Analytica Chimica Acta 887 (2015) 216–223

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Tungsten disulfide nanosheet and exonuclease III co-assisted amplification strategy for highly sensitive fluorescence polarization detection of DNA glycosylase activity

Jingjin Zhao [a, 1], Yefei Ma [a, 1], Rongmei Kong [b], Liangliang Zhang [a, *], Wen Yang [a], Shulin Zhao [a]

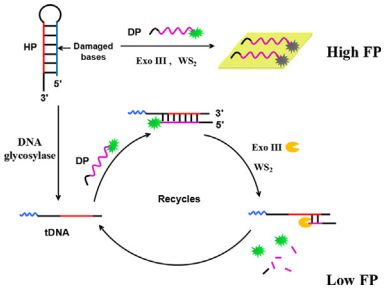
[a] Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources of Education Ministry, Guangxi Normal University, Guilin, 541004, China

[b] The Key Laboratory of Life-Organic Analysis, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu, Shandong 273165, China

HIGHLIGHTS

- A fluorescence polarization strategy for DNA glycosylase activity detection was developed.
- The present method was based on WS_2 nanosheet and exonuclease III co-assisted signal amplification.
- A high sensitivity and desirable selectivity were achieved.
- This method provides a promising universal platform for DNA glycosylase activity detection and related biological studies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form
28 July 2015
Accepted 30 July 2015
Available online 8 August 2015

Keywords:
Tungsten disulfide nanosheet
Exonuclease III
Fluorescence polarization
DNA glycosylase

ABSTRACT

Herein, we introduced a tungsten disulfide (WS_2) nanosheet and exonuclease III (Exo III) co-assisted signal amplification strategy for highly sensitive fluorescent polarization (FP) assay of DNA glycosylase activity. Two DNA glycosylases, uracil-DNA glycosylase (UDG) and human 8-oxoG DNA glycosylase 1 (hOGG1), were tested. A hairpin-structured probe (HP) which contained damaged bases in the stem was used as the substrate. The removal of damaged bases from substrate by DNA glycosylase would lower the melting temperature of HP. The HP was then opened and hybridized with a FAM dye-labeled single strand DNA (DP), generating a duplex with a recessed 3′-terminal of DP. This design facilitated the Exo III-assisted amplification by repeating the hybridization and digestion of DP, liberating numerous FAM fluorophores which could not be adsorbed on WS_2 nanosheet. Thus, the final system exhibited a small FP signal. However, in the absence of DNA glycosylases, no hybridization between DP and HP was occurred, hampering the hydrolysis of DP by Exo III. The intact DP was then adsorbed on the surface of WS_2 nanosheet that greatly amplified the mass of the labeled-FAM fluorophore, resulting in a large FP value. With the co-assisted amplification strategy, the sensitivity was substantially improved. In addition, this method was applied to detect UDG activity in cell extracts. The study of the inhibition of UDG was also performed. Furthermore, this method is simple in design, easy in implementation, and selective, which holds potential applications in the DNA glycosylase related mechanism research and molecular diagnostics.

* Corresponding author.
E-mail address: liangzhang319@163.com (L. Zhang).

[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aca.2015.07.006
0003-2670/

1. Introduction

Fluorescence polarization (FP) is a convenient fluorescent analytical technique in the construction of homogenous bioassays [1]. The FP value is sensitive to the rotation rate of the fluorophore. Generally, the free fluorophore possesses a fast rotation rate, resulting in a low FP signal. But FP value will increase when the fluorophore is bound with another molecule to form a complex which slows down the rotation rate. As a result, the degree of FP change is relied on the mass or volume of the formed complex [1]. In addition, as a ratiometric approach, the FP is less sensitive to the photobleaching and the fluorescence fluctuation than other fluorescent techniques [2]. Thus, the FP has been widely used for the detection of various biomolecules including protein, nucleic acid and cancer cell [3–5]. Recently, to improve the sensing sensitivity, nanomaterials such as gold nanoparticle [6], carbon nanotube [7], carbon nanoparticle [8] and graphene oxide [9] were employed as FP amplifiers to achieve larger changes of FP signal for sensitive bioanalysis.

At present, two-dimensional transition-metal dichalcogenides (TMDCs) have attracted great attention due to their superior mechanical, thermal, and electronic properties, offering new opportunities for sensing, catalysis, and energy storage [10–12]. Layered tungsten disulfide (WS_2) nanosheet is an important TMDC, which consists of S–W–S sandwiches [13]. It has been reported that WS_2 nanosheet can adsorb single-stranded DNA (ssDNA) via the van der Waals force between nucleobases and the basal plane of WS_2 nanosheet [14–16]. More importantly, the WS_2 nanosheet, with size ranging from tens to thousands of nanometers, has a large mass. Obviously, based on these two properties, the fluorohpore that was labeled on the ssDNA will be captured on the surface of WS_2 nanosheet through the interaction between ssDNA and WS_2 nanosheet, resulting in an enhancement of FP signal due to the formation a large mass complex. That is to say, WS_2 nanosheet is able to act as a FP amplifier. However, up to now, using WS_2 nanosheet as an amplifier and a scaffold to develop amplified FP bioassays is still rare.

Exonuclease III (Exo III)-assisted cyclic enzymatic amplification is a simple and widely used nucleic acid-based signal amplification method. Exo III catalyzes the removal of mononucleotides from blunt or recessed 3′-termini of double-stranded DNA (dsDNA) [17]. Since no specific recognition site is required for Exo III which facilitates the DNA sequence design, Exo III-assisted cyclic signal amplification has been used in the construction of numerous sensitive biosensing methods [17–20]. Currently, combining the nucleic acid-based signal amplification methods with nanomaterial-amplifier to develop an ingenious co-amplified FP sensing strategy, which can further improve the sensitivity, is fascinating [21,22], and it still remains at a very early stage. To the best of our knowledge, there is no report about the nanomaterial and Exo III co-amplified FP detection strategy.

Base excision repair (BER) is an important DNA repair pathway, protecting genome from nucleotide base damage [23]. As the initial repair enzyme in BER pathway, DNA glycosylase catalyzes the removal of damaged bases and the generation of abasic (AP) sites for the downstream repair processes [24]. The DNA glycosylase is therefore of great significance in maintaining the genome integrity and has been demonstrated to be related with cancer susceptibility [25]. Accordingly, the monitoring DNA glycosylase activity plays an important role in the study of repair mechanism and development of tools for molecular diagnostics. Gel electrophoresis is the traditional detection methods for DNA glycosylase activity [26,27], which is time-consuming, complicated and of insufficient sensitivity. Consequently, several novel detection methods have been developed to overcome these limits, such as fluorescence resonant energy transfer based detection methods [28–31], G-quadruplex based fluorescent approaches [32] and gold nanoparticle based colorimetric assay [33,34]. Due to the low amounts of DNA glycosylase in real samples, researchers focused on the introduction of signal amplification strategies in the DNA glycosylase activity assays to achieve a high sensitivity, such as DNAzyme-based autocatalytic amplification [35,36], nicking enzyme-assisted signal amplification [37], DNA machine-based amplification [38], and alkaline phosphatase-based enzymatic electrochemical signal amplification [39]. However, these methods still suffer from some weakness. For examples, in DNAzyme-based assays, high background signals resulted in a low signal-background ratio. DNA machine-based method, which relied on the cyclic hybridization-strand displacement, required sophisticated DNA sequence design, and the competitive hybridization was existed in the process of strand displacement. In electrochemical method, tedious modification and washing steps were needed. To the best of our knowledge, up to now, FP detection technique has not been applied for sensitively monitoring the activity of DNA glycosylase.

Herein, we demonstrated that WS_2 nanosheet can be acted as an effective signal amplifier in FP sensing strategy, and proposed a highly sensitive and universal DNA glycosylase activity detection method based on the WS_2 nanosheet and Exo III co-assisted signal amplification. In this work, two DNA glycosylases, uracil-DNA glycosylase (UDG) and human 8-oxoG DNA glycosylase 1 (hOGG1), were detected respectively. UDG catalyzes the removal of commonly existent damaged uracil base from deoxyribose phosphate backbone [24], and it is reported to be related with human diseases such as Bloom syndrome and human immunodeficiency [40,41]. hOGG1 repairs 8-oxo-7,8-dihydroguanine (oxo-G) which is a oxidation product of guanine in DNA [24,42]. The expression level of hOGG1 is connected with several cancers including gastric cancer, lung cancer and bladder cancer [42–44]. With the co-assisted amplification by WS_2 nanosheet and Exo III, the sensitivity of the present work was improved with low detection limits of 0.00030 U mL^{-1} and 0.0070 U mL^{-1} for UDG and hOGG1, respectively. In addition, the applications of this method for real sample analysis and inhibition assay were also demonstrated.

2. Experimental

2.1. Reagents

E. coli uracil-DNA glycosylase, human 8-oxoG DNA glycosylase 1, uracil glycosylase inhibitor, Dam methyltransferase (Dam MTase), M.SssI methyltransferase (M.SssI), Dpn II restriction endonuclease (Dpn II) and Nb.BbvCI restriction endonuclease (Nb.BbvCI) were purchased from New England Biolabs (Beijing, China). Exonuclease III (Exo III) was bought from Thermo Fisher Scientific Co. Ltd. (Shanghai, China). Few-layered tungsten disulfide nanosheet was supplied by Nanjing XFNano Material Technology Co., Ltd. (Nanjing, China). Ultrapure water was purified with a Milli-Q plus 185 purification system (Bedford, MA) and used throughout the work. The oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and the sequences were as follows:

HP-U: 5′-GCUGUCUGUGAAGGAGGTAGATCACAGACAGCAAAA-3′
HP-G:5′-GCT-oxoG-TCT-oxoG-TGAAGGAGGTAGATCACAGACAGC AAAA-3′
DP: 5′-AAAAAGCTGTCTGTGATC-FAM-3′

The letter U in the HP-U and oxoG in HP-G denote the uracil deoxyribonucleotide modification and 8-oxo-7,8-dihydroguanine modification, respectively. The underlined letters indicate the stem sequence.