Talanta 154 (2016) 455–460

Contents lists available at ScienceDirect

Talanta

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

Proximity hybridization-regulated chemiluminescence resonance energy transfer for homogeneous immunoassay

Mengmeng Liu, Jie Wu*, Kaili Yang, Chen Zong, Jianping Lei, Huangxian Ju

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form
26 January 2016
Accepted 28 January 2016
Available online 29 January 2016

Keywords:
Immunoassay
Proximity hybridization
Chemiluminescence resonance energy transfer
Graphene oxide
CEA

ABSTRACT

Chemiluminescence resonance energy transfer (CRET) and the proximity ligation assay have been widely used in design of sensors for the bioanalysis. Here, a wash-free and homogeneous strategy was proposed to detect carcino-embryonic antigen (CEA) based on proximity hybridization-regulated CRET. The Cy5 demonstrated strong chemiluminescence (CL) via the oxidation of TCPO in the presence of H_2O_2 and energy transfer between excited TCPO and Cy5. Graphene oxide (GO) as an excellent quencher was used to produce the "Signal off" mode that little CL emission was observed through CRET between GO and the Cy5-labelled DNA3. Once CEA was introduced, the target-induced proximity hybridization occurred to form a proximate complex, which inhibited the CRET by preventing GO from absorbing Cy5-labelled DNA3. Furthermore, taking advantage of nicking endonuclease Nt.BbvCI for in situ recycling, the signal could be further amplified for highly sensitive CL detection. Our results showed that this strategy enabled a specific response to CEA with a detection range of 5 orders of magnitude, along with a detection limit of 3.2 pg mL^{-1}. Apart from its easy operation, high sensitivity and acceptable accuracy, the proposed method needed only 0.3 μL of sample, indicating its great opportunity for commercial application.

1. Introduction

The immunoassay is a key technology for clinical diagnostics and environmental testing due to the good specificity [1,2]. Generally, immunoassay methods, including chemiluminescence immunoassays (CLIA) [3,4], enzyme-linked immunosorbent assays (ELISA) [5] and fluoroimmunoassays [6] have been widely used. Combining the specificity of immunoassay and the sensitivity of CL, CLIA possesses have been well established for the quantitation of low concentration analytes in complex samples with the advantages of high sensitivity, high selectivity, low cost and pollution-free [7,8]. For example, our group previously developed a chemiluminescence imaging immunoassay method which could simultaneously and selectively detect 4 tumor markers with the detection limits down to fg mL^{-1} level by using gold nanoparticle-based multienzymatic amplification tags [9]. Traditionally, the analyte molecules in heterogeneous assay formats are accumulated on solid substrates, such as magnetic microparticles and disposable glass chips [10,11], which is time-consuming with washing and separation steps. Thus, no-wash and one-step homogeneous CLIAs are urgently needed for fast and high-throughput detection.

Similar to fluorescence resonance energy transfer (FRET), chemiluminescence resonance energy transfer (CRET) involves non-radiative energy transfer from a chemiluminescent donor to a suitable acceptor molecule [12]. Meanwhile, unlike the FRET, CRET occurs by the oxidation of a luminescent substrate, and then the excited substrate directly transfers the energy to the acceptor without an external excitation source, which avoids photobleaching and autofluorescence [13]. Various nanomaterias have been utilized as the energy acceptor, involving carbon nanoparticles [14–17], quantum dots (QDs) [18,19] and metallic nanomaterials [20–22]. As a single-atom-thick and two-dimensional carbon material, grapheme oxide (GO) has attracted an increasing interest owning to its remarkable characteristics [23]. Moreover, GO could serve as a good energy acceptor due to its excellent quenching efficiency with the long-range nanoscale energy transfer property [24,25]. To the best of our knowledge, there is still no report focusing on the proximity hybridization-regulated GO based CRET for homogeneous detection of protein.

The PLA, which relies on simultaneous recognition of a target molecule by a pair of affinity probes, is a newly developed homogenous DNA-assisted immunoassay [26,27]. Significantly, the bound probes can be functionalized to convert the target event to amplifiable tag sequences for subsequent real-time PCR quantification or localized rolling-circle amplification, which ensures PLA to be one of the most universal and sensitive protein assays [28,29]. In our previous work, several affinity ligand-based

* Corresponding author. Tel./fax: +86 25 83593593.
E-mail address: wujie@nju.edu.cn (J. Wu).

http://dx.doi.org/10.1016/j.talanta.2016.01.060
0039-9140/

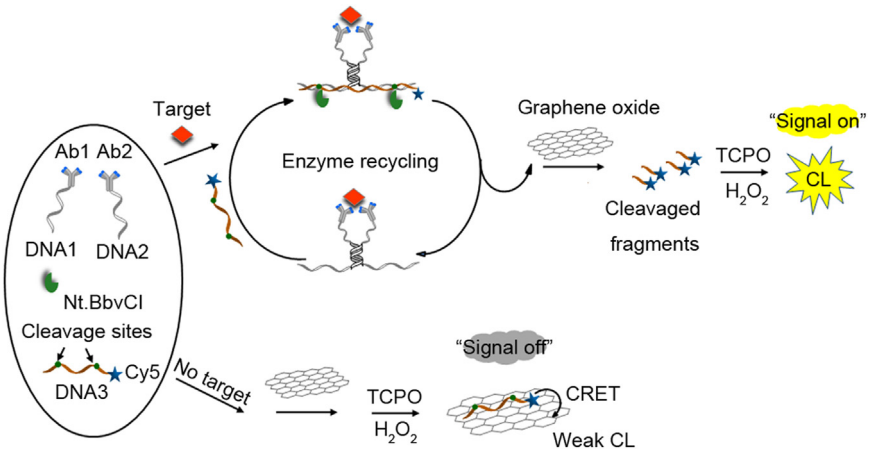

Fig. 1. Schematic illustration of proximity hybridization-regulated CRET for homogeneous detection of CEA.

proximity assay methods have been designed via target-induced DNA assembly and enzymatic amplification cycle for one-step and ultrasensitive detection of protein [30,31]. Yet, these strategies require the DNA to be multi-modified, leading to high-cost as well as complex operation, which makes their application in point-of-care testing remain challenging.

Here, taking advantages of proximity hybridization to regulate the efficiency of CRET, we developed a wash-free and homogeneous immunoassay for CEA detection for the first time (Fig. 1). Firstly, the CL of Cy5 was generated via the oxidation of TCPO in the presence of H_2O_2 and energy transfer between excited TCPO and Cy5. When GO was introduced into this system, the CL of Cy5 attached at the end of single-stranded DNA3 was quenched via CRET. With the adding of target CEA and the antibody-DNA complexes, the target-induced proximity hybridization occurred to form a proximate complex, which inhibited the CRET through desorbing DNA3 from GO. More importantly, a signal amplification strategy was realized by using nicking endonuclease Nt.BbvCI for in situ recycling of the proximity complex. At optimal conditions, this strategy showed a specific response to CEA with a detection range of 5 orders of magnitude, along with a detection limit at pg mL^{-1} level. Our method was successfully applied for determination of CEA levels in serum from cancer patients, and the results were in good agreement with commercial assays.

2. Experimental section

2.1. Materials and reagents

The oligonucleotides were synthesized and HPLC-purified by Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China). The oligonucleotide sequences were shown in Table S1. CEA, anti-CEA antibody (anti-CEA, mouse monoclonal antibodies, clone nos. Z-2011 and Z-2012) and α-fetoprotein (AFP) were purchased from Keybiotech Co. Ltd. (Beijing, China). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-car-boxylate (SMCC) was supplied by HeownsBiochem LLC (China), and dithiothreitol (DTT) was from Sangon Biotechnology Co. Ltd. (Shanghai, China). Nt.BbvCI as well as 10 × NEB 2.1 buffer (pH 7.9 at 25 °C) was obtained from New England BioLabs Inc. (USA). Bis(2,4,6-trichlorophenyl) oxalate (TCPO) was purchased from Tokyo Chemical Industry Co. Ltd. (Japan). GO was obtained from XFNano Materials Tech Co. Ltd. (Nanjing, China). All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all experiments. TE buffer (10 mM, containing 1 mM EDTA and 0.3 M NaCl, pH 7.9) was used as the stock solution for oligonucleotides. PBS1 (55 mM, containing 150 mM NaCl and 20 mM EDTA, pH 7.2) and PBS2 (55 mM, containing 150 mM NaCl and 5 mM EDTA, pH 7.2) were used to prepare DNA-labeled antibodies. NEB buffer 2.1 (1 ×) was used for the homogeneous CL detection of CEA. The clinical serum samples were from Jiangsu Cancer Hospital and stored at −20 °C before use.

2.2. Apparatus

An IFFM-E luminescent analyzer (Remax, Xian, China) was used to collect the CL signal. A F97XP fluorospectophotometer (Lengguang Tech., China) was used to record the FL signal. The gel electrophoresis was performed on the DYCP-31BN electrophoresis analyzer (Liuyi Instrument Company, China) and imaged on a BioradChemDoc XRS (Bio-Rad, USA). The ultraviolet-visible (UV–vis) absorption spectra were recorded with a Nanodrop-2000C UV–vis spectrophotometer (Nanodrop, USA).

2.3. Preparation of DNA-labeled antibody

The DNA-labelled antibody was prepared according to the previous work [32]. 2 mg mL^{-1} Anti-CEA was first reacted with a 20-fold molar excess of SMCC in PBS1 for 2 h at room temperature. At the same time, 12 μL of 100 μM thiolated oligonucleotide (DNA1 or DNA2) was reduced with 16 μL of 100 mM DTT in PBS1 at 37 °C for 1 h. Both products were purified by ultrafiltration (100 KD Millipore for anti-CEA-SMCC and 10 KD Millipore for the reduced oligonucleotide, 10000 rmp, 10 min). After mixing the two reaction products in PBS2 to incubate overnight at 4 °C, the unreacted anti-CEA and DNA were removed by ultra-filtration using a 100 KD millipore (10000 rmp, 10 min) for 3 times, and the obtained Ab-DNA was collected in PBS2.

2.4. Homogeneous CL detection of CEA

The detection was performed by mixing 0.3 μL of 10 μM DNA3 with 1.2 μL of 250 nM Ab1-DNA1 and Ab2-DNA2, 0.25 μL 10000 U mL^{-1} Nt.BbvCI, 0.3 μL of various concentrations of CEA or serum samples and 1 × NEB buffer 2.1 to a total volume of 29.3 μL, followed by a 30 min incubation at 37 °C. Then, 0.7 μL 1 mg mL^{-1} GO solution was added. After 3 min, 20 μL of CL substrate containing 8.5 mM TCPO and 25 mM H_2O_2 was added to record the CL intensity at gain 2 and 950 V.