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Short communication

Coupling DNA with enzyme activity: A complex electrochemical sensor with enhanced specificity

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ABSTRACT

We report here an electrochemical sensor which couples the detection of target DNAs with enzyme activity. A hairpin probe DNA complementary to both target DNAs was dually labeled with 5′-thiol and 3′-methylene blue (MB), and covalently immobilized to gold electrode. In the presence of both target DNAs, the hairpin structure shifted from "close" state to "open" state with the help of *Escherichia coli* (*E. coli*) DNA ligase, which can be characterized by alternating current voltammetry (ACV). For target DNA 1 and target DNA 2, the detection limits were 1 nM and 0.25 nM, respectively. Furthermore, the assay was coupled with the activity of lactate dehydrogenase (LDH), which catalyzes the conversion of NADH to NAD^+. The latter acted as the co-substrate of DNA ligase and produced current change in the presence of both target DNAs. The sensor can analyze the co-existence of multi-components, which efficiently improves the accuracy in the applications such as species identification.

1. Introduction

The sequence-specific DNA detection on the basis of hybridization with the complementary DNA probe has attracted wide interests and shown significant applications in the field of microbial identification, food quality control, disease diagnosis, environmental monitoring and drug analyses [1–3]. Among them, molecular beacon-based sensors are one of the most popular types [4,5]. Though those sensors are mainly based on fluorescent analysis, electrochemical DNA sensors showed advantages such as simple, rapid, environment-insensitive and low-cost [6–10].

Recently, the task of multi-component analysis emerged gradually to achieve high-accurate determination [11,12]. For example, to improve the accuracy of identification of microbial species or diagnosis of diseases, several biomarkers should be detected simultaneously. Enzyme activity analysis is widely employed in such areas besides DNA detection. LDH distributes widely in various organisms. The level of LDH was suggested to be species-specific or pathophysiology-dependent [13]. Therefore, it became a biomarker for disease diagnosis or species identification. Traditionally, a series of parallel single-component analyses followed with comprehensively evaluation are required to make a reliable conclusion. Taking advantages of rapid, high-efficient and high-throughput, multi-component simultaneous analysis is an ideal alternative in such tasks [14–16]. Among them, the electrochemical technique exhibits a broad prospect because of its simple operation, high sensitivity and the microchip-compatibility [17,18].

Herein, we report an electrochemical approach for the detection of dual-target DNA by employing methylene blue (MB)-labeled molecular probe complementary to both target DNAs. As shown in Scheme 1, the probe was dually modified with 5′-SH and 3′-MB and self-assembled on gold electrode by means of facile Au–S chemistry. In the original state, the probe forms a "close" hairpin structure, which forces MB group to approach the electrode surface and exchange electrons freely with the electrode. In the presence of both targets (target DNA 1 and target DNA 2), the hybridization takes place between the targets and the probe. However, the probe still maintains its hairpin structure until *E. coli* DNA ligase is introduced, which catalyzes the ligation of target DNAs and then breaks the hairpin. MB group is thus far away from the electrode due to the less flexibility of the complete double helix. Thus the electron transfer is blocked [19,20]. The detection of target DNAs is therefore achieved. Furthermore, *E. coli* DNA ligase is known as an NAD^+-dependent enzyme, which catalyzes the formation of phosphodiester bonds between 3′-hydroxyl and 5′-phosphoryl termini within complementary dsDNA along with the hydrolysis of NAD^+. The mechanism is utilized to design a complex assay which couples the detection of target DNAs with LDH activity. In the presence of LDH, NADH is converted to NAD^+, which in turn accelerates the ligation of target DNAs and produces the change of electrochemical signal.

2. Experimental

2.1. Chemicals

NAD^+ and NADH were purchased from Sangon Biotech Co., Ltd. L-lactate dehydrogenase (LDH) and 6-mercaptohexanol (MCH) were

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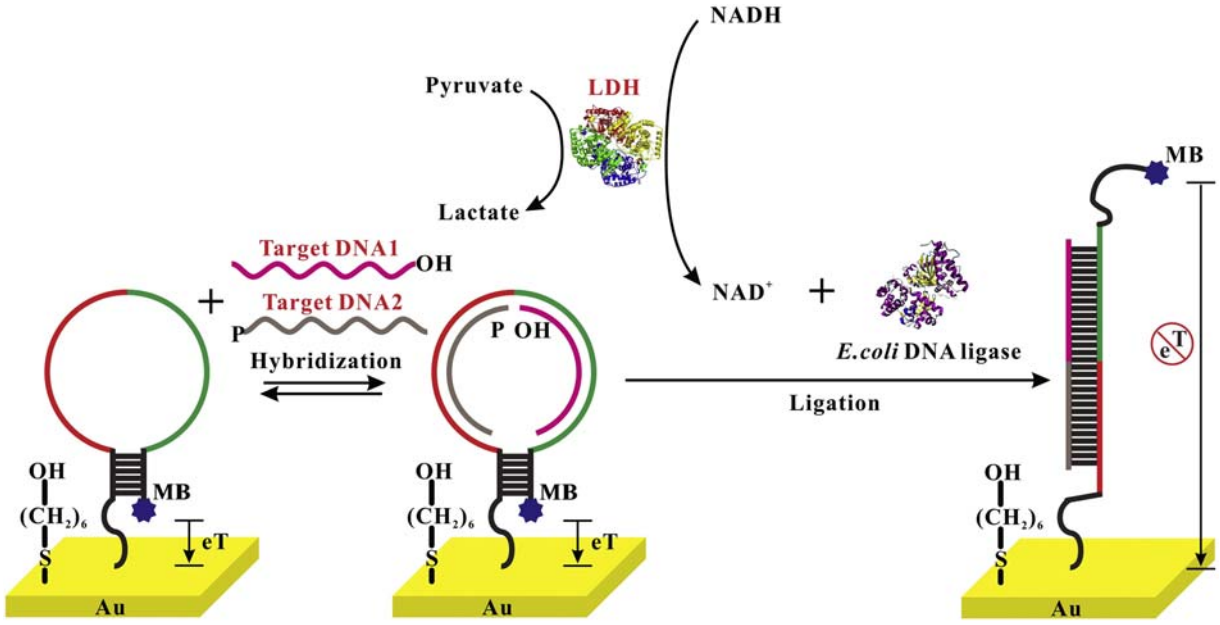

Scheme 1. Schematic illustration of simultaneous detection of dual-target DNA and LDH.

from Sigma-Aldrich. *E. coli* DNA ligase was purchased from Tarkara Biotechnology Co., Ltd. Other chemicals were of analytical grade. All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. and listed as below. The underlined sequence in the probe indicates the stem sequence. Letter "p" represents phosphate group and the italic letters indicate the mismatched bases.

Probe DNA: 5′-HS-$(CH_2)_6$-CCTCTTGGGCGATTTCGGGGGTTATGTATA ATGGA AGAGG-MB-3′

Target DNA 1 (T1): 5′-TATACATAACC-3′

Target DNA 2 (T2): 5′-p-CCCGAAATC-3′

Mismatched DNA sequences:

M11: 5′-TATAC*T*TAACC-3′
M12: 5′-TATACCTAACC-3′
M13: 5′-TATAC*G*TAACC-3′
M14: 5′-TATA*GT*AAACC-3′
M21: 5′-p-CCCG*T*AATC-3′
M22: 5′-p-CCCGCAATC-3′
M23: 5′-p-CCCGGAATC-3′
M24: 5′-p-CCCC*TT*ATC-3′

2.2. Preparation of the modified gold electrode

Gold electrode (2 mm diameter) was polished with 0.3 and 0.05 μm alumina powder, and ultrasonicated in ethanol and water for 5 min respectively. Then it was electrochemical activated in 0.5 M H_2SO_4, washed with ultrapure water and dried under nitrogen stream. 50 μL of 0.5 μM probe DNA in 10 mM PBS buffer (pH7.0) containing 1 M NaCl and 1 mM $MgCl_2$ was dropped onto the electrode and kept for 3 h at room temperature. After incubation, physically adsorbed probe DNA was removed via ultrapure water rinse. Dried in nitrogen, the probe modified electrode was immediately incubated in 2 mM MCH for 4 h to block the uncovered surface. The electrode was ready for use after rinsing and drying.

2.3. Detection of dual-target DNA

50 μL DNA hybridization solution (1 mM PBS containing 1 mM NaCl and 30 mM $MgCl_2$, pH7.0) containing different concentrations of target DNAs was applied onto the modified electrode. The hybridization between target DNAs and the probe was conducted for 30 min. Then the electrode was rinsed with 1 mM PBS to remove non-hybridized DNA. Finally, 20 μL solution containing 30 U *E.coli* DNA ligase and 0.1 mM NAD^+ was dropped onto the electrode and incubated at 40 °C for 2 h. Rinsed with PBS, the electrochemical detection was performed.

A conventional three-electrode cell was employed, involving a modified gold working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. The alternating current voltammetry (ACV) measurement was conducted in nitrogen-purged PBS containing 0.1 M NaCl using a CHI660c workstation with a step potential of 4 mV, an amplitude of 25 mV and a frequency of 100 Hz.

To verify the specificity of the assay, single-base and three-base mismatched oligonucleotides of target DNAs were hybridized with the modified electrode and the measurements were carried out as above.

2.4. Multi-component analysis for dual-target DNA and LDH

150 μL overall reaction system in PBS (pH7.5) contained 166.7 U mL^{-1} LDH, 1 mM pyruvate, 1 mM NADH, 1 μM target DNA 1 and 5 μM target DNA 2. The solution was incubated at 37 °C for 10 min. Then LDH was inactivated at 80 °C for 5 min. After addition of 30 U *E. coli* DNA ligase, the solution was applied onto the probe DNA modified electrode. After 2 h, the electrode was rinsed with 1 mM PBS and ACV was conducted.

3. Results and discussion

The basis of simultaneous detection of dual-target DNA is that the hairpin structure of the probe can only be broken in the presence of both targets with the help of DNA ligase, and therefore the electron transfer status between MB and the electrode can be changed. The feasibility of the electrochemical assay was first verified. As shown in Fig. 1A, no peak can be observed in ACV curve of bare gold electrode. However, the modified electrode exhibits an extremely high current at −0.28 V (vs. Ag/AgCl), which can be attributed to the electron transfer between MB and the electrode. The formation of the hairpin structure shortens the distance between MB and the electrode, and thus enables the interaction. Interestingly, after incubation with 0.5 μM target DNA 1 and 5 μM target DNA 2, the peak current shows negligible change, which means that the hybridization of two short target sequences with the probe is insufficient to break the stem portion of the hairpin. The phenomenon is in