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A novel enzyme biosensor for glucose based on rhodanine derivative chemiluminescence system and mesoporous hollow silica microspheres receptor

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ABSTRACT

In this work, hollow silica microspheres (HSMs), which have hollow interiors and nanoporous shells, were used in the immobilization of enzyme to establish a novel chemiluminescence biosensor. The immobilization behavior of enzyme in HSMs with different pore sizes has been studied. The results revealed that the pore size and the surface area of HSMs play important roles in their immobilization performance. Compared with traditional methods, this immobilization method not only provides tunable and consistent pore system and restricted microspaces for enzyme immobilization, but also exhibits a larger immobilization capacity and a higher adsorption rate. A rhodanine derivative-$KMnO_4$-HCl-H_2O_2 was used to replace traditional chemiluminescence system (luminol-horseradish peroxidase-H_2O_2) to reach the purpose of high sensitivity, simple operation and pH expansion for chemiluminescence biosensor. The linear range of this novel method is 7.72×10^{-6} to 2.54×10^{-2} mol L^{-1} ($r = 0.9994$). The detection limit is 8.0×10^{-7} mol L^{-1}. The Michaelis–Menten constant of glucose oxidase was 0.3 mmol L^{-1}. With glucose oxidase as a test enzyme, the proposed method gave an accurate and satisfactory result once applied to the determination of glucose in four different human serum samples.

1. Introduction

The immobilization of enzyme on solid inorganic supports has been a hot research area (Bickerstaff, 1997; Livage et al., 2001). Immobilized enzymes in solid supports, due to their potential to improve the stability of enzymes under extreme conditions, are quite useful for catalysis, sensor, and separation applications. Researchers have developed many approaches to immobilize enzyme, such as protein-polymer layer-by-layer assembly, crossing-linking crystals, sol–gel encapsulation and mesoporous materials adsorption (Gill et al., 1999; Jin and Brennan, 2002; Li et al., 2009; Yu et al., 2008). The microstructure of polymeric materials is normally amorphous and the catalytic activity of enzyme molecule can be deteriorated by deformation (DeLouise and Miller, 2005). Unlike silica gel, ordered mesoporous silica (OMS) materials provide tunable and consistent pore system, functionalizable surfaces, and restricted nanospaces for enzyme immobilization. OMS have become popular ever since they were synthesized and characterized by Mobil researchers in 1992 (Beck et al., 1992; Kresge et al., 1992). Recently, many research groups have immobilized enzymes on OMS and reported a significant improvement on enzyme stability, catalytic activity, products specificity, and resistance to extreme environmental conditions (Deere et al., 2002; Lee et al., 2006; Wang and Caruso, 2005). Hollow silica microspheres (HSMs) offer large internal spaces inside the shells and create a separated environment. The micro/mesopores in the shells provide a path for the substances going in and out of the interior hollows. Due to this unique structure, HSMs has not only special mechanic, acoustic, optic, electromagnetic, thermal properties, but also the immobilization property for enzyme (Han et al., 2006; Lim et al., 2002; Tartaj et al., 2001). HSMs have free silanol groups on their surfaces which can form hydrogen bonds with the free amino groups of enzymes. Besides this force, Van der Waals interaction also exists between enzyme and the surface of HSMs. Hydrophobic interactions contribute in adsorption by interaction of hydrophobic patches on the enzyme with the silicon network of HSMs. To our best knowledge, no reports about the immobilization of enzyme in HSMs have been published, its schematic representation of enzyme immobilized in HSMs with different pore size was shown in Fig. 1.

Diabetes is a worldwide health problem affecting hundreds of millions of people. Fortunately, with careful management of diet and medication, complication of diabetes can be reduced. Part of this treatment includes monitoring the glucose levels in the blood, so that proper action can be taken if its levels get too high. The glucose oxidase (GOx), which is an extremely stable enzyme and can survive wide excursions of pH, ionic strength, has made glucose measurement fast, easy, and inexpensive. The characteristics

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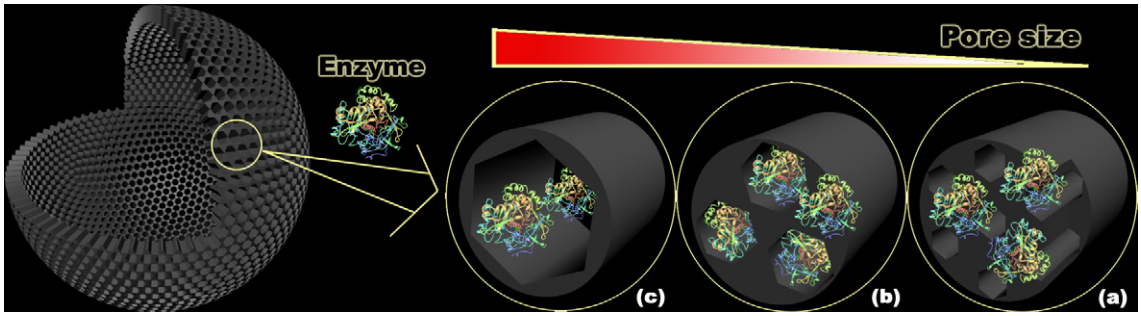

Fig. 1. Schematic representation of enzyme immobilized HSMs in different pore sizes.

of GOx in bulk solution are well documented (Bright and Gibson, 1967; Bergmeyer, 1974; Gibson et al., 1964; Rogers and Brandt, 1971). Therefore, in this work, we chose GOx as our test enzyme to establish a novel chemiluminescence biosensor.

In recent years, a number of studies have been conducted to develop new glucose monitoring methods (Fang et al., 1997; Li et al., 2008; Park et al., 2006; Zhang et al., 2005). Among them flow injection-chemiluminescence (FI-CL) biosensor is the most popular. Typical FI-CL biosensor for glucose is based on its enzymatic reaction with the GOx, producing gluconic acid and hydrogen peroxide (H_2O_2). Then the determination of glucose can be achieved via CL reaction between the enzymatic produced H_2O_2 and luminol-horseradish peroxidase (HRP) CL system (Li et al., 2008). In luminol CL system, an alkaline medium (pH 10–12) should be afforded, which limits the use of CL biosensor. In this paper, we demonstrate a novel rhodanine derivative CL system, which can be used in acid medium. Thus, the application of this rhodanine derivative CL system would expand the applicable pH range of CL biosensor. We replaced the traditional flow-though cell with a polymethyl methacrylate module and with the Y-shaped flow path drilled through it, by which the CL reactants can be injected simultaneously.

Rhodanine is a traditional chromogenic reagent (Savvin and Gur'eva, 1987). Its derivatives after substituting different groups in a rhodanine matrix also have excellent chemiluminescence and fluorescence performance which were found and studied in our lab (Yu et al., 2009; Yu et al., 2009a; Ge et al., 2010; Yu et al., 2009b; Yu et al., 2009c). To our best knowledge, no reports about monitoring of glucose by rhodanine CL system have been published. In this work, for the first time, a rhodanine derivative 3-p-nitrylphenyl-5-(4′-methyl-2′-sulfonophenylazo)rhodanine (M4NRASP) has been used in CL system to replace the traditional luminol-HRP CL system (Yu et al., 2009c). We found this derivative CL system has high sensitive CL response in the determination of H_2O_2.

In this work, the use of HSMs for enzyme immobilization was demonstrated and it was used as receptor to establish a biosensor. The adsorption capacity of HSMs with different pore sizes was compared. We found that the monitoring capacity of the biosensor was enhanced remarkably when the HSMs-enzyme receptor was combined with rhodanine derivative CL system. This novel method has a higher sensitivity, better selectivity and shorter response time, thus the goals of simplifying the experimental setup and expanding the applicable pH range of CL biosensor have been reached.

2. Material and methods

2.1. Chemicals and reagents

HSMs were synthesized as per method described below. Rhodanine derivative was obtained from our lab (Yu et al., 2009c). GOx (E.C. 1.1.3.4; Aspergillus Niger type; 146,000 U g^{-1}) was purchased from Sigma. All reagents were of analytical reagent grade or above. Oxygen-saturated phosphate buffer solution (PBS) (pH 7.4) is used in the experiments for glucose determination and Michaelis–Menten constant studies. A 1.0×10^{-2} mol L^{-1} stock glucose-PBS standard solution was obtained by dissolving 0.4954 g of glucose (Aladdin, Shanghai, China) with little doubly distilled-deionized water and then diluting to 250 mL with PBS (pH 7.4). Working glucose-PBS standard solutions was prepared by diluting of this stock standard solution with PBS (pH 7.4). A stock standard solution containing 5.0×10^{-3} mol L^{-1} M4NRASP was prepared by dissolving 0.5656 g of M4NRASP with anhydrous alcohol and then diluting to 250 mL with doubly distilled-deionized water. A working standard solution of M4NRASP was obtained by dilution of the stock standard solution with doubly distilled-deionized water. A 5.0×10^{-3} mol L^{-1} stock solution of potassium permanganate was prepared by dissolving 0.0790 g $KMnO_4$ (Aladdin, Shanghai, China) with 500 mL doubly distilled-deionized water.

2.2. Apparatus and manifold

Doubly distilled-deionized water was obtained by SYZ-550 quartz sub-boil high-purified water distiller (Jiang Su Jin Tan, Jiang Su, China). The pH measurements were performed by using a PHS-3C digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. A TU-1901 two-beam UV–vis spectrophotometer was used with 1 cm quartz cell (Perkin-Elmer, America). The nitrogen sorption and pore diameters measurements were performed on Micromeritics ASAP 2020 surface area and porosity analyzer. A CS 501 super-constant temperature bath (Chongqing, China) was used to keep temperature constant. The IFFM-D flow injection CL analyzer (Xi'an Remex Electronic Instrument High-Tech Ltd., China) was equipped with an automatic injection system and a detection system. Peristaltic pumps were used to deliver all solutions and PTFE tubing (0.8 mm id) was used to connect the flow system.

2.3. Preparation of HSMs

The synthesis details of the HSMs can be found in Ref. (Liu et al., 2008). 0.10 mol Tetraethyl orthosilicate (TEOS, Alfa Aesar) and 0.06 mol octyl amine (OA, Alfa Aesar) were first mixed for 3 min with a stirring rate of 600 rpm. Then 7.50 mol doubly distilled-deionized water containing 0.01 mol hydrochloric acid (conc.) was quickly added. After further reaction of 5 min under stirring, the resulting precipitate was collected by vacuum filtration and air-dried. Then it was calcined at 550 °C for 6 h with a heating rate of 1 °C/min, and encoded as A. For the pore expansion, uncalcined samples were hydrothermally treated at 80 °C and 100 °C respectively. After being calcined, they were encoded as B and C.