An E-DNA Sensor for Sequence-Specific DNA detection

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Abstract. In this work, a sensitive E-DNA sensor based on avidin modified electrode and DNA-functionalized Au nanoparticle (DFNP) was developed. The DNA-Functionalized Au 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 Au 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.

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 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 E-DNA sensor with hybridization occurred in one homogeneous solution that employed DNA-functionalized Au nanoparticle (DFNP) and avidin modified electrode. In the present study, we have developed a DNA-functionalized Au 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 DFNP is under stem-loop state, comparatively huge bulk of Au 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 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 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 Au nanoparticle (AuNP) 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.

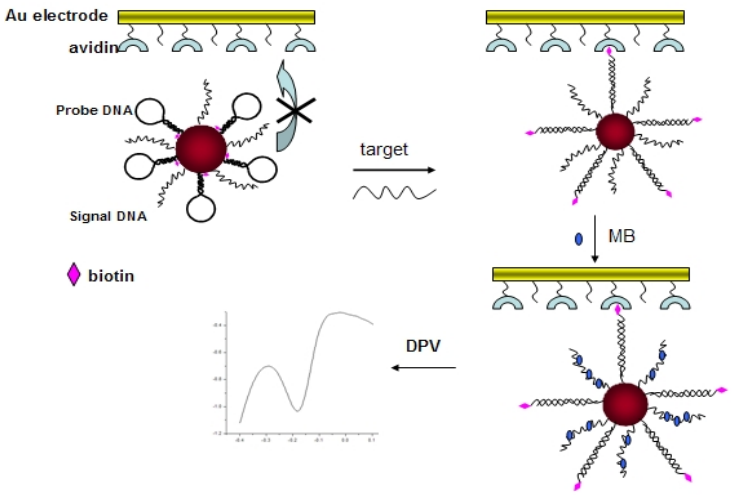


Fig.1 Scheme for the DNA-functionalized Au nanoparticle- based E-DNA sensor.

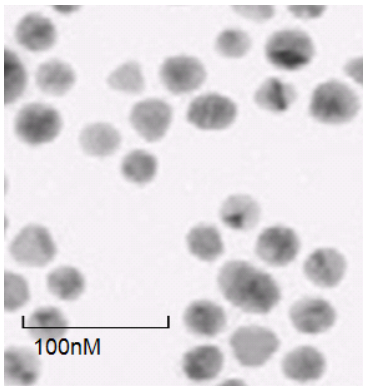
Experiment section

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

Preparation of Au nanoparticles Au nanoparticles were prepared by citrate reduction of $HAuCl_4$ according to the literature. In brief, 100 mL of 0.01g $HAuCl_4$ solution was brought to reflux with stirring, and 2.5 ml of 1% sodium citrate solution was introduced into this $HAuCl_4$ solution. The mixture solution was kept boiling for another 30 min and left to cool to room temperature. The diameter of such-prepared Au nanoparticle was determined ca.16±3 nm by using atomic force microscopy scanning with line scan mode and taking the tip convolution effects into account (The AFM image is in the Supporting Information Figure 2). The obtained Au nanoparticles were then stored in brown glass bottle at 4 °C for further use.

Preparation of the DNA-Functionalized Au 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 Au nanoparticles, and shaken gently overnight. Over the course of 16h, the DNA-AuNP 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 repeatly for three times. The resulting nanoparticles were dispersed into a buffer solution (0.1 M, containing 0.3 M NaCl and 3 mM Mg^{2+}, pH 8.1) and stored at 4 °C.



Results and discussion

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 Au nanoparticle 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 Au 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 Au nanoparticle. Due to the steric effect of the Au 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.

Conclusions

In summary, we completed a E-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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