Talanta 163 (2017) 140–145

Contents lists available at ScienceDirect

Talanta

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

DNA covalently linked to graphene oxide for biotin–streptavidin interaction assay

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ARTICLE INFO

Keywords:
Terminal protection
Small molecule–protein interaction
Graphene oxide
Covalent

ABSTRACT

Based on terminal protection of small molecule-linked DNA and the covalently linking DNA to graphene oxide (GO) strategy, a high resisting nonspecific probe displacement platform for small molecule–protein interaction assay is proposed in this work. Specifically, the small molecule-linked DNA (probe 1) can be protected from exonuclease-catalyzed digestion upon binding to the protein target of the small molecule, so the DNA strand may hybridize with another DNA strand (FAM and amino dual modified DNA, probe 2) that is previously covalently linked onto GO surface. Such hybridization will result in the fluorescence restoration of FAM. Taking biotin–streptavidin (SA) interaction assay as an example in this work, the linearity, stability and specificity of the covalent sensor were systematically studied and compared to the noncovalent sensor. The covalent sensor can determine the protein in a linear range from 0.15 to 12 nM with a detection limit of 0.08 nM, which is comparable with that of noncovalent sensor. Though both sensors have similar sensitivity, the covalent one is more resistant to nonspecific probe displacement by proteins. Furthermore, because the covalent sensor can be used for the assay of biotin–SA interaction in serum samples, this novel method is expected to have great potential applications in the future.

1. Introduction

Molecular diagnostics and therapeutics would greatly benefit from novel techniques for detecting small molecule–protein interactions because of the tremendous importance of small molecules in chemical genetics, molecular diagnostics, and drug development [1–4]. Numerous analytical platforms, such as surface plasmon resonance (SPR) [5,6], affinity chromatography [7], kinetic capillary electrophoresis [8,9], field effect transistors [10], protein-fragment complementation assay [11,12], fluorescence resonant energy transfer [13,14] and fluorescence anisotropy [15], have been established for detecting the small molecule–protein interactions. Although technically endowed with impressive results, these techniques generally require sophisticated instruments, specific labelling or immobilizing reagents, cumbersome assay procedures, limited throughput, and insufficient sensitivity, making them difficult to use widely. To get rid of the drawbacks of the existing methods, recently, Jiang's group has reported that protein binding to small molecules in DNA-small-molecule chimeras could protect the conjugated DNA from degradation by the 3′ single-strand-specific exonuclease I (Exo I) [16]. This finding, which is called terminal protection, represent an ideal option for accessing the field of small molecule-protein interaction [16–21]. In light of the unique characteristics of DNA, such as stability, specificity, site-specific labelling, and sequence coding, the DNA part provides the chimeras with versatile ability for signal transduction. And the protein-binding small molecule moiety offers the capacity of selective capturing the target proteins.

Recently, terminal protection strategies combine with nanomaterials-based biosensors have been developed to detect small molecule–protein interactions to achieve favourable detection limits [22–25]. Among all the nanomaterials, graphene, as a single atom-thick, two-dimensional carbon material, shows remarkable electronic, mechanical, and thermal properties [26,27]. Graphene oxide (GO), a derivative of graphene, can disperse in water on account of hydroxyl and carboxyl groups on its surface [28,29]. Due to its peculiar electronic properties, GO has superior fluorescence quenching ability [30,31]. In addition, GO can adsorb single-stranded DNA (ssDNA) strongly, but hardly interacts with rigid double stranded DNA (dsDNA) or well-folded DNA [32–38]. These properties of GO form a basis for constructing numerous biosensors for a variety of targets detection. Most of these

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http://dx.doi.org/10.1016/j.talanta.2016.10.096
Received 6 August 2016; Received in revised form 25 October 2016; Accepted 27 October 2016
Available online 29 October 2016

sensors were designed using a fluorescent probe DNA which is adsorbed by GO. Addition of its cDNA or target molecule induces probe desorption and fluorescence enhancement. While these physisorption methods are effective and simple, a potential problem is that the adsorbed probe might be displaced by non-target molecules, thus producing false positive signals. This is particularly problematic for serum sample detection. To compensate this problem, an effective method is to covalently link DNA probes on GO. Liu's group systematic researched the method of covalently link a fluorophore and amino dual modified DNA on GO [39–43]. Many target analytes have been successfully detected in complex sample matrixes, including complementary nucleic acids [39], small molecules [42] and metal ions [41]. As the covalent probe is highly resistant to nonspecific probe displacement. Thus it holds considerable potential for molecular detection in serum and cellular samples where high probe stability is demanded.

The results mentioned above are an excellent motivation for designing a covalent probe on GO for small molecule–protein interaction assay in serum samples. Herein, we covalently link a fluorophore and amino dual modified DNA on GO, combing with terminal protection strategy, a high resisting nonspecific probe displacement platform for small molecule–protein interaction assay is proposed in this work. Detection of small molecule–protein interaction assay in serum sample is demonstrated. Besides, we systematically compare this covalent sensor with noncovalent sensor in terms of small molecule–protein interaction assay.

2. Experimental

2.1. Reagents

The oligonucleotides probe 1, probe 2, probe 3, target DNA, and cDNA were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences are as follows: 5′-TCTTCACAGATGCGT-biotin-3′ (probe 1), 5′-FAM-ACGCATCTGTGAAGAGAACCTGGG-NH2-3′ (probe 2), 5′-FAM-ACGCATCTGTGAAGAGAACCTGGG- 3′ (probe 3), 5′-TCTTCACAGATGCGT-3′ (target DNA), and 5′-CCCAGGTTCTCTTCACAGATGCGT-3′ (cDNA). Graphene oxide (GO) was purchased from Sinocarbon Materials Technology Co., Ltd. (China). Exonuclease I (Exo I) was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimidehydrochloride (EDC.HCl), tris(hydroxylmethyl) aminomethane (Tris), 4-morpholineethanesulfonate (MES), and 2-[4-(2-hydroxyethyl)−1-piperazinyl]ethanesulfonicacid (HEPES) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA), lysozyme (Lyz), hemoglobin (HGB), cytochrome C (Cyto C) streptavidin (SA), were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The buffer solutions used were as follows: MES buffer consisted of 25 mM MES, pH 6.0; buffer A consisted of 25 mM HEPES, pH 7.6, 150 mM NaCl, and 1 mM $MgCl_2$; Tris buffer consisted of 5 mM Tris, pH 9.0; Exo I buffer consisted of 67 mM glycine–KOH (pH 9.5), 1 mM dithiothreitol (DTT), and 6.7 mM $MgCl_2$, and the buffer B consisted of 20 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 50 mM NaCl. Milli-Q purified water was used to prepare all the solutions.

2.2. Apparatus

Fluorescent emission spectra were performed on Varian cary eclipse fluorescence spectrophotometer, Varian Medical Systems, Inc. (Palo Alto, American). The sample cell is a 700-μL quartz cuvette. The luminescence intensity was monitored by exciting the sample at 480 nm and measuring the emission at 520 nm. The fitting of the experimental data was accomplished using the software Origin 8.0.

2.3. Covalent attaching DNA to GO

Amino and FAM (6-carboxyfluorescein) dual labeled DNA (probe 2) was covalently attached to GO via an amide linkage. The conjugation reaction was carried out for 3 h at room temperature under magnetic stirring in a glass vial with a final volume of 500 μL containing 100 μg/mL GO, 2 μM probe 2, 10 mM EDC·HCl (freshly prepared), 25 mM NaCl, and 25 mM MES (pH 6.0). The GO/probe 2 complex was purified by centrifugation at 15,000 rpm for 15 min followed by removing the supernatant. To remove noncovalently attached DNA, the sample was washed with 80% isopropanol followed by dispersing the sample in 5 mM pH 9.5 Tris. This procedure was repeated two more times. The sample was then dispersed in buffer A, containing 4 μm cDNA to fully desorb physisorbed DNA probes. After 1 h of incubation, the sample was centrifuged and washed. To remove the cDNA, the sample was soaked in 12 M urea and heated at 70 °C for 10 min twice. Finally, the sample was washed by water, dispersed in buffer A, and stored at 4 °C. To calculate the coupling efficiency, the fluorescence intensity of the supernatant of each washing step was measured in pH 8.5 Tris buffer (15 mM) and the fluorescence was compared against a standard curve.

To prepare the physisorbed sensor (noncovalent sensor), probe 3 (2 μM) and GO (100 μg/mL) were mixed in buffer A for 30 min at room temperature. The GO-probe 3 complex was purified by centrifugation at 15,000 rpm for 15 min followed by removing the supernatant. The noncovalent sensor was dispersed in buffer A and stored at 4 °C with a final GO concentration of 100 μg/mL.

2.4. Optimization of the concentration of Exo I

To optimize the concentration of Exo I, 5 μL probe 1 (10 μM) and 0, 2.5, 5, 7.5 and 10 U Exo I solution were mixed and incubated for 30 min at 37 °C with gentle shaking. Then 20 μL GO-DNA was added. The mixed solution was diluted with buffer B to 100 μL. Finally, the above prepared solution was incubated 60 min at room temperature. The fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

2.5. Optimization of the reaction time between probe 1 and Exo I

To optimize the reaction time between probe 1 and Exo I, probe 1 (5 μL, 10 μM) and Exo I (7.5 U) solutions were mixed. This solution was incubated for 0, 5, 10, 20, 30, 40, and 60 min at 37 °C with gentle shaking. Then 20 μL GO-DNA was added. The mixed solution was diluted with buffer B to 100 μL. Finally, the above prepared solution was incubated 60 min at room temperature. The fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

2.6. Performance of biotin-SA interaction assay

For quantitative measurement of biotin-SA interaction, a fixed concentration of probe 1 (500 nM) was treated with different concentrations of SA and shaken gently for 30 min at 37 °C in buffer B. After that, Exo I (7.5 U) was added to the solution, and the mixed solution was incubated for 30 min at 37 °C. Subsequently, 20 μL GO-DNA was added. The mixed solution was diluted with buffer B to 100 μL. Finally, the above prepared solution was incubated 60 min at room temperature. The fluorescence intensity of the incubated solution was measured at 520 nm with excitation at 480 nm.

3. Results and discussion

3.1. Covalent sensor preparation

In this work, to avoid nonspecific displacement of the probe by nontarget molecules, a covalent linkage was established using an amino and FAM (carboxyfluorescein) dual labeled single-stranded DNA (probe 2) to react with the carboxyl group on GO in the presence of EDC. Since not all the DNA molecules were covalently attached, the