Colloids and Surfaces B: Biointerfaces 140 (2016) 233–238

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Detection of adenosine triphosphate in HeLa cell using capillary electrophoresis-laser induced fluorescence detection based on aptamer and graphene oxide

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ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form
16 December 2015
Accepted 21 December 2015
Available online 28 December 2015

Keywords:
Graphene oxide
Aptamer
Capillary electrophoresis
Adenosine triphosphate

ABSTRACT

A method for ATP quantification based on dye-labeled aptamer/graphene oxide (aptamer/GO) using capillary electrophoresis-laser induced fluorescence (CE-LIF) detecting technique has been established. In this method, the carboxyfluorescein (FAM)-labelled ATP aptamers were adsorbed onto the surface of GO, leading to the fluorescence quenching of FAM; after the incubation with a limited amount of ATP, stronger affinity between ATP aptamer and ATP resulted in the desorption of aptamers and the fluorescence restoration of FAM. Then, aptamer-ATP complex and excess of aptamer/GO and GO were separated and quantified by CE-LIF detection. It was shown that a linear relation was existing in the CE-LIF peak intensity of aptamer-ATP and ATP concentration in range of 10–700 μM, the regression equation was $F = 1.50 + 0.0470C_{(ATP)}$ ($R^2 = 0.990$), and the limit of detection was 1.28 μM ($3S/N$, $n = 5$), which was one order magnitude lower than that of detection in solution by fluorescence method. The approach with excellent specificity and reproducibility has been successfully applied to detecting concentration of ATP in HeLa cell.

1. Introduction

Adenosine triphosphate (ATP) is an important energy currency in cell regulating various biological processes [1]. The variation of intracellular ATP concentration usually relates to cell living status. Thus, accurate and selective quantification of ATP concentration in cell is highly significant. Up to now, many methods have been developed to determine ATP, such as colorimetric [2], florescence [3], chemiluminescence [4], electrochemistry [5], high performance liquid chromatography [6] etc.

Since the discovery of aptamer in 1990 [7], it has been extensively studied and attracted great attention as newly emerging molecular recognition element [8]. Aptamers are single-stranded nucleic acids screened from random-sequence DNA or RNA libraries by an in vitro selection process called the systematic evolution of ligands by exponential enrichment (SELEX). Each of them has the ability to bind specifically to corresponding target, such as inorganic ion [9], small organic molecule [10], protein [11], cell [12] and microorganism [13]. Compared with antibodies, merits of aptamers, in terms of easily and repeatedly chemical synthesis, excellent temperature stability, lower molecular weights and high affinity, make them remarkable molecular receptors for sensing applications. For example, He et al. designed a dual-hairpin structure consisting of ATP aptamer and partly complementary ssDNA, and detected ATP through the change of electrochemical signal [14]. Ju's team developed an off-on electrochemiluminescence strategy for detecting ATP through the recognition of aptamers [15].

Recently, owing to uniquely structural, electrical and mechanical characteristics, carbon nanomaterials have been combined with specific molecular recognition elements, such as aptamers, to create new probes for life science. Carbon nanomaterials include carbon nanotube, graphene, graphene oxide, carbon dot and C_{60} [16–20]. Among them, graphene oxide (GO) which is the oxidative product of graphene, has excellent electronic, mechanical and

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http://dx.doi.org/10.1016/j.colsurfb.2015.12.043
0927-7765/

thermal properties similar to graphene; more importantly, GO is water-soluble for its oxygen-containing groups (as hydroxyl, carbonyl and epoxy group). It has been reported that GO could quench the emission of adjacent fluorophore through fluorescence resonance energy transfer (FRET) [21]. Taking advantage of this extraordinarily high fluorescence quenching efficiency, GO has been applied to biochemical analysis. For example, Chen et al. developed a biosensor for simultaneous determination of Human Enterovirus 71 and Coxsackievirus B3 according to antigen-antibody reaction hindering the FRET from the antibodies-associated quantum dots to GO [22].

It has been proved that GO can strongly adsorb single-stranded DNA (ssDNA) via a π-stacking interaction between the ring structures in nucleobases and the hexagonal cells of GO, while it rarely adsorbs double-stranded DNA (dsDNA) or a folded DNA structure [23]. Taking advantage of these properties of GO, aptamer/GO probes have been widely used for the detection of biomolecules [24,25]. The basic mechanism is, fluorescent aptamers can be adsorbed and quenched by GO, while binding with target molecules, the fluorescent aptamers can be released with the fluorescence restoration.

However, one question cannot be ignored in above works which were measured in a cuvette with fluorometer. That is the fluorescence of aptamer-target complexes are still affected by the quenching effect of GO existing in the same solution. As Dong et al. reported, more than 97.6% fluorescence of quantum dot-labelled molecular beacon (QD-MB) could be quenched by addition of GO; when the QD-MB was hybridized with target sequence in high concentration to form dsDNA, fluorescence recovery efficiency could only reach to 38.7% of original intensity [26], which could be as a result of the strong quenching effect by surrounding GO and the low hybridizing efficiency of target with the probe [27]. Thus, for a certain sensing platform, sensitivity of aptamer/GO based analysis will be adversely affected by the strong quenching effect of GO existing in same solution. For a small amount of recovered fluorescence of probe induced by trace target can hardly be detected as a result of re-quenching effect of GO.

The problem will be solved if fluorescent aptamers can be separated from system before detection. For example, Liu's group reported the immobilization of a fluorescent aptamer sensor on magnetic microparticles and the use of flow cytometry for separate detection of adenosine in serum [28]. Separation and detection methods not only can decrease the effect by quencher but also can minimize interference from other molecules in sample. Herein, we adopted capillary electrophoresis (CE), with merits of high separation efficiency, short analysis time and small sample consumption, which is extremely useful for biological samples analysis [29–32].

In presented work, we combined a sensitive and selective aptamer/GO system and a capillary electrophoresis-laser induced fluorescence (CE-LIF) detector to determine ATP concentration in HeLa cell. By capillary electrophoresis, the released fluorophore could be isolated from GO and other molecules in HeLa sample so that the recovered fluorescence would no longer be quenched and interfered by GO and other molecules. Therefore, the sensitivity and accuracy of detection were improved. Besides, small sample consumption also reduced using amounts of all kinds of reagents and lowered the detecting cost. This work expanded a new direction in biological analysis using CE.

2. Experimental section

2.1. Apparatus

CE-LIF analyses were carried out on a home-built system, consisting of a high voltage supply (0–30 kV) (Shanghai Nuclear Research Institute, China) and a IX71 inverted fluorescence microscope (Olympus, Japan) equipped with a 488 nm argon ion excitation laser (Melles Griot, USA), a dichromatic mirror (DM 510, Semrock, USA), an emission filter (LP 500, Semrock, USA) and a QE65000 fiber-optic spectrometer (Ocean Optics, USA). Uncoated 75 μm I.D. of fused-silica capillary (Yongnian Optical Fiber Factory, China) with 30 cm effective separation length (50 cm total) was used.

Ultrapure water (≥18.2 MΩ cm^{-1}) was obtained from a Rephile system (China). Transmission electron microscope (Tecnai G2 20 U-Twin, FEI, USA), FTIR spectrometer (VERTEX 70, Bruker, Germany), Zetasizer nano (ZS 90, Malvern, U.K.) and scanning probe microscope (NanoScope MultiMode, Veeco, USA) were used for characterization of GO. Fluorescence spectrophotometer (LS-55, Perkin-Elmer, USA) was applied to the measurements made in a cuvette. HeLa cells were broken through an ultrusonic cell disrupter system (ScienTZ, China).

2.2. Materials and solutions

The carboxyfluorescein (FAM)-labelled ATP aptamer with a sequence of 5′-FAM-AACCTGGGGGAGTATTGCGGAGGAAGGT-3′, and the FAM-DNA used for non-specific effect experiment with a sequence of 5′-FAM-TCCTCCTCCTTCACACGGAAC-3′ were both synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). ATP, guanosine 5′-triphosphate (GTP), cytosine 5′-triphosphate (CTP), uridine 5′-triphosphate (UTP) were also purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Tris base was obtained from The Dow Chemical Co., (USA). ATP detection kits were purchased from Nanjing Jiancheng Bioengineering Inst. (Nanjing, China). All other materials were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

CE buffer is 20 mM $Na_2B_4O_7$ solution (pH 9.2). Binding buffer consisted of 20 mM Tris–HCl (pH 7.4), 100 mM NaCl, 25 mM KCl and 5 mM $MgCl_2$. The 10 mM PBS (pH 7.4) used in experiment was prepared with 0.80 g NaCl, 0.020 g KCl, 0.29 g $Na_2HPO_4{\cdot}12H_2O$ and 0.025 g KH_2PO_4. FAM-aptamer stocking solution (20 μM) was prepared by PBS and reserved in refrigerator at 4 °C.

2.3. Synthesis of graphene oxide nano sheets (GO-nS)

GO was synthesized from graphitic powder according to modified Hummer's method [33]. Graphite powder (0.75 g) was put into an 80 °C solution consisting of concentrated H_2SO_4 (4.5 mL), $K_2S_2O_8$ (1.125 g) and P_2O_5 (1.125 g) and reacted for 5 h. The mixture was cooled down to room temperature and diluted with deionized water (100 mL) overnight. Then, the product was obtained by filtering using 0.2 micron Nylon film and dried naturally. Pretreated graphite powder was put into 0 °C concentrated H_2SO_4 (60 mL). Successively, $KMnO_4$ (10 g) was added gradually under string, while keeping the temperature less than 15 °C. The mixture was stirred at 38 °C for 5 h and diluted with deionized water (200 mL) by keeping the temperature under 50 °C. The reaction was ended by addition of 20 mL H_2O_2 (30%). At the end, the mixture was repeated washed with 1:10 (v/v) HCl aqueous solution (375 mL), and then deionized water. Exfoliation was carried out by sonicating graphene oxide (2 mg mL^{-1}) under ice bath for 2 h. The resulted product was taken for future experiments.

2.4. CE-LIF procedure

New capillary was treated with 1 M NaOH, 1 M HCl, water, and CE buffer each for 20 min. Between each run, the capillary was flushed with water (3 min) and CE buffer (5 min). When finished all measurement, the capillary was flushed with 0.1 M HCl and H_2O for