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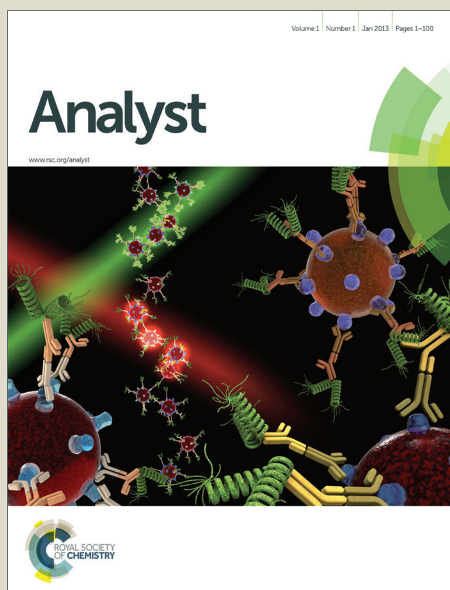

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Portable and Sensitive Quantitative Detection of DNA based on Personal Glucose Meters and Exonuclease III-Assisted Signal Amplification

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Abstract

A portable and sensitive quantitative DNA detection method based on personal glucose meters and Exonuclease III-assisted signal amplification was developed. In the presence of target DNA, a target recycling process which can release target DNA and linker DNA was obtained. The released linker DNA was used to link capture DNA on MBs and the DNA invertase. After the washing away of unbound target DNA and DNA-invertase conjugation, the bound DNA-invertase can be used to catalyze the hydrolysis of sucrose into glucose with millions of turnovers, which transformed the concentration of target DNA into the level of glucose for monitoring of PGM. There was a liner relationship between the signal of PGM and the concentration of target DNA in the range of 0.5 pM to 100 pM. A correlation coefficient of 0.989 was obtained and the relative standard deviation (RSD) was 4.1% for a concentration of 50 pM target DNA (n = 9). In addition, the method exhibited excellent sequence selectivity, being able to differentiate a single mismatch in the target DNA. What is more, there was almost none effect of biological complex to the detection performance, which suggested our method can be successfully applied to DNA detection in real biological samples.

Introduction

Simple, fast and highly sensitive detection of specific DNA sequences has becoming increasing important in the field of drug development[1], clinical diagnosis[2] and environmental science[3]. Many techniques and devices, such as, fluorescence[4], colorimetry[5], electrochemistry[6], surface enhanced Raman scattering (SERS)[7] together with other methods including surface plasmon[8], light scattering and force[9], have been developed to sensitive and selective detection of DNA. By employing those method, a highly selectivity and a low detection of limit can be obtained. However, most of the equipment used in above methods are expensive and require sophisticated instrument and operations, which are laboratory-based instrument and not easily available to the public. Some of the fluorescent or colorimetric methods can detection DNA through naked eyes without any instrument. However, only qualitative or semi-quantitative detections can be conducted and the color observation may be affected by many

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conditions, such as temperature, humidity, the laboratory technicians and light contrast of the surroundings. In order to obtain portable and quantitative DNA detection methods, expensive or customized portable spectrometers are stilled needed. Therefore, it is still a great challenge to develop portable and quantitative DNA detection method which is easily available to the public and with accessible resources at home.

Due to its low cost, simple operation and portability, personal glucose meter (PGM) has attracted worldwide attention[10]. It is available in stores with low price (as low as $10 for a meter), which has been integrated into cell phones for point-of-use. PGM is mainly used to monitor the glucose concentration in diabetic patients[11]. In 2011, Yi Lu linked PGM with functional DNA sensors to achieve portable, low-cost and quantitative detection of targets beyond glucose[12]. By introducing DNA-invertase conjugates to a DNA sandwich assay, DNA can be portably and quantitatively detected. However, A detection limit of about 40 pM was obtained, which was relative higher for ultralow detection of cancer related DNA molecules[13].

In order to improve the sensitive of DNA detection method, target amplification is widely used, especially for enzyme-assisted amplification[14]. Among them polymerase chain reaction (PCR) is the most widely used one, as its high sequence specificity and amplification yield[15]. However, in the procedure of PCR, small contaminants can also be amplified, which leads to false-positives and the requirement for thermal cycling and experimental expertise in PCR technique have ultimately restricted its widespread application[16]. Alternatively, a number of new methods of target amplification have been proposed. For example, nicking endonuclease signal amplification (NESA) is an isothermal amplification method, which can generates thousands of nicked probes by using only one target[17]. However, the target DNA used for amplification must possess certain specific sequences for recognition, which has limited its wider application. In order to widen the application of enzyme-assisted amplification, Exonuclease III (Exo III)-based target recycling has been developed. Exo III can remove the mononucleotides of duplex DNA from its 3'-terminus, which is not affected by the sequences.[18]

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In this paper, we developed a portable and sensitive DNA quantification method based on personal glucose meters and exonuclease III-assisted signal amplification. In the presence of target DNA, a target recycling process which can release target DNA and linker DNA was obtained. The released linker DNA was used to link capture DNA on MBs and the DNA invertase. After the washing away of unbound target DNA and DNA-invertase conjugation, the bound DNA-invertase can be used to catalyze the hydrolysis of sucrose into glucose with millions of turnovers, which transformed the concentration of target DNA into the level of glucose for monitoring of PGM.

Experimental section

Chemicals

All oligonucleotides were synthesized and purified by Sangon (Shanghai, China) and the sequences were showed in table 1. All DNA oligonucleotides were diluted by TE buffer (10 mM Tris-HCl and 1.0 mM Na_2EDTA, pH 8.0). They were denatured at 95 ℃ for 5 min naturally cooled down to room temperature before use. Exo III was also purchased from Sangon (Shanghai, China). Streptavidin-MNBs (350 nm in diameter, the aqueous suspension containing 0.05% Tween-20, 0.1% bovine serum albumin (BSA) and 10 μM EDTA at a concentration of 3.324×10^{11}beads mL^{-1}) were obtained from Bangs Laboratories Inc. (Fishers, IN). Grade VII invertase was obtained from baker's yeast (S. cerevisiae), Tween-20, sulfosuccinimidyl- 4-(N-maleimidomethyl) cyclohexane - 1 - carboxylate (sulfo-SMCC), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and bovine serum albumin (BSA) were purchase from Sigma (St. Louis, MO). Other chemicals were in analytical grade obtained from standard reagent suppliers and used directly. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from a Millipore system.

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Table 1 Oligonucleotides Designed in the Present Study.

ID	Sequence 5'to3'
Linker DNA	CTCTTCGAGGGTTTTGGGTTTTGGGTTTTGGGAGCTA
Template	AAAACCCAAAACCCAAAACCCGCGACGAGTCACAACAG
Target	CTGTTGTGACTCGTCGCAATAAC
1 base mismatch	CTGTTGTGACTC**C**TCGCAATAAC
3 bases mismatch	CTGTTGTGACTC**CAG**GCAATAAC
Capture DNA	CCCAAAACCCTCGAAGAGAAAAAAAAAAAA-Biotin
Detection DNA	HS-AAAAAAAAAAAATAGCTCCCAAAACCCAAAA

DNA-invertase conjugation

Firstly, a sum of 30 μL of detection DNA (1 mM), 2 μL of of sodium phosphate buffer (1 M, pH 5.5) and 2 μL of TCEP solution (30 mM) were mixed, which was then incubated for 1 h at room temperature. The mixture was purified by Amicon-10K for 10 times using buffer A (0.1 M NaCl, 0.05% Tween-20, 0.1 M sodium phosphate buffer, pH 7.3). Then, in order to conduct invertase conjugation, 1 mg of sulfo-SMCC was added into 400 μL of invertase solution (dissolved in buffer A), which was incubated for 1 h on a roller. The obtained mixture was purified through centrifugation and Amicon-100K using Buffer A by 10 times. Finally, the obtained sulfo-SMCC-activated invertase was mixed with the detection DNA, which was incubated for 48 h at room temperature. The mixture was purified by Amicon-100K for 10 times using Buffer A.

Exonuclease III-aided recycling process

Equal amount of Linker DNA (0.5 μM) and Template (0.5 μM) were mixed and allowed to incubate for 2 h under 37℃. Then, different concentration of target DNA were added and allowed to incubate for 0.5 h under 37℃. Finally, 0.25 μL (200 U/μL) ExoIII and 10 μL 10× reaction buffer were added. The recycling amplification process was performed for 2 h under 37℃. The mixture was then heated to 65℃ and cooled to room temperature.

Measurement of PGM signal

Firstly, 5 μL of Streptavidin-MNBs was washed three times by using buffer A to remove the surfactants. Then, 50 μL of buffer A was added into the

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Streptavidin-MNBs suspension. One microliters above Streptavidin-MNBs suspension was added into a tube and 10 μL of capture DNA was added to bind on the Streptavidin-MNBs, which was placed on a roller for 30 min. It was washed using buffer A for 5 times and the Streptavidin-MNBs were separated by a magnet. Then, the solution obtained from Exonuclease III-aided recycling process was transferred into the Streptavidin-MNBs solution and 100 μL of DNA-invertase conjugation was added. It was allowed to incubate for 2 h on a roller. The mixture was further washed 5 times using buffer A containing BSA (2 mg/mL). It was washed 5 times using buffer A. Finally, 100 μL of sucrose in Buffer A (0.5 M) was added into the system and allowed to incubate for 16 h. An amount of 5 μL obtained mixture was detected by a PGM.

Results and discussion

Principle of the portable and sensitive quantitative detection of DNA

The design principle of portable and sensitive quantitative detection of DNA is schematically described in Figure 1. Complex containing linker DNA was prepared by hybridizing target DNA with protect DNA. In the absence of target DNA, the complex had a protruding 3'-terminus, which could not be digested by Exo III. In the presence of target DNA, it will hybridize with the complex and generate a blunt 3'-terminus. With the aid of blunt 3'-terminus, Exo III could stepwise hydrolyze complex from its 3'-terminus. After the stepwise hydroxylation, target DNA and linker DNA were released. The target DNA used to hybridize with another complex and lead to a new target recycling process and generate more linker DNA. The mixture was transfer into a system which contained Magnetic Beads (MBs), modified by capture DNA, and DNA-invertase conjugation. The released linker DNA was further used to link capture DNA on MBs and the DNA invertase. Therefore, only in the presence of linker DNA, generated by the target hybridization, can the DNA-invertase conjugation be immobilized onto Streptavidin-MNBs. We assumed the concentration of target DNA was proportional to the amount of DNA-invertase bound to the Streptavidin-MNBs. After the washing away of unbound target DNA and DNA-invertase conjugation, the bound DNA-invertase can be used to catalyze the hydrolysis of sucrose into glucose

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with millions of turnovers, which transformed the concentration of target DNA into the level of glucose for monitoring of PGM.

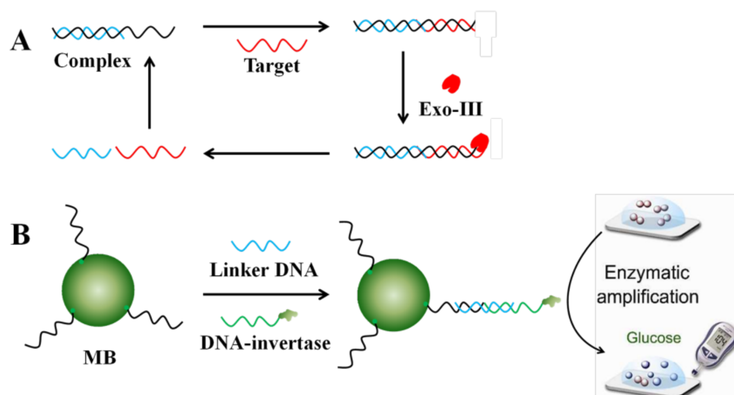

Figure 1. Schematic illustration of portable and sensitive quantitative detection of DNA based on personal glucose meters and Exonuclease III-assisted signal amplification.

Optimization of Experimental Parameters

The performance of our DNA detection method was determined by both the efficiency of target recycling process and the read out of PGM. In order to obtain an optimal performance, the dosage of Exo III and the incubation time for mixture and MBs were optimized.

Optimization for the dosage of Exo III

Exo III played a key role in the detection of DNA, which affect the efficiency of target recycling process. Therefore, the effective of dosage of Exo III to the signal of PGM was investigated. As shown in Figure 2, the signal intensity of PGM increased sharply with the increasing dosage of Exo III. There was a peak at the dosage of 50 U and the signal of PGM began to decrease when the dosage over than 50 U. Therefore, a dosage of 50 U of Exo III was selected for subsequent detection.

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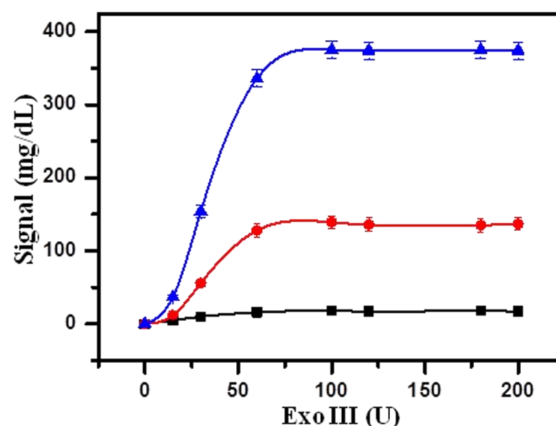

Figure 2 Relationship between the amount of Exo III and signal. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation). The plots with different color from up to below stand for 100, 50 and 10 pM of target DNA.

Optimization of incubation time for mixture and MBs

The performance of DNA detection was strongly affected by the incubation time of mixture and MBs. As shown in Figure 3, the signal of PGM elevated gradually with the increasing of incubation time (in the presence of 10, 50 and 100 pM target DNA) at the early stage and a maximum was obtained at 100 min. In order to obtain the the best signal-to-background level, 100 min was selected as the incubation time for sequent detection.

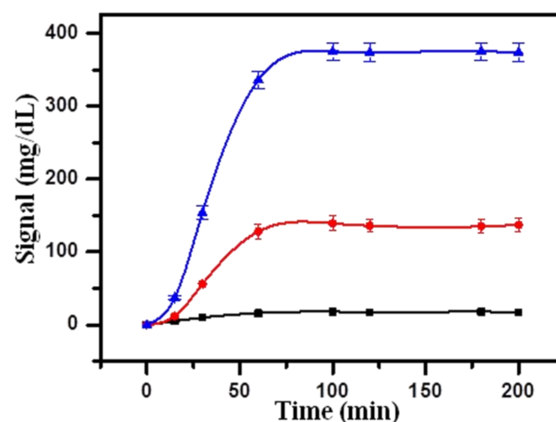

Figure 3 Relationship between the incubation time and signal. The concentration of

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Sensing Performance for target DNA

In order to evaluate the performance of our proposed method for target DNA detection, we challenged the proposed method with a series of concentrations of target DNA, covering a range of nearly 4 orders of magnitude (0.5 pM to 100 pM). Improved signal of personal glucose meter was observed with the increase of Hg^{2+} concentration and intensity of signal increased monotonically (nearly linearly) with the concentration of DNA (As shown in Figure 4). There was a liner relationship between the signal of PGM and the concentration of target DNA in the range of 0.5 pM to 100 pM. A correlation coefficient of 0.989 was obtained and the relative standard deviation (RSD) was 4.1% for a concentration of 50 pM target DNA (n = 9), which was comparable or even better than some of other reported methods. Such an attractive detection limit of our sensing strategy can be primarily attributed to three aspects: the amplification of target recycling process, the enrichment effect of Streptavidin-MNBs and the million turnovers of sucrose hydrolysis into glucose. With the aid of target recycling process, linker DNA can be released, which increased the sensitivity dramatically. Substantial target DNA and detection DNA in solution were collected onto the surface of PGM and one sucrose on the detection DNA turns into millions of glucose for monitoring of PGM.

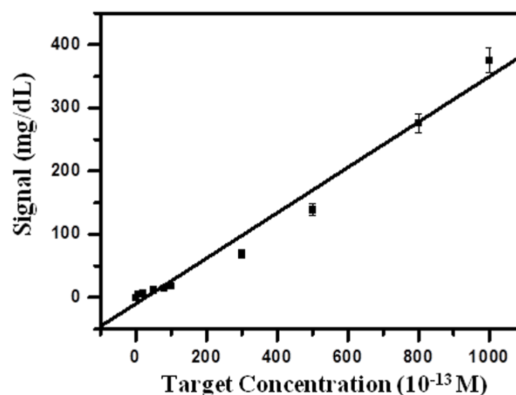

Figure 4 Relationship between the concentration of target DNA and signal. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

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Selectivity

In order to evaluate the specificity of this strategy, we then challenged our assay to different mismatched targets. As shown in Figure 4, the signal intensity of PGM for one base mismatch DNA, three bases mismatch DNA were similar to that of black hybridization buffer, demonstrating that the designed assay was specific for the target DNA.

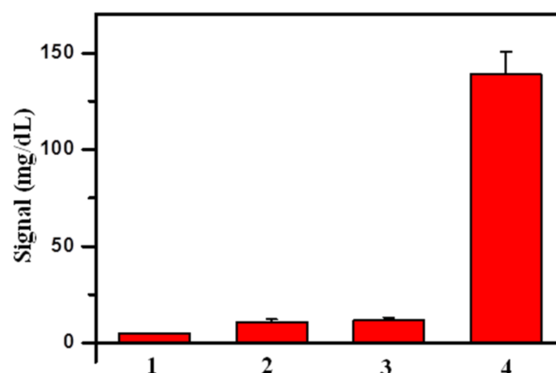

Figure 5 The signal intensity of PGM to (1) hybridization buffer, (2) single base mismatch DNA, (3) three bases mismatch DNA and (4) target DNA.

Feasibility in complex biological matrices

In order to evaluate the practical application of proposed DNA detection method, we challenged it to complex biological matrixes such as serum, using the same experimental procedures as those for target DNA detection in buffer solution. Serum is what remains from whole blood after coagulation. It does not contain target DNA. The signal of PGM detected in hybridization buffer, 20% human serum and 50% human serum did not show significant difference, indicating very little interference of complex matrices on the method. Furthermore, our method was challenged to different amount of target DNA in 20% human serum for recovery tests. The results were summarized in Table 1. Satisfactory values between 97.6 and 106% were obtained for the recovery experiments, which indicated that the possible interference from the serun on the target DNA detection was negligible. The above results demonstrate that our proposed DNA detection method can be successfully applied to DNA detection in real biological samples.

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Table 2 Recovery tests for different concentration of target DNA in human serum.

Sample ID	Added value of DNA /pM	Detected / pM	Recovery/ %
1	5	5.3±0.9	106.0%
2	80	78.1±4.5	97.6%
3	100	105.4±8.8	105.4%

Conclusion

In summary, a portable and sensitive quantitative detection of DNA based on personal glucose meters and Exonuclease III-assisted signal amplification was developed. The target DNA triggered target recycling process, which released linker DNA. The linker DNA was used to link the MBs and DNA-invertase conjugation. The DNA-invertase was used to catalyze the hydrolysis of sucrose into glucose for PGM readout. A low limit of detection was obtained owning to following reasons: the amplification of target recycling process, the enrichment effect of Streptavidin-MNBs and the million turnovers of sucrose hydrolysis into glucose. In addition, the method exhibited excellent sequence selectivity, being able to differentiate a single mismatch in the target DNA. What is more, there almost none effect of biological complex to the detection performance, which suggested our method can be successfully applied to DNA detection in real biological samples.

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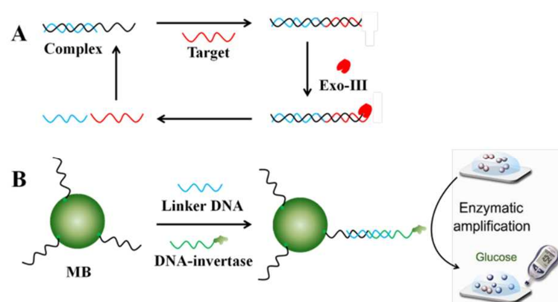

A portable and sensitive quantitative DNA detection method based on personal glucose meters and Exonuclease III-assisted signal amplification.

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