Biosensors and Bioelectronics 104 (2018) 65–71

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A novel electrochemiluminescent biosensor based on resonance energy transfer between poly(9,9-di-*n*-octylfluorenyl-2,7-diyl) and 3,4,9,10-perylenetetracar-boxylic acid for insulin detection

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ARTICLE INFO

Keywords:
Insulin
Electrochemiluminescence
Biosensor
PFO
PTCA

ABSTRACT

An electrochemiluminescencent (ECL) biosensor was designed for the determination of insulin using a novel ECL resonance energy transfer (ECL-RET) strategy. In this strategy, carboxyl poly(9,9-dioctyfluorenyl-2,7-diyl) dots (PFO dots) were worked as ECL donor and 3,4,9,10-perylenetetracar-boxylic acid (PTCA) exploited as ECL acceptor, and hydrogen peroxide (H_2O_2) employed as the coreactant. The ECL donor and ECL acceptor were separately labeled with primary antibody (Ab_1) and secondary antibody (Ab_2), forming a sensing interface to the analyte target, insulin. In this expected sandwich-type ECL biosensor, PFO dots acted as sensing platform and PTCA employed as labels to quench the ECL emission of PFO dots. During the determination process, ECL signal of PFO dots was decreased in a gradual way by the increase of insulin concentration, and the quenching mechanism was also investigated. Under the optimal experimental conditions, the constructed biosensor exhibited an excellent performance, including a wide linear range from 1.0×10^{-5} ng/mL to 1.0×10^{2} ng/mL, low detection limit of 3.0×10^{-6} ng/mL, good stability and selectivity for the detection of insulin. This pair of PFO-PTCA, as a new donor-acceptor pair in ECL-RET system, would provide a promising platform for bioanalysis in ECL field.

1. Introduction

Insulin is a peptide hormone, which is produced by beta cells of the pancreatic islets (Arvinte et al., 2010). As one of the important anabolic hormone of human body, insulin can stimulate the glucose in the blood circulation into the liver cells, muscle cells, adipocytes and other tissue cells, thus regulating the metabolism of carbohydrates, fats and protein (Schirhagl et al., 2012; Xiong et al., 2015; Yu et al., 2016). Insulin hormone controls the glucose level in blood within a narrow concentration range and its disorder causes severe illness and dysfunctions in humans which called diabetes mellitus (Abdollah et al., 2007). The sensitive and fast detection of insulin is of great importance because it not only served as a predictor of diabetes of insulinoma and trauma (Melloul et al., 2002) but also was used as a doping drug in competitive sports for cheating (Schirhagl et al., 2012). There have been massive methods employed for the insulin analysis, such as immunoassays (Neal and Han, 2008), high performance liquid chromatography (HPLC) (Mercolini et al., 2008), surface plasmon resonance (Frasconi et al., 2010), electrochemistry (Wang and Li, 2009) and flow injection analysis (Salimi et al., 2009). Among these reported methods, electrochemiluminescence (ECL), as a novel method integrated electrochemistry and luminescence, has arisen growing attention in recent years (Gao et al., 2016). It has been used in DNA, protein and biomolecules analysis and detection due to its high sensitivity, good facility, wide dynamic range, spatial and temporal control and simple instrumentation (Zhang et al., 2017). Thus, it is worth using this kind of excellent potential method for insulin studying.

Due to high sensitivity, non-background unselective photoexcitation and non-interference scattered light, ECL resonance energy transfer (ECL-RET) has drawn wide concern in biosensor field (He et al., 2013; Wu et al., 2014; Dong et al., 2014; Liu et al., 2015). The precondition of ECL-RET is the good spectra overlaps between the energy donors' ECL spectra and the energy acceptors' Uv–vis absorption spectra (Rajapakse et al., 2010; Zhu et al., 2017). While the ECL-RET has been applied into

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https://doi.org/10.1016/j.bios.2017.12.046
Received 24 September 2017; Received in revised form 20 December 2017; Accepted 26 December 2017
Available online 27 December 2017
0956-5663/

the test of protein (Ji et al., 2014), DNA (Jie et al., 2015) and some ions (Lei et al., 2015), there are still some challenges for searching the corresponding donor-acceptor pair. One of the big challenges is the lack of donor-acceptor system with good spectrum overlap (Ma et al., 2016). Among the reported ECL-RET system, the donor-acceptor pairs are often confined to the following categories, such as quantum dots-Ru $(bpy)_3^{2+}$ (Wu et al., 2012), quantum dots-metal nanoparticles (Zhang et al., 2015), quantum dots-luminol (Zhao et al., 2015), graphene carbonitride (g-C_3N_4)-luminol (Wang et al., 2016) or g-C_3N_4-$Ru(bpy)_3^{2+}$ (Feng et al., 2016a, 2016b). Above donor-acceptor pairs exhibited disadvantages more or less. For example, the quantum dots are commonly poisonous and bad biocompatibility, and the price of $Ru(bpy)_3^{2+}$ is expensive. So, it's significant to exploit efficient, nontoxic and oversimplified ECL-RET system with more types of donor-acceptor collocations.

Conjugated polymers have seen an ongoing interest due to their high fluorescence brightness and excellent photostability, and several views and perspectives about conjugated polymers have been reported in past decades (Wu et al., 2007; Wu and Chiu, 2013). Barbara and his co-workers investigated the ECL behavior of PFBT CPNs for the first time (Chang et al., 2008). Chi and co-workers studied the MEH-PPV CPNs' ECL performance in aqueous solution (Dai et al., 2015). Cheng's group prepared a silole-containing polymer dot, and studied its ECL properties with the coreactant TPrA (Feng et al., 2016a, 2016b). And for our group, we mainly studied poly(9,9-dioctyfluorenyl-2,7-diyl) (PFO). As one of the conjugated polymers, PFO has attracted considerable interest in optoelectronic device, ECL sensors, and other biological labels due to its pleased characters such as high electroluminescence quantum yields with bule light emission, high charge carrier and chemical stability (Zhang et al., 2017). In our previous work, the anodic ECL behavior of PFO was explored in the presence of coreactant $Na_2C_2O_4$. And following we found that H_2O_2 can serve as coreactant to greatly amplify the ECL signal of PFO, thus constructed an oxidase-based biosensor using PFO-H_2O_2 system (Zhang et al., 2017; Chen et al., 2016). We also constructed a ratiometric sensing for detecting organophosphorus pesticides based on carboxyl-PFO dots as anodic luminophore and reduced graphene oxide-CdTe quantum dots as cathodic luminophore (Chen et al., 2017a, 2017b). And all these sensors exhibited a good stability, high sensitivity and excellent selectivity. However, these researches were not mentioning the contents of energy transfer of PFO, and even the application of PFO as energy donor or acceptor. Consequently, investigating the energy transfer performance of PFO was valuable and of great significance since it can extend the application of PFO material in ECL analytical field. Furthermore, it can also expand the range of ECL-RET system, making an addition of energy donor and acceptor pair.

3,4,9,10-perylenetetracar-boxylic acid (PTCA) is the hydrolyzate of perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA). The ECL properties of perylene derivatives have been studied in recent years in our group. It can be exploited as cathodic luminescent material when worked with the co-reactant $S_2O_8^{2-}$ (Lei et al., 2016), or it worked as the accelerator of coreaction reagent and fixed stabilizer for luminous agent, thus presenting excellent ECL performance (Zhao et al., 2016). However, scanty studies of PTCA was related to the ECL-RET capability. Our group constructed a ratiometric aptasensor based on the energy transfer from peroxydisulfate/oxygen (O_2/$S_2O_8^{2-}$) to amino-terminated perylene derivative (PTC-NH_2). It showed that perylene derivatives such as PTCA may be well employed in ECL-RET system.

Encouraged by the excellent performance of above materials, an ECL biosensor was developed for insulin detection based on the energy transfer from PFO (donor) to PTCA (acceptor). In the present work, PFO dots were connected with the primary of insulin antibody (Ab_1) through the amide bond to synthesize the Ab_1-PFO biocomposites, which then could interact with insulin antigen via specific recognition between antibody and antigen. When the biosensor was modified with PTCA labeled secondary antibody (Ab_2-PTCA), the ECL-RET strategy for detecting insulin was completely achieved. Based on the RET from PFO to PTCA, a series quantitative detection of insulin could be well obtained. In this strategy, a new donor-acceptor pair was investigated, and the absorption spectra of PTCA showed great overlaps with the ECL spectra of synthesized PFO dots. Furthermore, the ECL acceptor PTCA exhibited excellent quenching capability to the ECL donor PFO dots. This new pair of donor-acceptor would extend the ECL-RET system and present their admirable potentiality in further ECL analysis.

2. Experiment

2.1. Reagents and materials

PFO (MW 20000) was provided by ADS Dyes, Inc. (Quebec, Canada). Insulin and insulin antibodies were purchased from Zhengzhou Humanwell Biocell Biotechnology Co. Ltd (Zhengzhou, China). Sigma Chemical Co. (St. Louis, MO, USA) provided the functional copolymer poly(styrene-*co*-maleicanhydride) (PSMA) with MW ~ 1700 and styrene content 68%. N-hydroxysulfosuccinimide (NHS), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. (Shanghai, China). PTCDA and H_2O_2 were provided by Lian Gang Dyestuff Chemical Industry Co. Ltd. (Liaoning, China) and Aladdin Ltd. (Shanghai, China), respectively. Na_2HPO_4 (0.10 M) and KH_2PO_4 (0.10 M) were used to prepare phosphate-buffered saline (PBS, 0.10 M) solutions with various pH. Human serum samples were provided by the 9 th People's Hospital of Chongqing.

2.2. Apparatus

The model MPI-A electro-chemiluminescence analyzer, which was provided by Xi'an Remax Electronic Science & Technology Co. Ltd. (Xi'an, China), was used to perform the ECL measurements. The voltage of photomultiplier tube (PMT) was set at 800 V. The CHI600D electrochemical work station was from Shanghai CH Instruments Co. China, and was used to perform cyclic voltammetry (CV) measurements. Both ECL and CV measurements were carried out using a three-electrode system, which contained a working electrode (modified glassy carbon electrode), a reference electrode (saturated calomel electrode) and a counter electrode (Pt wire). UV–vis absorption spectra and Fourier-transform infrared spectroscopy (FT-IR) were recorded at a UV-2450 UV–VIS Spectrophotometer from Shimadzu Corp. (Tokyo, Japan) and a Nexus 670 FTIR spectrophotometer from Nicolet Instruments (Nicolet Instruments, Tokyo), respectively. The scanning electron microscope (Hitachi, Japan) was used to record scanning electron micrographs (SEM), and the Transmission electron microscopy (TEM) images were obtained from a Hitachi H-800 microscope (Japan).

2.3. Preparation of PFO dots and Ab_1-PFO biocomposite

The carboxyl conjugated PFO dots were obtained according to the literature (Li et al., 2013). First, tetrahydrofuran (THF) was used to dissolve PFO and PSMA to obtain two polymer solutions, respectively. Then, two polymer solutions were blended and diluted with THF. In resultant mixture solution, the concentrations of PFO and PSMA were 50 μg/mL and 10 μg/mL, respectively. Afterwards, under the ultrasonic condition, 1 mL of the mixture solution was added into 1 mL of deionized water. By vacuum evaporation THF was well removed, and after that the carboxyl conjugated PFO dots solution was obtained. Then, insulin Ab_1 was bond to carboxyl group functionalized PFO dots by an EDC/NHS (molar ratio 4:1) coupling protocol and stirred for 6 h, followed by centrifuging to remove unreacted NHS and EDC. 100 μg/mL insulin Ab_1 was mixed with the formed solution and stirred at 4 °C for 24 h. After centrifuging, the resulting Ab_1-PFO was redispersed in pH 7.4 PBS. The obtained dispersion was stored in a refrigerator (4 °C) for further use.