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A terminal-block strategy for the amplified detection of DNA-break events

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ABSTRACT

DNA break, including DNA single-stranded break (SSB) and DNA double-stranded break (DSB), is the most serious DNA damage. Sensitive detection of DNA-break events would be helpful for the monitoring and understanding of DNA break as well as the screening and preventing of those chemical or physical damage factors. However, most of the current detection methods suffer from some disadvantages of complex, time-consuming, lowly sensitive and especially only available for SSB or DSB only. In this work, we developed a novel terminal-block strategy for the convenient detection of both SSB and DSB events. Rolling circle amplification (RCA), a well-known isothermal nucleic acid amplification technique, was also introduced to improve the sensitivity. In our strategy, an oligonucleotide is elaborately designed to work as the primer of RCA. As contrasted with conventional mono-functional primer, our primer has three functional domains: a reserved sequence at the 5′-terminal to hybridize with the circular probe, a double-stranded domain to be exposed to DNA break inducements, and a blocking sequence at the 3′-terminal to block the RCA reaction. The RCA reaction can be launched once the blocking sequence is cut off under DNA break. Thus, the DNA break signal can be translated into the amplified RCA readout. Fenton reaction and nicking endonuclease were adopted as two model inducements of the DNA break in our study. The former induces DSB and random break sites on DNA, whereas the latter induces SSB and only one specific break site. Results show that both of these two typical kinds of DNA-break events can be well analyzed using our method, suggesting the prospect for universal application in the detection of DNA break.

1. Introduction

DNA damage is an alteration in the chemical structure of DNA, which occurs in cellular processes per day due to organism aging or the accumulation of toxic substances [1–3]. Damage to DNA can be also caused by many environmental factors, including ultraviolet irradiation [4,5], ionization radiation [6,7], and many chemicals [8,9]. Most DNA damages could be normally repaired by nucleotide excision repair pathway (NER) in cell [10,11]. However, when NER is pathologically lacking, the damaged DNA can accumulate, lead to gene mutation, and may eventually develop into cancers. DNA break is the most serious kind of DNA damage, because the integrity of gene is even lost. DNA break usually can be categorized into two main kinds i.e. the single-stranded break (SSB) and the double-stranded break (DSB) [12]. It has been reported that DNA break has related to many diseases, such as cell carcinogenesis, respiratory system disease, and sperm malformation [13–16]. Thus, the study on DNA break and its inducement is of great importance for the monitoring and understanding of DNA break-induced diseases as well as the screening and preventing of those chemical or physical damage factors.

Qualitative and quantitative detection of DNA break is usually the prerequisite to the study on DNA break. Some techniques have been applied to detect DNA break, such as comet assay [17–20], unscheduled DNA synthesis test [21], sister chromatid exchange test [22], SOS-chromotest [23,24] and high performance liquid chromatography (HPLC) [25–28]. However, most of these methods are complex, time-consuming, and have no quantitatively analytical ability. In order to conquer these deficiencies,

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some novel methods have also been developed in recent years, e.g. genome-wide sequencing [29–31], fluorescence spectroscopy [32], image cytometry [33], electrochemistry [34–39], electrogenerated chemiluminescence [40–42] and photoelectrochemistry [43–45]. Nonetheless, because of the lack of signal amplification, the sensitivity is not satisfying for the low level of DNA break. The operation simplicity of these methods has also a space for improvement. More important, most of these methods have poor universality, and can be applied for only one kind of DNA break. For example, some methods are only applicable for the detection of SSB [34,41], and some others are only for DSB [35,36,38–40,43–45]. Thus, amplified and convenient detection of DNA break and its inducements with considerable universality is still urgently required.

Herein, we developed a new method for the detection of DNA-break events by using rolling circle amplification (RCA), which can be regulated by DNA-break events through an elaborately designed trifunctional primer. RCA is a high-efficient isothermal nucleic acid amplification technique and has been used in various fields [46–48]. Benefited from the signal amplification by RCA, we are able to detection break events with favorable sensitivity. Two model inducements of DNA break are introduced, one is the Fenton reaction, another is a nicking endonuclease. The former could induce DSB and random break sites on DNA, whereas the latter induces SSB and only one specific break site. Results show that both of them can be well detected, suggesting the prospect of our method for wide application in the detection of DNA break and its inducement.

2. Experimental section

2.1. Chemicals and materials

Bst DNA polymerase large fragment, 10 × ThermaPol reaction buffer, Nb.BsrDI enzyme, 10 × NEB buffer 2 and deoxynucleotide solution mixture (dNTPs) were purchased from New England Biolabs (Ipswich, MA). SanPrep column DNA gel extraction kit and agarose were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). SYBR Green I was bought from Solarbio Bioscience & Technology Co., Ltd. (Shanghai, China). Ferrous sulfate ($FeSO_4$) and hydrogen peroxide (H_2O_2) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ethylene diamine tetraacetic acid disodium salt (EDTA·2Na) was obtained from Sigma-Aldrich (St. Louis, USA). All oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences were showed in Table S1. Water used throughout all experiments was purified by a Milli-Q system (Branstead, USA) to a specific resistance of >18 MΩ cm.

2.2. DNA-break

The model of DNA-break was established *in vitro* using two methods: Fenton reaction and a nicking endonuclease. For the former, 1 μM primer DNA and 1 μM complementary DNA were mixed together to hybridize and form double-stranded DNA (dsDNA), followed by the addition of $FeSO_4$ and H_2O_2 with equivalent concentration. The mixture was incubated for 10 min at room temperature to allow the proceeding of Fenton reaction and the random damage to DNA sequences. After that, EDTA was added to terminate the Fenton reaction.

For the DNA-break events caused by nicking endonuclease, the experimental details are as follows: 1 μM primer DNA and 1 μM complementary DNA were mixed together to hybridize and form dsDNA. Then, Nb.BsrDI enzyme together with 1 × NEB buffer 2 was added. The enzymatic reaction proceeded at 65 °C for 2 h to allow the formation of a nick at the primer sequence. Finally, the nicking endonuclease was inactivated by incubation at 80 °C for 20 min.

2.3. Rolling circle amplification triggered by the products of DNA break

The products of DNA break caused by Fenton reaction or nicking endonuclease were used as the primer to trigger RCA. RCA reaction was performed by mixing 100 nM circular probe, 1 μM reverse primer, 400 μM dNTPs, 8 U Bst DNA polymerase, 1 × ThermaPol reaction buffer and the products of DNA break together. The mixture was then incubated at 65 °C for 1 h and subsequently inactivated at 95 °C for 10 min.

2.4. Gel electrophoresis analysis

Agarose gel electrophoresis was performed for the characterization of the products of RCA. 5 μL of the products of RCA together with 1 μL 6 × loading buffer was loaded onto a 1% non-denaturing agarose gel. The electrophoresis experiments were carried out in 1 × Tris-acetate-EDTA (TAE) at 100 V for 30 min. Subsequently, the gel was stained with SYBR Green I for 30 min. The imaging of the gel was performed using a Gel Doc XR Imaging System.

Polyacrylamide gel electrophoresis was performed for the characterization of the primer DNA. 10 μL of a sample together with 2 μL 6 × loading buffer was loaded onto a 10% non-denaturing polyacrylamide gel. The electrophoresis experiments were carried out in 1 × Tris- boric acid-EDTA (TBE) at 120 V for 90 min. Subsequently, the gel was stained with SYBR Green I for 30 min. The imaging of the gel was performed using the Gel Doc XR Imaging System.

2.5. Fluorescent measurements

The RCA products were extracted from the agarose gel using a SanPrep column DNA gel extraction kit according to the manufacturer's instructions. The purified RCA products and 20 × SYBR Green I were mixed and incubated at room temperature for 15 min and were ready for fluorescent detection. Fluorescent measurements were performed at ambient temperature on a SpectraMax M3 multimode microplate reader. The emission spectra were collected with the excitation wavelength of 470 nm. The fluorescence intensity at 528 nm is used for quantitative analysis.

3. Results and discussion

3.1. Principle

As is illustrated in Scheme 1, the primer of RCA contains three functional domains: a reserved sequence at the 5′-terminal (red), an intermediate sequence (green) and a blocking sequence at the 3′-terminal (blue). The reserved sequence and the intermediate sequence are complementary to a circular probe, which can work as an everlasting template for DNA polymerization to generate multiple single-stranded linear copies of the primer in a continuous head-to-tail series. This process is just referred as RCA. However, the blocking sequence at the 3′-terminal of the primer is not complementary to the circular probe, so the RCA is blocked unless the blocking sequence is cut off. In our scheme, DNA-break events are introduced to cut the blocking sequence off. Briefly, the primer DNA can hybridize with a complementary DNA through the intermediate sequence. This hybridized double-stranded domain works as the substrate of DNA break inducements. No matter where the DNA breaks, the reserved sequence together with the residual of the intermediate sequence can work as the primer to trigger the proceeding of RCA. So, it is expected that this strategy can be applied for both DSB and SSB, and both multi-break and single-break. By using fluorescent intercalator to indicate the RCA products quanti-