A New Electrochemical DNA Sensor for Sequence-Specific DNA Detection Based on DNA-functionalized CdS Nanoparticle

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Abstract. In this work, a sensitive electrochemical DNA sensor based on avidin modified electrode and DNA-functionalized CdS nanoparticle (DFCP) was developed. The DNA-Functionalized CdS nanoparticle contained two kinds of DNA, one was hairpin probe DNA with a biotin at the 3' terminal and a thiol at the 5' terminal, the other is linearity signal DNA. Without hybridized with target DNA, the loop of hairpin impeded biotin linked with avidin on electrode. However, after target hybridization, hairpin was opened and biotin was recognized by avidin resulting in DNA-functionalized CdS nanoparticle was brought on electrode surface. Electrochemical signals of methylene blue (MB) bound to the signal DNA were measured by differential pulse voltammetry (DPV). Introduction By using this new method, we demonstrate that this prototype sensor has been able to detect as low as picomolar DNA targets with excellent differentiation ability for even single mismatches.
Keywords: DNA, sensor, MB, CdS nanoparticle

1 Introduction

Nucleic acids are one of the most fundamental molecules for all life forms, and as a results the specific nucleic acid sequence quantification is essential in biological and biomedical studies, such as medical diagnostics, gene expression analysis, and the detection of infectious diseases. Until now, many approaches have been successfully developed for the sequence-selective DNA hybridization examination, including fluorescence, electrochemical and colorimetric etc. methods.[1-6]

The electrochemical DNA sensing technology has received particular attention mostly due to its high sensitivity, selectivity and easy operation with simple instrumentations. The DNA recognition event can be detected using

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different strategies, including intrinsic electroactivity of the nucleic acid, enzyme labels, DNA duplex intercalators, electroactive markers, and metal nanoparticles/quantum dots.

We herein reported a non-immobilizing DNAsensor with hybridization occurred in one homogeneous solution that employed DNA-functionalized CdS nanoparticle (DFCP) and avidin modified electrode. In the present study, we have developed a DNA-functionalized CdS nanoparticle, integrated DNA recognition; signal amplification and specific biotin- avdin link functional section, as probe for DNA detection and featured high sensitivity up to low pmtomolar. In target DNA detection, as Figure 1, when the probe DNA of DFCP is under stem-loop state, comparatively huge bulk of CdSnanoparticle and the loop of probe DNA would prevent biotin on the probe DNA be captured by the avidin on the electrode for the spatial effects. After the DFCP solution is incubated in a buffer solution containing target DNA, target DNA would combine probe DNA by forming double strand with complementary sequences, which caused hairpin open and eliminate steric hindrance to biotin. Afterwards, when avidin modified electrode was immersed in a solution after the hybridization event happened, the biotin of the DFCP could have attached to avidin on the electrode and thus was captured on the electrode. The electrochemical signals of MB of signal DNA were measured by differential pulse voltammetry (DPV). Taking advantage of amplification effects of the CdS nanoparticle and binding specificity of hairpin probe, this biosensor greatly simplifies the electrochemical detection method of DNA and displays higher specificity than the linear probe in DNA detection.

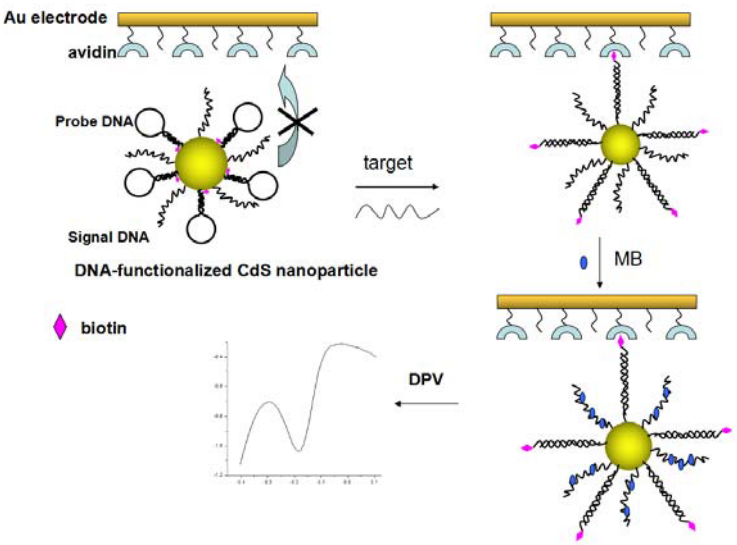

Fig.1 Scheme for the DNA-functionalized CdS nanoparticle- based DNA sensor.

2 Experiment Section

2.1Apparatus

All voltammetric experiments were performed using a CHI 660 electrochemical analyzer (CHI Instrument Inc, USA). Electrochemical experiments were carried out in a 3 ml electrochemical cell at room temperature (25 °C) by using three electrode configurations. A platinum wire served as a counter electrode and an Ag/AgCl as reference electrode with saturated KCl solution.

Unless otherwise noted, all chemicals were purchased from Dingguo Biotechnology Inc. (Shanghai, China) and of analytical reagent grade. The biotin and avidin were pur chased from Sangon Biotechology Inc. (Shanghai, China). All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

Materials

All the probes and target oligonucleotides were purchased from Takara Biotechnology Company. The oligonucleotides were purified via C18 reversed-phase HPLC and polyacrylamide gel electrophoresis (PAGE) and their sequences are shown in table 1. The stem-loop probe oligonucleotide (oligo 1) has a 5′-NH_2 label and a 3′-biotin, which will form the stem at appropriate ionic strength. The MB-labeled signal DNA (oligo 2). The sequence of the target (oligo 3) is perfectly matched to the loop sequence of the probe; oligo 4 contains a one-base mismatch, while oligo 5 has three-base mismatches. Oligo 6 is a random sequence unrelated to the probe sequence.

Table 1. Oligonucleotides Employed in This Work

oligo 1 (stem-loop probe)	5'-NH2-CCACGCTTGTGGGTCAACCC CCGTGG-Biotin-3'
oligo 2 (linear signal DNA)	5'-NH2-GGTGGAGGGACGAGG-MB-3'
oligo 3 (target)	5'-GGGGTTGAC CCACAAG-3'
oligo 4 (One base-mismatched DNA)	5'-GGGGTCGACCCACAAG-3'
oligo 5 (Three base-mismatched DNA)	5'-GGGGTCTTCCCACAAG-3'
oligo 6 (Non-complementary DNA)	5'-TTCGGCTCTATCAATC-3'

2.2 Preparation of nano CdS

$Cd(NO_3)_2$ and Na_2S solutions were filtered through a 22 μm microporous membrane filter prior to use. CdS nanoparticles were prepared according to the literature [7] by using mercaptoacetic acid as the stabilizer. In brief, 9.22 μl mercaptoacetic acid was added to 50 ml 0.4 mM $Cd(NO_3)_2$ solution, and then the pH was adjusted to 7 with 0.5 M NaOH. The solution was bubbled with nitrogen for 30 min, followed by the slow addition of 1.34 mM Na_2S to the mixture solution. The molar ratio of Na_2S to $Cd(NO_3)_2$ was kept at 2.5. The reaction was carried out for 24 h under nitro-gen protection and then gradually a brown colloid which is the CdS nanoparticles covered with a carboxyl group was obtained. As TEM images show, the diameter of CdS nanopatricles was about 7 nm (Figure 2).

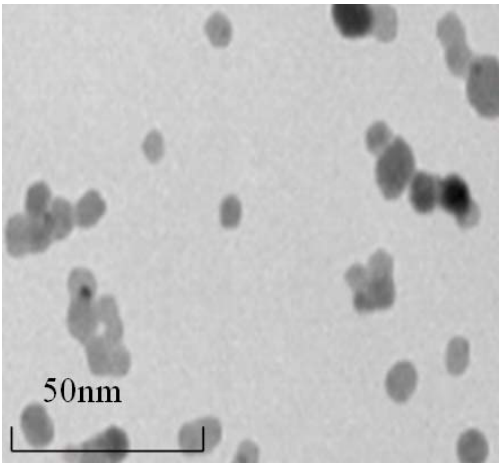

Fig. 2 TEM image of the synthesized CdS nanoparticles.

2.3Preparation of the DNA-Functionalized CdS Nanoparticles

200 μl of 0.1 mol/L imidazole was added to 2OD of 5-amido-capped detection probe DNA. After stirred for 30 min, 100 μl of 0.1 mol/L EDC and 5 ml of CdS colloids were added to the mixture. The resulting mixture was stirred for 12 h at room temperature and then continued to centrifugate for at least 25 min at 14,000 rpm to remove the excessive DP. The yellow DNA-CdS precipitate was washed with 0.1 mol/L PBS and re-dispersed in 0.1 mol/L PBS. Then, the resulting solution was stored in the refrigerator for further use.

2.4 Preparation of avidin-coated electrode surfaces

The gold electrode was exposed to an ethanolic 5 mM solution of 3,3’ -dithiodipropionic acid for 30 min, followed by water rinsing. 5 μl of 100 mg

mL^{-1} EDC solution was then placed on the surface, followed immediately by 5 μL of 100 mg mL^{-1} NHS solution. These solutions were allowed to interact with the electrode with 3,3’-dithiodipropionic acid modification for 30 min in a 100% humidity environment to prevent solution evaporation. The surface was then rinsed with water and immersed in an aqueous 0.2 mg mL-1 avidin solution for at least 120 min, after which the surface was rinsed again. The electrode was then exposed to a 1mM 2-aminoethanol solution (pH 8.0, adjusted using HCl) for 60 min, rinsed, and used in hybridization detection.

3 Results and Discussion

3.1 Principle of DNA detection

We employed a stem-loop DNA probe dually labeled with HS and biotin at the 5′- and the 3′- end, respectively, which could be facilely immobilized at CdSnanoparticle surfaces via the Au-S bridge and hybridized with target DNA. The signal DNA with HS at 5′ end, which could provided electrochemical signal. Two kinds of DNA have been immobilized at CdSnanoparticle to construct DFCP.

The detection strategy is demonstrated in Figure 1. Before the hybridization, the DFCP remained in the stem-loop structure, which forced the biotin to be closed to the CdS nanoparticle. Due to the steric effect of the CdSnanoparticle, the biotin was prevented from conjugating with the avidin on the electrode and resulting in that the DFCP could not be captured by the electrode. After hybridized with the target DNA, the DFCP’s loop-stem structure opened and then the biotin molecule was easily bound to avidin modified electrode and resulting in that the DFCP could be captured by the electrode and the capture efficiency was proportion with the concentration of the target DNA. The target hybridization event can be sensitively transduced via detecting the electrochemical reduction current signal of MB at the DFCP.

We found that as the concentration of the complementary target DNA sequence continuously increased (Figure 3A), which was logarithmically related to the target protein concentration from 3.3×10^{-12} to 3.3×10^{-9} M (Figure 3B). The equation for the resulting calibration plot was calculated as y=0.129logx+0.163 (x is the concentration of target DNA divided by pM, y is the DPV peak current value) with correlation coefficient of 0.9923 and detection limit of 2.7×10^{-12} M (>3 SD). This sensitivity reflected the high signal amplification of this method and the improved signal gain as a signal-on sensor used MB as indicators.

4 Conclusions

In summary, we completed a DNA sensing strategy, which allowed the hybridization between DNA probe and target DNA occur in homogeneous solution. This assay protocol is simple, convenient and cost-effective by using this novel method, we could conveniently detect as few as 4.2×10^{-13} M target DNA. We propose that it might be a promising approach to perform DNA-based diagnostics where resources are limited, such as small clinics in developing countries or field detection.

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