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Multiple signal amplified electrochemiluminescent immunoassay for brombuterol detection using gold nanoparticles and polyamidoamine dendrimers-silver nanoribbon

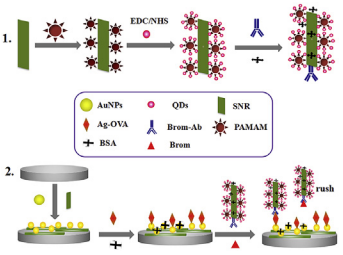
Tiantian Dong, Liuyi Hu, Kang Zhao, Anping Deng**, Jianguo Li*

The Key Lab of Health Chemistry & Molecular Diagnosis of Suzhou, College of Chemistry, Chemical Engineering & Materials Science, Soochow University, Suzhou 215123, China

HIGHLIGHTS

- A multiple signal amplification ECL immunosensor of eco-friendly CdSe QDs for brombuterol determination was developed.
- Besides substrates, AuNPs and PAMAM-SNR were creatively used to accelerate the electron transport between electrode and QDs.
- SNR-PAMAM with numerous amino groups also could be employed to bond abundant actived QDs to amplify ECL signal.
- Competitive immunoassay was performed with ECL to detect small molecules of brombuterol.
- It provided a method for detecting Brom and enlarged the usage of QDs, AuNPs and SNR-PAMAM in ECL biosensing.

GRAPHICAL ABSTRACT



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ABSTRACT

Electrochemiluminescent (ECL) immunosensor with multiple signal amplification was designed based on gold nanoparticles (AuNPs), polyamidoamine dendrimers (PAMAM) and silver-cysteine hybrid nanoribbon (SNR). Low toxic L-cysteine capped CdSe QDs was chosen as the ECL signal probe. To verify the proposed ultrasensitive ECL immunosensor for β-adrenergic agonists (β-AA), we detected Brombuterol (Brom) as a proof-of-principle analyte. Therein, AuNPs as the substrate can simplify the experiment process, accelerate the electron transfer rate, and carry more coating antigen (Ag-OVA) to enlarge ECL signal. On one hand, SNR on the surface of electrode can avoid the aggregation of AuNPs, and SNR-PAMAM-AuNPs also can be acted as a good accelerator for electron transfer. On the other hand, PAMAM (16 -NH_2) functionalized SNR (SNR-PAMAM) with numerous amino groups could be employed to bond abundant actived QDs to further amplify ECL signal. The new immunosensor can offer a simple, reliable, rapid, and selective detection for Brom, which have a dynamic range of 0.005–700 ng mL^{-1} with

* Corresponding author.
** Corresponding author.
E-mail addresses: denganping@suda.edu.cn (A. Deng), lijgsd@suda.edu.cn (J. Li).

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a low detection limit at 1.5 pg mL^{-1}. The proposed biosensor will extend the application of nanomaterials in ECL immunoassays and open a new road for the detection of Brom and other β-AA in the future.

1. Introduction

β-adrenergic agonists (β-AA) with perfect nutrition redistribution function have been used for the livestock industry such as ruminants and pigs to reduce adipose tissue accretion and increase muscle mass while enhancement of feed conversion and growth rate [1–3]. β-AA with stable properties can be easily deposited in human body after meat consumption, which triggered off the symptoms of serious health problems such as muscular pain, dizziness cardiac and palpitation [4]. Although the EU and China have listed the β-AA as directory of prohibited drugs for animals feed, β-AA was still used as feed additives by some unscrupulous businessmen for economic interests [5]. In order to elude the official controls, a number of new compounds have been developed from β-AA such as Brombuterol (Brom). Brom (4-amino-3,5-dibromo-α[(tert.-butyl-amino) methyl] benzyl alcohol) is certainly known a representative of the class of aryl amine β-AA [6]. Brom have received extensive attention for their illegally used, however, only few studies specialized on Brom were in the scientific literature. Therefore the effective means for detecting Brom or other β-AA residues in food and feed are badly in need of improvement.

Presently, various analytical methods of β-AA and related compounds have been developed in an effort to combat the illegal usage. These methods include high performance liquid chromatography (HPLC) [7], ultra-high performance liquid chromatography hyphenated to tandem mass spectrometry (UHPLC/MS/MS) [8], a liquid chromatography-mass spectrometry (LC-MS) [9,10], enzyme-linked immunosorbent assay (ELISA) [6,11], gas chromatography-mass (GC-MS) [6], etc. There are some disadvantages of the above methods, such as sample treatment for a long time, cumbersome detection process and difficult to operate, expensive instruments and phenomenon of false positives, which limit their practical applications. In this paper, electrochemiluminescent immunoassay (ECLIA) prepared by competition immune as an effective method has been used for detecting Brom.

ECL involved the generation of species via electron red-ox processes of substance on the surfaces of electrode, undergone electron-transfer reactions to form excited states and then caused emission of light [12,13]. Since the first putforward ECL studies, the ECL with many distinct characteristics (such as no radioisotopes useing, temporal and spatial controlling, rapid measurement and far low detection limits of label) has attracted much interest [14–16]. ECLIA as a powerful bioanalysis technique is composed of ECL and immunoassay with values of specific identification, stability, simplified optical setup, high sensitivity, wide dynamic range and weak background signal [13]. The ECLIA methods developed mainly on two mechanisms: consumption of ECL coreactant in reaction and steric hindrance produced from the formation of immunocomplex [17]. In this experiment, consumption of CdSe QD based immunosense (ECL coreactant) resulted from an immune reaction, which could be generated by changing the combination of the CdSe QDs-labeled antibody and coating antigen (Ag-OVA) through varying Brom standard solution.

In recent years, there are some novel labeling agents (semiconductor nanocrystals [18], metallic oxide semiconductors [19] and carbon nanocrystals [20], etc.) and immobilization support (grapheme [21], carbon nanotubes [22], ZnO nanotube arrays [23], etc.) for ECL immunosensors. Moreover, low toxic CdSe QDs for eco-friendly alternatives QD-ECL probes with great interest was prepared in aqueous phase L-cysteine as the stabilizer [24]. L-cysteine can avoid the aggregation of quantum dots, as well as it possesses a favorable biocompatibility, which would be as a good ECL luminophore for further extending the analytical performance of CdSe QD-based ECL biosensors.

According to its particularity properties of nanomaterials and some unique physicochemical property in catalytic chemistry, electronics, energy and biological fields, noble metal nanomaterial act as an important part of metal nanomaterial had widely application prospects and caused more and more attention of scientists. Gold and silver nanomaterials with abundant electrical and optical properties, good stability and low biological toxicity also have attributed widespread concern in the fields of physics, chemistry, materials, etc [25–28]. Due to their stability, high specific surface area, and biocompatibility, AuNPs have attracted tremendous attention in the area of applications like catalysis and bioanalysis [29]. In this work, AuNPs with negatively charged in weakly alkaline conditions can be combined with positive charge on the protein molecule groups by means of the electrostatic interactions, which do not affect the biological activity of the protein. Polyamidoamine dendrimers (PAMAM) with high geometric symmetry, precise molecular structure, a large number of functional groups, good biocompatibility, molecular memory in the cavity and the molecular chain growth controlled properties has attracted extensive attention in sensor fields. Since the amino groups possessed a high reaction activity and easily modified could be employed to bond abundant actived QDs to further amplify ECL signal. We synthesized silver-cysteine hybrid nanoribbon (SNR) by a simple and green one-step aqueous chemical synthetic strategy [30,31]. Firstly, the prepared SNR are monodispersed nanoribbon with diameter of 500 nm and length of 3 μm, have abundant carboxyl groups (-COOH) on their surfaces and fabricate amplified SNR-PAMAM-QDs ECL signal probe [32]. We have put forward a SNR amplified QDs competitive immunoassay for sensitive detection of Brom for the first time. Then, AuNPs were decorated on the surface of the SNR-PAMAM via Au-N bonds [33]. The formed AuNPs-PAMAM-SNR composite possessed larger specific surface area, which could effectively immobilize the abundant Ag-OVA to enhance the ECL signal. On one hand, SNR avoided the aggregation of AuNPs on electrode, and on the other hand, the excellent electric conductivity made AuNPs-PAMAM-SNR on the surface of GCE act as good accelerators for improving the electrochemical reaction efficiency of QDs and $K_2S_2O_8$. Therefore, PAMAM (16 -NH_2) functionalized SNR (SNR-PAMAM) with numerous amino groups would exhibit potential applications in immunosensors.

Herein, taking advantages of AuNPs, SNR-PAMAM and biocompatibility of CdSe QDs, we design a new multiple amplified ECL immunosensor, where Brom is detected as target, the SNR-PAMAM-AuNPs-Ag-OVA as substrate and Brom-Ab-SNR-PAMAM-CdSe QDs act as the ECL signal probes. The standard solutions of Brom compete with quantitative Brom Ag-OVA for limited specific binding sites of the Brom-Ab-SNR-PAMAM-CdSe QDs to form an immunocomplex. When Brom increased, the obtained Brom-Ab-SNR-PAMAM-CdSe QDs with competition bounding to the electrode surface was reduced, which result ECL signal decreased.