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Electrochemical detection of sequence-specific DNA based on formation of G-quadruplex-hemin through continuous hybridization chain reaction

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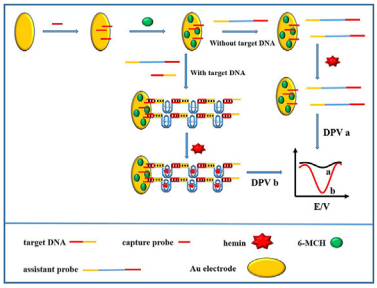
HIGHLIGHTS

- A DNA sensor based on hybridization chain reaction and G-quadruplex-hemin complex.
- High sensitive detection of HIV DNA was achieved with the detection limit down to fM level.
- The DNA sensor can discriminate mismatched sequences of HIV DNA sequence.
- The DNA sensor was applied in detection of the target DNA in serum.

GRAPHICAL ABSTRACT



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ABSTRACT

A high-sensitive detection of sequence-specific DNA was established based on the formation of G-quadruplex-hemin complex through continuous hybridization chain reaction (HCR). Taking HIV DNA sequence as an example, a capture probe complementary to part of HIV DNA was firstly self-assembled onto the surface of Au electrode. Then a specially designed assistant probe with both terminals complementary to the target DNA and a G-quadruplex-forming sequence in the center was introduced into the detection solution. In the presence of both the target DNA and the assistant probe, the target DNA can be captured on the electrode surface and then a continuous HCR can be conducted due to the mutual recognition of the target DNA and the assistant probe, leading to the formation of a large number of G-quadruplex on the electrode surface. With the help of hemin, a pronounced electrochemical signal can be observed in differential pulse voltammetry (DPV), due to the formation of G-quadruplex-hemin complex. The peak current is linearly related with the logarithm of the concentration of the target DNA in the range from 10 fM to 10 pM. The electrochemical sensor has high selectivity to clearly discriminate single-base mismatched and three-base mismatched sequences from the original HIV DNA sequence. Moreover, the established DNA sensor was challenged by detection of HIV DNA in human serum samples, which showed the low detection limit of 6.3 fM. Thus it has great application prospect in the field of clinical diagnosis and environmental monitoring.

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1. Introduction

With the development of medical science, DNA detection plays an important role in pathogen analysis [1], genetic disorder diagnosis [2] and forensic tests [3]. Sensitive and low-cost detection of sequence-specific DNA can facilitate early diagnosis, reduce the health risk of diseases or pollution, with affordable cost to the people in low income countries and regions [3]. Up to now, different techniques have been employed for DNA detection, such as fluorescence [4], electrochemistry [5], electrochemiluminescence [6], chemiluminescence [7], quartz-crystal microbalance [8], and surface plasmon resonance technique [9]. Among these techniques, electrochemical DNA sensors possess several outstanding characteristics, such as high-sensitive, high-selective, simple and easy to miniaturization, and therefore have been widely used in different scenarios [9–11].

To achieve sufficient sensitivity during electrochemical detection, an appropriate signal amplification strategy appears to be the most significant [12,13]. The commonly used signal amplification includes nanomaterials-based techniques and DNA amplification-based techniques. The former techniques generally utilize various nanomaterials as carriers of signal labels. The high-load signal labels owing to the high specific area of nanomaterials offers efficient amplification of detectable signals [14–16]. However, the stability of nanomaterials sometimes is not satisfactory and the steps for the preparation of nanomaterials can be tedious. The latter techniques including polymerase chain reaction [17], hybridization chain reaction [18,19], strand displacement amplification [20,21], and rolling circle amplification [22,23], amplify the output signals based on DNA hybridization and amplification reaction either under the catalysis of enzymes or in enzyme-free mode. Polymerase chain reaction requires specific enzyme to realize the rapid synthesis and amplification of the target DNA. Strand displacement amplification requires DNA polymerase and forms new DNA long chain under the action of primers, which can replace the target DNA. Rolling circle amplification utilizes circular DNA as template and stretches out many complement repetitive DNA sequence under the catalysis of DNA polymerase. Both these amplification techniques involve DNA polymerase. The certain reaction conditions of enzymes can limit the application of such techniques. Whereas hybridization chain reaction (HCR) can be conducted without the involvement of enzymes [24]. Using the target DNA as the evoked agent, hybridization chain reaction can promote signal amplification and improve detection sensitivity through proper design [25]. Different readout signals can be produced after hybridization chain reaction depending on the labels used [26,27], among which the electrochemical detection attracted wide interest owing to its outstanding characteristics as described above.

G-quadruplex is a guanine-rich DNA sequence that can fold into spatial structure in the presence of K^+ and the structure can strongly combine with hemin to form G-quadruplex-hemin complex [28]. Owing to its unique features, such as relatively easy to label, low cost, more stable against hydrolysis and heat treatment, low nonspecific adsorption, and typical electrochemical property, G-quadruplex-hemin complex that consists of hemin intercalated in a G-quadruplex structure becomes a frequently used signal label in biosensors [29–32].

Herein, we fabricated a simple yet ultrasensitive electrochemical DNA biosensor. Taking HIV DNA sequence as an example, the sensor coupled the hybridization chain reaction between the target sequence and the assistant probe with the formation of signal-generating G-quadruplex-hemin complex. The rationally designed probes triggered the signal amplification procedure. Thus high sensitivity as well as high specificity of the sensor were achieved.

2. Experimental

2.1. Materials and reagents

6-mercaptohexanol (6-MCH) and hemin were purchased from Sigma-Aldrich. Human serum was purchased from Beijing Solarbio Science & Technology Co., Ltd. 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid sodium salt (HEPES) was obtained from Sangon Biotech Co., Ltd. Other chemicals were of analytical grade. All solutions were prepared with ultrapure water (18.2 MΩ cm) obtained from a Millipore water purification system.

All oligonucleotides were synthesized and HPLC-purified by Sangon Biotech Co., Ltd. They were diluted to 100 μM with 10 mM PBS buffer (pH7.4) containing 1 M NaCl and 1 mM $MgCl_2$. The sequences of the oligonucleotides were as below.

Capture probe: 5′-HS-$(CH_2)_6$-TT*TAGTACTGGTG* -3′

Assistant probe: 5′-AAATTGCTGCCTTTGGGTAGGGCGGGTTGGG C*TTAGTAC TGGTG*-3′

HIV DNA: 5′-GGCAGCAATTT*CACCAGTACTA*-3′

Single-base mismatched HIV DNA: 5′-GGCAGCAATTT**G**ACCAG-TACTA-3′

Three-base mismatched HIV DNA: 5′-GGCAGCAATT**AGT**CCAG-TACTA-3′

The italic letters and the underlined letters are the complementary sequences, and the bold letters are the mismatched bases.

2.2. Preparation and modification of Au electrode

Au electrode (3 mm in diameter) was polished carefully with alumina slurry (0.3 and 0.05 μm) and then sonicated in ultrapure water and ethanol for 3 min, respectively. Then the polished electrode was electrochemically activated by cyclic scanning in the potential range from −0.2 to +1.5 V in 0.5 M H_2SO_4. Thereafter the electrode was dried at nitrogen atmosphere.

The pretreated Au electrode was immersed in 100 μL of 0.3 μM capture probe for 6 h, allowing the capture probe to be attached to the surface of Au electrode via Au-S bond. Then the capture probe-modified electrode was washed with 10 mM PBS buffer, dried with nitrogen, and then immersed in 200 μL of 2 mM 6-MCH for 4 h to block the nonspecific adsorption sites on electrode surface. Finally, the electrode was thoroughly washed with 10 mM PBS buffer and dried with nitrogen.

2.3. Fabrication of the electrochemical DNA biosensor

Samples with different concentration of HIV DNA were mixed with 0.8 μM assistant probe in 100 μL hybridization solution, which were heated to 95 °C for 5 min, and cooled down to room temperature. The capture probe-modified Au electrode was then immersed into the hybridization solution and incubated for 2 h to allow the complete hybridization among the complementary sequences of the capture probe, the target DNA and the assistant probe. After hybridization, the electrode was immersed into 200 μL G-quadruplex-forming solution (10 mM HEPES containing 50 mM KCl, pH 8.0) for 30 min to allow the formation of the spatial structure of G-quadruplex. After that, 2 μL of 20 mM hemin (dissolved in DMSO) was added into the G-quadruplex-forming solution and incubation for additional 1 h to form G-quadruplex-hemin complex.

The electrochemical measurements were conducted on a CHI 660e electrochemical workstation. Differential pulse voltammetry (DPV) was conducted in 20 mM HEPES buffer containing 20 mM KCl (pH 8.0) in the potential range from −0.6 to −0.15 V.

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