Analytica Chimica Acta 799 (2013) 36–43

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Signal-on impedimetric electrochemical DNA sensor using dithiothreitol modified gold nanoparticle tag for highly sensitive DNA detection

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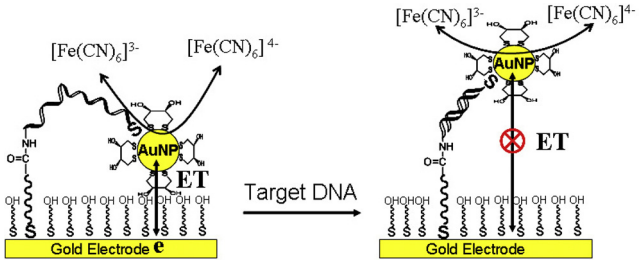
HIGHLIGHTS

- Simple signal-on E-DNA sensor using AuNPs as tag was developed.
- Electrochemical impedance spectroscopy was utilized to determine hybridization-induced distance changes between AuNP tag and the electrode surface.
- The design of the E-DNA sensor is simple and the assay is fast and user-friendly.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form 3 September 2013
Accepted 9 September 2013
Available online 14 September 2013

Keywords:
DNA
E-DNA sensor
Impedimetric
Au nanoparticle
Signal-on

ABSTRACT

A signal-on impedimetric electrochemical DNA (E-DNA) sensor using gold nanoparticles (AuNPs) as tag was developed for highly sensitive detection of DNA hybridization. A probe ssDNA (PDNA) was immobilized by forming an amide between the —NH_2 moiety at the 5′-terminus of PDNA and the —COOH group at self-assembled 11-mercaptoundecanoic acid on a gold electrode. Subsequently, AuNPs were attached to the —SH moiety at the 3′-terminus of the immobilized PDNA by S–Au interaction, and then functionalized with —OH by immersing the electrode in dithiothreitol solution. In the absence of the target DNA, the flexible single-stranded PDNA supports efficient contact between AuNP tag and electrode, ensuring a low electron transfer resistance (Ret) of the E-DNA sensor using the $[Fe(CN)_6]^{3-/4-}$ redox probe. Upon hybridization, a rigid probe-target duplex is formed, which pushes the AuNP tag away from the electrode and increases the distance between AuNP tag and the electrode, thereby increasing the Ret of the E-DNA sensor. Based on hybridization-induced conformational changes, the E-DNA sensor shows an increased Ret response when the target DNA concentration is increased from 5 fM to 500 pM. Furthermore, the E-DNA sensor showed differentiation abilities for single-base mismatch.

1. Introduction

The development of sensor for sensitive DNA detection is extremely important in clinical diagnostics, gene therapy, and a variety of biomedical studies [1]. A number of biosensor approaches have been suggested in the recent past for the identification and quantification of DNA molecules [2–6]. Among the sensing technologies, the electrochemical method is one of the widely adopted detection platforms for nucleic acid analyses and shows great potentiality in future decentralized DNA analysis, because of its cost-effectiveness, simplicity, and portability [7]. However, the majority of these electrochemical methods require the addition of exogenous, label-containing secondary probes [8,9]. Thus, electrochemical DNA (E-DNA) sensors, operate by detecting hybridization-induced conformational changes in a redox-modified and electrode bound DNA probe, have been developed [10,11].

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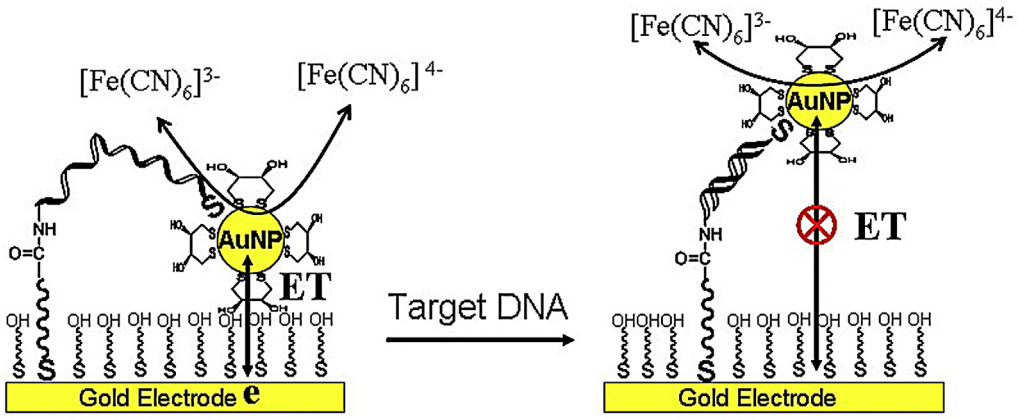
http://dx.doi.org/10.1016/j.aca.2013.09.024



Scheme 1. Schematic representation of the DNA sensor preparation and the analysis of the target DNA.

As a new class of reagentless DNA biosensor, E-DNA sensors include both signal-on and signal-off sensors. The characteristics of signal-on sensors are usually superior to that of the signal-off sensors [12,13]. A number of signal-on E-DNA sensor architectures, including a DNA pseudoknot [14]; triblock structure [15]; hybridization-based, double-stranded sensor [16]; adjunct probe [17], and asymmetric hairpin probe [18], have been developed. However, most of the these E-DNA sensors involve complicated structural designs of the capture probes, as well as complex pretreatment of the electrodes. For the electrochemical measurement, appropriate electrochemical-active indicators, such as methylene blue or ferrocene, are utilized as tags and labels to probe DNA [19]. Gold nanoparticles (AuNPs) are widely used as tags in electrochemical DNA sensors because of their high stability, low-cost, and labeling convenience [20], it also used in the fabrication of the impedimetric DNA sensors [21]. It was reported that AuNPs can mediate electron transfer (ET) across the self-assemble monolayer (SAM) on the electrode through the electron tunneling [22]. Electrochemical impedance spectroscopy (EIS) is highly sensitive to changes in interfacial ET [23]. Therefore, a simple signal-on impedance E-DNA sensor based on hybridization-induced distance changes in AuNPs tag and electrodes is developed here.

Nearly one in eight US women will develop breast cancer in their lifetime. Most breast cancer is not associated with a hereditary syndrome, occurs in postmenopausal women, and is estrogen and progesterone receptor-positive [24]. Estrogen exposure is an epidemiologic risk factor for breast cancer and estrogen is a potent mammary mitogen. Studies indicate that genes other than the well-mapped regions act as modifiers of breast cancer risk. Steroid hormone receptor gene variants in ESR1, ESR2, and PGR that might be associated with risk of breast cancer, perhaps leading to accelerated or slower rates of neoplastic transformation [25]. Here, we proposed an impedimetric electrochemical DNA sensor for the detection of PGR gene (rs1255998) related to estrogen and progesterone receptors.

As shown in Scheme 1, the gold electrode was functionalized with —COOH group by mixed assembly of 11-mercaptoundecanoic acid (MUA) and 6-mercaptohexanol on the electrode. A probe ssDNA (PDNA) was immobilized by forming an amide between $-NH_2$ moiety at the 5′-terminus of PDNA and —COOH group at MUA on a gold electrode. Subsequently, AuNPs were attached to the —SH moiety at the 3′-terminus of the immobilized PDNA via S–Au interaction, and then functionalized with dithiothreitol. In the absence of the target, the flexible single-stranded PDNA supports efficient contact between the AuNPs tags and electrodes, ensuring a low electron transfer resistance (Ret) of the E-DNA sensor. Upon hybridization, the formation of a rigid probe-target duplex pushes AuNP tag away from the electrode surface and increases the distance between the AuNP tag and electrode. This leads to an increase in Ret of the E-DNA sensor. The fabrication and performance of the DNA sensor is discussed in this study.

2. Materials and methods

2.1. Chemicals and reagents

All oligodeoxynucleotides were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). Gene sequences (*PGR* gene rs1255998) related to estrogen and progesterone receptors were used as target [26]. A 21-mer DNA complementary to the wild type of *PGR* gene rs1255998 was utilized as a probe DNA (PDNA). The oligonucleotide sequences for both the probes and the targets are shown in Table 1.

$HAuCl_4{\cdot}3H_2O$, 11-mercaptoundecanoic acid (MUA), 6-mercaptohexanol (MCH), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHS) and thionine were obtained from Sigma–Aldrich (Shanghai, China) Trading Company Limited. Dithiothreitol (DTT) was purchased from Bio Basic Incorporation (Shanghai, China). AuNPs with diameter of approximately 13 nm and 20 nm were prepared via the citrate reduction of $HAuCl_4$ [27]. Citrate capped AuNPs with diameter of approximately 5 nm were prepared based on reported method [28] The PDNA was diluted with 10 mM Tris–HCl buffer (pH 7.4, 1 M NaCl). Unless otherwise stated, all DNA solutions were prepared using 10 mM Tris–HCl buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$. Other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. and were of analytical grade. Water was obtained from Millipore Milli-Q purification system.

Table 1
DNA probes and their targets.

Name	Sequence
Probe DNA	5′-NH_2-$(CH_2)_6$-AGTCGACACGGGACGGTTGTC-$(CH_2)_6$-SH-3′
Target DNA (T1, 27-mer)	5′-GTAGACAACCGTC**C**CGTGTCGACTG GT-3′
Target DNA (T2, 21-mer)	5′-GACAACCGTCCCGTGTCGACT-3′
Target DNA (T3, 17-mer)	5′-CAACCGTCCCGTGTCGA-3′
Target DNA (single mismatch)	5′-GTAGACAACC***G***TCGCGTGTCGACTG GT-3′
Noncomplementary DNA	5′-CAGGATCATGGTGATGCTCTACGTGCCGTAGCC-3′
Probe DNA for PCR products	3′-NH_2-$(CH_2)_6$-CAACCTCGACCACCGCATCCG-$(CH_2)_6$-SH-5′