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Voltammetric DNA Biosensor using Gold Electrode Modified by Self Assembled Monolayer of Thiol for Detection of *Mycobacterium tuberculosis*

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Abstract

Mycobacterium tuberculosis is an infectious agent that causes tuberculosis (TB). TB is one of the major causes of death worldwide, mainly in the developing country. Early and rapid diagnosis of TB will be of great help to isolate the patients and control the disease. The aim of this research is to detect *Mycobacterium tuberculosis* using voltammetric DNA biosensor by using gold electrode modified by self assembled monolayer with thiol. Single-stranded probe DNA was immobilized on the surface of self assembled monolayer gold electrode with the assistance of cysteamine and glutaraldehyde, which was further used to hybridize with the target DNA sequence and non-complementary target sequence. Differential Pulse Voltammetry (DPV) was used to study the immobilization of DNA probe and hybridization with the target DNA. The hybridization reaction on the gold electrode surface was detected by monitoring a guanine oxidation signal at +0.2 Volt. Voltammetric DNA biosensor using gold electrode modified with thiol can be used to determine hybridization between probe DNA and target DNA sequence of *M. tuberculosis* with sensitivity value is 0.5175 for target DNA in concentration range 0- 30 µg/mL; detection limit is 2.7046 µg/mL and quantification limit is 9.0155 µg/mL, accuracy is 99.22%, precision 99.86%

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Keywords: Biosensor DNA; Self-Assembled Monolayer; gold electrode; Mycobacterium tuberculosis

1. Introduction

Mycobacterium tuberculosis (M. tuberculosis) is a very dangerous pathogenic bacterium that causes tuberculosis (TB), one of the leading infectious diseases causing of the death. Currently, about one-third of the human population is infected with TB worldwide. The infection of TB is a serious public health concern because it may emerge as a complication of acquired immune deficiency syndrome (AIDS) infection. The rapid diagnosis and

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treatment of infectors is considered crucial for the effective control of TB because one patient is known to transmit the disease to 12–15 people/year on average through respiratory tract infection[1].

The development of biosensor DNA has attracted considerable attention due to their potential applications, including gene analysis, clinical diagnostics, forensic study and more medical applications. Among the various methods for DNA detection, electrochemical techniques offer great advantages with simplicity, rapidness, relatively low cost, and high sensitivity, and are suitable for the development of inexpensive and portable devices[2,3].

Issa *et al.*[4] was used electrochemical DNA biosensor using methylene blue on Screen Printed Carbon Electrode (SPCE) for the detection of *M. tuberculosis*. The difference voltammetric signal of methylene blue between DNA probes and *M. tuberculosis* amplified DNA indicated that the electrochemical biosensor is simple, sensitive and economical method for detecting *M. tuberculosis*. Hamdan *et al.*[5] was used a graphite pencil electrode and methylene blue to detect *M. tuberculosis* using electrochemical biosensors. Both methods are using electro active indicators. Nowadays, increased attention is given to direct label-free hybridization detection involving the intrinsic electro activity of nucleic acids. This hybridization method, based on oxidation signal of probe on modified gold electrodes in electrochemical DNA biosensor, seems to be a simple, less time consuming and more applicable strategy in comparison with active indicators [6].

Silva *et al.*[7] was used non-labelling electrochemical DNA biosensor based on Self Assembled Monolayer (SAM) modified gold electrode for single strand DNA (ssDNA) detection, using the covalently coupling of complex cysteamine (Cys)/ glutaraldehyde (Glu) as a cross-linking structure amino-functional, which allowed the DNA to hybridized efficiently. These new electrochemical DNA based-biosensors offer new opportunities in the analytical field, increasing the applications of DNA-based molecular diagnostics, taking full advantage of the existing modified electrode technologies.

In this study label-free voltammetric DNA biosensor using gold electrode modified by SAM of thiol has been used to determined the hybridization of DNA target synthetic and amplified DNA by using Polymerase Chain Reaction (PCR) method from blood sample of TB sufferer.

2. Material and Methods

Differential pulse voltammetry was carried out using potensiostat Metrohm® µAutolab type III connected to PC with NOVA software. Three electrode system were used, consisted of an Ag/AgCl as reference electrode, platinum electrode as an auxiliary electrode and gold electrode (0.5 mm) as working electrode.

All oligonucleotides DNA (probe, target and non-complementary sequences) were synthesized by First Base Asia. forward primer 5'- GAACTGGGCTTCGACATGAT -3'; reverse primer 5'- ATCAGGTGGGCTACCAAATG -3’[7]; Probe DNA sequences:5‘-IACIIICAATCCAIIIC-3' ; DNA Target sequences: 5‘- GCCCTGGATTGCCCG TC -3'. Glutaraldehyde (Glu) (25%), 2-aminoethanethiol or cysteamine (Cys) (95%) were purchased from Sigma. Alumina, ethanol (99.5%), hydrogen peroxide (30%), propanol, sulfuric acid were from Merck. The phosphate buffer (0.1 mol/L; pH 7.0) was used as electrolyte.

2.1. Pretreatment of the gold electrode.

The gold electrode was mechanically polished with alumina slurry followed by rinsing with distilled water and sonication in pure ethanol and water (1:1), for 2 minutes. After mechanical cleaning, the gold electrode suffered a chemical treatment by immersion in a ‘piranha solution’ (H_2SO_4/H_2O_2, 1 : 3 v/v) for 10 minutes at room temperature. Afterwards, the gold electrode was immersed in phosphate buffer (PB) and 10 cycles were carried out between + 0.2 and + 1.5 V at 50 mV/s. Finally, the electrode was rinsed thoroughly with distilled water for 10 minutes and after exposed to UV radiation for 15 minutes[7].

2.2. Self-assembled monolayer and DNA immobilization.

The pretreated electrodes were immersed into 25 mmol/L ethanolic solution of Cys for 2 hours at room temperature (25°C). After that, the electrode was washed with distilled water and incubated in a Glu solution (2.5% of Glu in 0.1 mol/L PB pH 7.0 at 4°C for 50 minutes). The Au-SAM electrode was then exhaustively washed with ultrapure water and incubated with DNA probe. The Au-SAM-DNA probe was incubated for 1 hours at room temperature (25°C). Then, the electrode was washed for 2 minutes twice with PB (pH 7.0) to remove the non-

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