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Carcino-embryonic antigen detection based on fluorescence resonance energy transfer between quantum dots and graphene oxide

Zi-Ming Zhou [a], Jun Zhou [b], Jun Chen [a], Rong-Na Yu [a], Ming-Zhen Zhang [a], Ji-Tao Song [a], Yuan-Di Zhao [a,*]

[a] Britton Chance Center for Biomedical Photonics at Wuhan National Laboratory for Optoelectronics—Hubei Bioinformatics & Molecular Imaging Key Laboratory, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China

[b] Department of Clinical Laboratory, Wuhan Commercial Staff Hospital, Wuhan 430021, PR China

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ABSTRACT

The mixture of graphene oxide (GO) and aptamer labeled fluorophore is widely used in developing fluorescent sensors for the analysis of biomolecules, according to the light signal 'off–on' procedure. Moreover, the laser-induced fluorescence-coupled affinity probe capillary electrophoresis (APCE) technique has been broadly applied for the separation of micromolecules. Here, a strategy is proposed for analysis of content of carcino-embryonic antigen (CEA) based on the combination of GO and quantum dots labeling aptamer (QD–aptamer) by capillary electrophoresis (CE). The method has three advantages: (i) combined with CE, only few samples are required and efficiency of separation is high, (ii) fluorescent detection can be carried out after separation of GO and fluorescence probe combined with targets by CE, while fluorescence detection sensitivity had been greatly improved, and (iii) the issues of APCE, including the effect of excess fluorescence probe and maximizing separation between analytes, could be solved by introducing GO. It has been proved that QD–aptamer–CEA complex can completely dissociate from GO. Results show that the fluorescence intensity has a linear relationship with the concentration of CEA in the range from 0.257 to 12.9 ng/mL, and the limit of detection is approximately 5 pg/mL ($S/N=3$). The proposed method with high specificity has been applied for the accurate analysis of content of CEA in patient's serum.

1. Introduction

Graphene oxide (GO) has quickly sparked tremendous interest in materials science because of distinctive characteristic in the physicochemical and structural properties (Feng et al., 2013; Liu and Zhao et al., 2012). Many oxygen-containing functional groups, such as hydroxyl group, epoxide group, and carbonyl group, are attached on the surface of GO, and make it soluble in water (Ramesha et al., 2011; Ding et al., 2012). It has been found that this kind of water-soluble nano-material has a vast potential for future biochemical application (Lu et al., 2010; Wang et al., 2012). For example, it was indicated that the fluorescence could be quenched by effective fluorescence resonance energy transfer (FRET) between GO and fluorophores (Mohanty and Berry, 2008; Lu et al., 2009; Zhang et al., 2011).

Recently, some reports indicated that single-stranded nucleic acid could adsorb strongly on GO by π-stacking interaction between carbon cycle and nucleic acid chain, but duplex DNA or aptamer combined with targets could not bind to GO stably (Liu and Dong et al., 2012; Wang et al., 2011). Therefore, the single-stranded nucleic acid could be dissociated from GO as a result of competition in the presence of its complementary chain or target (Dong et al., 2010; Chang et al., 2010; He et al., 2011; Wang et al., 2010; Liu and Aizen et al., 2012). These phenomena inspired the design of novel fluorescent sensors. The basic strategy was that single-stranded DNA conjugated with fluorophore was stacked onto GO firstly, and then the fluorescence was quenched by FRET. The FRET would be inhibited in the presence of complementary chain or target and then the fluorescence was restored. According to this light signal 'off–on' switch, the combination of GO and fluorophore labeling aptamer was used to develop fluorescent sensors for biomolecules, like triphosadenine (ATP) (Chang et al., 2010; He et al., 2011), thrombin (Wang et al., 2010), and inorganic micromolecules, Pb^{2+} (Li et al., 2013), Ag^{+} (Wen et al., 2010), etc.

* Corresponding author. Tel.: +86 27 8779 2235; fax: +86 27 8779 2202.
E-mail address: zydi@mail.hust.edu.cn (Y.-D. Zhao).

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Up till now, fluorescence recovery amounts resulting from targets have been collected in the solutions containing GO in almost all existing reports (Wang et al., 2010; Liu and Aizen et al., 2012; Li et al., 2013; Wen et al., 2010). However, there is an insurmountable obstacle, which is that no matter whether fluorophore is attached on GO or dispersed freely in solution, its fluorescence has been consumingly quenched by GO, and the fluorescence quenching efficiency is very high. Ju's group reported when QD-molecular beacon was added in the GO solution, the fluorescence quenching efficiency was 97.6%, and only 39.7% of the original fluorescence could be maintained after addition of excess target DNA (Dong et al., 2010). Meanwhile, the decrease was 80% even if only free QDs were added in GO solution, indicating the fluorescence quenching efficiency was still very strong by GO to free the fluorescent molecules. This phenomenon is terrible for the analysis of content of target molecules by fluorescence recovery amounts, because little fluorescence recovery amount resulting from low content will be quenched easily by GO, which has a great influence on the limit of detection. Therefore, it could not be ignored by this negative effect.

As a separating technique, the laser-induced fluorescence-coupled affinity probe capillary electrophoresis (APCE) has been broadly used in protein- or aptamer-based assay methods in a variety of application fields, such as biochemical and environmental areas by virtue of its simple and rapid operation, little dosage of samples, and high efficiency of separation (German et al., 1998; Zhang et al., 2008). However, the issues involved in the aptamer-based assay methods include the effect of excess fluorescence probe and maximizing separation between analytes (Zhu et al., 2010; Jong and Krylov, 2011). In this context, if the fluorescent probe (aptamer labeled fluorophore as usual) is initially quenched, the issues can be solved.

Compared with organic fluorophore, QDs have unique optical characteristics, including good chemical stability, broad absorption and narrow emission wavelengths, which made it widely used in fluorescence probe and medical imaging (Huang et al., 2007; Chan et al., 1998; Wu et al., 2012). Specially, it could be used easily to improve limit of detection for biosensor based on FRET (Bogomolova and Aldissi, 2011). QDs are also used as probe for target detection combined with GO, aptamer or molecular beacon (Wang et al., 2010; Li et al., 2013).

Carcino-embryonic antigen (CEA) is one of the most widely used cell-surface tumor markers. According to its content, CEA can indicate a variety of tumors and estimate curative effect, patient's condition, and prognosis for some cancers (Denk et al., 1972; Boehm et al., 1996; Sun et al., 2013). Therefore, it is important to analyze the content of CEA. At present, the aptamer that can recognize CEA specially has been screened out (Tabar et al., 2010), and applied to analyze the content of CEA (Lin et al., 2012). In this paper, a strategy is proposed for analysis of content of CEA based on the combination of GO and aptamer labeled QDs by CE. It had been proved that high sensitivity and specificity had been obtained and accurate analysis of content of CEA in serums of patients had been achieved by the proposed method. It was a meaningful technique for fluorescence analysis of biomolecules in biosensing field.

2. Materials and methods

2.1. Chemicals and materials

Bis(trimethylsilyl) sulfide ($(TMS)_2S$), hexadecylamine (HAD, 90%), tri-n-octylphosphine (TOP, 90%), tri-n-octylphosphine oxide (TOPO, 90%), glutathione (GSH), fluorescein isothiocyanate (FITC, 98%), and thrombin were obtained from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). 1-Ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC · HCl, 98.5%), N-hydroxy succinimide sulfur generation (Sulfo-NHS) were purchased from Aladdin Chemistry (Shanghai) Co., Ltd. (Shanghai, China). Ultrafilter was supplied by Millipore (Bedford, MA, America). CEA was purchased from Meridian Life Science, Inc. (Memphis, USA). Human immune globulin (IgG, 99%), human immunoglobulin (HSA, 99%) were obtained from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). CEA aptamer, the base sequences as follow: 5′-NH_2-$(CH_2)_6$-ATACCAGCT-TATTCAATT-3′, was synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai. China), and then diluted to 100 μM by PBS (0.01 M, pH 7.4). Fused-silica capillary (75 μm of inner diameter) was supplied by Yongnian Optical Fiber Factory (Hebei, China). All other materials and reagents were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water ($\geq$ 18.2 MΩ) from a Milli-Q system (Millipore, Bedford, MA, USA) was used for all solutions. The synthesis of graphene oxide was referred from other works and details are provided in the Supporting information. Water-soluble CdSe/ZnS core–shell QDs were synthesized and characterized according to previous reports, and detailed in the Supporting information.

2.2. Apparatus

The absorption spectra were measured by UV–vis spectrophotometer (UV-2550, Shimadzu, Japan) and the fluorescence spectra were recorded by a luminescence spectrometer (LS-55, Perkin-Elmer, USA). Atomic force microscopy (AFM) measurements were carried out on scanning probe microscope (NanoScope MultiMode, Veeco, USA). High-resolution transmission electron microscopy (HRTEM) images were obtained with a transmission electron microscope (Tecnai G^2 20 U-Twin, FEI, USA). The Fourier transform infrared spectroscopy (FTIR) spectra were recorded by a FTIR spectrometer (VERTEX 70, Bruker, Germany) and the Raman spectra were obtained with a fluorescence spectrum tester (HR800UV, JY Horiba, France). The size and zeta-potential were measured on a ZS90 nanosizer (Malvern, UK) at 25 °C by the dynamic light scattering (DLS) and laser doppler electrophoresis methods, respectively. A chemiluminescent immunoassay system (LIASON, DiaSorin S.p.A, Italy) with standard CEA kit (DiaSorin S.p.A, Italy) was used to standardize the concentrations of CEA in patient's sample.

2.3. Preparation of CdSe/ZnS and aptamer (QD–aptamer) probe

Water-soluble CdSe/ZnS QDs (30 μL) were added into EDC solution (30 μL, 5 mg/mL) followed by the addition of Sulfo-NHS (20 μL, 1.5 mg/mL), and the solution was continuously stirred for 0.5 h. After that, CEA aptamer (50 μL, 100 μM) solution was added and the mixture was whirled. Following the reaction for 2 h, the solution was centrifuged using an ultrafilter at 10,000 rpm for 20 min to remove excess aptamer. Finally, the obtained residue was dissolved in PBS buffer (100 μL) for future experiments. The characterization of QD–aptamer is shown in the Supporting information.

2.4. Preparation of GO/QD–aptamer biosensor for detection of CEA

QD–aptamer (5 μL) was added into the GO solution (5 μL) and then whirled. After reaction for 4 h, the GO/QD–aptamer complex was obtained. The final concentration of GO was 45 μg/mL. Then, a range from 0 to 256 ng/mL of CEA was added and continuously stirred for 4 h. The measurements for final solutions were performed by CE at 25 °C. CE analyses were carried out on a home-built system followed by previous report (Li et al., 2011), and details are provided in the Supporting information.