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Ultrasensitive nucleic acid biosensor based on enzyme–gold nanoparticle dual label and lateral flow strip biosensor

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ABSTRACT

In this article, we describe an ultrasensitive nucleic acid biosensor (NAB) based on horseradish peroxidase (HRP)–gold nanoparticle (Au-NP) dual labels and lateral flow strip biosensor (LFSB). The results presented here expand on prior work (Mao et al., 2009a) by optimizing the preparation of HRP–Au-NP–DNA conjugates. It was found that sodium dodecyl sulfate (SDS) and the immobilization sequence of thiolated DNA and HRP on the Au-NP surface played very important roles to improve the sensitivity of the assay. After systematic optimization, the detection limit of current approach is 1000 times lower than that in prior work. Deposition of insoluble enzymatic catalytic product (red colored chromogen) on the captured Au-NPs at the test zone of LFSB offers a dramatic visual enhancement. Combining enzyme catalytic amplification with unique optical properties of Au-NPs, the NAB was capable of detecting of 0.01-pM target DNA without instrumentation. The NAB thus provides a rapid, sensitive, low-cost tool for the detection of nucleic acid samples. It shows great promise for in-field and point-of-care diagnosis of genetic diseases and for the detection of infectious agents.

1. Introduction

Nucleic acid biosensors (NABs) are of considerable recent interest due to their tremendous promise for obtaining sequence-specific information in a faster, simpler, and cheaper manner compared to traditional hybridization assays (Wang, 2006). Recent advances in developing such devices open up new opportunities for gene diagnostics, drug discovery, detection of infection agents, and biowarfare agents (Sassolas et al., 2008). A large number of NABs, in connection with different transducers, have been reported in the literatures (Odenthal and Gooding, 2007). However, the practical application of NABs in gene diagnosis is still in its early stage because of the high detection limit, expensive instrumentation, and complex detection procedures (Soper et al., 2006). Comparing with the low detection limit (copies of nucleic acids) of a polymerase chain reaction (PCR), the detection limit (DL) of most of NABs is relatively high and cannot meet the requirement for detecting the extremely low concentration of nucleic acids. Recently, scientists have made significant progress to improve the DL of NABs. Copies of DNA or RNA could be detected by the use of nanomaterial labels and novel signal amplification strategies (Zhang et al., 2004; Nam et al., 2004). For these highly sensitive NABs to become widely used for both clinical and research applications, reduction in the complexity of the tests (i.e., number of steps and skill base required), together with a reduction in the instrumentation and cost per test, is required. Particularly, for these NABs to be applicable at the near patient (point-of-care) or near process level, simple, easy-to-use, cost-competitive systems are required (Soper et al., 2006).

Recently, our group and others have reported lateral flow nucleic acid biosensors (LFNABs) that enable visual detection of DNA segments (Mao et al., 2009a,b; Baeumner et al., 2004). The concept was derived from a traditional immunochromotograph strip test. In this case, DNA hybridization reactions were carried out on the lateral flow device, and Au-NPs were used as labels. The captured Au-NPs on the test zone can be visualized as a red band for qualitative and quantitative detection of nucleic acid segments. Compared with other types of NABs (electrochemical, optical, and gravity), the LFNAB eliminates multiple incubation and washing steps and also minimizes the requirements for highly qualified personnel. However, the poor detection limit (nM to sub-nM) prevents its application in gene diagnosis.

Because of signal amplification, enzymes (horseradish peroxidase, alkaline phosphatase, and glucose oxidase) have been widely used as labels in the development of biosensors and bioassays (Chen et al., 2010; Patolsky et al., 2001; Hajdukiewicz et al., 2010; Wang et al., 2002). Enzymes have been also used as a label to

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prepare cross-flow chromatography devices for the detection of proteins (Cho et al., 2005, 2009). In these studies, the enzyme was labeled with streptavidin (or antibody) and captured on the sensor surface through DNA hybridization events (or antibody–antigen immunoreactions) and subsequent streptavidin–biotin interaction. A catalytic reaction in the presence of enzymatic substrate produced a large number of products, which would be detected with electrochemical or optical transducers. Recently, the sensitivities of assays have been enhanced by loading multiple enzymes on nano-size carriers, such as carbon nanotubes (CNT) (Wang et al., 2004) and gold nanoparticles (Au-NPs) (Li et al., 2009; Lai et al., 2009; Tang et al., 2008; Ambrosi et al., 2007). These nanocarrier-enzyme probes have significantly improved sensing performance in a variety of optical and electronic biosensing systems. In those enzyme–Au-NP-based bioassays, the localized Plasmon absorption property of Au-NP has not been utilized.

In this paper, we present an ultrasensitive LFNAB based on HRP–Au-NPs dual labels and lateral flow strip biosensor. Combining the unique optical properties of Au-NPs and enzyme catalytic amplification, which produce red colored chromogen and deposit it on the captured Au-NP and test zone of LFNAB, the LFNAB was capable of detecting of 0.01-pM target DNA without instrumentation. The promising properties of the approach are reported in the following sections.

2. Materials and methods

2.1. Apparatus

Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator, and the Guillotine cutting module CM 4000 were from Biodot Ltd. (Irvine, CA). Eppendorf Biophotometer (Eppendorf, Hauppauge, NY) was used to determine ss-DNA concentration. The portable strip reader (DT1030) was purchased from Shanghai Goldbio Tech. Co., Ltd. (Shanghai, China).

2.2. Reagents

Streptavidin, horseradish peroxidase (HRP), sucrose, sodium dodecyl sulfate (SDS), hydroxylamine, Tween 20, $HAuCl_4$, 3-amino-9-ethylcarbazole (AEC)/H_2O_2 substrate solution, 3,3′,5,5′-tetramethylbenzidine/H_2O_2 substrate solution, Triton X-100, trisodium citrate, bovine serum albumin (BSA), sodium chloride–sodium citrate (SSC) buffer 20× concentrate (pH 7.0), and phosphate buffer saline (PBS, pH 7.4, 0.01 M) were purchased from Sigma–Aldrich. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100), and nitrocellulose membranes (HFB18004) were purchased from Millipore (Billerica, MA). DNA oligonucleotide probes used in this study were obtained from Integrated DNA Technologies, Inc. (Coralville, IA). The oligonucleotide sequences are as follows:

Target DNA: 5′-AGA CCA TCC TGG CTA GTC TGT TGT CTC TAC TAA AAA TA-3′;

Thiolated detection probe (used to prepare HRP–Au-NP–DNA conjugates): 5′-Thio/MC6-D/GGG GTT TCA CCG TGT TAG CCA GGA TGG TCT-3′;

Biotinylated capture probe 1 (used to prepare test line): 5′-biotin/CGC CCG GCT AAT TTT TTG TAT TTT TAG TAG AGA C-3′;

Biotinylated capture probe 2 (used to prepare control line): 5′-biotin/ATG AGA CCA TCC TGG CTA ACA CGG TGA AAC CC-3′;

2.3. Preparation of HRP–Au-NP–DNA conjugates

Au-NPs with an average diameter of 15 ± 3.5 nm were prepared according to the reported methods with slight modifications (Mao et al., 2009a). Three methods were used to prepare HRP–Au-NP–DNA conjugates.

2.3.1. Method 1

1 mL of tenfold-concentrated Au-NP solution was adjusted to pH 6.5 by adding 0.2 M NaOH; then, 25 μL of 10 mg mL^{-1} HRP were added to the above solution and the mixed solution was incubated for 2 h at 4 °C. Subsequently, the thiolated DNA (1.0 OD) was added, and the solution was kept for another 24 h at 4 °C. After incubation, 20 μL of 1% sodium dodecyl sulfate (SDS) were added to stabilize the Au-NPs with shaking at room temperature for 1 h. Finally, 75 μL of 2 M NaCl was added slowly into the solution up to a final concentration of 150 mM to "age" the DNA. The solution was kept for another 24 h at 4 °C, and the excess reagents were removed by centrifugation for 13 min at 12,000 rpm. After discarding the supernatant, the red pellets were washed twice with 0.01 M PBS (pH 7.4); centrifuged; dispersed in 1 mL of an aqueous solution containing 20 mM $Na_3PO_4{\cdot}12H_2O$, 5% BSA, 0.25% Tween, and 10% sucrose. The resulting HRP–Au-NP–DNA conjugate solution was stored at 4 °C before further use.

2.3.2. Method 2

Thiolated DNA (1.0 OD) and 25 μL of 10 mg mL^{-1} HRP were added to 1 mL of tenfold-concentrated Au-NP solution simultaneously (pH adjusted to 6.5 as stated in Method 1), and the mixture was kept for 24 h at 4 °C. Other procedures, including the addition of SDS and salts, followed those described in Method 1.

2.3.3. Method 3

Thiolated DNA probe (1.0 OD) was added to 1 mL of the tenfold-concentrated Au-NP solution. After standing at 4 °C for 24 h, the pH of the solution was adjusted at 6.5; then, 25 μL of 10 mg/mL HRP were added to the solution and the mixture was kept at 4 °C for an additional 2 h. Other procedures were the same as described in Method 1.

2.4. Sample assay procedure

The HRP–Au-NP–DNA-based LFNAB was prepared by the procedure developed by our group (Mao et al., 2009a). In a typical DNA test on the HRP–Au-NP–DNA-based LFNAB, 80 μL of sample solution containing a desired concentration of target DNA, which was prepared in a running buffer (fourfold-diluted SSC buffer containing 4% BSA) were applied to the sample application zone. After waiting for 10 min, the biosensor was washed twice by adding 20 μL of running buffer on the sample pad at 3-min intervals. Red bands were observed on the LFNAB due to the accumulation of HRP–Au-NP on the test zone and the control zone. For enzymatic amplification, 60 μL of HRP substrate solution containing 0.05% 3-amino-9-ethylcarbazole (AEC) and 0.03% H_2O_2 in 0.05 M sodium acetate buffer (pH 5.5) was added. The enzymatic reaction proceeded for 6 min to deposit red enzymatic products on the test zone and control zone of the LFNAB. The intensities of the red bands were recorded using the portable strip reader combined with the "AuBio strip reader" software. The total assay time was about 30 min.

3. Results and discussion

3.1. Principle of HRP–Au-NP dual-label-based LFNAB

HRP–Au-NP dual-label-based LFNAB combines the unique optical properties of Au-NPs with catalytic amplification of an enzyme tracer for ultrasensitive detection of nucleic acid on a lateral flow device. Our previous LFNAB (Mao et al., 2009a,b) was based on Au-NP labels, which suffered from a poor detection limit (nM or sub-nM). In the current study, we introduced an enzyme tracer,