

A study on sugarcane disease diagnosis at Khoozestan region in South of Iran

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Abstract : The aim of this research work is to study the direct electrochemical DNA sensor for rapid diagnosis of the most destructive plant disease of Khoozestan (means land of sugarcane) Province(47°40', 50°33') with area of 67282km² in South of Iran, sugarcane white leaf (SCWL) disease. Formation of a self-assembled monolayer on glassy carbon electrode was well done by the incorporation of 1% oligochitosan. The target ssDNA was stably immobilized on this oligochitosan modified electrode. Hybridization detection was accomplished by Volta metric measurement in buffer using a pair of 21 bp each known sequence ssDNA based on SCWL 16S DNA as a complimentary DNA probes. The established biosensor is capable to completely discriminate between healthy and SCWL diseased samples. Thus, this first successful real sample detection would allow great advantages towards an effective, convenient, and cheap method for ultra speed detection of diseases and various gene targets.

Keywords: Electrochemical, DNA sensor, sugarcane white leaf disease diagnosis, oligochitosan modified electrodes.

Introduction

DNA biosensor technologies has recently opened up a new era of biotechnology research due to their great promise for the simple, user , rapid and low cost. Development of electrochemical DNA sensors could provide also high sensitivity and specific detection of DNA sequences that are required for several fields including genetic identification, forensic medicine, environmental testing, rapid biological warfare agents and diagnosis of various diseases. A series of Volta metric approached electrochemical DNA sensors with either labeled or label-free methods for sequence specific DNA detection have been reviewed¹. Most biosensors commonly rely on the immobilization of single stranded DNA on a transducer surface and the self-assembly mono-layer techniques using amphiphilic molecules such as alkanethiol and chitosan are widely used for the formation of functionalized surfaces in the detection by hybridization of their complementary DNA²⁻³⁻⁴. Sugarcane white leaf (SCWL) disease caused by a phytoplasma is the most

important plant disease in Khoozestan. The disease causes several hundred million Rials losses to the Thai sugarcane industry each year⁵. Rapid, convenient and cheap diagnostic technique is urgently needed for reducing such economic loss. Electrochemical hybridization biosensors for the SCWL phytoplasma may greatly suite for this purpose. A preliminary investigation on Volta metric behavior of simple DNA biosensor using label-free DNA probe and oligochitosan, a natural cationic polymer with excellent DNA affinity as immobilization matrix are demonstrated in this present study.

Experimental Methods

Total DNA of SCWL diseased and healthy plants was extracted from sugarcane leaves by the CTAB method⁶ and stored in buffer (10mM TrisHCL and 1mM EDTA, pH 8.0). Detection of SCWL phytoplasma DNA was simply achieved by hybridization treatment with the two synthetic oligonucleotides probes primarily used as SCWL 16S

DNA primers in previous researches⁷⁻⁸⁻⁹. Two oligonucleotides with their sequences based on the 16S rRNA of the phytoplasma, 5'-GTT TGA TCC TGG CTC AGG ATT-3' and 5'-AAC CCC GAG AAC GTA TTC ACC-3' were used as DNA probes (PssDNAs) for specific detection of the phytoplasma DNA extracted from sugarcane plants.

The self assembled monolayer of oligochitosan modified glassy carbon electrode (Och/GCE) was prepared by coating a freshly polished glassy carbon electrode with 2.0 μl of 1% oligochitosan solution and it was dried at room temperature. The coated electrode was immersed in 0.1M NaOH for 30 min and was dried naturally prior to use.

Total DNA were extracted from healthy (H) and sugarcane white leaf (SCWL) diseased plants. Single strand DNA was prepared by heating the extracted DNA in boiling water bath for 10 min followed by rapid cooling in an ice bath. Immobilization of ssDNA was formed by immersing the oligochitosan modified electrode in 1.0 ml buffer containing 10 n mole ssDNA and stirring at room temperature for 3 hours. The electrode was then washed with 0.1% SDS phosphate buffer pH 7.0 three times and kept in buffer. The ssDNA immobilized oligochitosan modified electrodes (ssDNA/Och/GCE) were immersed in 1 ml hybridization buffer (2 x SSC, 0.3M NaCl and 0.03 M sodium citrate pH 7.0) containing 0.1 mg/ml mixture of the two DNA probes and stirred in water bath at 45°C for one hour. After the hybridization reaction was completed, the electrodes were washed three times with a washing solution containing 0.4M NaOH and 0.1% SDS prior to electrochemical detection.

Cyclic voltammeter (CV) were carried out with an ECO Chemise Auto lab PSTAT 30 potentiostat using the software package GPES 4.9 (General purpose Electrochemical System) in a 10 ml electrochemical cell using the ssDNA or dsDNA as working electrode, an Ag/AgCl/KCl as reference electrode and a platinum wire as counter electrode.

Measurements were performed in 0.01 M blank, buffer at 100 mV/s with the scan range from -0.2 to +0.6 (vs. Ag/AgCl/KCl). Charging current density (Jc) was measured at 0.3842 mV. The double layer capacitance of electrodes was calculated for F/cm² by the equation: $C_d = i/\nu S$, where i is the capacitance current, ν is the scan rate, and, S is the surface area of electrode.

Results and Discussion

The new DNA biosensor system was successfully developed by a regular concept of the DNA hybridization between their complementary

sequences and measurement of electrochemical transduction of the recognition reaction. There by, detection of SCWL phytoplasma in diseased leaf extract using the 16S DNA probes for specific hybridization was accomplished by electrochemical biosensor on oligochitosan modified glassy carbon electrode, for the first time. Although some of the works indicated high probability for practical use such as the detection of short sequences related to hepatitis B virus, the detection of cyan bacteria, *Microcystis* spp short DNA sequence and the detection of yeast DNA¹⁰⁻¹¹⁻¹².

The charging current and total capacitance of bare glassy carbon electrode (bare GCE) was appeared at 8.6000×10^{-8} JcA/ cm² and 260.28 $\mu\text{F}/\text{cm}^2$ using, buffer as an electrolyte. While the values obtained from oligochitosan self assembled monolayer-modified glassy carbon electrode were 4.6520×10^{-8} and 152.12 $\mu\text{F}/\text{cm}^2$, respectively. So, the electrochemical response of oligochitosan modified electrode (Och/GCE) was decreased to almost half value from the bare electrode. The ssDNA from each DNA extract sample was stably immobilized on oligochitosan modified electrode as indicated by decreasing of charging current down to 1.404×10^{-8} JcA/ cm² and total capacitance down to 44.12 $\mu\text{F}/\text{cm}^2$. The signals obtained from both immobilized ssDNA of healthy and SCWL diseased plants were similarly appeared. The electrochemical response was increasing up after performing the hybridization reaction with single-stranded 16S DNA probes (PssDNAs). Hybridization of the ssDNA from healthy plant sample (HssDNA) yielded 6.1290×10^{-8} JcA/ cm² charging current and 164.17 $\mu\text{F}/\text{cm}^2$ total capacitance. These electrochemical values from HssDNA could be considered as hybridization background signal commonly found in most DNA detection system. When the hybridization of ssDNA from SCWL diseased plant sample yielded significantly higher response values at 6.7300×10^{-8} JcA/ cm² charging current and 199.20 $\mu\text{F}/\text{cm}^2$ total capacitance. Thus, discrimination between healthy and diseased samples were obviously seen and confirmed by voltammogram records and their double-layer capacitance values as shown in Table1. Cyclic volt ammograms recorded in 0.01 M buffer at 100mV/s scan from -0.20 to +0.60 on, bare glassy carbon electrode(GCE), chitosan modified GCE (Och/GCE), SCWLssDNA immobilized Och/GCE (SCWLssDNA/Och/GCE), immobilized healthy plant ssDNA after hybridization treatment with SCWL ssDNA probe PssDNAs/HssDNA/Och/GCE), immobilized SCWL diseased plant ssDNA after hybridization treatment with SCWL ssDNA probes(PssDNAs/SCWLssDNA/Och/GCE).

Table1. Charging Current and total capacitance

Electrodes	Charging Current $j_c/A/cm^2$	Total Capacitance $\mu F/cm^2$
Bare GCE	8.6000×10^{-8}	260.28
Och/GCE	4.6520×10^{-8}	152.12
ScwlssDNA/Och/GCE	1.404×10^{-8}	44.12
PssDNAs/HssDNA/Och/GCE	6.1290×10^{-8}	164.17
PssDNA/SCWLssDNA/Och/GCE	6.7300×10^{-8}	199.20

Table1 shows charging current and total capacitance of bare GCE, Och/GCE, SCWLssDNA/Och/GCE, PssDNAs/HssDNA/GCE, PssDNAs/SCWLssDNA/Och/GCE under the scan rate 100 mV/s in buffer at 0.3842 mV measurement.

Conclusions

The sample single strand DNA prepared from healthy and SCWL diseased plant extracts was successfully immobilized onto an oligochitosan modified glassy carbon electrode. This immobilization method is relatively simple, convenient and avoids the costly and tedious chemical detribalization of DNA. Moreover, it is certainly cheap and worthwhile to deal with as all the chitosan formulas including oligochitosan are domestic products of Khoozestan. Since only the complimentary ssDNA of SCWL phytoplasma from diseased plant could hybridize the ssDNA probes to form double-strand DNA sequences on modified

electrode as shown by the corresponding signals such as charging current and total capacitance that were significantly higher than the signals from hybridization reaction with healthy plant ssDNA.(Table1). The DNA biosensor systems reported to date focus almost on the analysis of purified DNA sample or short synthetic DNA sequences to prove the effectiveness of specific DNA detection and mismatch DNA clarification. But neither of them had performed the detection with crude extracts nor with the real samples. Here, we are reporting for the first time, a direct and simple DNA detection system with real diseased plants without any DNA purification process before hybridization with the probes. This successful real sample detection would offer a number of advantages in the way towards an effective, cheap and rapid method for early diagnosis of diseases and genotypic identification.

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