Biosensors and Bioelectronics 30 (2011) 241–248

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Ultrasensitive DNA detection by cycle isothermal amplification based on nicking endonuclease and its application to logic gates

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ARTICLE INFO

Article history:
Received 3 August 2011
Received in revised form
15 September 2011
Accepted 16 September 2011
Available online 22 September 2011

Keywords:
DNA
Cycle isothermal amplification
Logic gates
Nicking endonuclease
Sensor

ABSTRACT

In recent years, an intense interest has grown in the DNA logic gates having high potential for computation at literally the "nano-size" level. A limitation of traditional DNA logic gates is that each target strand hybridizes with only a single copy of the probe. This 1:1 hybridization radio limits the gain of the approach and thus its sensitivity. The exponential amplification of nucleic acids has become a core technology in medical diagnostics and has been widely used for the construction of DNA sensor, DNA nanomachine and DNA sequencing. It would be of great interest to develop DNA-based logic systems with exponential amplification for the output signal. In the present study, a series of three-input DNA logic gates with the cycle isothermal amplification based on nicking endonuclease (NEase) are designed. Very low concentrations of the analytes were sufficient to initiate an autocatalytic cascade, achieving a significant improvement of the detection limit, 100-fold improvement compared to the non-autocatalytic system. This was achieved by engineering a simple and flexible biological circuit designed to initiate a cascade of events to detect and amplify a specific DNA sequence. This procedure has the potential to greatly simplify the logic operation because amplification can be performed in "one-pot".

1. Introduction

In recent years, an intense interest has grown in the logic gates made of nano-molecules having high potential for computation at literally the "nano-size" level (Pischel, 2007). In particular, nucleic acids (DNA and RNA) have proven to be highly useful building blocks for the construction of molecular logic gates and computational devices due to their characteristic ability to hybridize with their sequence-specific recognition properties (Seelig et al., 2006; Frezza et al., 2007; Miyoshi et al., 2006; Stojanovic et al., 2002; Penchovsky and Breaker, 2005). Nucleic acid-based logic devices operate using three different approaches: nonenzymatic Watson–Crick type interaction (strand displacement hybridization) (Seelig et al., 2006; Frezza et al., 2007), recognize corresponding ligands (Ogawa and Maeda, 2009), and catalyze specific chemical reactions (deoxyribozymes or ribozymes) (Chen et al., 2006; Lederman et al., 2006), and interaction of nucleic acids with metal ions (Park et al., 2010; Bi et al., 2010b).

Since the output of any gate can serve as the input to another, gates can be electrically wired together to form multi-level circuits that produce the desired functional and information processing characteristics. A critical feature that contributes to the success of modern electronic circuits is input–output signal homogeneity (Lake et al., 2010). Substantial efforts have been undertaken to design chemical logic gates that can be combined to construct large, reliable circuits (Frezza et al., 2007). In the previous report, a series of DNA logic gates based on supermolecular DNAzymes as functional components have been constructed (Bi et al., 2010a). A limitation of traditional DNA logic gates is that each target strand hybridizes with only a single copy of the probe. This 1:1 hybridization radio limits the gain of the approach and thus its sensitivity.

The sensitive and selective detection of nucleic acids is important in biological studies, clinical diagnostics since they are routinely used as biomarkers to help diagnose pathogenic infections and genetic disorders. Specific nucleic acids indicating the presence of a disease are often found in only trace amounts in a complex biological extract, it is necessary to develop amplification techniques that enable the detection of trace levels of a specific sequence. The amplified detection of DNA has spurred substantial research efforts and the existing DNA-amplification techniques can be summarized as: polymerase chain reaction (PCR) (Saiki et al., 1988; Weissenborn et al., 2010), ligase chain reaction (LCR) (Barany, 1991; Du et al., 2005), rolling circle amplification (RCA) (Zhao et al., 2008), strand-displacement amplification (SDA) (Connolly and Trau, 2010; Ding et al., 2010), hybridization chain reaction (HCR) (Huang et al., 2011; Niu et al., 2010).

The exponential amplification of nucleic acids has become a core technology in medical diagnostics and has been widely used for the construction of DNA sensor, DNA nanomachine and DNA

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doi:10.1016/j.bios.2011.09.019

sequencing. Winfree et al. reported enzyme-free nucleic acid logic circuits with hybridization-based system as input amplifier for signal restoration and amplification (Seelig et al., 2006). To the best of our knowledge, there are few reports on the combination of the signal amplification and DNA logic gate (Elbaz et al., 2010). It would be of great interest to develop DNA-based logic systems with exponential amplification for the output signal. In the present study, a series of three-input DNA logic gates with the cycle isothermal amplification based on nicking endonuclease (NEase) are designed.

2. Experimental

2.1. Apparatus

The electrochemiluminescence (ECL) measurement was performed with a Model MPI-A Electrochemiluminescence Analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature. A commercial cylindroid glass cell was used as an ECL cell. A three-electrode system composed of a disk gold electrode (diameter 2.0 mm) as working electrode, a platinum plate as counter electrode, and an Ag/AgCl (saturated KCl) as reference electrode. The spectral width of the photomultiplier tube (PMT) was 200–800 nm and the voltage of the PMT was −400 V to −800 V in the detection process. Non-denaturing polyacrylamide gel electrophoresis was performed on DYCZ-28C electrophoresis power supply equipped with WD-9413A gel documentation & analysis systems from Beijing Liuyi Instrument Factory (Beijing, China). Fluorescence emission spectra were recorded on a FL-4600 FL spectrophotometer (Tokyo, Japan) equipped with 1 cm quartz cells. The excitation was made at 450 nm with recording emission range of 550–700 nm. All excitation and emission slits were set at 5 nm. The CL measurements were performed by using a BPCL ultraweak luminescence analyzer (Institute of Biophysics Academic Sinica, Beijing, China) with the sample cell of a 2-mL quartz cuvette.

2.2. Chemicals

All oligonucletides used in the present study were purchased from SBS Genetech Co., Ltd. (Beijing, China), and their sequences were provided in Supplementary Table S1. The nucleic acid with one adenine ribonucleotide (rA) was obtained from Takara Biotechnology (Dalian) Co., Ltd. (Dalian, China). 2.5 U Nb.BbvCI nicking endonuclease (NEase) and polymerase Klenow fragment exo− were purchased from Sigma (U.S.A.) and were used without any further purification. The deoxynucleotide solution mixture (dNTPs) was obtained from Takara Biotechnology (Dalian) Co., Ltd. (Dalian, China). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide solution (EDC), N-hydroxysuccinimide (NHS), 2,2′-bipyridyl-4,4′-dicarboxylic acid (dcbpy), N,N′-dicyclohexyl carbodiimide (DCC), N,N′-dimethylformamide (DMF), 6-mercapto-1-hexanol (MCH), and sodium hexafluorophosphate were purchased from Sigma (U.S.A.). Dithiothreitol (DTT), tripropylamine (TPA), 2,2′-bipyridine, $NaPF_6$, and mercaptohexanol were obtained from First Reagent Corporation of Shanghai (China). Other chemicals employed were all of analytical grade and double distilled water was used throughout the experiments.

2.3. Construction of the logic gates

Logic gates are formed in a course of slow annealing. A stoichiometric amounts of corresponding sequences (**G1**, **G2** for ECL gate, **G3**, **G4** for CL gate, **G5**, **G6** for fluorescence gate, 1.0×10^{-8} M for each) are mixed in phosphate buffer solution (pH 7.4, 0.1 M) and reacted for 5 min at 90 °C and cooled to 25 °C at which the solution was held for 40 min.

2.4. Non-denaturing polyacrylamide gel electrophoresis

The DNA samples with 0.2 μM concentration were loaded onto gels contained 30% polyacrylamide (acrylamide/N,N′-methylenebisacrylamide, 29:1) in 50× TAE buffer (Tris–Acetate–EDTA, pH 8.5) followed by electrophoresis separation at 150 V for 1 h at 25 °C. After staining the gel with ethidium bromide (EB), a photograph was taken with WD-9413A gel documentation and analysis systems.

2.5. Experimental operation for ECL logic gate

2.5.1. Synthesis of ECL probe

The ECL probe, bis(2,2′-bipyridine) (2,2′-bipyridine-4,4′-dicarboxylic acid) N-hydroxysuccinimide-DNA ($Ru(bpy)_2$(dcbpy)-NHS-DNA), was synthesized according to our previously reported reference (Zhang et al., 2008). Briefly, 100 μL of 5.8×10^{-4} M $Ru(bpy)_2$(dcbpy)NHS and 5 μL of 0.1 M tetraborate buffer (pH 8.5) was added to 5 μmol **P1**, shaking at low speed over 8 h at room temperature. Then, by addition of 100 μL of 3 M NaAc and 2 mL of ethanol to the mixture, the precipitate reaction was carried out in refrigerator at −20 °C over 12 h. The mixture was centrifuged at 12,000 r/min for 30 min, and the precipitate was rinsed with cold 70% ethanol twice and dried. The precipitate was re-dissolved in 200 μL of 1.0 M phosphate buffer saline (PBS, pH 7.0, 0.1 M NaCl + 1.0 M KH_2PO_4/K_2HPO_4) and stored at −16 °C for use.

2.5.2. ECL probe DNA self-assembly at gold electrode

The gold electrode (diameter 2.0 mm) was polished with a 0.05 μm alumina powder and soaked in an ultrasonic bath successively with distilled water, absolute alcohol, and distilled water for 5 min each. Then, the gold electrode was dipped in piranha solution (H_2SO_4/H_2O_2, 7:3, v/v) for 5 min at 90 °C and electrochemically treated by cycling the potential between 0.1 and 1.5 V in 0.1 M H_2SO_4 until a stable gold oxide cyclic voltammogram was obtained. The pretreated Au electrode was immersed into a immobilization buffer (IB: 20 mM Tris–HCl, 0.1 M NaCl, 5 mM $MgCl_2$ at pH 7.4) containing 1.0×10^{-5} M RuDNA probes for 4 h at 100% humidity. The resulting electrodes were thoroughly rinsed with 10 mM PBS to remove the unbound ECL probe on the surface of the electrode, and further treated with 1 mM MCH for 30 min to obtain well-aligned DNA monolayers and to block the uncovered surface of the electrode.

2.5.3. Cycle operation of the logic gate and ECL detection

The operation of the logic gate and the cycle signal amplification were performed in "one-pot". The formed gate and different concentrations of input DNA strands (**I1**, **I2**, **I3**), as well as dNTPs in NEB buffer solution (NaCl (50 mM), (hydroxymethyl)aminomethane hydrochloride (Tris–HCl; 10 mM), $MgCl_2$ (20 mM), DTT (1 mM); pH 7.9), the N.BbvC IA endonuclease, and polymerase Klenow fragment exo− were introduced to the solution of ECL probe immobilized on the Au electrode. Hybridization and scission reaction were performed at 37 °C for 90 min. The ECL measurement was performed before and after the introduction of the logic units at a constant potential of 800 V in 2.0 mL of 0.10 M PBS containing 25 mM TPA.

For control, the ECL measurement was also performed without the cycle amplification.

2.5.4. Fluorescence experiments

Fluorescence measurement was made using **P1** modified with FAM and BHQ1 at its 5′ and 3′ ends, respectively. Fluorescence