An Electrochemical DNA Sensor for p53 Tumor Suppressor Gene Detection

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Abstract. In this work, a sensitive electrochemical DNA sensor based on avidin modified electrode and DNA-functionalized Cds nanoparticle (DFNP) 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 p53 tumor suppressor gene with excellent differentiation ability for even single mismatches.

1 Introduction

The p53 tumor suppressor gene, well known as a transcription factor of cell regulation, is the most commonly mutated gene in human tumors. About 50% of all malignancies contain a mutation in p53 and aggressive growth of several types of cancer has been attributed to mutations in this gene. Moreover, p53, as the “master switch” for the control of cell proliferation, plays an important role in inducing cell cycle arrest for DNA repair or apoptosis to eliminate severely damaged cells. Sequence-specific analysis of the p53 gene could help early diagnosis of cancer development and consequently increase the success of the treatment Therefore, sensitive and rapid detection of p53 gene and the mutations in the p53 gene are of great value. [1-6]

The electrochemical DNA sensing technology has received particular attention mostly due to its high sensitivity, selectivity and easy operation with simple instrumentations. [7-10] The DNA recognition event can be detected using 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 electrochemical DNA sensor with hybridization occurred in one homogeneous solution that employed DNA-functionalized Cds nanoparticle (DFNP) and avidin modified electrode. In the present study, we have developed a DNA-functionalized Cds nanoparticle, integrated DNA recognition; signal amplification and specific biotin-avidin 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 DFNP is under stem-loop state, comparatively huge bulk of Cds nanoparticle 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 DFNP solution is incubated in a buffer solution containing target DNA, target DNA would combine probe DNA by forming double strand with complementary sequences, which ccdssed 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 DFNP 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 (CdsNP) 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.

2 Experiment section

2.1 Apparatus

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 purchased from Sangon Biotechnology Inc. (Shanghai, China). All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

2.2 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). The stem-loop probe oligonucleotide (oligo 1) has a 5′-HS 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. Unless otherwise noted, all chemicals were purchased from Dingguo Biotechnology Inc. (Shanghai, China) and of analytical reagent grade. The biotin and avidin were purchased from Sangon Biotechnology Inc. (Shanghai, China). All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

2.2 Preparation of nano CdS

Cd(NO₃)₂ and Na₂S solutions were filtered through a 22 μm microporous membrane filter prior to use. CdS nanoparticles were prepared by using mercaptoacetic acid as the stabilizer. In brief, 9.22 μl mercaptoacetic acid was added to 50 ml 0.4 mM Cd(NO₃)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 Na₂S to the mixture solution. The molar ratio of Na₂S to Cd(NO₃)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 nanoparticles was about 7 nm.

2.3 Preparation of the DNA-functionalized CdS nanoparticles

The process of probe DNA and signal DNA labeling was performed according to paper as follows: The mixture of 5.6 nM probe DNA and 2.8 nM signal DNA was activated with acetate buffer (pH 5.2) and 1.5 μL of 10 mM TCEP for 1 h, then added to 1 mL of freshly prepared CdS nanoparticles, and shaken gently overnight. Over the course of 16 h, the DNA-CdS conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for another 24 h. Excess reagents were removed by centrifuging at 16000 rpm for 30 min. The red precipitate was washed and centrifuged repeatedly for three times. The resulting nanoparticles were dispersed into a buffer solution (0.1 M, containing 0.3 M NaCl and 2 mM Mg²⁺, pH 8.1) and stored at 4 °C.

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 Optimization of testing conditions

Many experimental parameters, such as hybridization temperature, hybridization time, and Mg²⁺ ion strength, capture time, can affect the detection sensitivity of the
assay. A series of optimizations of these parameters were carried out to improve the sensitivity and selectivity of the DNA assay. Figure 3 shows the effect of the capture time on the signal of DNA detection.

In this study, a 1.2×10^{-11} M complementary DNA target was incubated with DFNP for 30 min at a series of temperatures ranging from 25 to 45 °C. The highest signal is obtained at 37 °C. Therefore, a hybridization temperature of 37 °C was selected in the following experiments. The effect of the hybridization time has also been investigated. The target DNA was incubated with DFNP at 37 °C for different times from 10 to 50 min. The signal responses of the assay to the different hybridization times are shown in Supporting Information Figure S6. It can be seen from this figure that the signal increases rapidly when the hybridization time increases from 10 to 30 min and then increases slightly from 30 to 50 min, indicating that the hybridization reaches equilibrium at 30 min. As a result, a reaction time of 30 min was selected for the DNA hybridization.

The cation Mg^{2+} plays an important role in DNA hybridization. For a hairpin DNA probe-based DNA assay, there is hybridization competition between intramolecular stem part hybridization and intermolecular target DNA-probe hybridization. Therefore, Mg^{2+} concentration in the hybridization buffer should be balanced. Although a high concentration of Mg^{2+} can enhance target DNA hybridized to the loop of the structure, it may also strengthen the binding of the stem part of the probe, which may adversely affect the performance of the assay. The effect of Mg^{2+} concentration in the hybridization buffer on the response signal of the assay. The assay produces the highest signal when the Mg^{2+} concentration is 2 mM. Accordingly, a 2 mM Mg^{2+} concentration was used throughout the experiments to obtain the highest signal.

3.2 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 Cds nanoparticle surfaces via the Cds-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 Cds nanoparticle to construct DFNP.

The detection strategy is demonstrated in Figure 1. Before the hybridization, the DFNP remained in the stem-loop structure, which forced the biotin to be closed to the Cds nanoparticle. Due to the steric effect of the Cds nanoparticle, the biotin was prevented from conjugating with the avidin on the electrode and resulting in that the DFNP could not be captured by the electrode. After hybridized with the target DNA, the DFNP’s loop-stem structure opened and then the biotin molecule was easily bound to avidin modified electrode and resulting in that the DFNP 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 DFNP.

Indeed, in stem-loop state we only found a relatively small and stable background current (Figure 2 curve a). As we challenged the sensor with 1.1×10^{-11} M target DNA, which was expected to open the stem-loop, a large signal (Figure 4 curve b) was observed. We found that as the concentration of the complementary target DNA sequence continuously increased, which was logarithmically related to the target protein concentration from 8.3×10^{-12} to 8.3×10^{-9} M. The equation for the resulting calibration plot was calculated as y=0.139logx+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 6.2×10^{-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 electrochemical 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 6.2×10^{-12} 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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