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Ultrasensitive photoelectrochemical biosensor for the detection of HTLV-I DNA: A cascade signal amplification strategy integrating λ-exonuclease aided target recycling with hybridization chain reaction and enzyme catalysis

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ABSTRACT

Sensitive and specific detection of DNA is of great significance for clinical diagnosis. In this paper, an effective cascade signal amplification strategy was introduced into photoelectrochemical (PEC) biosensor for ultrasensitive detection of human T-cell lymphotropic virus type I (HTLV-I) DNA. This proposed signal amplification strategy integrates λ-exonuclease (λ-Exo) aided target recycling with hybridization chain reaction (HCR) and enzyme catalysis. In the presence of target DNA (tDNA) of HTLV-I, the designed hairpin DNA (h_1DNA) hybridized with tDNA, subsequently recognized and cleaved by λ-Exo to set free tDNA. With the λ-Exo aided tDNA recycling, an increasing number of DNA fragments (output DNA, oDNA) were released from the digestion of h_1DNA. Then, triggered by the hybridization of oDNA with capture DNA (cDNA), numerous biotin-labeled hairpin DNAs (h_2DNA and h_3DNA) could be loaded onto the photoelectrode via the HCR. Finally, avidin-labeled alkaline phosphatase (avidin-ALP) could be introduced onto the electrode by specific interaction between biotin and avidin. The ALP could catalyze dephosphorylation of phospho-L-ascorbic acid trisodium salt (AAP) to generate an efficient electron donor of ascorbic acid (AA), and thereby greatly increasing the photocurrent signal. By utilizing the proposed cascade signal amplification strategy, the fabricated PEC biosensor exhibited an ultrasensitive and specific detection of HTLV-I DNA down to 11.3 aM, and it also offered an effective strategy to detect other DNAs at ultralow levels.

1. Introduction

Highly sensitive detection of DNA is of great significance for clinical diagnosis, gene therapy, mutation analysis and pathogen detection (Diao et al., 2018; Liu et al., 2009; Hänsel-Hertsch et al., 2017; Shaw et al., 2017; Wang et al., 2011). Human T-cell lymphotropic virus type I (HTLV-I) is a human retrovirus, which is associated with the clinical diagnoses of rare lymphocytic neoplasms, progressive neurodegenerative disease, and adult T-cell lymphoma/leukemia. Nevertheless, HTLV-I DNA is so fractional that more ingenious analytical strategies were necessary for detection (Kalyanaraman et al., 1982; Bhagavati et al., 1988; Poiesz et al., 1980).

Over the past decades, various techniques have been developed for sensitive detection of the specific DNA sequences, such as microarrays, colorimetric, electrochemcial, electrochemiluminescence, photoelectrochemical (PEC) biosensors, and so on (Schena et al., 1995; Teengam et al., 2017; Freeman et al., 2011; Low et al., 2017; Zhang et al., 2012; Zhao et al., 2014). Among these methods, PEC biosensor is a newly emerged yet vibrantly developing technique for biological sensing. PEC biosensors inherited the advantages of conventional electrochemical and optical methods, and offered a potential biosensing platform with high sensitivity and reduced background signal (Li et al., 2016; Zeng et al., 2014; Fan et al., 2016; Zhao et al., 2015).

In order to detect ultralow levels of DNA, the most important aspect of a sensitive PEC biosensor is to employ an excellent signal amplification strategy. To date, the signal amplification strategies for

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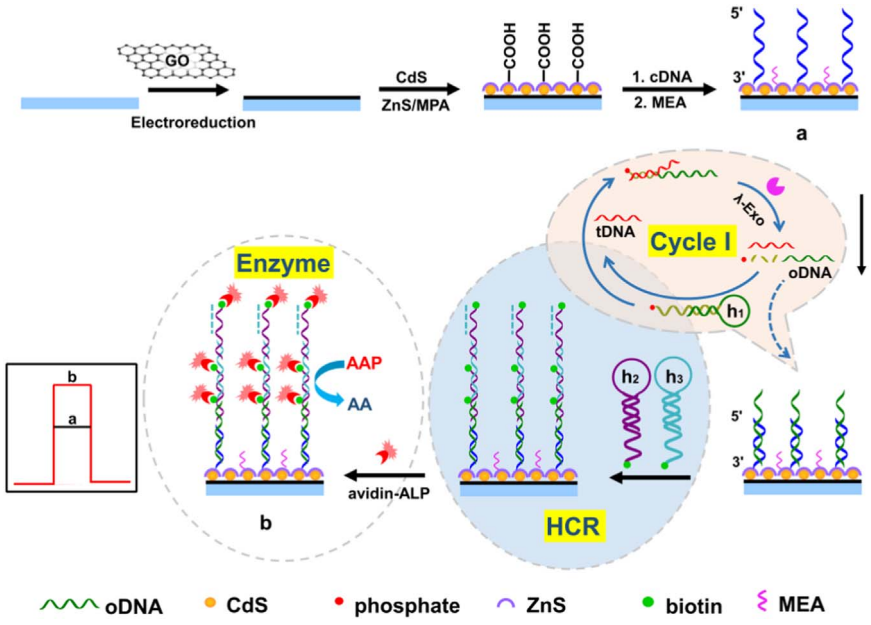
ultrasensitive DNA detection have attracted more and more attention, because they can overcome the inherent limitation of target-to-signal ratio of 1:1 in the traditional hybridization assay and acquire higher sensitivity (Tao et al., 2015; Wang et al., 2015; Wu et al., 2015; Song et al., 2015). So far, a number of amplification methods have been developed, such as rolling circle amplification (RCA) (Song et al., 2015; Murakami et al., 2008; Wang et al., 2014), polymerase chain reaction (PCR) (Cheglakov et al., 2006; Du et al., 2013), hybridization chain reaction (HCR) (Choi et al., 2010; Chen et al., 2011a; Yang et al., 2015), and enzyme-assisted target recycling (Tao et al., 2015; Zuo et al., 2010; Bi et al., 2012; Ren et al., 2015). Among them, enzyme-assisted target recycling amplification has been widely adopted for the development of DNA biosensor. As one of the exonuclease, λ-exonuclease (λ-Exo) can catalyze the stepwise digestion of a 5′ phosphorylated strand in the 5′ to 3′ direction in a double-stranded DNA (dsDNA) (Kovall and Matthews, 1997). Thus, λ-Exo provides a universal platform for the target recycling amplified DNA assay. Further development in DNA signal amplification technique is the HCR technique. HCR is a complementary base paring reaction, which is triggered by a single-stranded DNA initiator and propagates a chain reaction of hybridization events to yield nicked double helices analogous to alternating copolymers, making HCR a promising strategy in signal amplification (Choi et al., 2010)

Inspired by the works discussed above, we have for the first time designed a novel ultrasensitive PEC biosensor for HTLV-I DNA detection by combining λ-Exo aided target recycling, HCR and enzyme catalysis for cascade signal amplification, as illustrated in Scheme 1. The h_1DNA consists of two regions, including the recognition region which is complementary to target DNA (tDNA) at the 5′-terminus and the output region which is partly complementary to capture DNA (cDNA). In the absence of tDNA, h_1DNA remains intact to λ-Exo because the overhang 5′-phosphorylated terminus could not be cleaved by λ-Exo. However, h_1DNA could form a dsDNA with a blunt 5′-phosphorylated terminus in the presence of tDNA (tDNA). The blunt 5′-phosphorylated terminus dsDNA could be stepwise digested by λ-Exo, and then releases the tDNA and output DNA (oDNA). The released tDNA could further take participate in the next cycles of hybridization and cleavage process (Cycle I). The introduction of HCR into the PEC DNA biosensor could further improve the detection sensitivity. The released oDNA could be captured by the cDNA on the electrode, and then open h_2DNA through hybridization, trigger the subsequent hybridization reaction by the sequential introduction of h_3DNA, and realize the downstream HCR amplification (Yang et al., 2015). The formed dsDNA chains on the electrode with the biotin-tagged h_2DNA and h_3DNA provide multiplex biotin sites, which could conjugate with avidin-alkaline phosphatase (Av-ALP) through the combination between biotin and avidin. The enzyme of ALP, which exists extensively in mammalian organisms and catalyzes the hydrolysis and transphosphorylation of diverse phosphoryl esters (Zhao et al., 2012a, 2012b), could dephosphorylate the substrate of 2-phospho-L-ascorbic acid trisodium salt (AAP) and generate the product of ascorbic acid (AA), a commonly used electron donor in PEC biosensor (Yin et al., 2015; Zhou et al., 2014). In the proposed cascade signal amplification strategy, the presence of tDNA could trigger the cycle I, releasing plenty of oDNA, and then produce more biotin sites through HCR. With the biotin sites, more Av-ALP could anchor on the DNA chains. Given the enzyme catalysis amplification of ALP, the photocurrent response could be greatly enhanced by the AA generated from AAP by ALP. The intensity of the photocurrent signal was proportional to the concentration of tDNA by this cascade signal amplification strategy. The designed PEC system exhibited excellent analytical performance, which would have promising perspective for sensitive and selective DNA detection.

2. Experimental

2.1. Materials and reagents

ITO electrodes (type JH52, ITO coating 30 ± 5 nm, sheet resistance ≤10 Ω/square) were ordered from Nanjing Zhongjingkeyi Technology Co., Ltd. (China). Graphite powder, potassium peroxydisulfate ($K_2S_2O_8$), potassium permanganate ($KMnO_4$), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphorus pentoxide (P_2O_5), cadmiumnitrate ($Cd(NO_3)_2{\cdot}4H_2O$, sodium sulfide(Na_2S), zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), sodium hydroxide (NaOH), and sodium chloride (NaCl) were obtained from Nanjing Chemical Reagent Co. Ltd. (China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), monoethanolamine (MEA), 3-mercaptopropionic acid (MPA), and 2-phospho-L-ascorbic acid trisodium salt (AAP) were received from Sigma-Aldrich (USA). Ascorbic acid (AA) and hydrogen peroxide (H_2O_2, 30 wt%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Avidin labeled alkaline phosphatase (adivin-ALP) was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Lambda Exonuclease (λ-Exo) was obtained from



Scheme 1. Fabrication process of the PEC DNA biosensor with cascade signal amplification.