very low, even negligible (see Fig. 4) in the absence of QDs. QDs treated before and after BSA solution were respectively dropped onto the lysozyme bound electrode in the absence of cDNA oligonucleotides. When QDs were directly dropped onto the electrode without cDNA oligonucleotides, which means without biotin, a relatively strong signal could still be observed. And after BSA treatment for 1 h, this signal became weak in a great extent. According to above the experiments, the conclusion can be obtained. First, the ECL signal of this biosensor was emitted by QDs. Second, avidin-QDs were mainly bound to modified electrode via biotin-avidin-system with the help of biotin modified cDNA oligonucleotides. Third, the BSA treatment could reduce the most QDs nonspecific absorption.

Furthermore, different proteins were used to measure the selectivity of lysozyme aptamers. Under the same concentration, when thrombin and human Immunoglobulin G (HIgG) were employed for combining with the probes, the ECL signals are much stronger than the signal for lysozyme combine. This suggested the biosensor held a good selectivity to its target protein lysozyme. When the biosensor was scanned continuously, stable and high ECL signals could be still observed, which meant that the biosensor possessed excellent stability.

4. Conclusion

QDs ECL in aqueous solution was employed for the aptamer-based lysozyme detection with high selectivity and stability. Through biotin-avidin-system, avidin-QDs could be bound tightly to the biotin-modified cDNA oligonucleotides. This is the first time QDs ECL assay in aptamer-protein detection. As a DNA oligonucleotide, aptamer was artificially synthesized and could be modified with the desired specific function group in the synthesis process. it expands the possibility of using QDs ECL for the aptamer-based detection of other proteins like thrombin and DNA sequence detection.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (20635020, 20821063, 90606016), and National Basic Research Program of China (2006CB933201).

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