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A novel room temperature nucleic acid detection method based on immobilization of adenosine-based molecular beacon

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ABSTRACT

We developed a novel room temperature fluorescence DNA biosensor based on immobilization of well designed adenosine-based molecular beacon (ABMB). In the presence of coralyne, a small molecule which can react with adenosines, the ABMB would form a hairpin structure just like traditional molecular beacon used extensively. In our experiments, two kinds of adenosine-based molecular beacon probes with twelve adenosines bases at each end (A_{12}-MB1-A_{12} and A_{12}-MB2-A_{12}), and their complementary target DNA, non-complementary target DNA and single base mismatched DNAs were used as models to demonstrate the proof-of-concept. Under optimized conditions, the detection limit was estimated to be 50 pM based on $S/N = 3$. The ability of our developed biosensor for discriminating the single base mismatched DNA related to a human genetic disease, hereditary tyrosinemia type I, was improved undoubtedly once A_{12}-MB2-A_{12} was used as capture probe comparing with previous report. It is worth to mention that the whole assay procedures were conducted under room temperature.

1. Introduction

DNA hybridization has been extensively studied based on current biosensing technologies such as surface plasmon resonance (SPR) [1], DNA microarrays [2], X-ray photoelectron spectroscopy (XPS) [3], quartz crystal microbalance (QCM) [4], and microcantilever biosensors [5–9], for genome research, gene expression, disease diagnosis and applied drug discovery and synthesis. All of these techniques depend on a surface that is modified with single strand DNA (ssDNA) monolayer with the ability of interacting with complementary target DNA in solution, giving rise to a physical response owing to the hybridization between target and surface-bound ssDNA.

Tyagi and Kramer [10] first reported the fluorescent molecular beacon (MB) in 1996 as nucleic acid probe, being able to initiate conformational change spontaneously due to the hybridization with complementary nucleic acid target. Conventional molecular beacon has a fluorophore at 5′ end and a quencher at 3′ end, with 85–97% quenching efficiency. In order to tackle this shortage, Yang and co-workers designed a super-quenchers MB with a three-quencher assembly (SQ-MB) as quencher, with the quenching efficiency increased to 99.7% [11]. However, Lukhtanov, developed a novel probe, in comparison to common MB, which had a fluorophore and molecular groove beacon (MGB) at 5′ end. When there is no target, MGB can also quench fluorophore like a quencher [12]. Afterwards, researchers discovered some metal ions can be used as efficient quenchers. For example, Brunner synthesized copper ion compounds as quencher, with higher fluorescence intensity (15 times) than traditional one [13]. In recent years, nanomaterials including gold nanoparticle and carbon nanotube were employed as substrates to immobilize molecular beacons. Duberttre made use of high quenching efficient gold nanoparticles to design a nano-beacon above 90% quenching efficiency, for the real-time detection of nucleic acid and single nucleotide polymorphism detection [14]. Bockisch et al. developed a novel DNA stem–loop structured probe for enzymatic detection of nucleic acid target based on the molecular beacon immobilized on the surface of microplate and electrode [15,16]. This method also can be used in electrochemical fields. To our best knowledgements, there are some researchers have tried to study the interactions between Thymine-Hg-Thymine [17,18], Uricil-Ag-Uricil [19] in detail, and applied these reactions for detection of mercury ions and silver ions, since then, such protocols were employed in detection of glutathione, cysteine [20] and DNA. The study of interaction between adenosine and coralyne was first reported by Ren and Chaires [21]. Lin and Tseng utilized coralyne, a special molecule able to interact with adenosine, to design a novel molecular beacon. In the report, the fluorescence method was used to realize quantitative detection of target DNA molecules

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Table 1
DNA sequences of ABMB and tested DNA.

Name	Sequence(5′ to 3′)
A_{16}-MB1-A_{16}	Dig-A_{16}GTCCGTGGTAGGGCAGGTTGGGGTGACTA_{16}-Biotin
A_{12}-MB1-A_{12}	Dig-A_{12}GTCCGTGGTAGGGCAGGTTGGGGTGACTA_{12}-Biotin
A_6-MB1-A_6	Dig-A_6GTCCGTGGTAGGGCAGGTTGGGGTGACTA_6-Biotin
DNA_1	AGTCACCCCAACCTGCCCTACCACGGACTT
DNA_2	TCAGTGGGGTTGGACGGGATGGTGCCTGAA
A_{12}-MB2-A_{12}	Dig-A_{12}CCAGATACTCACCGGA$_{12}$-Biotin
DNA_3	CCGGTGAGTATCTGG
DNA_4	CCGGTGAATATCTGG
DNA_5	CCGGTGATTATCTGG
DNA_6	CCGGTGACTATCTGG

successfully [22]. However, results for discrimination of single base mismatched target DNA were not satisfied. According to some previous studies, molecular beacon has the ability to discriminate single base mismatched target DNA sequences [23–27].

In our design, we immobilized the ABMB on microplate well through streptavidin and biotin bindings, combining with optimization of the number of adenosine bases on each end of the ABMB, the performance of the biosensor was comparable with the reported DNA biosensor [22] based on homogeneous reaction and improved its ability for discrimination of single base mismatched DNA. A hairpin structure capture probe just like typical molecular beacon used extensively would form once ABMB reacted with a small molecule, coralyne. Upon the addition of target DNA, the conformation of hairpin structure capture probe will be changed to release the digoxin at 5′ end. Following the specific interaction between the conformation-triggered digoxin and monoclonal anti-digoxin-ALP antibody, subsequent quantification of DNA is realized by fluorescence detection of enzymatic products in the presence of substrate. A_{12}-MB2-A_{12} has ability to recognize the fumarylacetoacetate hydrolase gene mutation for human genetic disease, hereditary tyrosinemia type I [28]. It was worth mentioned that the whole assay was conducted at room temperature in less than 1.5 h, which greatly simplify the assay procedures.

2. Experimental

2.1. Materials and reagents

Albumin bovine serum (BSA) was supplied by Wolsen. Streptavidin from Streptomyces Avidin and 4-methylumbelliferyl phosphate disodium salt (Substrate) were purchased from Sigma-Aldrich (Shanghai, China). Monoclonal Anti-Digoxin-Alkaline Phosphatase antibody (2.0 mg/ml) produced in mouse was purchased from Sigma-Aldrich (American). DNA sequences (see Table 1) were supplied by Sangon Biotech (Shanghai, China). Mouse serum was purchased from Dingguo Biological Products (Beijing, China). The buffer of 0.05 M Na_2CO_3–$NaHCO_3$ (pH 9.6) and 0.1 M Tris–HCl (pH 9.0) were used as incubating buffer. 0.1 M HEPES (pH 7.0, 0.2 M NaCl) was used as incubating and washing buffer. All other chemicals were of analytical reagent grade. All buffer solutions were prepared using ultrapure water.

2.2. Apparatus

Fluorescence intensity was recorded at a RF-5301PC fluorophotometer (Shimadzu Corporation, Kyoto, Japan) with 1.5 nm excitation slit width and 1.5 nm emission slit width. Basic pH meter was purchased from Sartorius (Beijing, China). Sterilization kettle was purchased from Shanghai Boxun Industry & Commerce Co. Ltd. (Shanghai, China). Thermostat water bath and high pure water distiller were purchased from Changzhou Guohua electric appliance Co. Ltd. (Jiangsu, China).

2.3. Immobilization of streptavidin in microplate well

Before the experiment, microplate wells should be rinsed thoroughly by 70% ethanol and ultrapure water. To each well 40 μl of a 200 nM streptavidin in Na_2CO_3–$NaHCO_3$ buffer (0.05 M, pH 9.6) was added to incubate overnight at 4 °C. For blocking the active sites of the microplate wells, 60 μl of 2% BSA (dispersed in mouse serum) was pipetted to each microplate well to incubate for 1.5 h at 37 °C in a humidified chamber. Wells were washed four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl) prior to use.

2.4. Pre-treatment of adenosine-based molecular beacon and its immobilization

Biotin-modified hairpin structures (A_{12}-MB1-A_{12}, or A_{16}-MB1-A_{16}, or A_6-MB1-A_6 probes) were immobilized on microplate wells precoated with streptavidin. It is worthy to mention that 4 μl ABMB probes used should be treated subsequently at 95 °C and ice-cold water bath for 10 and 30 min, respectively before adding to each microplate well, followed by adding 10 μl of 2 mM coralyne and 986 μl of 0.1 M HEPES (pH 7.0, 0.2 M NaCl). To each well, 40 μl such solution was added to incubate for 30 min. Wells were washed four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl) prior to use.

2.5. Assay procedure

Hybridization reaction between the immobilized ABMB probes and DNA targets was performed by dropping 40 μl of their full complement (DNA_1), or non-complement (DNA_2), or 0.1 M HEPES (pH 7.0, 0.2 M NaCl) in each microplate well. The hybridization procedure was conducted at 25 °C for 30 min. The digoxin groups labeled on ABMB were then triggered while hybridization occurred. After washing four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl), 40 μl of the monoclonal anti-digoxin-ALP conjugate diluted in 1:7000 in 0.1 M HEPES (pH 7.0, 0.2 M NaCl) was added, following incubation for 30 min, wells were washed four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl) and 100 μl solution of 125 μM 4-MUP (0.1 M Tris–HCl, pH 9.0) was added to each well to incubate for 8 min in dark. Emission of each well at 450 nm was measured by fluorophotometer.

2.6. Detection of complementary target DNA in human serum

To test complementary target DNA in human serum, 40 μl sample solution of 1 nM complementary target DNA (DNA_1) spiked in 100% human serum, 50% human serum, and 0.1 M HEPES (pH 7.0, 0.2 M NaCl) were added to each well during the hybridization procedure. The hybridization procedure were conducted at 25 °C for 30 min. Wells were washed four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl) before conducting following experiment. Other steps were followed by Section 2.5.

2.7. The discrimination of single base mismatched DNA

For discrimination of single base mismatched DNA sequences, a well designed capture probe, called A_{12}-MB2-A_{12} was used. All procedures were similar with above mentioned protocols except the hybridization step. During hybridization step, a 40 μl sample solution containing complementary target DNA (DNA_3), non-complementary target DNA (DNA_2) and single base mismatched target DNA sequences (DNA_4, DNA_5 and DNA_6) were added to each well to incubate for 15 min. Wells were washed four times with 0.1 M HEPES (pH 7.0, 0.2 M NaCl) prior to use. Other steps were followed by Section 2.5.