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An enzyme-assisted electrochemiluminescent biosensor developed on order mesoporous carbons substrate for ultrasensitive glyphosate sensing

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ABSTRACT

In this paper, a strategy of developing a late-model and sensitive electrochemiluminescence (ECL) biosensor for glyphosate detection based on enzyme-assisted in situ generation of ZnS quantum dots (QDs) on ordered mesoporous carbons (OMC) substrate was proposed. OMC, as a typically ordered mesoporous carbon material, not only provides a protective microenvironment for enzyme to retain its structure and activity but also has a synergistic effect with chitosan for the absorption of Zn^{2+} ions by virtue of its high surface area and high pore volume and thus employed as the matrix of proposed biosensor. Then horseradish peroxidase (HRP) was introduced to expedite the generation of ZnS QDs via accelerating the reduction of $Na_2S_2O_3$ with H_2O_2 to yield H_2S that reacted with Zn^{2+} ions. Glyphosate, as a kind of organic pesticide with amine, carboxyl and phosphonate group which would coordinate strongly to metal ions, possessed the potential to inhibited the activity of HRP, because HRP contain iron (III) protoporphyrin IX (ferriprotoporphyrin IX) as the prosthetic group which would react with the amine, carboxyl and phosphonate group of glyphosate. Accordingly, the proposed ECL QDs biosensor was employed to determine the Gly and shown a wide linear range from 0.1 nM to 10 mM with excellent sensitivity, reproducibility and selectivity. Further, the half maximal inhibitory concentration (IC_{50}) and the Michaelis–Menten constant were studied, and all the results indicated that the proposed strategy is practicable and provide a new opportunity to develop novel ECL sensing platforms in various applications.

1. Introduction

Electrochemiluminescence (ECL) is a versatile analytical technique that coupling excellent temporal and space control inherited from electrochemistry and low background from chemiluminescence [1]. Accordingly, ECL is widely used in many fields such as clinical diagnostics, food safety testing and environmental detection [2]. And ECL emitters can be generally classified into three broad categories, organic, inorganic luminophore and semiconductors such as TiO_2 and quantum dots (QDs). QDs, owing to lots of merits as the high quantum yield, high stability against photobleaching, size or surface trap-control luminescence and simultaneous excitation of multiple fluorescence colors, have been extensively used in constructing ECL biosensors [3]. And the effective synthesis of the QDs have been regarded as an significant factor to develop an efficient ECL QDs biosensor for its application due to the prepared process determine the size, color and property of QDs. To the best of our knowledge, there are two main kind of chemical approaches to synthesize QDs, the organometallic synthesis and aqueous synthesis [4]. Compared with the organometallic synthesis, aqueous synthesis could avoid the demerits of hydrophobic QDs, and the prepared QDs have high stability and biological compatibility [5]. However, the procedure of aforementioned method is tedious and prolix. To address the disadvantages of above methods, a strategy of enzyme-assisted in situ generation of QDs was proposed.

Enzymes, with the inherent characteristics of efficient catalysis, have often being utilized to develop sensitive and amplified biosensors [6]. Howbeit, efficient catalysis of enzyme is often

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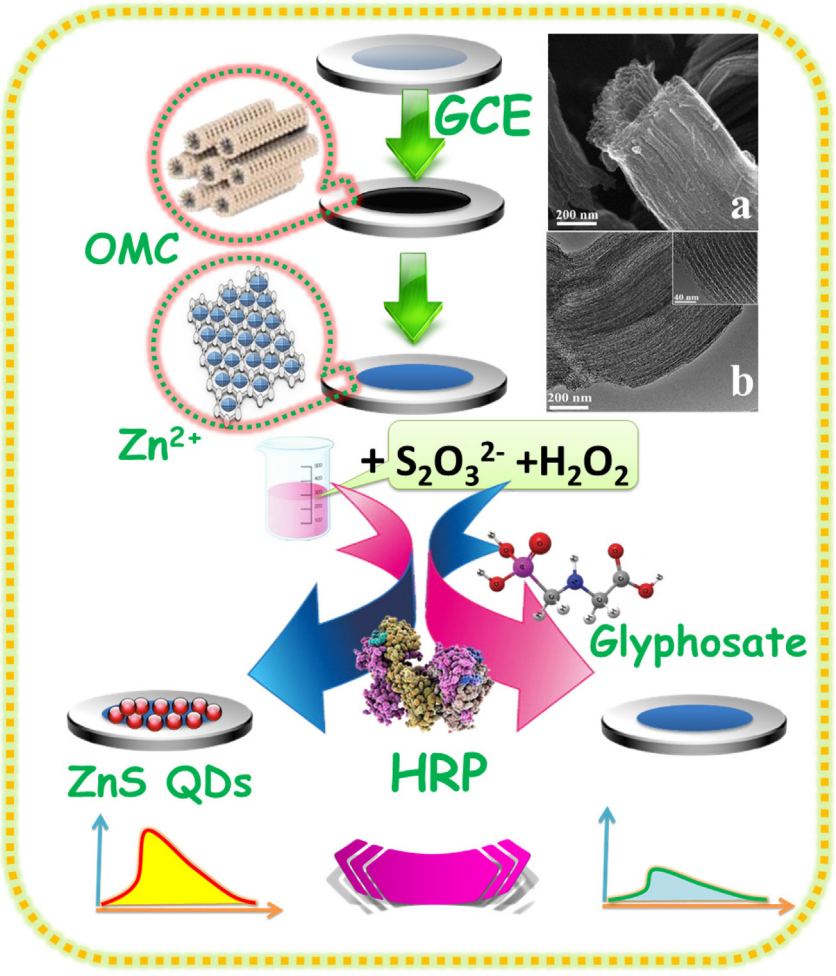
accompanied by a higher possibility of enzyme inactivation [31]. Therefore, the nanomaterial which is employed as enzyme immobilization substrates need to conserve the enzymatic activity via providing an efficient electron transfer intimate enzyme attachment. And among various nanomaterials, ordered mesoporous carbon (OMC) has attracted a great deal of interest in the fabrication of enzyme-based biosensor.

Ordered mesoporous carbon (OMC), as a kind of porous carbon materials, possess high pore volume which would be in favor of metal molecular and biomolecular adsorption [22]. And it has an excellent biocompatibility and the porousness could provide a protective microenvironment for enzyme and other biomolecules to retain their enzymatic stability and activity. Besides, the high surface area of OMC allows more active molecules to be loaded to facilitate the binding affinity. In addition, its straight and short mesochannels provid a highway for the faster transportation of electron [17]. Possessing such exceptional attributes make OMC a very attractive material for the carbon support in the in situ enzymatic reaction.

Glyphosate (Gly) is a kind of organic pesticide with amine, carboxyl and phosphonate group, known as a broad-spectrum, nonselective herbicide, which is widely used in controling of long grasses and broad-leaved weeds [7,8]. Some studies suggested Gly ingestion might affect the central nervous system, resulting in respiratory, myocardial, neuromuscular malfunctions [1] and potential endocrine disruptor [9], and a great quantity even led to death [10]. Several methods and strategies have been employed to detect the Gly, such as High performance liquid chromatography [23], capillary electrophoresis [12], mass spectrometry, florescence, gas chromatography, colorimetry [11], and electrochemical method [8]. Nevertheless, these methods are laborious, time consuming, expensive and have a high requirement on the instrument although they are credible, efficient and could determine Gly in low abundance. However, no reports have focused on ECL sensing platform based on enzyme-assisted in situ generation of QDs employed to detect Gly [13].

Herein, the in situ generation of ZnS QDs have been successful fulfilled with the assistance of HRP and employed in ECL detection. Here, the OMC was chosen as the substrate and confirmed that it has a synergistic effect on the adsorption of Zn^{2+} ions with chitosan. The detailed synthesis procedure is illustrated in Scheme 1, the mixture of OMC and CS were coated on GCE, then immersed in the solution of Zn^{2+} ions, subsequently, the resultant electrode was soaked in the mixture of HRP, H_2O_2 and $Na_2S_2O_3$, and the HRP would facilitate H_2O_2 and $S_2O_3^{2-}$ to generate H_2S that bind with Zn^{2+} to synthesize ZnS QDs on the electrode [14]. The potassium persulphate as the coreactant of ZnS QDs was performed to evaluate the proposed ECL biosensor and verified that ZnS QDs resulted in a noteworthily enhanced and stable ECL emission [15]. The amine, carboxyl and phosphonate group of Gly should coordinate strongly to iron (III) from ferriprotoporphyrin IX of HRP [8], inspired by the above observation, the sensitive ECL biosensor for determining Gly based on in situ formation of ZnS QDs assisted by HRP was developed.



Scheme 1. Schematic representation of the fabrication procedures for the biosensor and its sensing mechanism for detection of GLY. (Illustration: SEM image (a) and TEM image (b) of OMC).