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One-step modification of various electrode surfaces using diazonium salt compounds and the application of this technology to electrochemical DNA (E-DNA) sensors

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ABSTRACT

In this work, we have demonstrated that 4-carboxy phenyl groups based modification could be performed on different electrodes, such as indium tin oxide (ITO), gold (Au), and glassy carbon electrode (GCE). The electrode modification has been performed through cyclic voltammetry by sweeping the potential between +1.0 V and −1.0 V for '*n*' number of potential cycles. The 4-carboxyphenyl modification at different electrode surfaces was confirmed by cyclic voltammetry, contact angle measurements, atomic force microscopy, and X-ray photoelectron spectroscopy analysis. Subsequently, the electrochemical DNA sensor (E-DNA sensor) based on the 4-carboxy phenyl modified GCE was fabricated by the immobilization of probe DNA. The fabricated E-DNA sensor can detect the influenza virus (type A). The current density of the E-DNA sensor was also evaluated by cyclicvoltammetry when the probe DNA and target DNA were hybridized.

1. Introduction

Genetic testing requires the development of easily constructed, simple, low cost, and miniaturized analytical methods as well as fast-detection methods. Traditional methods for detecting deoxyribonucleic acid (DNA) hybridization, such as polymerase chain reaction (PCR), RT-PCR, or electrophoresis, are slow and labor intensive. The DNA biosensor offers a promising alternative for faster, cheaper, and simpler nucleic acid assays. DNA hybridization commonly relies on immobilization of probe DNA onto a transducer surface to recognize its complementary sequences. The binding of the probe on the surface and to its target sequence was translated into a useful electrical signal [1]. There have been various types of highly sensitive and selective DNA biosensors developed over the years. These biosensors have been based on electrochemical [2–4], optical [5], and micro-gravimetric detection methods [6]. Among them, electrochemical DNA (E-DNA) biosensors have attracted considerable attention to the detection of DNA hybridization. Their high sensitivity, compatibility with modern micro-fabrication technologies, low cost, portability, and label-free design make them excellent candidates for a wide variety applications in areas such as medical diagnostics [7], drug screening [8,9], food safety [10,11], and many other fields.

Influenza virus (type A) (IV) infections are a major cause of disease ranging from an asymptomatic infection to an acute, fatal disease in poultry. So far, a variety of methods have been developed for the detection and identification of Influenza virus (type A) (IV), such as enzyme-linked immunosorbent assays (ELISA) and PCR test. However, it must be pointed out that those methods are labor-intensive and time-consuming for large numbers of clinical samples. Recently, increasing interest has focused on the development of DNA electrochemical biosensors for a high sensitivity and inexpensive diagnosis method of infectious agents [12]. DNA electrochemical biosensors were fabricated through immobilization of DNA utilizing electrochemical mediators or with modified electrode surface.

Tam et al. [13] reported an E-DNA biosensor based on multi-wall carbon nanotubes (CNTs) for label-free detection of influenza virus (type A) by covalent immobilization. However, problems, such as dispersion and binding on the electrode surface were reported while using CNT as the support matrix [14]. The detection of DNA has been performed through capacitance and resistance changes of the CNT coated modified electrodes. In addition, the fabrication of E-DNA biosensor electrode, involves coating a layer of Nafion®,

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chitosan or other polymer based binders on the surface of support matrix [15]. As a result, a portion of the probe DNA molecules on the surfaces of the CNT is covered with the polymer coating that hampers complete matching of the target DNA. Therefore, it would be beneficial to immobilize probe DNA directly on the surface of an electrode, without using any additional binder. This would enhance matching between the probe DNA and target DNA in an aqueous electrolyte. In this study, we have adopted a direct electrode modification approach without any binder to enhance the sensitivity of E-DNA sensor.

The chemical modification of electrode surfaces for immobilization of biomolecules has been extensively studied. Surface functional groups (thiol, carboxyl, amino etc.) were formed on glass or indium tin oxide [16,17] and alkanethiol monolayers on Au [18] through various chemistries. The modification of electrode surface through reduction of diazonium salts has been proved to be an efficient approach. The advantages and mechanistic aspects of covalent attachment of electroactive moieties through reduction of aryl diazonium salts on carbon surface have been well documented [19]. In general, electrochemical reduction of a diazonium salt involves generation of aryl-based radicals through elimination of dinitrogen. The aryl radicals subsequently make covalent linkage to electrode surface to result in highly stable modified electrode surface. Diazonium salt reduction approach could be used to fabricate modified electrodes with various functional groups and can be targeted to different applications [20,21]. Diazonium modified electrodes are also suitable to bind macromolecules and target materials of diverse nature and shapes via grafting from [22] and grafting onto approaches [23,24].

The immobilization of biomolecules based on avidin–biotin conjugation has been studied in recent periods due to its excellent robustness and simplicity. Commercial biochips have been developed using this technology for reliable immobilization of biotinylated ligands. The avidin-biotin conjugation methods have various benefits, such as: (1) do not depend on the isoelectric point of the protein, (2) require very low quantities of biotinylated ligand, (3) commercial availability of biotinylated reagents, (4) ease of immobilization, (5) ability to control accurate concentration of bound conjugates, (6) lower electrostatic charges compared to COOH chips, (7) and the versatility of biotinylation reaction to yield product.

It is envisioned that sensitive detection of DNA can be improved by stratergies involving surface modification and/or effective immobilization of DNA. Several reports are available on the surface modification of electrodes using different substituted diazonium salts [25]. In this work, we intended to utilize the effective electrode surface modification strategy via incorporation of substituted phenyl groups by the electrochemical reduction of an aryl diazonium salt (4-carboxyl phenyl diazonium salt). We have demonstrated that 4-carboxy phenyl groups based modification could be performed on different electrodes, such as indium tin oxide (ITO), gold (Au), and glassy carbon electrode (GCE). The electrode modification has been performed through cyclic voltammetry by sweeping the potential between +1.0 V to −1.0 V for '*n*' number of potential cycles ($n = 10$). The electrodes (ITO, Au and GCE) were modified by applying constant number of potential cycles. The 4-carboxyphenyl modification at different electrode surfaces was confirmed by cyclic voltammetry, contact angle measurements, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) analysis. After ascertaining the relative advantages of electrode modification with carboxy phenyl groups, we have fabricated an electrochemical DNA biosensor (E-DNA) using GCE-carboxyphenyl modified electrode through avidin-biotin reaction of biotinylated probe DNA. The experimental results showed that the E-DNA fabricated with COOH–GCE has good selectivity to detect influenza virus (type A).

2. Experimental

2.1. Materials

4-Aminobenzoic acid, sodium nitrite ($NaNO_2$), hydrochloric acid, tetrabutylammonium tetrafluoroborate (NBu_4BF_4), acetonitrile, potassium ferrocyanide, potassium ferricyanide, potassium chloride (KCl), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and Avidin from egg whites were purchased from Sigma-Aldrich Korea Ltd. Phosphate buffer solution (PBS) was prepared by mixing stock solutions of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH. All solutions for the experiments were prepared with water purified in a Milli-Q plus water purification system (Millipore Co. Ltd.; the final resistance of the water was 18.2 M Ω cm^{-1}) and it was degassed prior to each measurement.

The 24-base oligonucleotides biotinylated probe DNA, its complementary sequence DNA (target DNA, namely a 24-base fragment of an influenza virus gene sequence), and two-base mismatch DNA were purchased from the Bionics Company (Korea), with the following base sequences: biotinylated probe DNA 5′-biotin-ATG AGT CTT CTA ACC GAG GTC GAA-3′; complementary target DNA 5′-TTC GAC CTC GGT TAG AAG ACT CAT-3′; two-base mismatch DNA 5′-TTC GAC AGC GGT TAT AAG ACT CAT-3′. All oligonucleotides were dissolved in Tris-EDTA buffer solution (pH 8.0) and were kept frozen.

2.2. Synthesis of 4-carboxyphenyl diazonium salt

0.01 mol of 4-aminobenzoic acid was dissolved in 20 mL of warm aqueous hydrochloride solution (0.22 M). After cooling to 0 °C, 0.011 mol of aq $NaNO_2$ was added slowly to the reaction mixture with stirring. After filtration of the solution, 0.01 M of $NaBF_4$ was added. The solution was cooled to below 0 °C, filtered, washed with ice water and cold ether and dried under vacuum.

2.3. Electrode modification

ITO was precleaned using acetone and ultra-sonicated for 10 min before use. Au and GCE surfaces were cleaned by polishing with 0.3 μm α-Al_2O_3 powder. After each polishing, the electrodes were washed with ultrapure water. Prior to electrodeposition, the electrodes were once treated with acetonitrile. Modification of the electrode (ITO/Au/GCE) was independently done by electrochemical reduction of diazonium salt in deaerated acetonitrile containing 4-carboxyphenyl diazonium salt (5 mM) and NBu_4BF_4 (0.1 M) on the respective electrode. Cyclic votammograms were recorded in the potential range from +1.0 to −1.0 V (vs. Ag/AgCl). After electrochemical modification, the electrodes were washed with acetonitrile and dried. The carboxy phenyl modified electrodes (ITO/Au and GCE) are designated as COOH-ITO, COOH-Au and COOH-GCE respectively.

DNA biosensor was fabricated using 4-carboxyphenyl diazonium salt as follows. The COOH-GCE was activated by immersion in 0.1 M PBS (pH 7.0) containing 30 mM EDC and 30 mM NHS for 2 h at room temperature [26]. The linker/COOH-GCE was rinsed with 0.1 M PBS to wash off the excessive or unbound EDC and NHS. Then, the electrode was functionalized with avidin in a PBS containing 200 μg/mL avidin for 2 h at room temperature, and rinsed with 0.1 M PBS [27].

2.4. Immobilization of the probe DNA onto the electrode and its hybridization for target DNA

In order to immobilize the biotinylated probe DNA using the avidin–biotin interaction, the avidin-modified electrode was immersed in 0.1 M PBS (pH 7.0) containing 10 pM biotinylated