Biosensors and Bioelectronics 85 (2016) 8–15

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A sensitive electrochemiluminescent aptasensor based on perylene derivatives as a novel co-reaction accelerator for signal amplification

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ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form
14 April 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Perylene derivatives
Co-reaction accelerator
Graphene-CdTe
Electrochemiluminescence
Thrombin

ABSTRACT

Herein, a novel signal amplification strategy was designed using the perylene derivative as the co-reaction accelerator toward graphene-CdTe quantum dots (G-CdTe)/$S_2O_8^{2-}$ system to construct a highly sensitive electrochemiluminescent (ECL) aptasensor for thrombin (TB) detection. Firstly, the G-CdTe nanocomposites were prepared by one-step method of *in situ* generating CdTe quantum dots onto the surface of the graphene oxide by using 3-mercaptopropionic acid as the CdTe QDs stabilizer. Then, a kind of perylene derivative (PTC-Lys), was synthesized by covalently binding L-lysine to 3,4,9,10-perylenetetracarboxylic acid, which was further immobilized onto the G-CdTe by the π-π* stacking and cross-linked the detection thrombin aptamer (TBA II) to obtain the TBA II/PTC-Lys/G-CdTe signal probes. It is worth pointing out that PTC-Lys acting as an efficient co-reaction accelerator interacted with the co-reactant of $S_2O_8^{2-}$ rather than G-CdTe to promote the more oxidant mediators of $SO_4^{\bullet -}$, which could further react with G-CdTe to produce excited state species G-CdTe* for emitting light. Compared with the G-CdTe/$S_2O_8^{2-}$ ECL system, our proposed strategy with the introduction of co-reaction accelerator of PTC-Lys exhibited ultra-high sensitivity to quantify the concentration of TB from 1.0×10^{-7} nM to 10 nM with a detection limit of 34 aM.

1. Introduction

As a class of organic π-conjugated materials with a perylene aromatic core, perylene derivatives, such as 3,4,9,10-perylenetetracarboxylic acid (PTCA) and perylenetetracarboxylic diimide (PDI), have been widely utilized in fluorescent sensors (Wang et al., 2014b; Lv et al., 2015), electrophotographic devices (Feng et al., 2013) and organic solar cells (Vercelli and Zotti, 2012) due to their strong absorption and fluorescence, electroactive and photoactive properties (Krieg and Rybtchinski, 2011; Zhan et al., 2015). Recently, perylene derivatives have been expanded to apply in electrochemical or electrochemiluminescence (ECL) sensors fields (Niu et al., 2013; Gao et al., 2014; Wang et al., 2014a) owing to their large specific surface area, good membrane-forming property, and low manufacturing cost. Zhu's group has reported that PTCA with pyrenyl group and carboxyl groups was regarded as a cross-linking reagent to achieve the β-cyclodextrin non-covalently functionalized single-walled carbon nanotubes for highly selective electrochemical detection of 9-anthracenecarboxylic acid and achieved a sensitivity of 0.65 nM (Zhu et al., 2012). Xiao et al. has also prepared an ECL immunosensor based on PTCA linked ruthenium(II) complex on the graphene sheets with a limit of detection of 0.2 pg · mL^{-1} (Xiao et al., 2014). In our previous work, we have developed electrochemical biosensors using hemin or o-phenylenediamine functionalized perylene derivative as electrochemical redox probes to detect thrombin (TB) with detection limit of 0.001 nM and 0.05 pM, respectively (Yuan et al., 2012; Chang et al., 2015). Later, we found the perylene derivative of PTCA/thiosemicarbazide could act as a co-reactant to significantly promote the ECL response of the peroxydisulfate ($S_2O_8^{2-}$)/O_2 system and achieved a sensitivity of 3.3 fM for TB (Ma et al., 2015a), and the amino-terminated perylene derivative could also be explored as a high-efficiency ECL luminophor for specific detection of Pb^{2+} with a limit of detection of 0.35 pM (Lei et al., 2015). Therefore, it is meaningful to expand the new function of perylene derivatives for significantly amplifying ECL signal in the application of biosensors.

With controllable size and emission wavelength, high photoluminescence yield, and good chemical stability (Hesari et al., 2015; Zhou et al., 2015), quantum dots (QDs) have been widely used as a kind of ECL emitters for biomarkers analysis (Lei and Ju, 2011). Previous studies have demonstrated that the immobilization of QDs was the most important step in solid-state QDs based-ECL biosensors

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http://dx.doi.org/10.1016/j.bios.2016.04.088
0956-5663/

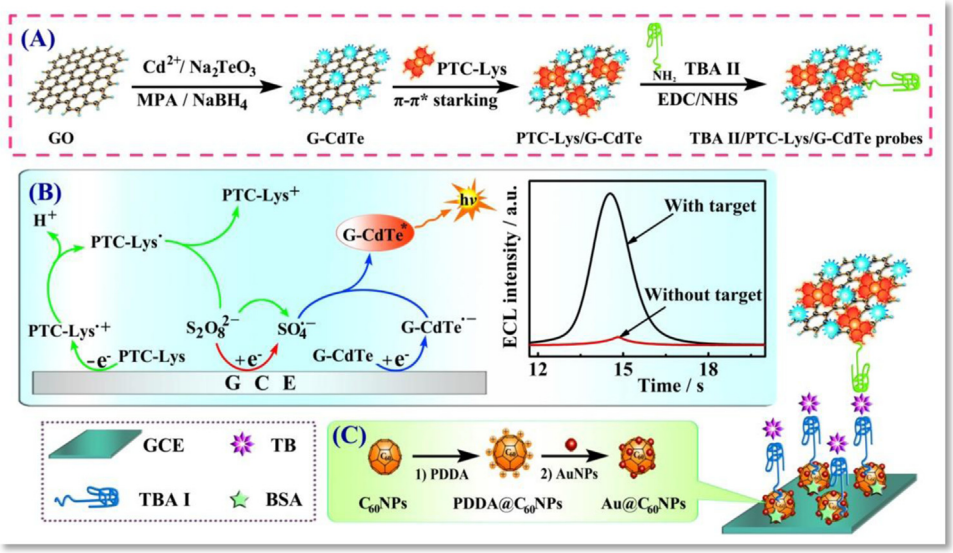
fabrication (Algar et al., 2010). Generally, the QDs were immobilized by entrapping QDs in some membrane-forming materials, such as nafion (Dai et al., 2012) or chitosan (Liu et al., 2015). Nevertheless, the QDs thin-film prepared by the entrapment method would suffer from the swelling property and steric hindrance effect of the membrane-forming materials, which was difficult to obtain a stable and strong ECL signal (Petryayeva and Krull, 2012). Recently, a promising approach to solve these problems was developed by the introduction of a matrix for QDs immobilization to obtain QDs nanocomposites with enhanced ECL intensity and stability (Divsar and Ju, 2011; Wang et al., 2012; Huang et al., 2015). Due to the remarkable conductivity, high electron transfer rate, and large specific surface area, graphene was a desirable matrix to anchor QDs for developing ECL biosensor (Deng et al., 2011). Li' group has reported a simple method to immobilize the CdSe QDs onto the surface of poly(diallyldimethylammonium chloride)-protected graphene (PDDA-G) through electrostatic interaction (Li et al., 2011). Subsequently, Yu et al. (2012) reported a mild one-step process for synthesis of graphene-CdSe nanocomposites using aminoethanethiol as the QDs stabilizer and the linker of graphene and CdSe QDs. Thus, one-step synthesis of graphene-CdTe (G-CdTe) nanocomposites with use of 3-mercaptopropionic acid (MPA) as the CdTe QDs stabilizer was developed in our work and then G-CdTe nanocomposites were employed as an ECL emitter with stable and strong ECL response. Moreover, the L-lysine covalently binded PTCA (PTC-Lys), as a kind of perylene derivatives, was further loaded on the surface of the G-CdTe nanocomposites *via* $\pi-\pi^*$ stacking for the bio-recognition element immobilization to achieve a multifunctional signal probe. Herein, the PTC-Lys could not only provide the carboxy group to immobilize bio-recognition element, but also exhibit a new function as a co-reaction accelerator to promote the ECL response. Specifically, the co-reaction accelerator was a type of substance which could react with the co-reaction reagent rather than the luminophor to amplify the ECL intensity (Ma et al., 2015b). In this work, PTC-Lys could accelerate the reduction of $S_2O_8^{2-}$ to produce the more oxidant mediators of $SO_4^{\bullet -}$, which further enhanced production of excited states of G-CdTe to emit light. Thus the ECL luminous efficiency of G-CdTe/$S_2O_8^{2-}$ system was significantly promoted.

In this study, a sandwich-type ECL aptasensor for TB detection was designed by utilizing PTC-Lys functionalized G-CdTe nanocomposites as signal probe. Briefly, the G-CdTe nanocomposites were synthesized *via* one-step method of *in situ* reduced CdTe QDs onto the surface of graphene by using MPA as the CdTe QDs stabilizer. Then the G-CdTe nanocomposites were used as nanocarriers for PTC-Lys loading to obtain the carboxyl groups functionalized PTC-Lys/G-CdTe nanocomposites, which were further used to immobilize amido-terminated detection thrombin aptamer (TBA II) with the aid of EDC and NHS as coupling agents to form the multifunctional signal probes of TBA II/PTC-Lys/G-CdTe. Besides, AuNPs functionalized C_{60} nanoparticles (Au@C_{60}NPs) nanocomposites were applied to construct a functional interface of the electrode for the thiol-terminated capture thrombin aptamer (TBA I) immobilization. When the developed biosensor was successively incubated with TB and the TBA II/PTC-Lys/G-CdTe probes, a considerably enhancement of ECL signal was obtained in the $S_2O_8^{2-}$ solution. The schematic diagram of the stepwise assembly procedure of the aptasensor and the ECL detection principle was shown in Scheme 1.

2. Experiment

2.1. Reagents and material

Cadmium chloride hemipentahydrate ($CdCl_2 \cdot 2.5H_2O$) and sodium tellurite (IV) (Na_2TeO_3) were purchased from Alfa Aesar Chemical Co. (Tianjin, China). Graphene oxide (GO) and fullerene (C_{60}, 99.5%) were purchased from Pioneer Nanotechnology Co. (Nanjing, China). Thrombin (TB), hemoglobin (HB), gold chloride ($HAuCl_4 \cdot 4H_2O$), bovine serum albumin (BSA, 96～99%), 3-mercaptopropionic acid (MPA), poly-(diallyldimethylammonium chloride) (PDDA, 20%, *w*/*w* in deionized water) and human serum albumin (HSA, ≥ 96%) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). α-1-fetoprotein (AFP) was obtained from Biocell Co. (Zhengzhou, China). 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA) was gotten from Lian Gang Dyestuff Chemical Industry Co. (Liaoning, China). Trisodium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2\,H_2O$) was gotten from Chongqing Chuandong Chemical Co. (Chongqing, China). N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimidehydrochloride (EDC) were purchased from Shanghai Medpep Co. (Shanghai, China). Sodium persulfate ($Na_2S_2O_8$) was purchased from Chengdu Chemical Reagent Co. (Chengdu, China). L-lysine (Lys) was obtained from shanghai regal biotech Technology, Inc. (Shanghai, China).



Scheme 1. The schematic diagrams of the proposed ECL aptasensor. (A) The preparation of TBA II/PTC-Lys/G-CdTe probes. (B) The possible ECL mechanism of the PTC-Lys as co-reaction accelerator in G-CdTe/$S_2O_8^{2-}$ system for TB detection. (C) The preparation of Au@C_{60}NPs nanocomposites.