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Preliminary study of electrochemical DNA sensor for Cucumber Mosaic Virus

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Abstract

An electrochemical DNA (EC-DNA) sensor using screen printed gold electrode (SPGE) was developed to detect CMV. Cyclic Voltammetric (CV) analysis was performed to characterize the working gold electrode surface using potassium ferrocyanide. A direct self assemble monolayer (SAM) format was applied. The modification of SPGE surfaces was conducted using 5mM thiol solution. Immobilisation of CMV DNA probe was carried out by activating carboxylic acid groups of thiol monolayer with 20µL of EDC-NHS mixture. Various concentration of CMV DNA probe solution (1ng/µL, 10ng/µL, and 100ng/µL) was applied to the gold surface. The hybridization process was carried out by applying CMV complementary DNA solution on top of CMV DNA probe layer. The DNA hybridization reaction occurred on the electrode surface was then electrochemically measured using CV analysis. The measurements were performed using Autolab potentiostat with NOVA 1.11 software. The significant potential of 0.1V was selected. The oxidation activity was occurred at + 0.1 ± 0.02mV and reduction activity was observed at range –ve 0.075 ± 0.025 mV.

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Keywords: Electrochemical DNA Sensor; Cucumber mosaic virus; Cyclic voltametry; DNA hybridization, Screen printed gold electrode

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1. Introduction

1.1. Cucumber mosaic virus

Cucumber mosaic virus (CMV) is the type member of the Cucumovirus genus in the family Bromoviridae. This virus found to be able to infect over 1000 plant species from 85 families, including dicotyledons, monocotyledons, herbage and woody plants, horticultural crops and wild plants [1],[2],[3]. It is spread naturally by more than 60 aphid species in a nonpersistent manner [1]. In Malaysia, CMV has been reported to be present in many important economic crops [4],[5],[6] and weeds [7]. CMV was found to be the most common viruses infecting and damaging chilli in Malaysia which results in the losses of yield ranges from 10%-15 % if infection came in late and may reach up to 60% if plants were infected at early stage [8]. The effective treatments have not been reported to prevent or eliminate CMV infection [9]. Therefore, early detection of infection is of the utmost importance and key to the successful management of CMV. Many serological and molecular detection techniques have been developed and exploited to identify and quantify viral plant pathogens [10].

1.2 Electrochemical DNA sensor

DNA based biosensors have recently gained popularity and much importance for detection of target genes responsible for diseases, in food industry, environment and in agriculture [11]. The determination using electrochemical biosensor methods has attracted much interest because of their simple instrumentation, high specificity, sensitivity, rapid, and is inexpensive with potential for applications in molecular sensing devices [12]. The idea in this research is to develop the detection method for CMV using electrochemical DNA nanosensor approach.

2. Material and Methods

Specific DNA probe and DNA complementary size about 18-mer for CMV coat protein were successfully designed and synthesis. The screen printed gold electrode (SPGE) was treated using thiol [11-Mercaptoundecanoic acid: 11-Mercapto-1-undecanol (prepared in absolute ethanol)]. The suitable fix potential for chronoamperometry assay was determined by immobilizing DNA probe on SPGE and then hybridized using CMV complementary DNA at different step potential from +600 mV to -600 mV within 100s. Immobilisation of CMV DNA probe was carried out by activating carboxylic acid groups of thiol monolayer with 20 µL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysulfosuccinimide (EDC-NHS) mixture previously prepared in HPLC grade ultrapure water. Ten microlitres of the 10mM CMV DNA probe solution was applied to the gold surface and was incubated for 2 hours at ambient temperature. The electrode was then washed with 0.05M Phosphate buffer pH 7.4 and ultra pure water to remove excess DNA probe. Then the similar process was applied for CMV DNA complementary for hybridization processes. The current signal produce from the hybridization process was measured using electrochemical analyzer (NOVA 1.11 software).

3. Result and Discussion

The applied potential was selected through the study of the electrochemical behaviour of the immobilized CMV DNA probe with a hybridized CMV complementary DNA on SPGE using chronoamperometry analysis. Figure 1 (a) indicates the result of different set potential applied to SPGE for 100s using electrochemical NOVA 1.11 software. The significant potential of 0.1V was selected due