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Enzyme-mediated amperometric biosensors prepared via successive surface-initiated atom-transfer radical polymerization

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ABSTRACT

The development of enzyme-mediated amperometric biosensors on the indium–tin oxide (ITO) glass electrode via surface-initiated atom-transfer radical polymerization (ATRP) was investigated. A trichlorosilane coupling agent, containing the sulfonyl halide ATRP initiator, was immobilized initially on the ITO electrode surface for consecutive surface-initiated ATRP of ferrocenylmethyl methacrylate (FMMA) and glycidyl methacrylate (GMA). Glucose oxidase (GOD) was subsequently immobilized on the modified ITO electrode surface via coupling reactions between the epoxide groups of GMA and the amine groups of GOD. The surface composition after each functionalization step was ascertained by X-ray photoelectron spectroscopy (XPS). With the introduction of redox-P(FMMA) block as the electron-transfer mediator, the enzyme-mediated ITO electrode exhibits high sensitivity, as revealed by cyclic voltammetry measurement. The sensitivities of the ITO-*g*-P(GMA–GOD)-*b*-P(FMMA) and ITO-*g*-P(FMMA)-*b*-P(GMA–GOD) electrodes are about 3.6 μA/(mM cm^2) (in the linear concentration range 0–5 mM of glucose) and 10.9 μA/(mM cm^2) (in the linear concentration range of 0–17 mM of glucose), respectively. For both biosensors, the steady-state response time and the detection limits are estimated to be less than 20 s and 0.4 ± 0.1 mM of glucose concentration, respectively. Furthermore, the spatial effect of the redox mediator on the electrode surface is revealed by the fact that the block copolymer brush-functionalized ITO electrode with P(FMMA) as the inner (first) block is more sensitive to glucose than that with P(GMA) as the inner block.

1. Introduction

Enzyme-based biosensors have been used extensively in chemistry and biology because of their high sensitivity and good selectivity (Sulak et al., 2006; Shankaran et al., 2003; Colombari et al., 2007; Heller, 1990; Cass et al., 1984; Mikeladze et al., 2002; Lee et al., 2006; Akram et al., 2006; Razumiene et al., 2003; Laurinavicius et al., 2004), in addition to their low-cost potential and possibility of their miniaturization/automation. Among the enzymatic biosensors, glucose sensors have been of considerable interests owing to the growing need for diagnostic analysis of diabetes (Sulak et al., 2006; Shankaran et al., 2003). Generally, the amperometric glucose biosensors are based on enzymatic oxidation mediated by glucose oxidase (GOD), a well-known biological sensing material for the quantitative determination of β-D-glucose in solution (Colombari et al., 2007). The electrical communication between redox enzymes (such as GOD) and electrode surfaces represents the basic process for activating the redox-active biocatalysis (Heller, 1990; Cass et al., 1984). Among electron-transfer mediators, monomeric and polymeric ferrocene are superior candidates as redox catalysts in electrochemical assays, due to their high chemical stability, low redox potentials, low interferences, good antifouling property, and good electrochemical response (Akram et al., 2006; Razumiene et al., 2003; Laurinavicius et al., 2004; Nagel et al., 2007). The spatial immobilization of enzymes and electron mediators on the electrode surface is another key factor for the development of an effective amperometric biosensor. The common techniques reported for immobilization of enzymes and electron-transfer mediators on electrode substrates include coating (Garcia et al., 2007a,b) and layer-by-layer deposition (Deng et al., 2007).

Films with enzymes and redox polymers "wired" in their structures will have high sensitivity toward molecular recognition and electrical communication in a single sensing device (Cass et al., 1984; Mikeladze et al., 2002; Lee et al., 2006; Akram et al., 2006). Tethering of polymer brushes via surface-initiated radical polymerization on solid substrates is an effective method for modifying their surface properties (Royea et al., 2000; Royea et al., 2000; Braunecker and Matyjaszewski, 2007; Kato et al., 2003). Atom-transfer radical polymerization (ATRP) is a recently developed "living" or "controlled" radical polymerization method, and has

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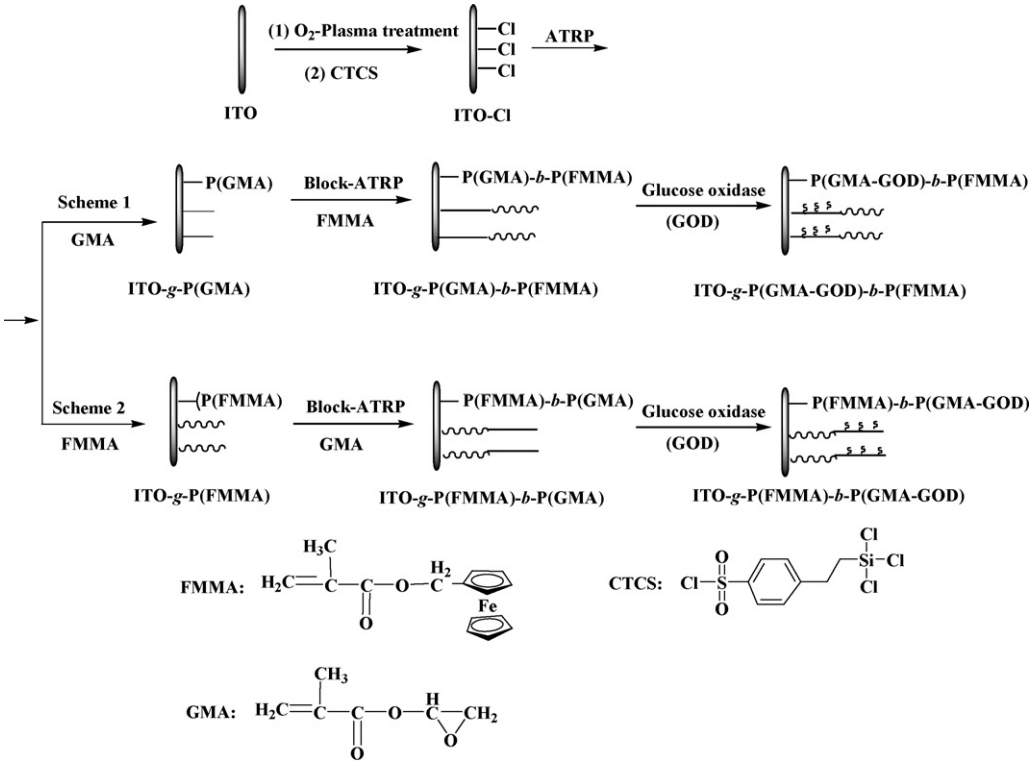

Fig. 1. Schematic diagram illustrating the processes of immobilization of trichlorosilane coupling agent on the ITO surface (the ITO–Cl surface), surface-initiated ATRP of GMA from the ITO–Cl surface to produce the inner block, followed by block copolymer of FMMA and the coupling of GOD to the epoxide groups of the P(GMA) block to produce ITO-*g*-P(GMA–GOD)-*b*-P(FMMA) biosensor (Scheme 1). In Scheme 2, P(FMMA) was first grafted from the ITO–Cl surface, and the P(GMA) segment was then grafted as the outer block of the copolymer in the ITO-*g*-P(FMMA)-*b*-P(GMA–GOD) biosensor.

been employed to prepare well-defined polymer brushes on different substrates (Pyun et al., 2003; Iwata et al., 2004; Matayjaszewski et al., 1997; Qiu and Matayjaszewski, 1997; Davis et al., 1999).

The present study attempts to provide an alternative approach to the development of GOD-mediated amperometric biosensors on the indium–tin oxide (ITO) glass electrode via consecutive surface-initiated ATRP of ferrocenylmethyl methacrylate (FMMA) and glycidyl methacrylate (GMA). The electrochemically active ferrocene moieties in the FMMA block were introduced on the ITO surface to act as electron-transfer mediator. Poly(glycidyl methacrylate) (P(GMA)) is a known surface linker and spacer for biomolecules (Stears et al., 2003; Nishiyama et al., 2002), and is used as anchoring sites for the enzyme, glucose oxidase (GOD), via chemical reactions between the epoxide groups of GMA and amine groups of GOD (Xu et al., 2005). The use of ATRP technique in the fabrication of biosensors allows easy tuning of the amount of the functional polymer chains and adjustment of the spatial distribution of electron-transfer mediators. The surface functionalization process is shown schematically in Fig. 1.

2. Experimental

2.1. Materials

Indium–tin oxide (ITO)-coated glass substrates (10 Ω/m^2, 1.1 mm in thickness) were purchased from Hua'yi Conducting Glass Co. Ltd (China). The ITO glass plates of 10 mm × 33 mm in area were cleaned by ultrasonication in deionized water, acetone, and isopropanol for 10 min each, in that order. Glycidyl methacrylate (GMA, >99%) was passed through a silica gel column to remove the inhibitor and was then stored under an argon atmosphere at −10 °C. The silane coupling agent containing the chlorosulfonyl atom-transfer radical polarization (ATRP) initiator, 2-(4-chloro-sulfonylphenyl)-ethyltrichlorosilane (CTCS, 50% in toluene solution), was obtained from ABCR GmbH and Company KG (Karlsruhe, Germany), and was used as received. The ligand for the transition metal, *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%) was obtained from BASF Chem. Co. (Ludwigshafen, Germany). Glucose oxidase (GOD Type II, 15,500 units g^{-1} from *Aspergillus niger*) was purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI). Dulbecco's phosphate buffered saline solution (PBS, pH 7.4, containing NaH_2PO_4 at 1.17 g L^{-1} and Na_2HPO_4 at 2.166 g L^{-1}) was prepared afresh and used for the GOD immobilization. PBS (10 mM) at pH 7.0 was employed as the supporting electrolyte. Ferrocenylmethyl methacrylate (FMMA) was prepared according to the method reported previously (Lai et al., 1971). Solvents, such as acetone, tetrahydrofuran (THF), toluene (99.8%), *N*,*N*′-dimethylformamide (DMF, 99.8%) and other chemicals were of reagent grade and were used as received.

2.2. Immobilization of ATRP initiator onto ITO glass surface

It has been reported that the ITO glass surface has a finite amount of hydroxyl groups associated with metal hydroxides (Huang et al., 2008; Wróblewski et al., 2004). The hydroxyl groups allow the introduction of an trichlorosilane coupling agent to the surface via self-assembly reactions to provide the halide ATRP initiators (Wróblewski et al., 2004). Oxygen-plasma pretreatment of the ITO glass was performed between two parallel-plate aluminum electrodes in a glow-discharge quartz reaction chamber (Model SP100, Anatech Co. of Springfield, VA) to further rid of carbon contaminants on the substrate surface (Sakakiyama et al., 2005; Lu et al., 2003; Wu et al., 1997; Kawai et al., 2007; Zhong and Jiang, 2006). The plasma power supply was set at 50 W at a frequency of 40 kHz. The ITO glass substrates were placed on the bottom electrode and exposed to the glow-discharge at an oxygen flow rate of 30 sccm and a pressure of