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Fabrication of self-assembled oligophenylethynylenethiol monolayer for electrochemical glucose biosensor

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ABSTRACT

An electrochemical glucose biosensor was developed using a gold (Au) electrode, which was composed of self-assembled oligophenylethynylenethiol monolayer and glucose oxidase (GOx) structure. Oligophenylethynylenethiol was used as a chemical linker for the immobilization of GOx on Au electrode, which facilitates the transfer of electron produced by enzyme reaction to the Au electrode. The electrical property of self-assembled oligophenylethynylenethiol monolayer was investigated by using cyclic voltammetry (CV). The formation of self-assembled oligophenylethynylenethiol monolayer and GOx layer on Au surface was verified by using atomic force microscopy (AFM) and surface plasmon resonance (SPR). The electrochemical glucose biosensor exhibited a linear relationship between target concentration and oxidation current in the range of 2–30 mM and its detection limit was 2 mM.

1. Introduction

Electrochemical detection of physiological species, such as glucose, insulin, prostate-specific antigen, has been extensively employed in biology and applications in diagnostics [1]. Great efforts have been devoted to the fabrication and characterization of a large number of enzyme-based electrochemical biosensors [2–4]. The enzymes immobilized on a solid electrode such as glucose oxidase (GOx), horseradish peroxidase (HRP), and alkaline phosphatase (ALP) serve as redox center and reacts selectively with biological species. The reaction product may be used directly or by mediation of a redox couple to determine the biological species of interest. Generally the immobilization of enzyme on a solid electrode makes it possible that the reduction–oxidation of substrate by enzyme occurred near or inside the diffusion layer on the electrode, which, in turn, increases the sensitivity of the electrochemical biosensor [5,6].

The enzyme may be immobilized in a thin layer at the solid surface in different ways [7–10]. Among them, the immobilization through covalent attachment of enzyme to the functionalized self-assembled monolayers (SAMs) is especially useful where miniaturization of the biosensor is required [11]. The functionalized SAMs formed on gold (Au) surface are well-ordered molecular assemblies, which are extensively used for the immobilization of biomolecules such as enzymes in biosensors fabrication [12–14].

The most general system of the SAMs is to use alkanethiols containing carboxylic acid in the tail group. In the long hydrocarbon chains structure, one side is reacted with solid substrate and the other side is reacted with target protein. However, alkanethiols-based SAM have a drawback for the application to electrochemical biosensor because it can function as an insulator [15,16]. Aromaticthiols have been extensively used as complementary candidates for the effective SAMs of electrochemical biosensors [17–19]. SAM of aromaticthiol has attracted considerable interest, as electrons are more delocalized in the benzene rings than in the alkane chains, which means a higher electrical conductance through aromaticthiol [20]. When aromaticthiol derivatives as chemical linker are used instead of alkanethiols, the sensitivity of electrochemical biosensor can be improved.

Previously, we reported that electrical conductivity of aromaticthiol such as 4-(2-(4-acetylthio)phenyl)ethynyl) benzoic acid (APBA), one of oligophenylethynylenethiol species, was higher than that of alkanethiol owing to the increase of coplanarity of π-conjugated structure [21]. We hypothesized that

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if APBA is used as chemical linker material for the fabrication of electrochemical biosensor, the transfer of electron produced by enzyme reaction to the electrode can be improved, which, in turn, increases the sensitivity of the electrochemical biosensor can be improved. Herein, we demonstrate the fabrication of self-assembled APBA monolayer for effective electrochemical glucose biosensor, which has not been reported in any publications. Glucose was used as a model protein due to the great demand for blood glucose measurements, particularly for treatment and control of diabetes [22]. The electrode was composed of Au/APBA/GOx structure. The electrical property of APBA SAM was investigated by using cyclic voltammetry (CV). The formation of APBA SAM and GOx layers on Au surface was verified by using atomic force microscopy (AFM) and surface plasmon resonance (SPR).

2. Experimental

2.1. Materials

Glucose oxidase (EC 1.1.3.4. Type II: from *Aspergillus niger*), 11-mercaptoundecanoic acid (MUDA), beta-D-glucose, ferrocenemethanol (Fc), (*N*-morpholino)ethane sulfonic acid hydrate (MES), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Chemical Co. (USA). 4-(2-(4-(Acetylthio)phenyl) ethynyl)benzoic acid (APBA) was synthesized in our lab [21]. Other chemicals used in this study were obtained commercially as a reagent grade.

2.2. Fabrication of APBA SAM and GOx layer on Au electrode

Au electrode was cleaned using piranha solution composed of 30 vol% H_2O_2 and 70 vol% H_2SO_4 at 70 °C for 5 min and then it was rinsed with ethanol and deionized water. The Au electrode was immersed in 10 mM APBA tetrahydrofuran (THF) solution containing 15 μl of concentrated H_2SO_4 at room temperature for 24 h. The electrode was thoroughly rinsed with THF, ethanol and deionized water, and finally was dried under a nitrogen stream. The carboxylic terminal group of APBA SAM was activated by the immersion of the electrode into a solution of MES buffer (25 mM, pH 5.5) containing 50 mg/mL EDC and 50 mg/mL NHS for 1 h. After vigorous washing, GOx layer was fabricated by the immersion of the electrode in the MES buffer solution (25 mM, pH 5.5) containing 5 mg/ml GOx for 2 h. The schematic diagram of GOx immobilization on Au surface via APBA SAM is shown in Fig. 1.

2.3. Surface plasmon resonance

The formation of APBA SAM and GOx layer on Au surface was verified by using SPR. A cover glass composed of BK7 (18 mm × 18 mm, Superior, Germany) was used as a solid support. Chromium (Cr) was sputtered onto the glass substrate initially, as an adhesion material, to a thickness of 20 Å and followed by Au sputtering to a thickness of 430 Å. SPR spectroscope with built-in He–Ne laser beam of 632.8 nm (Multiskop™, Optrel Gbr, Germany) was used. The p-polarized light beam was measured by photo multiplier tube (PMT) sensor. A glass prism (BK 7) with 90° angle was used as a Kreschmann ATP coupler [23]. The plane face of the 90° glass prism was coupled to cover glass via index matching fluid (Benzyl benzoate, Merck, Germany). The resolution of the angle reading of the goniometer was 0.001°.

2.4. Atomic force microscopy

The surface morphology of the APBA SAM/GOx layer was investigated by using atomic force microscopy (XE-100, Dark system Co. Ltd., Korea). AFM was operated in non-contact mode at room temperature. Images were acquired at a scan rate of 1–2 Hz with a silicon cantilever (XE-CAT-NCSI, PSIA Co., Korea), and the scan size used was 0.3 μm × 0.3 μm.

2.5. Cyclic voltammetry

Cyclic voltammetry measurement was performed with a potentiostate measurement analyzer (IviumStat, IVIUM Technologies, Netherlands). The Ag/AgCl (3.0 M KCl) reference electrode, Au working electrode, and platinum (Pt) wire counter electrode were used and the scan rate was 0.01 V/s.

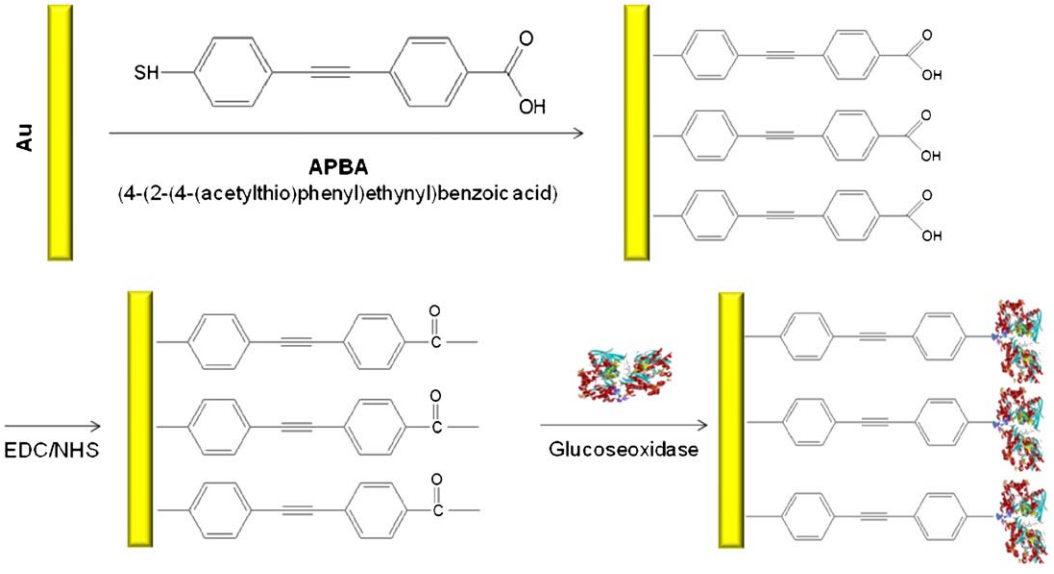

Fig. 1. Schematic diagram of APBA/GOx-modified Au electrode.

3. Results and discussion

3.1. Electrical property of self-assembled APBA layer on Au electrode

Prior to evaluating the electrochemical glucose biosensing scheme using the electrode with APBA/GOx structure, we characterized the electrical property of APBA SAM in 0.1 M phosphate buffer solution (pH 7.0) containing a variety of concentration of ferrocenemethanol, compared with that of MUDA SAM in the same condition. In the previous work, we showed the *I–V* characteristic of APBA SAM at Au electrode, compared with that of other chemical linkers such as 4-mercapto-biphenyl-4-carboxylic acid (MBCA), 3-mercaptopropanoic acid (MPA), and MUDA [21]. The electrical conductivity of APBA SAM was higher than that of MBCA, MPA, and MUDA SAMs. Fig. 2A shows the cyclic voltammetric responses of 0.1 mM ferrocene-methanol at Au electrode modified with MUDA and APBA SAMs. It should be noted that the electrical activity of APBA SAM was higher than that of MUDA SAM because of low gap resistance due to the conjugated backbone structure of APBA molecules. Fig. 2B shows the effective electron transfer through the oriented π-conjugated backbone of the APBA molecules at Au electrode, compared with that of MUDA SAM in the same condition. As a result, it could be found that the coplanarity of π-conjugated backbone structure of APBA plays a role in the increase of electrochemical activity in a forrocenemethanol solution.

3.2. Fabrication of GOx layer through APBA SAM

For the fabrication of the electrode with Au/APBA/GOx structure, APBA was self-assembled on Au surface. The carboxylic terminal group of APBA SAM was activated using EDC and NHS. Moreover, GOx was covalently attached on APBA SAM. The change of SPR curves by the adsorption of APBA, and by chemical binding of GOx on APBA-modified Au surface are shown in Fig. 3. As a result, the SPR angle was shifted from 43.00° to 43.07° by the adsorption of APBA on Au surface. The SPR angle was shifted from 43.07° to 43.72° by chemical binding between GOx and the activated carboxyl group of APBA with EDC and NHS. In principle, a SPR is extremely sensitive to the interfacial architecture. An adsorption process leads to a shift in the plasmon resonance and allows monitoring the mass coverage at the surface with a high accuracy. Therefore, the shift in the SPR angle could be verified that the formation of APBA SAM and chemical binding of GOx molecules on APBA-modified Au surface.

AFM images of the GOx layer on Au surface modified with APBA in comparison with that of bare Au are shown in Fig. 4. It could be observed that GOx molecules were adsorbed onto the APBA-modified Au substrate as an aggregated pattern in solid-like state with keeping its random cloud-like structure as in bulk solution. From the above results, it could be concluded that the GOx layer was well-formed on the Au substrate modified with APBA.

3.3. APBA SAM-based electrochemical glucose biosensor

Fig. 5A shows the cyclic voltammetric responses of the APBA SAM and GOx-modified Au electrode in 0.1 M phosphate buffer solution (pH 7.0) containing 0.1 mM ferrocenemethanol in the absence and presence of 20 mM glucose. In the absence of glucose, a pair of well-reversible redox wave with formal potential at 0.22 V (versus Ag/AgCl) was observed, which was assigned to one-electron reversible redox reaction of Fc+/Fc. Adding glucose to above solution, a well-defined catalytic wave was developed as a consequence of the GOx catalytic oxidation of glucose.

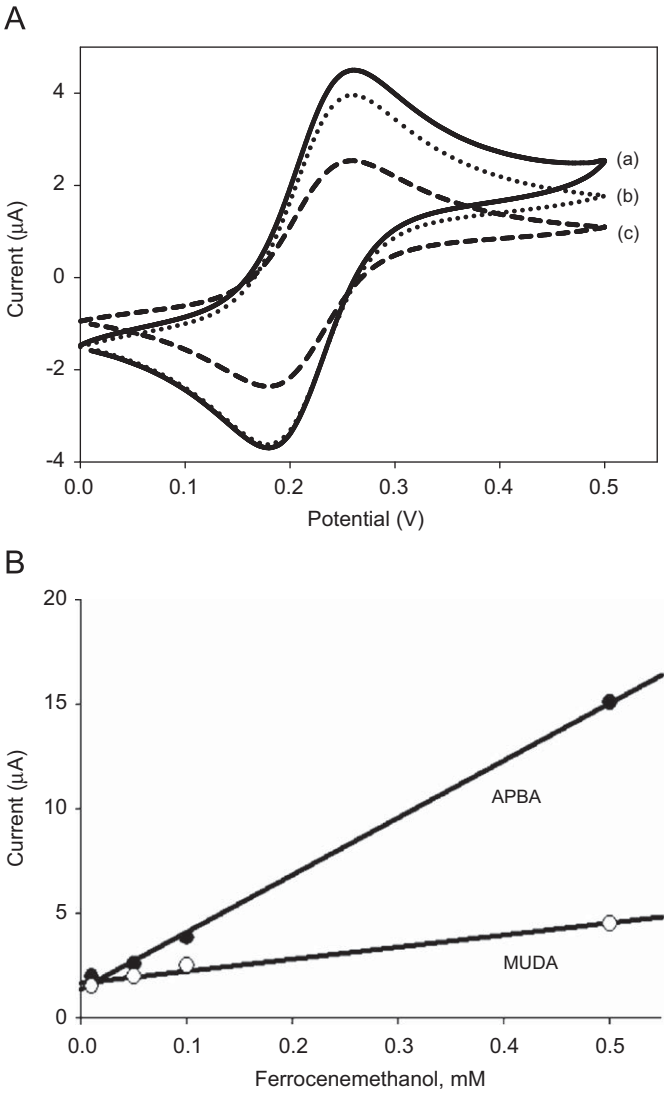

Fig. 2. (A) Cyclic voltammetric responses of 0.1 mM ferrocenemethanol at (a) Au electrode, (b) Au electrode modified with APBA SAM, and (c) Au electrode modified with MUDA SAM. (B) Oxidation current profile as a function of ferrocenemethanol concentration at Au electrodes modified with MUDA and APBA SAMs.

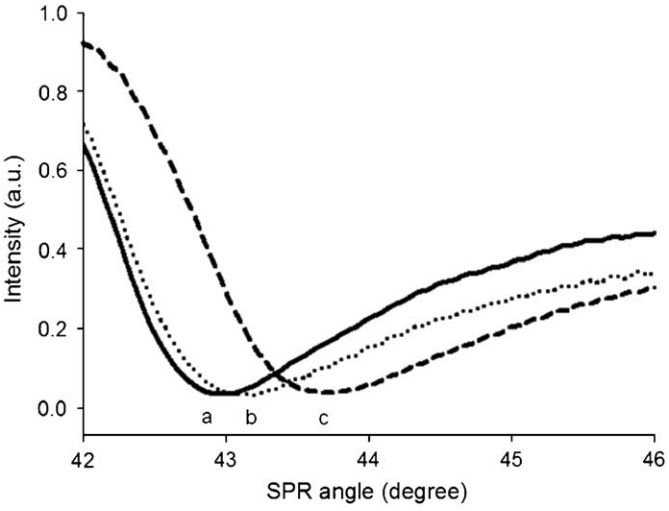

Fig. 3. SPR curves: (a) bare Au, (b) APBA SAM, and (c) GOx on APBA SAM.

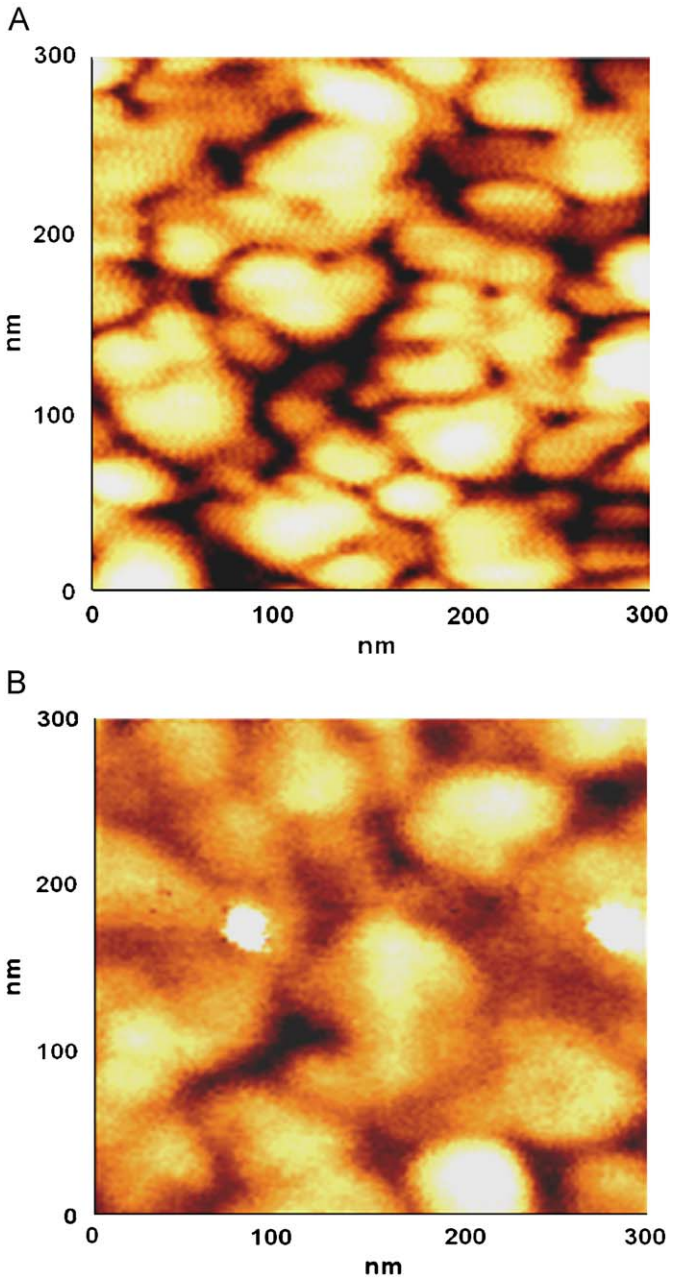

Fig. 4. AFM images: (a) bare Au and (b) GOx on APBA SAM.

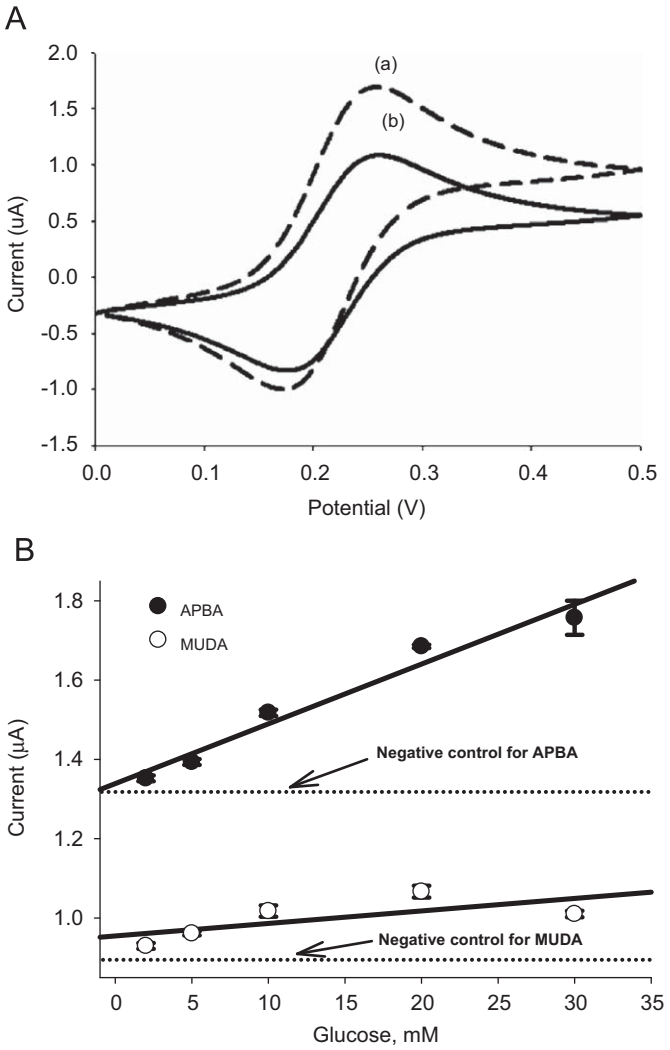

Fig. 5. (A) Cyclic voltammetric responses of the APBA/GOx-modified Au electrode in phosphate buffer solution containing 0.1 mM ferrocenemethanol in the presence (a) and absence (b) of 20 mM glucose. (B) Oxidation current of glucose from the ferrocenemethanol-mediated cyclic voltammogram obtained at APBA/GOx-modified Au electrode and MUDA/GOx-modified Au electrode.

Fig. 5B shows the oxidation current of glucose from the ferrocenemethanol-mediated cyclic voltammogram obtained at the APBA/GOx-modified Au electrode, compared with that of glucose at the MUDA/GOx-modified Au electrode. The oxidation current of glucose by GOx at APBA/GOx-modified Au electrode was higher than that of glucose by GOx at MUDA/GOx-modified Au electrode. As the concentration of glucose increases, the oxidation current at APBA/GOx-modified Au electrode increases more dramatically than that at MUDA/GOx-modified Au electrode because of better electrical conductance of APBA SAM by the conjugated backbone structure of APBA molecules.

All target concentrations over the 2–30 mM concentration range could be easily differentiated from the negative control using electrochemical bioassay system based on APBA/GOx-modified Au electrode. The assay exhibits a linear relationship between target concentration and oxidation current in the range 2–30 mM. It is shown that the electrochemical biosensor's response to glucose is well within the clinical range of glucose levels in human blood [22].

4. Conclusion

An electrochemical glucose biosensor was developed using APBA/Gox-modified Au electrode. APBA was used as a chemical linker for the immobilization of GOx on Au electrode, which facilitates the transfer of electron produced by enzyme reaction to the Au electrode. The electrical property of APBA SAM was investigated by CV. The formation of APBA SAM and GOx layer on Au surface was verified by AFM and SPR. The electrochemical glucose biosensor exhibits a linear relationship between target concentration and oxidation current in the range of 2–30 mM and its detection limit was 2 mM. The proposed APBA SAM can be used as an effective chemical linker for other electrochemical biosensors and bioelectronic devices.

Acknowledgments

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