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Binding-induced DNA walker for signal amplification in highly selective electrochemical detection of protein

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ABSTRACT

A binding-induced DNA walker-assisted signal amplification was developed for highly selective electrochemical detection of protein. Firstly, the track of DNA walker was constructed by self-assembly of the high density ferrocene (Fc)-labeled anchor DNA and aptamer 1 on the gold electrode surface. Sequentially, a long swing-arm chain containing aptamer 2 and walking strand DNA was introduced onto gold electrode through aptamers-target specific recognition, and thus initiated walker strand sequences to hybridize with anchor DNA. Then, the DNA walker was activated by the stepwise cleavage of the hybridized anchor DNA by nicking endonuclease to release multiple Fc molecules for signal amplification. Taking thrombin as the model target, the Fc-generated electrochemical signal decreased linearly with logarithm value of thrombin concentration ranging from 10 pM to 100 nM with a detection limit of 2.5 pM under the optimal conditions. By integrating the specific recognition of aptamers to target with the enzymatic cleavage of nicking endonuclease, the aptasensor showed the high selectivity. The binding-induced DNA walker provides a promising strategy for signal amplification in electrochemical biosensor, and has the extensive applications in sensitive and selective detection of the various targets.

1. Introduction

Signal amplification strategies have been widely implanted in electrochemical biosensor to achieve low detection limit and high sensitivity. Generally, there are two types of signal amplification strategies on the basis of nanomaterials (Wu et al., 2014) or biomolecules (Deng et al., 2015). Different from nanomaterial-based strategies, biomolecule-based strategies have excellent biocompatibility in physiological conditions, especially for DNA-based signal amplification tactics such as polymerase chain reaction, rolling circle amplification (Ji et al., 2016), hybridization chain reaction (HCR) (Li et al., 2017; Shuai et al., 2017b), catalytic hairpin assembly (CHA) (Shuai et al., 2016; Zhao et al., 2017) and so on. For example, Li's group designed a two-step signal amplification strategy by combination CHA and HCA for electrochemical detection of DNA with the assistant of external signal molecules and auxiliary probe (Liu et al., 2013). Zhang's group reported a sandwich DNA structure triggered by target DNA, and then initiated HCR reaction to generate electrochemical signal (Yang et al., 2016a). Huang's group developed a series of electrochemical sensing strategies combined nanomaterials with nicking enzyme for signal amplification (Huang et al., 2016; Shuai et al., 2017a). Although the biomolecule-based amplifications have been achieved for electrochemical analysis, these strategies still suffer from some limitations, such as slow electron transfer of amplified DNA structures and low turnover of catalytic cycle. Recent emergence of DNA walker strategy might provide new approach to solve the problem.

DNA walker as a type of molecular machine has been demonstrated to manipulate specific DNA (walking strand) to move autonomously along programmed oligonucleotide tracks such as double stranded DNA (Liu et al., 2016), DNA origami (Zhou et al., 2015), and DNA monolayer (Qu et al., 2017; Yehl et al., 2016). Usually, DNA walkers are propelled by DNAzymes (Pan et al., 2015), protein enzymes (Awaja et al., 2015) and strand displacement (Jung et al., 2016). The movements of walker can destruct the tracks and generate single-stranded product for signal amplification, which can be used as transducer in analytical and diagnostic applications (Peng et al., 2017; Li et al., 2015). In fact, a series of DNA walkers were constructed along a three-dimensional DNA-Au nanoparticle track and cleaved DNA substrates with a nicking endonuclease to release payloads for fluorescence assays (Yang et al., 2016b; Zhang et al., 2015). A free-running DNA walker was also proposed as electrochemical aptasensor formed by DNA self-assembly to detect breast cancer cell via chronocoulometry measure-

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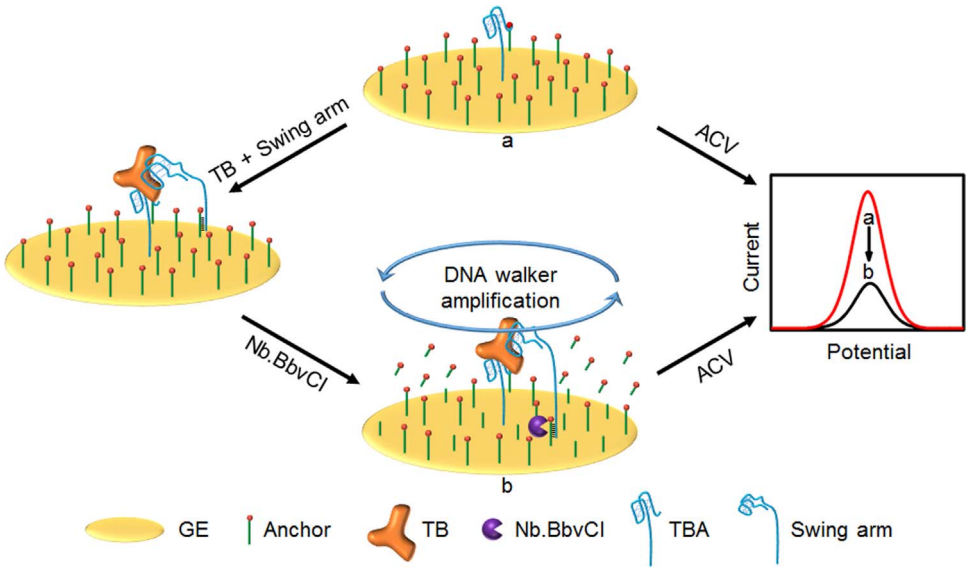

Fig. 1. Schematic illustration of design and analytical procedure of binding-induced DNA walker-assisted signal amplification for electrochemical detection of protein.

ment (Cai et al., 2016). Distinct from the DNA self-assembled walker, a binding-induced DNA walker harnesses specific target binding to trigger assembly of separate DNA components for movement. Therefore, the binding-induced DNA walkers initiate hundreds of signal molecules in response to a single binding event with high specificity, providing a promising tool to design signal amplification method.

In this work, a binding-induced DNA walker is for the first time proposed in the electrochemical aptasensor on the gold electrode (GE). Firstly, the aptasensor was constructed by self-assembly of ferrocene (Fc) labeled anchor as both DNA walker's track and signal molecule on GE with a certain number of thrombin aptamer 1 (TBA) (Fig. 1). Meanwhile, a swing arm DNA was designed consisting of a thrombin aptamer 2 (HD22) and walking strand with repeated T bases as the spacer. Due to only 7 complementary nucleotides between swing arm and anchor so that their hybrid is unstable at ambient temperature. When adding thrombin (TB) and swing arm DNA on the aptasensor, a sandwich structure was formed through the binding of thrombin with the two aptamers, which places the swing arm onto the GE surface. Therefore, the complementary sequences of swing arm and anchor are brought into close proximity, allowing for intramolecular binding-induced assembly to form the duplex. Then nicking endonuclease Nb. BbvCI can specifically cut the hybridized anchor, resulting in releasing Fc molecule from electrode surface to solution and liberate walking strand as free end again. Subsequently, the free walking strand would bind with another anchor to repeat the Nb.BbvCI nicking across the successive interface of electrode to release multiple Fc molecules for signal amplification. Therefore, a highly sensitive and selective strategy was achieved for the detection of target protein. The proposed binding-induced DNA walker provides a promising tool to construct efficient and rapid electrochemical detection strategy for a variety of biomarkers.

2. Experimental

2.1. Chemicals

Nb. BbvCI and CutSmart Buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg mL^{-1} BSA) were obtained from New England Biolabs Inc. (USA). Thrombin, hemoglobin (Hb), bovine serum albumin (BSA), L-cysteine (L-cys), 6-mercapto-1-hexanol (MCH), hexaammineruthenium(III) chloride (RuHex, 98%), ethylene diamine tetraacetic acid (EDTA) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich Inc. (USA). TE buffer (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 7.4) and Tris-HCl buffer (20 mM, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) were prepared in this work.

Oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these oligonucleotides were as follows from 5′ end to 3′ end:

Anchor: HS-TTTTTTTTTTTGTCGTGCTGAGGTT-Fc
Control anchor: HS-TTTTTTTTTTTGTCGTGCTGAGGTT
TBA: HS-$(T)_{25}$**GGTTGGTGTGGTTGG**
Blocker: GAGGACACG
Swing arms (n=2, 12, 22, 32, 42, 52):

AGTCCGTGGTAGGGCAGGTTGGGGTGACT$(T)_n$GTCGTGT*CCTCAGC*

The italic and bold bases represent walking strand and thrombin aptamers, respectively.

2.2. Preparation of aptasensor

Before modification, GE was cleaned by exposing into piranha solution (H_2SO_4/H_2O_2, 3:1) for 10 min, polished with 0.05 μm α-Al_2O_3 slurry, and electrochemically cleaned in 1 M H_2SO_4 by potential scanning between −0.2 and +1.6 V at a scan rate of 4 V s^{-1} for 40 scans until a reproducible cyclic voltammogram (CV) signal was obtained. 50 μL of anchor (2.0 μM) and 2.5 μL of TBA (2.0 μM) were incubated with 5.0 μL of TCEP (10 mM) for 1 h to allow reduction of disulfide bonds and finally diluted to a total volume of 100 μL to obtain a mixture of 1.0 μM anchor and 0.05 μM TBA. 5 μL of the above solution was dropped on GE and incubated at 37 °C for 2 h. After rinsing with phosphate buffer (PBS) and drying with nitrogen, 5 μL of 1 mM MCH was dropped on the electrode for 1 h at 37 °C to block the unmodified sites. Finally, the electrochemical DNA aptasensor was washed with PBS thoroughly to remove unfixed MCH and DNA.

2.3. Measurement procedure

The electrochemical measurements were automatically performed on a CHI 660D electrochemical workstation (Shanghai CH Instrument, China) with a conventional three-electrode system composed of a platinum wire as counter, saturated calomel electrode as reference