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Cascade signal amplification for electrochemical immunosensing by integrating biobarcode probes, surface-initiated enzymatic polymerization and silver nanoparticle deposition

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ABSTRACT

A cascade signal amplification strategy through combining surface-initiated enzymatic polymerization (SIEP) and the subsequent deposition of strepavidin functionalized silver nanoparticles (AgNPs) was proposed. The first step of constructing the electrochemical immunosensor involves covalently immobilizing capture antibody on a chitosan modified glass carbon electrode, which then catalyzes DNA addition of deoxynucleotides (dNTP) at the 3′-OH group by terminal deoxynucleotidyl transferase (TdT), leading to the formation of long single-stranded DNAs labeled with numerous biotins. Following the deposition of numerous strepavidin functionalized AgNPs on those long DNA chains, electrochemical stripping signal of silver was used to monitor the immunoreaction in KCl solution. Using α-fetoprotein as a model analyte, this amplification strategy could detect fetoprotein down to 0.046 pg/mL with a wide linear range from 0.1 pg/mL to 1.0 ng/mL. The achieved high sensitivity and good reproducibility suggest that this cascade signal amplification strategy has great potential for detecting biological samples and possibly clinical application.

1. Introduction

Highly sensitive and feasible methods for the determination of tumor markers in blood or tissue are critical in obtaining essential information for cancer screening and earlier disease diagnosis (Sidransky, 2002; Wulfkuhle et al., 2003). There has so far been a great difficulty to directly employ conventional methods to detect tumor markers due to their low abundance in body fluids and tissue (Schweitzer et al., 2000). Signal amplification is therefore becoming a popular strategy for developing various ultrasensitive immunoassay techniques. The amplification pathway is generally designed through loading high ratio of signal or signal-related molecule to reporting element on one nanocarrier to trace the immunoreactions (Wang et al., 2004; Zhuo et al., 2009; Du et al., 2011). Such an approach is unfortunately often accompanied by complicated and costly procedures. Meanwhile, some synthesized probes may not be stable over extended period of time. The in situ formation of assemblies on the immunocomplex, on the other hand, has shown some promising properties for signal amplification.

Assemblies based on DNA amplification techniques have recently been explored as an alternative strategy for sensitive protein assays (Mason et al., 2006; Stoeva et al., 2006; Zhang et al., 2012). Rolling circle amplification (RCA) is a conventional approach due to its easy quantification and validation by using a circular DNA template under isothermal conditions (Schweitzer et al., 2000; Cheng et al., 2010). However, the steps required to prepare and isolate the circular DNA template are complicated and time-consuming (Zhang et al., 2012; Wan et al., 2013). In contrast, surface-initiated enzymatic polymerization (SIEP) can also play the transduction role through an amplification method (Tjong et al., 2011; Wan et al., 2013). This assay uses terminal deoxynucleotidyl transferase (TdT), which is a template-free DNA polymerase that catalyzes the sequential addition of deoxynucleotides (dNTPs) at the 3′-OH group of an oligonucleotide primer and incorporates biotinlated 2′-deoxyadenosine 5′-triphosphate (biotin-dATP) into the long single-stranded DNA (ssDNA) chain. Through the binding between biotin and strepavidin, a large number of signal tags could be assembled for ultrasensitive detection of protein.

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Due to its good biocompatibility with biomolecules and easy functionalization, gold nanoparticle (AuNP) has been frequently utilized in immunoassays (Katz and Willner, 2004; Malhotra et al., 2010). AuNP is an attractive electroactive label to develop enzyme-free immunosensing strategy since it can be directly measured by electrochemical stripping analysis (Dequaire et al., 2000). The electrochemical oxidation of AuNP, however, occurs at a relatively more positive potential (Ho et al., 2010). In comparison to AuNP, silver nanoparticle (AgNP) can be directly detected by anodic stripping analysis in KCl at a more negative potential and produces a relatively sharper peak (Ting et al., 2009). Silver nanoparticle probes have indeed been used earlier to detect DNA (Taton et al., 2000) and proteins (Kim et al., 2009). Some previous work has designed a signal amplification strategy by using AuNPs or functionalized AgNPs probe to catalyze Ag deposition for ultrasensitive electrochemical immunoassay of biomarker (Lai et al., 2011; Lin et al., 2014).

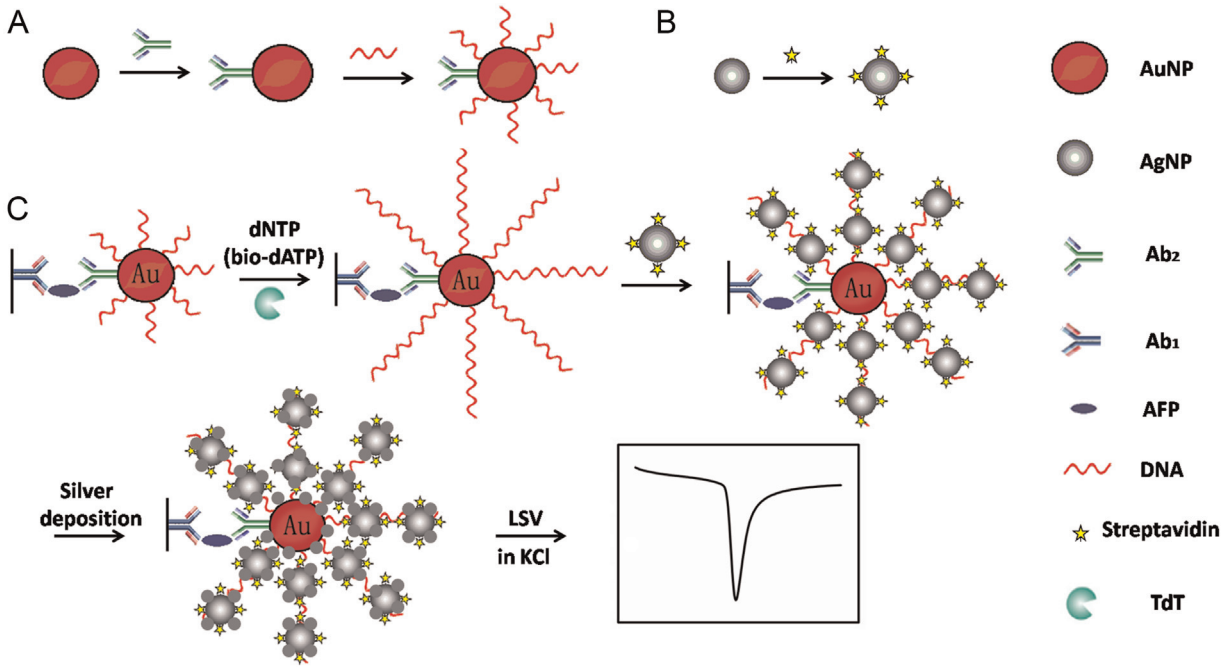
Herein, this work designed a novel and effective immuno-SIEP assay strategy for electrochemical determination of tumor markers. It was accomplished by integrating an advanced amplification technique, SIEP, with biobarcode nanoparticle probe, and strepavidin functionalized silver nanoparticles (AgNPs) (Scheme 1C). In the first step of constructing the immunosensor capture antibody was covalently immobilized on a chitosan modified glass carbon electrode. With a sandwich immunoassay, the biobarcode nanoparticles with numerous oligonucleotides were captured on the immunosensor surface, which catalyzed at the 3′-OH group of DNA addition of deoxynucleotides (dNTP) containing biotinlated 2′-deoxyadenosine 5′-triphosphate (biotin-dATP) by terminal deoxynucleotidyl transferase (TdT). The above prcoess results in the formation of many long single-stranded DNAs labeled with numerous biotins. Though the binding between biotin and strepavidin, a large number of strepavidin functionalized AgNPs were assembled. Anodic stripping voltammetric detection of Ag was performed in KCl solution for the cascade signal amplification, in which the method showed remarkable amplification efficiency, very little nonspecific adsorption and low background signal. This new strategy avoids the deoxygenation procedure for usual electrochemical detection and harmful solution for dissolution of tracing tag. A low detection limit down to sub pg/mL level has been achieved here, which corresponds to RCA or nanomaterial-based multienzyme amplification strategies (pg/mL).

2. Materials and methods

2.1. Reagents

Mouse monoclonal capture (Ab_1), signal (Ab_2) anti-AFP antibodies (clone no. bsm-1622M and bsm-1621M) and streptavidin were purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (China). TdT and the synthetic oligonucleotide were purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. Silver enhancer solutions were purchased from Sigma-Aldrich (USA). Bovine serum albumin (BSA), chloroauric acid ($HAuCl_4 \cdot 4H_2O$), trisodium citrate, sodium borohydride ($NaBH_4$), chitosan, and silver nitrate were obtained from Aladdin (Shanghai, China). AFP standard solutions were from AFP ELISA kit supplied by Fujirebio Diagnostics AB (Göteborg, Sweden). Ultrapure water was obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) and was used in all assays. The clinical human serum samples were obtained from Jiangsu Institute of Cancer Research. All human serum samples were used after the patients and healthy volunteers had provided informed consent and the institution provided authorization. The present study was approved by the Ethics Committee of Jiangsu Cancer Hospital. All other reagents were of analytical grade and used as received.

Tris-HNO_3 buffer (0.1 M, pH 7.4) containing 0.05% (w/v) Tween-20 was used as the washing solution. 0.1 M Tris–HNO_3 containing 5% BSA was used as blocking solution for the preparation of immunosensor. The mixture of silver enhancer solutions A and B was freshly diluted 20 times for the silver deposition.



Scheme 1. Schematic representation of (A) the preparation of Ab_2-biobarcode AuNP probe, (B) the preparation of streptavidin functionalized AgNPs, and (C) the SIEP signal amplification strategy.