ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 8820–8822

www.rsc.org/chemcomm

COMMUNICATION

A target-activated autocatalytic DNAzyme amplification strategy for the assay of base excision repair enzyme activity†

Liangliang Zhang, Jingjin Zhao, Jianhui Jiang and Ruqin Yu*

Received 25th June 2012, Accepted 16th July 2012
DOI: 10.1039/c2cc34531e

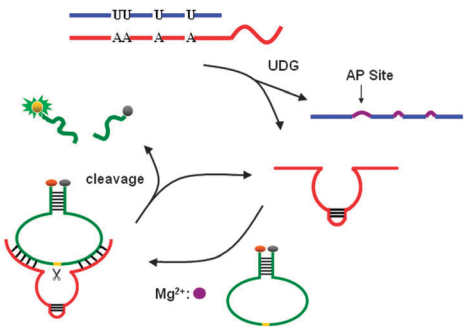
Based on a target-activated autocatalytic DNAzyme amplification strategy, novel fluorescence sensing platforms were constructed for highly sensitive and selective assay of base excision repair enzyme activity. By using a rolling circle amplification (RCA)-coupled amplification cascade, an extremely low detection limit (0.002 U mL^{-1}) was achieved.

Repair of DNA lesions has important implications in maintaining the integrity of the genomes.[1] There are several specific DNA repair pathways to counteract the deleterious effects of DNA damage, including base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR).[2] BER is the major pathway that protects cells from nucleotide base damage and it primarily repairs the damaged base moieties with a relatively small change in the chemical structure. The repair process in BER is initiated by a specific glycosylase that catalyses the cleavage of the *N*-glycosidic bond, liberating the damaged base and generating an abasic site (AP site). Then the repair is completed by AP endonucleases, deoxyribophosphodiesterases, DNA polymerases and DNA ligases.[2a] As a result, the activity of BER enzyme is important in DNA lesions repair and is connected to both individual and population disease susceptibility.[3] For instance, a damaged 8-oxoguanine induced by oxidation is mainly repaired by the BER pathway. The expression level of OGG1, a specific DNA *N*-glycosylase that initiates repair of 8-oxoguanine, was associated with the risk of lung cancer.[4] The assay of BER enzyme activity represents a critical step toward the understanding of DNA damage repairing. Therefore, the development of rapid and high-throughput screening assays for BER enzyme activities is of fundamental importance.

Traditional methods for BER enzyme assay are mainly radioactive labeling, gel electrophoresis, chromatography and paramagnetic beads.[5] These methods are time-consuming, of low sensitivity and indirect since they are based on separation techniques. Generally, DNA substrates are radioactively labelled to measure BER enzyme activity in a quantitative manner. In order to overcome these limits, current efforts for BER enzyme assay focus on the development of highly sensitive fluorescent sensors.[6]

Autocatalytic DNAzyme has attracted great interest in the sensor design used as a new nonenzymatic biocatalytic tool.[7] It has been successfully applied to detect metal ions, small molecules and nucleic acids.[8] Due to the easy implementation of DNAzyme, the combination of this biocatalyst with other protein enzyme-based nucleic acid amplification techniques, such as polymerase chain reaction (PCR), rolling circle amplification (RCA) and strand displacement amplification (SDA), may conceptually create a more efficient signal amplification strategy in bioanalysis.

Herein, we have explored the potential use of an autocatalytic DNAzyme-base signal amplification method for simple and highly sensitive assay of base excision repair enzyme activity. Using uracil-DNA glycosylase (UDG) as a model analyte, we report for the first time the target-activated autocatalytic DNAzyme amplification strategy for the detection of BER enzyme activity. UDG is a highly conserved damage repair protein, which catalyses the cleavage of the *N*-glycosidic bond between a uracil base and the deoxyribose phosphate backbone of DNA, initiating the BER to repair the commonly existent damaged uracil base in DNA.[9] In this work, the UDG treatment will trigger a conformational switch or generate multiple biocatalytic units to activate the autocatalytic activity of DNAzyme. A Mg^{2+}-dependent E6 DNAzyme[10] was introduced to construct this target-activated autocatalytic DNAzyme



Scheme 1 Schematic diagram of the UDG activity assay based on the autocatalytic DNAzyme amplification using a double-stranded substrate. U and A denote the uracil and adenine deoxyribonucleotide respectively.

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China. E-mail: rqyu@hnu.edu.cn; Fax: +86 731-88822782; Tel: +86 731-88822577

† Electronic supplementary information (ESI) available: Experimental details, optimization of experimental conditions and supporting figures. See DOI: 10.1039/c2cc34531e

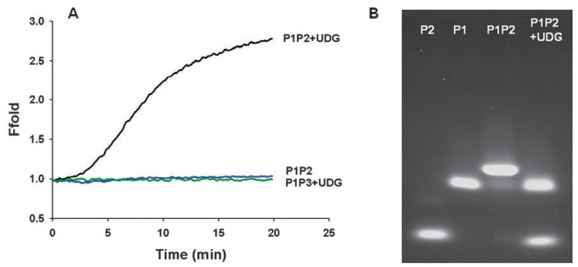


Fig. 1 (A) Time-dependent fluorescence enhancement of the DNAzyme-based strategy using a double-stranded substrate with or without 2 U mL^{-1} UDG. (B) Agarose gel (5%) electrophoresis images of dissociation of the double-stranded substrate in the presence of UDG.

for the amplified detection of BER enzyme. Scheme 1 outlines the principal design of the first target-activated DNAzyme strategy for UDG assay. E6 DNAzyme (P1) is partially hybridized with a single strand (P2) containing four uracil bases to form a double-stranded substrate (P1P2). The double-stranded substrate shows a relatively high melting temperature, making the substrate a stable hybrid and blocking the E6 DNAzyme. In the presence of UDG, uracil bases are removed from the deoxyribose phosphate backbone of P2, resulting in a lowered melting temperature of the substrate and liberating the E6 DNAzyme. The liberated E6 DNAzyme can then initiate the autocatalytic amplification process by repeating the hybridization with a MB probe and catalyzing the cleavage of the MB probe in the presence of Mg^{2+} as a cofactor. In this way, one released DNAzyme will cleave multiple MB probes, creating substantial amplification of the fluorescence signal for the UDG assay.

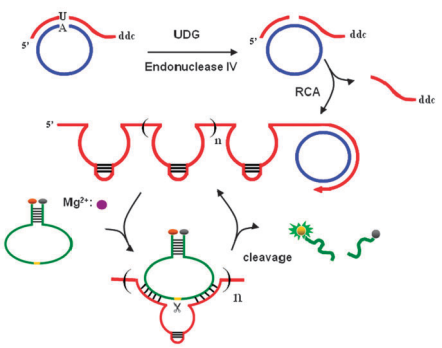
As shown in Fig. 1A, in the absence of UDG, negligible fluorescence enhancement was observed, indicating that the activity of DNAzyme was suppressed. In the presence of UDG, an obvious enhancement was observed in 20 min, suggesting that the DNAzyme was released and was free to catalyze the cleavage of the MB probe. To further confirm that the fluorescence increase was due to the uracil removal, a P1P3 double strand probe without a uracil base was investigated as a control experiment. No obvious enhancement was observed even in the presence of UDG (Fig. 1A). These results proved that the fluorescence enhancement only depended on the uracil removal by UDG. Agarose gel electrophoresis was also used to verify the dissociation of the double-stranded substrate. After the UDG treatment, two obvious single strand products were observed (Fig. 1B). Taking advantage of the present strategy, convenient and sensitive assay of the UDG activity can be realized.

Under the optimized conditions (all details can be found in ESI†), different concentrations of UDG were tested. The rate of fluorescence enhancement increased with increasing UDG concentration (Fig. S2, ESI†). The calibration curve for detection of UDG activity is shown in Fig. 3A. In the range from 0 to 1 U mL^{-1}, the velocity was directly proportional to the concentration of UDG. According to the 3σ rule, the detection limit for UDG was estimated to be 0.023 U mL^{-1} which is lower than that previously reported.[11] This high sensitivity could be attributed to enzymatic multiple turnover of DNAzyme, which gave rise to significantly increased detection signal. Hence, this strategy holds great promise as an effective method to detect the activity of UDG.

To demonstrate the feasibility of this method in assaying the inhibition of UDG, uracil DNA glycosylase inhibitor (UGI) was selected as a model inhibitor for this study. UGI which is produced by the bacteriophage PBS1 can form an extremely specific and exclusively stable complex with UDG with a 1 : 1 stoichiometry.[12] The influence of UGI on the E6 DNAzyme activity was first evaluated. The result indicated that UGI had no obvious effect on the activity of E6 DNAzyme under the experimental conditions (Fig. S3A, ESI†). However, in the presence of equal concentration of UGI, the activity of UDG was almost completely inhibited (Fig. S3B, ESI†). These results demonstrate that the proposed method can be used to monitor the UDG inhibitors simply and rapidly.

Combining the autocatalytic DNAzyme with other nucleic acid amplification methods may create an enhanced sensitivity for UDG assay. To prove this hypothesis, a rolling circle amplification (RCA) process is incorporated into the DNAzyme-based strategy. RCA is an isothermal amplification method that uses a short DNA primer to generate long single-stranded tandem repeated sequences of a circular DNA template, and has become an ideal strategy for ultrasensitive detection of nucleic acids, protein, or small molecules.[13] The principle of the proposed RCA-assist strategy is depicted in Scheme 2. A ligation probe with a uracil base is hybridized with a padlock probe that contains a complementary sequence to E6 DNAzyme, forming a circular substrate for UDG. The ligation probe is designed to have a dideoxynucleotide modification at the 3′ terminal with a six-deoxyadenosine overhang. This design disables the ligation probe to be the primer to initiate RCA in the absence of UDG. Upon UDG treatment, the uracil base was removed to generate an AP site that can be cleaved by endonuclease IV (Endo IV).[14] This cleavage produces a new primer probe with a 3′ hydroxyl end that can initiate a linear RCA reaction in the presence of Bst DNA polymerase and dNTPs. The RCA product has a tandem repeated sequence of E6 DNAzyme, and each E6 DNAzyme sequence can cyclically cleave the MB probe and generate an increased fluorescence signal. Due to the dual signal amplification cascade, it is expected to offer a substantially improved sensitivity and a much lower detection limit.

As shown in Fig. 2A, in the presence of UDG and Endo IV together, an obvious fluorescence enhancement was observed. However, there was negligible fluorescence enhancement when the circular substrate was treated with UDG or Endo IV alone.



Scheme 2 Schematic illustration of the UDG activity assay based on RCA-assisted DNAzyme strategy. U and A denote the uracil and adenine deoxyribonucleotide respectively.

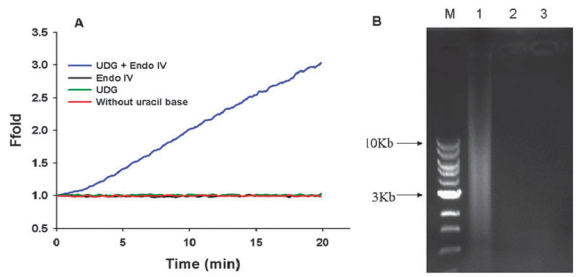


Fig. 2 (A) Time-dependent fluorescence enhancement of the RCA-assisted DNAzyme strategy in the presence of UDG or Endo IV. (B) Agarose gel (0.8%) electrophoresis images of RCA process obtained in the presence of UDG and Endo IV together (lane 1), UDG (lane 2) or Endo IV (lane 3) alone. Lane M: DNA size marker. The concentrations of UDG and Endo IV were 0.05 U mL^{-1} and 250 U mL^{-1}, respectively.

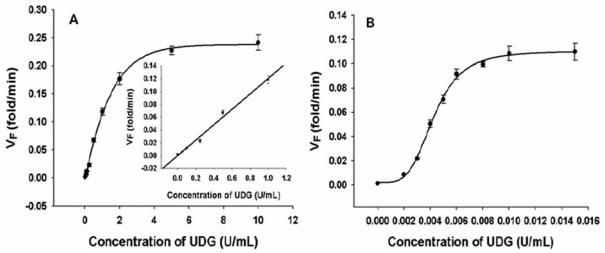


Fig. 3 (A) Calibration curve of the UDG detection using a double-stranded substrate. The inset shows the linear responses at low UDG concentrations. (B) Calibration curve of the UDG detection based on the RCA-assisted DNAzyme strategy. The curves were plotted with the initial rate of fluorescence enhancement *vs.* UDG concentration.

Additionally, a ligation probe 2 containing no uracil base was used to demonstrate the principle of the proposed strategy. No obvious fluorescence enhancement was observed after the treatment with UDG and Endo IV. These results reveal that uracil removal by UDG and Endo IV cleavage were essential in the UDG-triggered RCA process. Agarose gel electrophoresis was also used to further verify the RCA amplification process of this proposed method, as shown in Fig. 2B. It was observed that in the presence of UDG or Endo IV alone, no RCA product was obtained. When the circular substrate was treated with UDG and Endo IV together, low mobility products were obtained, indicating a large molecular weight of RCA products and a high efficiency of RCA reaction.

The time-dependent fluorescence response over background fluorescence with different concentration of UDG using the RCA-assisted method was studied (Fig. S4, ESI†). As the concentration of UDG increased, more RCA products were obtained to cleave the MB probe, causing the increase in the rate of fluorescence enhancement. As shown in the calibration curve (Fig. 3B), the detection limit of UDG in this RCA-assisted method is 0.002 U mL^{-1}, which is much lower than that of the aforementioned method based on a double-stranded substrate. Comparing calibration curves of two proposed DNAzyme-based methods, the results obviously indicated that the RCA-assisted method can remarkably improve the sensitivity of the UDG assay because of the dual signal amplification cascade. Similar to other RCA reactions, this method can be expected to achieve a lower detection limit and a wider dynamic range by further optimizing the RCA or DNAzyme reaction conditions, such as concentration of the circular substrate and enzymes or the reaction time of RCA and MB cleavage.

In summary, we have developed an autocatalytic DNAzyme-based amplification strategy for simple and sensitive detection of base excision repair enzyme activity. Taking UDG as a model analyte, the feasibility of this target-activated DNAzyme strategy is first demonstrated using a double-stranded substrate. This strategy can be facilely implemented. It can be completed by simply mixing the target UDG and the substrate in less than 20 min. At the same time, this method exhibits high sensitivity. The detection limit is much lower than those previously reported. This strategy can also be used to evaluate the inhibition of the UDG activity. Furthermore, taking advantage of the powerful signal amplification capability of RCA, a RCA-coupled amplification cascade was used to improve the sensitivity. We achieved a significant improvement in the detection limit down to 0.002 U mL^{-1}. Moreover, these methods can be further expanded easily to detect other BER enzymes by simply altering the related damaged bases which can be repaired by different BER enzymes. Thus, these strategies provide sensitive and selective platforms for BER enzyme assay. These advantages also endow the described methods with great potential to be applied in clinical diagnostics.

Financial support from the Natural Science Foundation of China (21025521, 21035001, 21190041), National Key Basic Research Program (2011CB911000), CSIRT Program and NSF of Hunan Province (10JJ7002) is gratefully acknowledged.

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