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Label-free colorimetric assay for base excision repair enzyme activity based on nicking enzyme assisted signal amplification

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ABSTRACT

Specific and sensitive detection of base excision repair enzyme activity is essential to many fundamental biochemical process researches. Here, we propose a novel label-free homogeneous strategy for visualized uracil DNA glycosylase (UDG) activity assay based on nicking enzyme assisted signal amplification. In this method two hairpin probes were employed for the colorimetric detection, namely hairpin probe 1 (HP 1) carrying two uracil residues in the stem, and hairpin probe 2 (HP 2) containing a G-riched DNAzyme segment, and the recognition sequence as well as the cleavage site for the nicking enzyme. In the presence of UDG, the uracil bases in the stem of HP 1 can be specifically recognized and hydrolyzed by UDG, which leads to the destabilization of its stem containing abasic sites (AP sites), and then results in the opening of HP 1 to form a single strand. The opened HP 1 hybridizes with HP 2 to form a DNA duplex, which initiates the specific cleavage of HP 2 by the nicking enzyme, leading to the release of G-riched DNAzyme segments. As a result, HP 1 is released and able to hybridize with another HP 2 to induce the continuous cleavage of HP 2, generating enormous amount of G-riched DNAzyme segments. Finally, the G-riched DNAzyme segments bind hemin to form a catalytically active G-quadruplex–hemin DNAzyme which can catalyze the H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$) to the colored $ABTS^{-}$, providing a visible signal for UDG activity detection. This assay exhibits several advantages such as simplicity, low-cost, high selectivity and desirable sensitivity, which shows great potential of providing a promising platform for convenient and visualized analysis of UDG or other biomolecules.

1. Introduction

As the first glycosylase discovered, uracil-DNA glycosylase (UDG) is one of the most studied base excision repair (BER) enzymes functioning as a sentry guarding against undesired uracil to prevent uracil lesions of DNA in almost all known organisms (Lindahl, 1974). UDG initiates the DNA BER by catalyzing the hydrolysis of the N-glycosidic bond that links the base to the deoxyribose backbone, removing the uracil and generating an abasic site (AP site) (Krusong et al., 2006; Cole et al., 2010). Human UDG is able to remove uracil from both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), whether it is in an U–A base pair or any type of base mismatches, but UDG is not active against uracil in RNA or any normal bases in DNA owing to its active site pocket being highly specific for uracil (Verri et al., 1992; Berramy and Baldwin, 2001; Slupphaug et al., 1995). Upon the removal of undesired uracil, the DNA repair process is then completed by AP endonucleases, deoxyribophosphodiesterases, DNA polymerases and DNA ligases (David and Williams, 1988). Since the BER process plays a very important role to maintain genetic integrity and in related diseases such as human immunodeficiency virus type 1 and Bloom syndrome (Zharkov et al., 2010; Seal et al., 1988), sensitive assays for UDG activity are crucial for the study of mechanisms and functions of many fundamental biochemical processes. Therefore, it is desirable to develop sensitive and selective assays to monitor UDG activity.

Classic strategies for UDG activity assay are primarily gel electrophoresis, mass spectrometry, and radioactive labeling (Krokan and Wittwer, 1981; Ischenko and Saparbaev, 2002; Tchou et al., 1991). However, all of these methods are known to be time-consuming and indirect due to the requirement of sophisticated instrumentation, additional multi-step separation techniques, and costly labeled reagents. Therefore, to overcome the drawbacks of the traditional assays, some fluorescence-based strategies performed in homogeneous phases have been developed to realize simple, robust and rapid assay of UDG activity (Zhang et al., 2012; Bellamy et al., 2007; Zhou et al., 2013; Xiang and Lu, 2012; Ono et al., 2012; Yang et al., 2011; Liu et al., 2007; Hu et al., 2011). For example, Liu et al. (2007) utilized dsDNA probes modified with multiple uracil residues for real-time monitoring of uracil removal by UDG. Qu's group

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(Hu et al., 2011) developed a label-free fluorescence turn-on strategy to assay UDG activity based on the unique strong interaction between N-methyl mesoporphyrin IX and the folded G-quadruplex upon removal of uracil by UDG. Quite recently, a colorimetric approach for assaying UDG activity was proposed based on the enzyme-catalyzed assembly of gold nanoparticles decorated with DNA probes (Nguyen et al., 2012). To further increase the sensitivity of UDG activity assays, current interests have been focused on the incorporation of signal amplification mechanisms into more sensitive UDG activity detection.

DNA enzymes (DNAzymes) represent a class of catalytic nucleic acids selected by the systematic evolution of ligands by exponential enrichment process (SELEX) (Willner et al., 2008). The G-quadruplex is a unique higher-order structure consisting of square-planar arrangements of guanine nucleobases stabilized by Hoogsteen hydrogen bonding and monovalent cations, and it is capable of intercalating hemin to form the G-quadruplex–hemin DNAzyme complex (Parkinson et al., 2002; Travascio et al., 1998). As one of the most studied DNAzymes, G-quadruplex–hemin DNAzyme exhibits superior peroxidase-like activity and can effectively catalyze the H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$) to generate a colorimetric signal as the readout (Li et al., 2010). This working mechanism of G-quadruplex–hemin DNAzyme has been widely used in the development of sensing biomolecules and metal ions by tethering it to a variety of DNA sequences with particular recognition features (Xiao et al., 2004; Liu et al., 2013; Elbaz et al., 2009; Kong et al., 2010). For instance, the recognition of cocaine by its aptamer leads to the activation of DNAzyme for cocaine detection (Elbaz et al., 2009). Kong's group proposed a strategy for Ag^+ detection based on Ag^+-mediated formation of G-quadruplex–hemin DNAzyme combining with C–Ag^+–C base pairs (Kong et al., 2010). Previous studies have demonstrated that nicking endonucleasesc (NEases) can recognize specific nucleotide sequences in dsDNA and catalyze the cleavages of only one strand of a dsDNA at a fixed position relative to the recognition sequence (Higgins et al., 2001). Quite recently, to take advantage of these fascinating characteristics of NEases, many researches have been focused on the integration of DNAzyme and NEases to develop nicking enzyme signal amplification detection platforms for different analytes to improve the sensitivity (Wen et al., 2012; Huang et al., 2013).

Herein, we propose a novel nicking enzyme assisted signal amplification strategy for sensitive label-free colorimetric analysis of UDG activity. This approach relies on the removal of uracil by UDG triggering the formation of G-quadruplex structures which result in the increase of catalytic activity of G-quadruplex–hemin DNAzyme. Specifically, the UDG activity sensing strategy was examined in the H_2O_2/$ABTS^{2-}$ system which generates a color change signal observed by naked eyes or detected by UV–vis spectroscopy. Furthermore, this combination of DNAzyme and the nicking enzyme signal amplification could have higher sensitivity than the traditional colorimetric strategies. Additionally, this method for UDG activity detection has the advantages of convenience and cost-efficiency, because no chemical modification or immobilization for the DNA probe is required. All of these advantages of the proposed strategy for the detection of UDG activity make it a very promising candidate to be applied in UDG functional studies in the future.

2. Experimental

2.1. Reagents and instruments

EDTA, NaCl, $MgCl_2$, KCl, sodium 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES) and [tris(hydroxymethy-1)aminomethane] (Tris) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2), hemin, disodium 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) were obtained from Sigma-Aldrich. The reagents were of analytical grade and were used without further purification or treatment. Double distilled water (DDW) was used throughout the experiments. 8-Oxoguanine DNA glycosylase (hoGG1), bovine serum albumin (BSA), and nicking enzyme Nb.BbvCI were purchased from the New England Biolabs, Inc. (Ipswich, MA, USA). *Escherichia coli* uracil DNA glycosylase (UDG) and 10 × UDG reaction buffer (200 mM Tris–HCl, 10 mM EDTA, 100 mM NaCl, pH 8.2) were purchased from Fermentas China Co., Ltd. All oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences were listed in Table 1. The hairpin probe 1 (HP 1) and hairpin probe 2 (HP 2) were used as provided and diluted in 20 mM Tris–HCl buffer solution (pH 8.0, containing 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM EDTA) to give the stock solutions of 10 μM. Each oligonucleotide was heated to 95 °C and maintained at this temperature for 10 min, and slowly cooled down to room temperature before use.

UV–vis absorbance measurements were performed using a Shimadzu UV-2600 UV–visible spectrophotometer at room temperature. The wavelength at the maximum absorbance (λ_{max}) of the peroxidase-mimicking reaction was 418 nm.

2.2. Assay of UDG activity

The uracil base removal experiments were performed in 1 × UDG reaction buffer (20 mM Tris–HCl, 1 mM EDTA, 10 mM NaCl, pH 8.2) containing 40 nM HP 1, 200 nM HP 2, and a varying amount of UDG at 37 °C for 30 min. Then, Nb.BbvCI (4 μL, 5 U/μL) and 2 × concentrated CutSmart™ Buffer (34 μL) were added and allowed to incubate for 1 h at 37 °C. After the reactions were completed, 2 μL of 5 mM hemin (prepared in DMSO), 30 μL of 10 × HEPES solution (250 mM HEPES, 2 M NaCl, 200 mM KCl, 0.5% Triton X-100, pH 8.0), and 140 μL of H_2O were added and the mixtures were incubated at room temperature for 1.5 h to form the hemin/G-quadruplex structures. Finally, 30 μL of 20 mM ABTS and 30 μL of 20 mM H_2O_2 substrates were added to initiate the biocatalyzed oxidation of $ABTS^{2-}$. UV–vis absorption spectra were obtained 5 min later and the absorbance at 418 nm was used for quantitative analysis. All other control experiments were performed in a similar way. All experiments were repeated three times.

3. Results and discussion

3.1. Principle of the analytical process for amplified detection of UDG activity

In the present work, we developed a novel simple homogeneous colorimetric sensor to investigate the UDG activity using nicking enzyme assisted signal amplification, and the principle of the strategy is illustrated in Fig. 1. The DNA probes utilized here

Table 1
Sequences of the oligonucleotides used in the experiments.

Name	Sequence
HP 1	5′-C**U**G**U**AAGGAGGGA***CCTCAGC***ACAG-3′
HP 2	5′-TGGGTAGGGCGGGTTGGGTTTTTTGT***GC***↓***TGAGG***TCCCAACCC-3′

The underlined bold letters in HP 1 are deoxyuridines. The italic bold letters in HP 1 and HP 2 are the recognition sequences for Nb.BbvCI, and the arrow indicates the nicking position.