Sensors and Actuators B 252 (2017) 477–482

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A sensitive and real-time assay of restriction endonuclease activity and inhibition based on photo-induced electron transfer

Yaocai Wang, Luzhu Yang, Yanjun Wang, Wei Liu, Baoxin Li, Yan Jin*

Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry Chemical Engineering, Shaanxi Normal University, Xi 'an, 710062, China

ARTICLE INFO

Article history:
Received 18 February 2017
Received in revised form 10 May 2017
Accepted 5 June 2017
Available online 6 June 2017

Keywords:
EcoRI endonuclease
Photo-induced electron transfer
Fluorescence
Real-time

ABSTRACT

DNA digestion by endonucleases is significant in various biological and medicinal processes. Herein, we describe a facile and sensitive fluorescent assay for real-time studying endonuclease activity and inhibition based on the photo-induced electron transfer (PIET) between deoxyguanosines and a fluorophore. The carboxyfluorescein-labeled DNA (FDNA) hybridizes with its complementary DNA (cDNA) to form substrate of EcoRI endonuclease. Then, the fluorescence of FDNA was quenched by nearby deoxyguanosines due to PIET. In the presence of EcoRI endonuclease, DNA substrate was digested into short fragments. The fluorophore is far away from deoxyguanosines, leading to a recovery of FDNA fluorescence. The fluorescence enhancement efficiency was linear with logarithm of EcoRI concentration over the range of 5×10^{-5}–1×10^{-2} U/μL, and the detection limit of was 4×10^{-5} U/μL, which was more sensitive than many previous reports. The influence of inhibitor on the activity of EcoRI endonuclease was facilely and reliably studied. More importantly, the activity of EcoRI endonuclease can be real-time monitored, which is vital for studying functionality and inhibition of endonuclease. The ingenious design of DNA probes ensures high specificity and low cost. Therefore, it offers a fast, sensitive, cost-effective and specific homogeneous analysis protocol for studying the activity and inhibition of EcoRI endonucleases.

1. Introduction

Restriction endonucleases play many significant roles in various biology processes involving DNA replication, repair, genotyping, enzymatic amplification technique and nanostructures/nanodevices fabrication and so on [1]. They can recognize and digest the phosphodiester bond in the nucleic acid sequences with high specificity. Furthermore, they also have been regarded as important targets in the antimicrobial and antiviral drugs development [2]. Researchers have paid more attentions to detect endonuclease activity and discover endonuclease inhibitors. So far, several methods have been developed, including enzyme-linked immunosorbent assay [3], fluorometry [4–8], colorimetry protocols [9–11] and microarray-based resonance light scattering (RLS) assay [12] and so on. Although great progress has been made, it is still in great demand to develop cost-effective, facile and real-time strategy for detecting endonuclease activity and screening endonuclease inhibitors.

Photo-induced electron transfer (PIET) is a process in which the light irradiation causes electron transfer from electron-rich species to electron deficient species [13,14]. Many previous reports had demonstrated that most of fluorescent dyes can be quenched by the deoxyguanosines via PIET mechanism, called usually as G-quenching [13–15]. In the PIET process, fluorophores and guanosine act as electron receptors and electron donor respectively. So far, PIET had been used for detection of metal ion [16,17], amino acid [18], DNAzyme activity [19], screening of telomere-binding ligands [20] and so on. By using deoxyguanosines as an effective quencher to carboxyfluorescein, our previous studies use a hairpin DNA probe to highly specific and sensitive detect lead (II) and high-throughout screen telomere-binding ligands [17,20]. Due to the diversity of nucleic acid and flexibility of conformational switch, it has the great potential to explore G-quenching for achieving cost-effective and specific bioanalysis and biosensing.

Herein, a facile, sensitive and real-time PIET assay was proposed for studying restriction endonuclease activity and inhibition. As a widely studied endonuclease, EcoRI endonuclease was utilized as a model endonuclease to explore the feasibility of PIET strategy. In this work, one singly fluorophore-labeled oligonucleotide (FDNA) hybridizes with label-free complementary oligonucleotide to form double-stranded DNA (dsDNA) substrate of endonuclease.

* Corresponding author.
E-mail address: jinyan@snnu.edu.cn (Y. Jin).

http://dx.doi.org/10.1016/j.snb.2017.06.025
0925-4005/

Three deoxyguanosines at the 5′-termini of cDNA are ingeniously designed as signal transfer element. In the absence of EcoRI endonuclease, the fluorescence of FDNA is quenched by nearby deoxyguanosines. In the presence of EcoRI endonuclease, dsDNA substrate was cleaved into short fragments which cannot form stable dsDNA. So, fluorophore is far away from deoxyguanosines, resulting in a recovery of fluorescence. Therefore, activity and inhibition of endonucleases can be easily assessed based on the change in fluorescence intensity. All reactions are performed in homogeneous solutions, which is beneficial to real-time monitor endonuclease activity. Therefore, it offers a novel way to study activity and inhibition of endonucleases by one-step, which is of great importance in bioanalysis and discovery of endonucleases-targeted antimicrobial and antiviral drugs.

2. Experimental sections

2.1. Chemicals and materials

DNA oligonucleotides used in this work were synthesized and HPLC-purified by Sangon Biotechnology Inc. (Shanghai, China). The sequences are listed as follows: carboxyfluorescein (FAM)-labeled single-stranded DNA (5′-TCACCTAGTC*GAATTC*CC-FAM-3′, FDNA), single-stranded DNA (5′-GG*GAATTC*GACTAGGTGA-3′, cDNA), FAM-labeled random DNA (5′-FAM-CCCTAACCCTAACCCTAA-3′, F-RDNA) and single-stranded DNA (5′-TTAGGGTT AGGGTTAGGG-3′, R-cDNA). EcoRI endonuclease, BamHI endonuclease, T4 polynucleotide kinase (T4 PNK) and bovine serum albumin (BSA) were obtained from Sangon Biotechnology Inc. (Shanghai, China). Nicking endonuclease (Nt.BbvCI), exonuclease I (Exo I) and lambda exonuclease (λ exo) were purchased from New England Biolabs (NEB, U.K.). Lysozyme (Lys) and immunoglobin G (IgG) were purchased from Beijing Dingguo Biotechnology Co., Ltd (Beijing, China). All other chemicals were of analytical grade and used as received without further purification. 50 mM Tris–HCl buffer (50 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$, 0.05 mg/mL BSA, pH 7.5) was employed to prepare the oligonucleotide stock solutions. Ultrapure water (≥18.2 MΩ, Milli-Q, Millipore) was used throughout the work.

2.2. Instrumentation

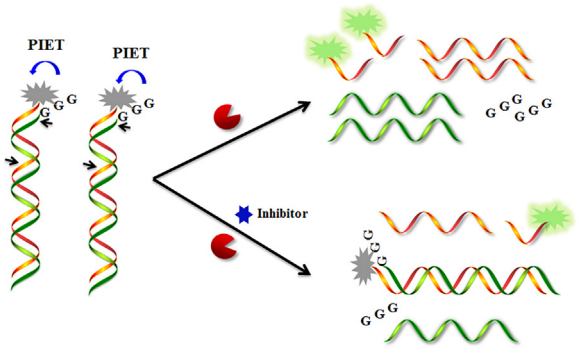
For assay of endonuclease activity and inhibition, the fluorescence spectra were collected on a Hitachi F-7000 fluorescence spectrophotometer (Japan). For real-time monitoring of enzymatic reaction, the fluorescence signal was measured by using a fluorometer (Fluoromax-4, HORIBA Jobin Yvon, Inc., NJ, USA). Gel electrophoresis analysis was performed on the vertical electrophoresis system (Bio-Rad Laboratories, Inc. USA). Photo of gel was taken on the Molecular Imager system (Shanghai Peiqing Science & Technology. Co., Ltd, Shanghai, China) with a Canon Camera.

2.3. Probe preparation

FDNA/cDNA and F-RDNA/R-cDNA mixture were annealed at 90 °C for 5 mins, then slowly cooled to room temperature and incubated overnight at 4 °C.

2.4. Gel electrophoresis

500 nM FDNA/cDNA or F-RDNA/R-cDNA was incubated with 0.5 U/μL EcoRI at 37 °C for 90 mins to perform specific DNA cleavage. A 12.5% native polyacrylamide hydrogel was prepared by utilizing 1 × TBE buffer (100 mM Tris-HCl, 83 mM boric acid, 1 mM EDTA, pH 8.0). The gel electrophoresis was performed at 100 V in TBE buffer for 45 mins followed by silver staining for 30 mins. The visualization and photography of gel were carried out using the Molecular Imager system (Shanghai Peiqing Science & Technology. Co. Ltd., Shanghai, China).



Scheme 1. Schematic illustration of the fluorescence turn-on assay for studying endonuclease activity and inhibition based on photo-induced electron transfer.

2.5. Assay of endonuclease activity and inhibition

To detect EcoRI activity, 100 μL 5 nM FDNA/cDNA duplex incubated with a varying amount of EcoRI at 37 °C for 1 h. Then, fluorescence spectra were recorded by using fluorometer (F-7000, Hitachi) with excitation at 480 nm and an emission range from 500 to 600 nm. Slits for both the excitation and the emission were set at 10 nm for all samples.

To assess the inhibition efficiency of EcoRI inhibitor, 5-fluorouracil was selected as inhibitor and co-incubated with 5 nM FDNA/cDNA for 15 mins at room temperature. Then, 0.005 U/μL EcoRI was introduced and further incubated at 37 °C for 60 mins. The fluorescence measurement was the same as previously mentioned. To perform polyacrylamide gel electrophoresis, the process of sample preparation was the same as previously mentioned. Then, 5 volume DNA samples were mixed 1 volume 6 × loading buffer. Finally, 2 μL samples were added into each gel lane. The remained process was the same as that aforementioned in Gel electrophoresis.

2.6. Real-time monitoring of enzymatic reaction

To real-time monitor interaction between DNA substrate and EcoRI endonuclease, FDNA/cDNA solutions (50 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 0.05 mg/mL BSA, pH 7.5) were preheated for 10 mins at 37 °C. Then, the fluorescence intensities of these solutions at 520 nm were continuously monitored for 180 s. Next, different concentrations of EcoRI were rapidly added into FDNA/cDNA solutions and the fluorescence intensities at 520 nm were continuously monitored until 1200 s. Excitation slit was set at 0.5 nm. Emission slit was set at 5 nm.

3. Results and discussion

3.1. Sensing principle

In this work, a PIET strategy was proposed to real-time study the activity and inhibition of endonucleases. The principle was illustrated in Scheme 1. FAM-labeled FDNA hybridized with its cDNA to form a stable duplex substrate, resulting in effective PIET between FAM and three deoxyguanosines of cDNA. In the presence of EcoRI endonuclease, FDNA/cDNA duplex was specifically cleaved at GAATTC palindrome, making FAM far away from three deoxyguanosines. Therefore, the PIET between FAM and deoxyguanosines no longer exists, leading to fluorescence restora-