Biosensors and Bioelectronics 63 (2015) 178–184

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Nicking enzyme and graphene oxide-based dual signal amplification for ultrasensitive aptamer-based fluorescence polarization assays

Yong Huang [a,b,*], Xiaoqian Liu [a,b], Liangliang Zhang [a], Kun Hu [a], Shulin Zhao [a,b,*], Baizong Fang [b], Zhen-Feng Chen [a], Hong Liang [a,b,*]

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

ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form
16 July 2014
Accepted 17 July 2014
Available online 22 July 2014

Keywords:
Nicking enzyme
Graphene oxide
Fluorescence polarization
Aptasensors
Amplified analysis

ABSTRACT

In this work, two different configurations for novel amplified fluorescence polarization (FP) aptasensors based on nicking enzyme signal amplification (NESA) and graphene oxide (GO) enhancement have been developed for ultrasensitive and selective detection of biomolecules in homogeneous solution. One approach involves the aptamer-target binding induced the stable hybridization between an aptamer probe and a fluorophore-labeled DNA probe linked to GO, and forms a nicking site-containing duplex DNA region due to the enhancement of base stacking. The second analytical method involves the target induced the assembly of two aptamer subunits into an aptamer-target complex, and then hybridizes with a fluorophore-labeled DNA probe linked to GO, forming a nicking site-containing duplex DNA region. The formation of the duplex DNA region in both methods triggers the NESA process, resulting in the release of many short DNA fragments carrying the fluorophore from GO, generating a significant decrease of the FP value that provides the readout signal for the amplified sensing process. By using the NESA coupled GO enhancement path, the sensitivity of the developed aptasensors can be significantly improved by four orders of magnitude over traditional aptamer-based homogeneous assays. Moreover, these aptasensors also exhibit high specificity for target molecules, which are capable of detecting target molecule in biological samples. Considering these qualities, the proposed FP aptasensors based NESA and GO enhancement can be expected to provide an ultrasensitive platform for amplified analysis of target molecules.

1. Introduction

The development of aptasensors has attracted substantial research efforts, and a variety of techniques have been exploited to develop aptasensors (Citartan et al., 2012; Liu et al. 2009; Tan et al., 2013) for detection of various targets. Among these methods, fluorescence polarization (FP) analysis offers an appealing approach for the detection of aptamer substrates due to its simplicity and rapidness (Deng et al., 2006, 2007; Zhang et al., 2011a). Many FP aptasensors have been developed for the detection of proteins (Zhang et al., 2011b, 2012a; Zou et al., 2012), small molecules (Perrier et al., 2010; Ruta et al., 2009; Zhang et al., 2012b; Zhao et al., 2014), and cancer cells (Deng et al., 2010). However, as with other signaling transduction approaches, the development of FP aptasensors is often accompanied by unsatisfactory sensitivities of the systems that are controlled by relatively low association constants of aptamers to their substrates. Several amplification strategies to improve the sensitivities of FP aptasensors have been developed. These include the use of gold nanoparticles (Ye and Yin, 2008), silica nanoparticles (Huang et al., 2012), graphene oxide (Liu et al., 2013a, 2013b; Yu et al., 2013), proteins (Cui et al., 2012; Zhu et al., 2012), and DNA-protein hybrid nanowires (Yang et al., 2013) as FP enhancers. Although enhanced sensitivities were demonstrated by these amplification strategies, greater sensitivity and specificity are frequently required, particularly when working with limited amounts of sample material or when target level is extremely low.

Nicking enzymes (NEs) are a special group of restriction endonucleases that can cleave one strand of a duplex DNA. This function has been used to develop different amplified NEs-based detection platforms that involve cyclic NEs-catalyzed cleavage of DNA by target recognition. For example, the cyclic NEs-catalyzed

* Corresponding authors at: Guangxi Normal University, School of Chemistry and Pharmacy, Yucai Road 15, Guilin 541004, China.
Tel.: +86 773 5845973; fax: +86 773 5832294.
E-mail addresses: huangyong_2009@163.com (Y. Huang), zhaoshulin001@163.com (S. Zhao), hliang@gxnu.edu.cn (H. Liang).

http://dx.doi.org/10.1016/j.bios.2014.07.036
0956-5663/

cleavage of specific DNA by nucleic acids was used to amplify nucleic acid detection (Bi et al., 2010; Kong et al., 2011; Liu et al., 2011; Lin et al., 2011; Zou et al., 2011; Yin et al., 2013). Also, the NEs-catalyzed recycling of aptamer-substrate complexes was used for the amplified fluorescence and colorimetric sensing of biomolecules (Huang et al., 2013; Hun et al., 2013; Li et al., 2012; Xue et al., 2012; Zheng et al., 2012). In addition, NEs were also used as biocatalytic amplifiers for monitoring enzyme activities (Chen et al., 2013; Liu et al., 2014). However, to the best of our knowledge, such NEs-assisted signal amplification systems have not been investigated in any attempts for their use in a FP biosensor.

Graphene oxide (GO) has found versatile applications in biological studies due to its unique optoelectronic properties and excellent biocompatibility (Chen et al., 2010a, 2010b; Loh et al., 2010). Specifically, GO has been regarded as a kind of superquencher that can effectively quench the fluorescence of a range of dyes (Chang et al. 2010; Lu et al., 2010a, 2010b). Based on this superquenching effect, GO has been employed to develop nanosensors for sensitive detection of nucleic acids (Dong et al., 2010; He et al., 2010; Liu et al., 2013a, 2013b; Lu et al., 2009; Zhang et al., 2013), aptamer substrates (Hu et al., 2013; Li et al., 2013; Lu et al., 2010a, 2010b; Wang et al., 2013; Zhang et al., 2011c), and enzyme activities (Li et al., 2011; Zhang et al., 2011d; Zhu et al., 2013), etc. Besides this property, recent studies suggest that GO can be used as an effective FP enhancer due to its extraordinarily larger volume. For example, Yang's group first reported the use of GO as an enhancer for the development of FP aptasensors for sensitive detection of ATP (Liu et al., 2013a, 2013b). Similarly, a GO-enhanced FP strategy was used for DNAzyme-based assay of metal ions by Huang and co-workers (Yu et al., 2013). However, exploration of GO with FP analysis still remains at a very early stage.

In this work, we report a new strategy for the development of amplified FP aptasensors for ultrahighly sensitive and selective detection of target molecules in homogeneous solution based on nicking enzyme signal amplification (NESA) and GO enhancement. Compared with traditional homogeneous aptasensors, the detection sensitivity of the developed aptasensors can be significantly improved by four orders of magnitude by using the NESA coupled GO enhancement approach. Moreover, the proposed aptamer-based sensing assays are conducted in aqueous solution, and not requiring separation and other troublesome procedures, which is very simple and convenient. With the use of adenosine (A) and thrombin (Tb) as model analytes, these new sensing platforms exhibit very high detection sensitivity, high specificity and wide dynamic ranges over six orders of magnitude. Furthermore, the suitability of the proposed method for biological sample analysis has also been demonstrated.

2. Experimental

2.1. Materials and reagents

Adenine (A), thymine (T), guanine (G), cytosine (C), thrombin (Tb), human serum albumin (HSA), human immunoglobulin G (IgG), human immunoglobulin E (IgE), thrombin (Tb), and graphene oxide (GO) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Nicking endonuclease Nb.BbvCI and human factor Xa were obtained from New England Biolabs (NEB, U.K.). All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. The sequences of the involved oligonucleotides were as follows:

A aptamer (AAA): 5′-GGGACCTCAGC***ACCTGGGGGAGTTGCGGAGGAAGGT***-3′

Tb aptamer subunit-1 (ATA-1):5′-GGGACCTCAGCGC***AGTCCGTGGTAGGGC***-3′

Tb aptamer subunit-2 (ATA-2): 5′-***TAGGTTGGGGTGACT***GC-3′

DNA-1: 5′-FAM-GC↓TGAGGATTTTTTTTTTT-3′

DNA-2: 5′-FAM-GC↓TGAGGTTTTTTTTTTTT-3′

DNA-3: 5′-FAM-GC↓TGAGGTCTTTTTTTTTT-3′

DNA-4: 5′-FAM-GC↓TGAGGTCCTTTTTTTTT-3′

The italic bold sequences of the above oligonucleotides probes were the aptamer nucleotides, and the underlined sequences of AAA and ATA-1 were partly complementary to DNA-1, DNA-2, DNA-3, and DNA-4, respectively. The arrow indicated the nicking position of Nb.BbvCI. Other chemicals were of analytical grade. Water was purified by using a Milli-Q plus 185 equip from Millipore (Bedford, MA).

2.2. Apparatus

Fluorescence polarization (FP) measurements were carried out using an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA) with 300 μL cuvette. The FP of the sample solution was monitored by exciting the sample at 490 nm and measuring the emission at 520 nm. And slits for both the excitation and the emission were set at 5 nm.

2.3. Adenosine assay with FP aptasensors

In a typical adenosine (A) assay, the AAA probe (20 nM) and the DNA-2 probe (85 nM) were incubated with GO (100 μg/mL) in 240 μL Tris–HCl buffer (20 mM Tris–HCl, 50 mM NaCl, 10 mM Mg^{2+}, and 50 mM K^{+}, pH 7.9) for 1 min at room temperature. After that, 10 μL the above Tris–HCl buffer containing Nb.BbvCI (0.5 U/μL) and different concentrations of A was added, and incubated the mixture at 37 °C for another 1 h. The obtained sample solution was used for FP measurements. Control experiments were performed under otherwise identical conditions but in the absence of GO or Nb.BbvCI, or in the absence of both GO and Nb.BbvCI. All experiments were repeated three times. FP of the sample solutions was measured by using the L-format configuration and FluorEssence™ software with constant wavelength analysis to achieve a FP value. The G factor was initially set to zero, to let the system measure G automatically. The FP value was also calculated automatically by the instrument. The integration time was set to 3 s for the FP measurements. Over five FP measurements were taken each time, and they were then averaged for further data processing.

2.4. Thrombin assay with FP aptasensors

In a typical thrombin (Tb) assay, the ATA-1 probe (20 nM), the ATA-2 probe (20 nM), and the DNA-2 probe (85 nM) were incubated with GO (100 μg/mL) in 240 μL Tris–HCl buffer (20 mM Tris–HCl, 50 mM NaCl, 10 mM Mg^{2+}, and 50 mM K^{+}, pH 7.9) for 1 min at room temperature. Afterward, 10 μL the Tris–HCl buffer (20 mM Tris–HCl, 50 mM NaCl, 10 mM Mg^{2+}, and 50 mM K^{+}, pH 7.9) containing Nb.BbvCI (0.5 U/μL) and different concentrations of Tb was added, and incubated the mixture at 37 °C for another 1 h. The resulting sample solution was used for FP measurements. Control experiments were performed under otherwise identical conditions but in the absence of GO or Nb.BbvCI, or in the absence of both GO and Nb.BbvCI. All experiments were repeated three times. The procedure for FP measurements was the same as that of A detection described above.