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In situ hybridization chain reaction mediated ultrasensitive enzyme-free and conjugation-free electrochemcial genosensor for BRCA-1 gene in complex matrices

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ABSTRACT

In this study, we report an enzyme-free and conjugation-free electrochemical genosensor enabling an ultrasensitive readout of BRCA-1, a breast cancer susceptibility gene. The sensor employs a target-responsive hybridization chain reaction (HCR) to significantly amplify the detectable current signals. By means of a functional auxiliary probe pair and a versatile initiator sequence, a linear DNA concatemer structure can be formed via spontaneous and continuous polymerization of DNA oligomers in the presence of target sequence. Such a DNA nanoassembly endows the genosensor an ultrahigh sensitivity up to 1 aM, which is higher than that of the nanomaterials-based or enzyme mediated amplification approaches by several orders of magnitude. More importantly, the sensor's responsive peak current exhibits a favorable linear correlation to the logarithm of the concentrations of target sequence ranging from 1 aM to 10 pM. In addition, the sensor is highly selective, and can discriminate a single mismatched sequence. This HCR-based genosensor is also capable of probing low-abundance BRCA-1 gene sequence directly in complex matrices, such as 50% human serum, with minimal interference. These advantages will make our tailor-engineered HCR-based electrochemical genosensor appealing to genetic analysis and clinical diagnostics.

1. Introduction

The accurate readout of naturally low abundant and specific DNA sequences is critically important to identification of viruses, pathogenic microorganisms and human genetic diseases, such as cancer (Ferguson et al., 2011; Chung et al., 2013; Lam et al., 2013; Liong et al., 2013; Vasilyeva et al., 2011). Note that BRCA-1 (breast cancer gene 1) has been proved to be closely related to the occurrence of familial breast cancer (Sifri et al., 2004). For example, the mutation of BRCA-1 can lead to an increased risk in breast cancer with hereditary breast-ovarian cancer syndrome, and it is responsible for more than 80% of inherited breast and ovarian cancers (Sifri et al., 2004; Tutt and Ashworth, 2002). Therefore, the accurate detection of BRCA-1 gene in disease-related sequence is significant towards genetic analysis and clinical diagnosis of breast cancer. Although PCR and gene sequencing technologies can decode the sequence information in part for such prone-to-mutate genes, they often require either enzyme involvement and thermal recycling, or relatively large-volume sample and complex sample treatment (Ozcelik et al., 2012). In contrast, genosensors can directly readout the target sequences (e.g., BRCA-1 gene fragment) at sub-picomolar or femtomolar level by means of custom-tailored sensing interface based on multiple transducers, including optical and electronic sensors (Wang et al., 2003; Mavrogiannopoulou et al., 2009; Li et al., 2009; Mohan et al., 2010; Xu et al., 2012; Li et al., 2012; Rasheed and Sandhyarani, 2014a; Rasheed and Sandhyarani, 2015a). Despite attractive high sensitivity, these genosensors require temperature-controlled enzyme-triggered target recycling or time-consuming multi-step conjugation with nanomaterials to amplify the output signals, which would degrade the sensor's appealing superiority, particularly in the point-of-care diagnostics. Thus, it is highly desirable to engineer a universal, enzyme-free and easy-to-assembly genosensor to identify the low-abundance target BRCA-1 sequence.

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DNA nanoassembly is a promising candidate to meet the requirement. Since the recognition of DNA as a nanoscale structural assembly material, DNA nanotechnology has achieved a rapid development and derives multiple nucleic acid signal-amplification strategy using nonenzymatic DNA self-assembly approaches (Seeman, 2003; Pinheiro et al., 2011; Dirks and Pierce, 2004). Hybridization chain reaction (HCR) is a typical enzyme-free, self-catalytic strategy to construct one-dimensional DNA nanostructure for DNA detection with PCR-like sensitivity, in which the hybridization event is initiated by a target sequence and ultimately forms a linear concatemer by continuous and ordered polymerization of a partially complementary probe pair (Dirks and Pierce, 2004). Due to the initiator-responsive hybridization mechanism of HCR, it produces target-dependent signal with an inherently low background. Comparably, each copy of the initiator can trigger a cascade assembly of probe sequences into a long polymer that has a potential to significantly amplify the detectable signals. Despite these advantages, the HCR proceeds without stringent temperature restriction, enzyme participation and complex conjugation. By integrating such a powerful HCR signal amplification strategy with simple, cost-effective electrochemical sensor, several groups realized femtomolar and even attomolar DNA detection (Chen et al., 2012a, 2012b; Ge et al., 2014). However, these methods require either exquisite probe design or enzyme conjugation. By contrast, a facile, enzyme-free signaling design will be more appealing.

Herein, we construct a simple, attomolar genosensor towards BRCA-1 gene by using a versatile initiator sequence to guide the long-range self-assembly of DNA nanostructure as a signal amplification carrier. The unique initiator probe consists of two functional domains. One domain hybridizes with the complementary region of target gene, and the other triggers polymerization of the hairpin probe pair. Such a simple design renders our genosensor more attractive than the existing electrochemical HCR sensor (Chen et al., 2012a, 2012b), because it can be easily extended to any potential target sequence by only modifying the target-complementary domain. In addition, the electroactive $\left[Ru(NH_3)_6\right]^{3+}$ (RuHex, a redox complex that can directly reflect the amounts of DNA strands localized at the electrode surface) can electrostatically interact with the DNA phosphate moieties in a stoichiometric approach, which allows a label-free biosensing (Yang et al., 2014). By synergizing these merits, our HCR-based genosensor can accurately readout the BRCA-1 gene in complex matrices such as human serum with minimal interference.

2. Experimental methods

2.1. Materials

All oligonucleotides (Table S1) were synthesized and purified by Sangon Inc. (Shanghai, China) and used without further purification. Capture probe is a 22-base sequence, which is thiolated with a -$(CH_2)_6$- spacer at the 5′ end. Target DNA (BRCA-1 gene fragment) is a 46-base sequence that contains a 22-base sequence complementary to capture probe. Initiator (I) is a 48-base sequence that contains 24 bases at its 5′ end complementary to the unhybridized target sequence. Two auxiliary probes, H1 and H2, are 48 base hairpin structures, which are half complementary to each other. One-mismatched (M1), two-mismatched (M2) and three-mismatched (M3) DNA sequences are the base mutated counterparts of target DNA, which contains a single-nucleotide, two-nucleotide and three-nucleotide mismatch to the capture probe, respectively. Noncomplementary DNA (NC) is a random sequence that is noncognate to target DNA.

Hexaammineruthenium(III) chloride ($\left[Ru(NH_3)_6\right]^{3+}$, RuHex) was purchased from Sigma-Aldrich (St. Louis, MO). 6-Mercapto-1-hexanol (MCH), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Aladdin Inc. (Shanghai, China). Other chemicals employed were all of analytical grade. The following buffer solutions were employed in this study: DNA immobilization buffer (I-buffer, 10 mM Tris–HCl + 1 mM EDTA + 500 mM NaCl + 10 mM TCEP, pH 7.4), DNA hybridization buffer (H-buffer, 10 mM Tris–HCl + 1 mM EDTA + 500 mM NaCl + 1 mM $MgCl_2$, pH 7.4), electrochemistry buffer (E-buffer, 10 mM Tris–HCl, pH 7.4), MCH solution (2 mM MCH in water), and RuHex solution (50 μM $\left[Ru(NH_3)_6\right]^{3+}$ in 10 mM Tris–HCl, pH 7.4). All solutions were prepared with Milli-Q water (18 MΩ cm resistivity) from a Millipore system.

Human serum samples (supported by the Judicial Identification Center of Henan University of Science and Technology) were used in this study to investigate the sensor's probing capability to the target DNA in complex biological fluids. Firstly, the serum samples were centrifuged about 5 min at 12000 rpm. Then, the supernatants were collected and mixed with equal volume of target DNA.

2.2. Electrode pretreatment

The detailed experimental procedures can be found in the literature (Zhang et al., 2007). Briefly, the gold electrodes (2 mm in diameter, CH Instruments Inc., Austin, TX) were polished on a microcloth (Buehler) with 0.05 μm alumina slurry for 5 min to obtain a mirror surface, followed by sonicating electrodes in ethanol and water for 5 min, respectively, to remove residual alumina powder. Then electrodes were electrochemically cleaned in 0.5 M H_2SO_4 solution to remove any remaining impurities. After being thoroughly rinsed with Milli-Q water and dried with nitrogen, the cleaned electrodes should be immediately used for DNA immobilization.

2.3. Gold electrode surface-based DNA self-assembly, hybridization and amplification.

First, 6 μL of I-buffer containing 1 μM thiolated capture probes was dropped on the cleaned gold electrode surface and incubated for 2 h at 37 °C to form self-assembly monolayers (SAMs). The electrode was then thoroughly rinsed with ultrapure water and dried under a stream of nitrogen gas. The DNA modified electrodes were subsequently treated with MCH solution (2 mM) for 1 h to fill the unoccupied region and remove the nonspecifically adsorbed DNA on the gold surface, and thus to assist the SAMs maintaining a favorable interfacial orientation. Then, the electrode surface was rinsed thoroughly and dried in nitrogen for next hybridization. BRCA-1 gene fragment of varied concentrations were pipetted to the functional electrode surface, allowing for probe-target binding for 90 min in H-buffer. After hybridization, the electrodes were extensively rinsed with H-buffer and dried under nitrogen gas. The mismatched DNA hybridization also followed the same procedure as the above-mentioned. Prior to assembling the complementary probe pair, an initiator-contained solution (1 μM) was first added to the hybridized surface to form sandwiched hybridization mode for 1 h and the initiating domain was thus left to trigger the subsequent polymerization between H1 and H2 (both at 100 nM). The two probes were heated separately to 95 °C for 5 min, allowing to cooling down at room temperature for 15 min, and then being mixed together in H-buffer to the final concentration of 100 nM before use. After reaction for 2 h, the electrode was thoroughly rinsed again by H-buffer and dried