Electrochimica Acta 271 (2018) 173–179

Contents lists available at ScienceDirect

Electrochimica Acta

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

A sensitive ratiometric electrochemiluminescence biosensor for hypoxanthine detection by in situ generation and consumption of coreactants

Fumei Zuo [a], Han Zhang [a], Ji Xie [b], Shihong Chen [a, *], Ruo Yuan [a]

[a] Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] Chongqing No. 8 Secondary School, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 1 January 2018
Received in revised form
27 February 2018
Accepted 21 March 2018
Available online 23 March 2018

Keywords:
Ratiometric electrochemiluminescence
Hypoxanthine
Enzyme
CdTe quantum dots
Luminol

ABSTRACT

In this work, a ratiometric electrochemiluminescence (ECL) biosensor was constructed for testing hypoxanthine (Hx). The reduced graphene oxide-CdTe quantum dots (rGO-CdTe QDs) and luminol were chose as the cathodic and anodic probes, the reactant (dissolved O_2) and the product (H_2O_2) in enzymatic reaction served as their coreactants, respectively. The enzymatic reaction triggered an opposite changes in the concentration of the reactant and the product, thus resulting in a reverse change trend in two ECL signals. The logarithmic value of two ECL signals intensity ratio showed a good linear relationship with the logarithmic value of the concentration of Hx from 0.020 nM to 2.0 μM. The limit of detection was 0.007 nM. Such a construction strategy decreased the interference from environment and improved the detection accuracy of Hx. The integration of CdTe QDs and luminol provides more choice for constructing ratiometric ECL biosensors, especially for oxidase-based ECL sensing system.

1. Introduction

Hypoxanthine (Hx) is an essential metabolite of adenine nucleotide, which is mainly accumulated in biological tissues [1]. The concentration levels of Hx in human serum and urine reflect the human pathological conditions such as gout and renal failure [2,3]. Furthermore, Hx is an indicator for the quality control of meat or fish products in food industries. With the increase in fish productions, the freshness of fishes is the most concern from consumers [4,5]. The freshness indicates the degree of various physical, chemical, biochemical, and microbiological changes in fish and meat. Therefore, the rapid and sensitive assay of Hx is very important, because it not only can estimate fish/meat freshness but also can realize early diagnosis of related diseases. Nowadays, Hx detection has been achieved by various methods, including capillary electrophoresis [6], high-pressure liquid chromatography (HPLC) [7], and electrochemistry technology [8,9]. Electrochemiluminescence (ECL), with high sensitivity, low background signal and rapid response, has been paid more attention in various fields [10–12], also applied in biological analysis for Hx detection. For example, Chen et al. constructed an ECL biosensor with luminol as emitter for Hx detection [13]. Ju et al. constructed an ECL enzyme sensor using CdS quantum dots (QDs) achieving the analysis of Hx [4]. However, these single-signal based ECL analytical methods are easy suffered from the interference by the instrument or some environment factors.

Recently, ratiometric ECL assays, which can eliminate most ambiguous inference by self-calibration of two emission bands, were applied in biological analysis to obtain more precise results under variable external environmental [14,15]. The first requirement for ratiometric ECL assays is to find two potential-resolved luminous substances. Thus, various cathodic and anodic ECL emitter groups have been investigated, such as CdS nanocrystals and luminol [16], graphitic carbon nitride and Ru (II) [17], and graphene QDs and luminol [18]. Unfortunately, in reported ratiometric ECL assays, two emitters commonly shared the same coreactant such as H_2O_2, $K_2S_2O_8$ or tri-n-propylamine. Furthermore, reported strategy usually need adding the coreactant into testing solution to achieve signal amplification. However, some of coreactants are unstable and volatile in aqueous solution, which would affect the luminous stability and luminous efficiency [19,20]. Additionally, using the same coreactant also greatly limited the

* Corresponding author.
E-mail address: cshong@swu.edu.cn (S. Chen).

https://doi.org/10.1016/j.electacta.2018.03.132
0013-4686/

choice of two emitters. Thus, it is meaningful to develop a ratiometric strategy excluding above limitations. Inspired by this idea, in our previous work, we proposed a novel double-potential ECL ratiometry for detecting organophosphorus pesticides [21]. Herein, rGO-CdTe QDs and poly (9,9-dioctylfluorene) dots (PFO) served as the cathodic and anodic emitters, and the reactant and product of enzymatic reaction as their coreactant, respectively. Such a construction strategy undoubtedly overcomes the limitations of the traditional ECL ratiometric method. However, the poor water-solubility of PFO would limit its luminous efficiency, thus limiting the applications of PFO in ECL analysis fields. It is very significative to find an anodic emitter with water-solubility and H_2O_2 as coreactant for constructing oxidase-based ratiometric ECL sensor.

As is well known, luminol and its derivatives exhibited a chemical stability and water-solubility, thus have been widely applied in various fields. In ECL system, luminol showed a strong ECL emission at a lower anode potential (0.6 V) using H_2O_2 as coreactant [14]. In this work, a ratiometric biosensor was constructed using the rGO-CdTe QDs as the cathodic probe and luminol as the anodic probe. The dissolved O_2 and H_2O_2 were used as the coreactants of two probes, respectively. In the presence of Hx, the xanthine oxidase (XOD) could catalyze Hx. With the occurrence of enzymatic reaction, the dissolved O_2 is consumed, and H_2O_2 is generated, thus triggering a reverse change in two ECL signals (Scheme 1). As a result, the ratio detection of Hx was achieved with a low detection limit. Such a construction strategy well avoided the false positive or negative results, and exhibited a great potential for detecting other biological molecules. And the integration of CdTe QDs and luminol provided a choice for constructing ECL ratiometric biosensors, especially for oxidase-based ECL sensing system.

2. Experimental

2.1. Reagents and chemical

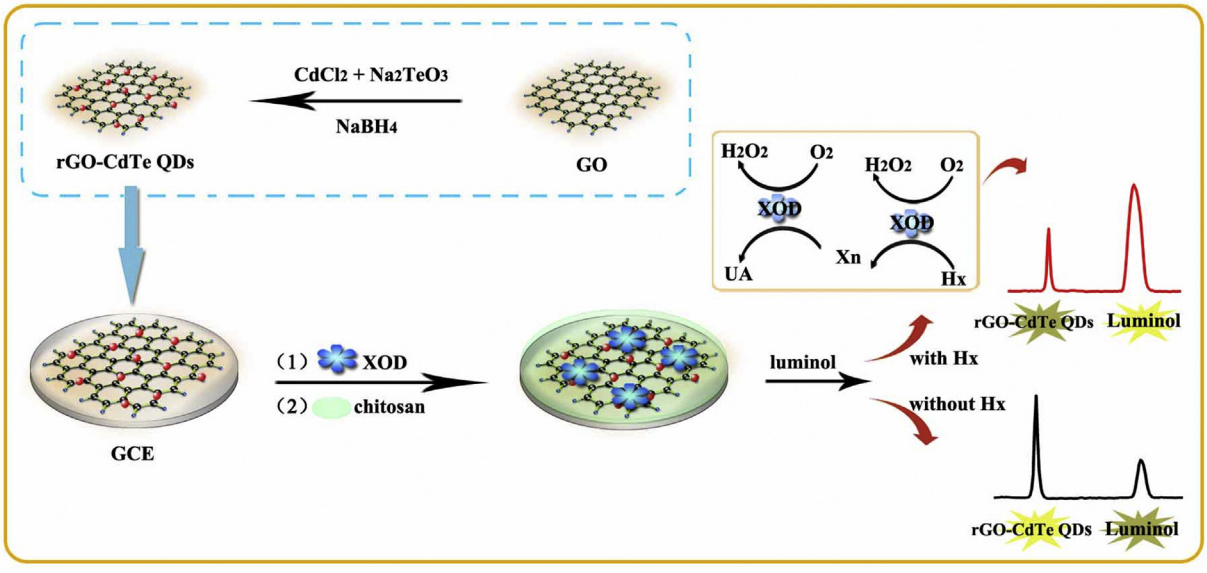
Xanthine oxidate (from bovine milk, 1.0–2.0 U/mg), hypoxanthine (≧99%), luminol (98%) and mercaptopropinonic acid (MPA) were obtained from Sigma Chemical Co. (MO, U.S.A.). Cadmium chloride hemipentahydrate ($CdCl_2 \cdot 2.5H_2O$) and Sodium tellurite (IV) (Na_2TeO_3) were purchased from Alfa Aesar Chemical Co., Ltd. (Tianjin, China). $NaBH_4$ and trisodium citrate dihydrate were purchased from Kelong Chemical Co. (Chengdu, China). Phosphate-buffered saline (PBS) solutions containing 0.10 M KCl as supporting electrolyte were used, which were prepared using KH_2PO_4 (0.10 M) and Na_2HPO_4 (0.10 M). The Ninth People's Hospital (Chongqing, China) provided the Human serum samples for experiments.

2.2. Apparatus

Xi'an Remax Electronic Science & Technology Co., Ltd. (Xi'an, China) provided MPI-A electrochemiluminescence analyzer for ECL measurements with a voltage of 800 V for photomultiplier tube. The 610A electrochemical work station was from Shanghai CH Instruments Co. China, which was used to perform the electrochemical impedance spectroscopy (EIS) measurements at a bias potential of 0.22 V. The alternative voltage was 5 mV and the frequency range is 10^{-1} -10^5 Hz. Transmission electron microscopy (TEM, JEM-1200EX) was used to characterize the morphology and size of various nanomaterials. UV–vis spectrophotometer (UV-2450) was from Shimadzu, Tokyo, Japan, which was used to recorded UV–vis absorption spectra from the range of 200–800 nm.

2.3. Preparation of rGO-CdTe QDs

rGO-CdTe QDs composites were prepared through following method [22]. Briefly, 220.0 μL of graphene oxide (GO) (1.0 mg/mL) and 36.89 mg of $CdCl_2 \cdot 2.5H_2O$ were added in 50 mL of dionized water for stirring 1 h. Then, 1.0 mL of Na_2TeO_3 (0.010 M), 50.0 mg of trisodium citrate dihydrate, 33.0 μL of MPA, and 100.0 mg $NaBH_4$ were added gradually at room temperature and kept stiring. Next, the mixture solution was refluxed in the round bottom flask for 10 h at 130 °C. Finally, the resultant rGO-CdTe QDs were collected by centrifugation and washed with ethanol/water (1:1, v/v) for three times. The obtained rGO-CdTe QDs were redispersed in dionized water and stored in a refrigerator (4 °C) for further use. Our previous work has confirmed that GO has been reduced to rGO during the formation of nanocomposites rGO-CdTe QDs due to the presence of $NaBH_4$ [21].



Scheme 1. Schematic diagram of the fabrication process of the ECL biosensor and the principle of hypoxanthine detection.