Sensors & Actuators: B. Chemical 270 (2018) 277–282

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A time-dependent fluorescent biosensor for uracil-DNA glycosylase detection based on the uracil inhibition effect towards archaebacterial DNA polymerases

Xuzhe Zhang[a,1], Tongbo Wu[c,1,2], Hongbo Wang[d], Yufan Zou[a], Wei Chen[c], Meiping Zhao[c], Shanshan Wang[b,*], Xianjin Xiao[a,*]

[a] Family Planning Research Institute/Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China
[b] Key Laboratory of Agro-product Safety and Quality, Institute of Quality Standards & Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China
[c] Beijing National Laboratory for Molecular Sciences, MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PR China
[d] Department of Obstetrics and Gynaecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, PR China

ARTICLE INFO

Keywords:
Uracil-DNA glycosylase
Archaebacterial DNA polymerase
Uracil inhibition
Time-dependent
Fluorescent detection
Exponential amplification

ABSTRACT

Uracil-DNA glycosylase (UDG) plays important role in DNA lesion repair. It can remove the uracil lesion in DNA and is involved in multiple biological processes. UDG activity detection methods based on DNA probe are developed fast recently due to its high sensitivity and simplicity. They typically use two strategies: the conformational change or the local environmental change of the DNA probe after UDG cleavage. Here we proposed a new strategy for UDG activity detection, which utilized uracil-containing oligo to inhibit archaebacterial DNA polymerases, and after UDG treatment the archaebacterial DNA polymerases could be activated to generate linear or exponential amplification fluorescent signal. The exponential methods showed a time-dependent signal output and achieved limit of detection of 0.0025 U/mL. More importantly, the feature of time-dependence allowed us to easily modulate the resolution or the dynamic range of our method to meet different demands in various occasions. The strategy was also demonstrated to be highly selective toward DNase and exonucleases and had been applied to detect the UDG activity in cell lysate.

1. Introduction

DNA lesion repair is of great significance in maintaining the integrity of genetic information [1,2]. Uracil lesion in DNA mainly results from deamination of cytosine which will cause dangerous mutagenesis arising from U:G mismatch and lead to diseases such as cancers [3,4]. Base excision repair (BER) plays the most crucial role in protecting cells from nucleotide base damage and repairing it with relatively small change in the chemical structure [5,6]. Uracil-DNA glycosylase (UDG) is one of the most important enzymes in BER pathway existing in most organisms from bacteria to human [7,8]. It is responsible for the removal of a damaged uracil base in DNA and initiates the BER pathway of uracil excision repair [5,9]. UDG is proven to be involved in cell cycle regulation, apoptosis and cancer stage [10–12]. Therefore, developing the assays of accurate detection in UDG activity is desirable and meaningful.

Traditional methods for UDG activity detection are based on gel electrophoresis [13], electrochemical assay [14] and mass spectrometry [15]. However, most of these methods are time-consuming, of poor sensitivity, indirect and tedious. Recently, DNA probe based methods for UDG activity detection have drawn great attention due to their advantages of simplicity and sensitivity over traditional methods. The majority of the DNA probe based methods use uracil-containing double-stranded DNA (dsDNA) [16–25] or hairpin [26–31] as the initiative probe. Once the uracil is removed by UDG, the resultant abasic site will destabilize the dsDNA or hairpin structure and directly generate fluorescent [27] or electrochemical signal [23] or release a free DNA to trigger subsequent reactions such as G-quadraplex formation [16,19,21,22,25,26,29,32], toehold mediated strand displacement [21,24–26,33], DNAzyme based amplification [17], rolling cycle

* Corresponding authors.
E-mail addresses: wangshanshan@caas.cn (S. Wang), xiaoxianjin@hust.edu.cn (X. Xiao).
[1] These authors contributed equally to this work.
[2] Present address: Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, T6G 2G3, Canada.

https://doi.org/10.1016/j.snb.2018.05.041
Received 22 February 2018; Accepted 9 May 2018
0925-4005/

amplification [17,29] and exponential amplification reaction [30,31,34]. To ensure obvious structure change, the initiative probe always contains multiple uracil sites [16–28,32,33]. However, little signal will be generated if only a part of the uracil sites are removed in one DNA substrate because it may be insufficient for conformational change and this limits the sensitivity of these methods. Some methods also use enzymatic cleavage such as endonuclease IV [18,29,31], exonuclease I [28] and endonuclease EcoRI [34] to assist the conformational change of the DNA substrate but this will further complicate the reaction system. An alternative strategy is using the small fluorescent molecule, which is linked in the DNA probe near the uracil [35,36] or is bound with uracil [37]. Uracil can quench these molecules and when uracil is removed, as the environment around the fluorescent molecule is changed, the fluorescent signal will turn on. This strategy uses only one uracil in each of the DNA probe to increase the sensitivity. However, they cannot be adapted to DNA based signal amplification methods and the sensitivity still need to be improved. Moreover, most of these DNA probe based methods are signal intensity dependent, that is, the results are measured after procedures in specific time and distinguished by fluorescence intensity, which is hard to be differentiated from the background signal and has difficulties in quantifying with small amounts. Herein we present a completely new strategy for UDG activity detection without using conformational or environmental change in the initiative DNA probe but using the inhibition ability of the uracil-containing oligo to the archaebacterial DNA polymerases.

Archaebacterial DNA polymerases are reported to tightly bind with the uracil in DNA oligo [38]. Single uracil in DNA oligo is strong enough to inhibit the activity of archaebacterial DNA polymerase. UDG can remove the uracil in DNA oligo and the resultant dU and the DNA oligo with abasic site could not inhibit archaebacterial DNA polymerases. Thus after UDG treatment, the activity of archaebacterial DNA polymerase is released and can be used for DNA elongation and uracil detection [39] Here, we used the inhibitor effect of uracil-containing DNA towards archaebacterial DNA polymerase to develop a new strategy for UDG detection. In this strategy, we can transfer the measurement from UDG activity detection to archaebacterial DNA polymerase activity detection. This method is simple, time-dependent output and adapts to multiple DNA based signal amplification to further improve the sensitivity. As a proof of concept, we chose Vent (exo-) DNA polymerase (Vent) which is a wildly used archaebacterial DNA polymerase [38,40] to show the feasibility of our novel strategy.

2. Material and methods

2.1. Reagents and chemicals

The DNA oligonucleotides (oligos) used in this work were synthesized and purified by Sangon Biotech Co. (Shanghai, China). The sequences of all the oligos were listed in Table S1. UDG, Vent (exo-) DNA polymerase, Nt. AlwI nicking enzyme, Thermopol Reaction Buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$ and 0.1% Triton X-100, pH 8.8 @ 25 °C) and UDG Reaction Buffer (20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 8.0 @ 25 °C) were all obtained from New England Biolabs (Beijing, China). dNTPs and 5-fluorouracil (5-Fu) were obtained from Sangon Biotech Co. (Shanghai, China). 20 × EvaGreen dye was obtained from Biotium Inc. (CA, USA). Ultra-pure water (18.2 MΩ cm^{-1}) purchased from Sangon Biotech Co. was used during all the experiments. All the DNA oligos were dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA pH 8.0) and stored at −20 °C for further use.

2.2. Apparatus

All fluorescence measurements were carried out on a StepOne Real-Time PCR Systems (ThermoFisher Scientific, Shanghai, China). The concentration of DNA oligonucleotides is measured by NanoDrop 2000 UV–vis Spectrophotometer (ThermoFisher Scientific).

2.3. Uracil-containing DNA inhibition effects on Vent (exo-) DNA polymerase

For the linear amplification reaction, to a 200 μL PCR tube, 2 μL of 10 × Thermopol Reaction Buffer, 2 μL of dNTPs (250 μM, the final concentration in 20 μL reaction solution, the same definition for other oilgos mentioned in the text), 1 μL of Template oligo (150 nM), 1 μL of Trigger oligo (150 nM), 1 μL of uracil-containing oligo of different concentration (6.25 nM, 25 nM, 37.5 nM and 100 nM, respectively), 1 μL 20 × EvaGreen dye and 11 μL of Ultra-pure water were added and mixed well. The solution was heated to 85 °C and then gradually cooled down to 37 °C. Then, 1 μL of Vent (exo-) DNA polymerase (0.25 U) was added and the fluorescent signal was measured at 48 °C using StepOne Real-Time PCR Systems. The excitation and emission wavelengths were set to 492 nm and 518 nm, respectively.

For the exponential amplification reaction, to a 200 μL PCR tube, 2 μL of 10 × Thermopol Reaction Buffer, 2 μL of dNTPs (250 μM), 1 μL of Template oligo (150 nM), 1 μL of Trigger oligo (150 pM), 1 μL of uracil-containing oligo of different concentration (6.25 nM, 25 nM, 37.5 nM and 100 nM, respectively), 1 μL 20 × EvaGreen dye and 10 μL of Ultra-pure water were added and mixed well. The solution was heated to 85 °C and then gradually cooled down to 37 °C. Then, 1 μL of Vent (exo-) DNA polymerase (0.25 U) and 1 μL of Nt. AlwI nicking enzyme (5 U) were added and the fluorescent signal was measured as mentioned above.

2.4. Detection of UDG activity

After the concentration of uracil-containing oligo is optimized (final concentration of 100 nM for linear and 7.5 nM for exponential amplification reaction), multiple amount of UDG (0.5 U, 0.05 U, 0.005 U, 0.0005 U, 0.00005 U) was added for activity detection. UDG was diluted with UDG Reaction Buffer. Other conditions were the same as described above in the Vent inhibition experiments.

To regulate the resolution of the exponential amplification reaction, uracil-containing oligo was used as 12.5 or 20 nM or the primer was used as 15 pM for comparison with the initial experiment condition.

2.5. Measurement of the selectivity of the method

To a 200 μL PCR tube, 2 μL of 10 × Thermopol Reaction Buffer, 2 μL of dNTPs (250 μM), 1 μL of Template oligo (150 nM), 1 μL of Trigger oligo (150 pM), 1 μL of uracil-containing oligo (7.5 nM), 1 μL 20 × EvaGreen dye and 9 μL of Ultra-pure water were added and mixed well. The solution was heated to 85 °C and then gradually cooled down to 37 °C. 1 μL of Vent (exo-) DNA polymerase (0.25 U) and 1 μL of Nt. AlwI nicking enzyme (5 U) were added. Then, 1 μL of UDG (0.02 U), or DNase I (0.2U), or BSA (0.2U), or Lambda Exonuclease (0.2U), or Exonuclease I (0.2U), or UDG with 5-fluorouracil (final concentration 12 mM) were added respectively and the fluorescent signal was measured as mentioned above for exponential amplification reaction.

2.6. UDG activity assay in biological samples

Approximately 1.5×10^8 Ishikawa cells samples were pelleted by centrifugation (3000 rpm, 5 min, 4 °C) and re-suspended in 90 μL of lysis buffer (10 mM Tris-HCl, pH 7.0) on ice. The mixture solution was then centrifuged at 12000 rpm for 30 min at 4 °C to remove insoluble material.

To a 200 μL PCR tube, 2 μL of 10 × Thermopol Reaction Buffer, 2 μL of dNTPs (250 μM), 1 μL of Template oligo (150 nM), 1 μL of Trigger oligo (150 pM), 1 μL of uracil-containing oligo (7.5 nM), 1 μL 20 × EvaGreen dye and 9 μL of Ultra-pure water were added and mixed well. The solution was heated to 85 °C and then gradually cooled down