Journal of Chemistry, Vol. 45 (2), P. 241 - 244, 2007

ELECTROCHEMICAL DNA SENSOR FOR LABEL - FREE SOYBEAN TRANSGENIC DETECTION

Received 3 July 2006

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SUMMARY

This paper describes the development of electrochemical label- free DNA sensor for detection of transgenic in Round–up Ready Soybean (herbicide tolerance). Two pairs of electrodes which the optimized dimension is 70μm x 30μm (the width of electrode x inter-distance between fingers), were fabricated on a chip for differential measures. The DNA probe (35S 5'-GCT CCT ACA AAT GCC ATC A-3) was designed in such a way that it can hybridize with the CaMV 35S promoter of Roundup Ready soybean. The DNA was immobilized on the surface of the sensor by the mean of (3-aminopropyl)triethoxysilane (APTS) polymer. The response time of DNA sensor is less than 1 second; in case of 100% matching level, the sensitivity is 2 mV/μM. The activity of DNA sensor remains unchanged after 9 months of storage at -4°C in DI water. Some random soybean samples were collected, extracted and tested by the DNA sensor and the results were well matched with the of PCR method.

I - INTRODUCTION

The detection of specific DNA/RNA sequences is great important in numerous applications, such as clinical diagnosis, food safety, pharmacogenetic, criminology, and many other fields [1]. DNA sensors are based on single-stranded oligonucleotide (ODN) probe (designed DNA sequence) immobilized on a physical substrate. The immobilization of tagged double stranded ODNs provides the basis of sample detection and the resulting pattern is detected by (for example) fluorescence, chemiluminescance or from the tag [2]. The main drawbacks of such detection methods are cost, complexity and inefficiency of tagging reactions. A number of alternative 'label-free' methodologies have also been developed, such as quartz crystal microbalance (QCM) [3] or electrochemical sensors with the aim to detect the hybridization event directly [3, 4]. The electrochemical sensor is found to be well suited in realizing DNA sensors because of the rapid and inexpensive detection procedure involved [5]. In this paper, we report the development of label-free electrochemical DNA sensor based on microelectrodes to detect the transgenic soybean.

II - EXPERIMENTAL

1. Sensor fabrication

Used as transducer in biosensor system [3], the microelectrodes were designed to be compatible with PCI standard for easy connections. Some model of electrodes with various configurations were designed and fabricated at ITIMS. Our "first version" sensor consists of a pairs of microelectrodes on the surface of silicon substrate, one of which acts as working sensor and other as a reference

electrode. The optimal dimension was 70 µm x 30 µm. The fabrication process of this kind of sensors was detailed here [6].

2. Design DNA probe

The analysis conducted by means of DNA hybridization is very specific combination which resulting in the changing in electric density. Thus, to detect the transgenic in plants, we must understand, firstly, the structure of DNA target sequence by searching and alignment it with the reported sequences in gene bank with the aim to find out the information of inserted foreign DNA strand which changes the plant properties. Based on the information of DNA target strands, the sequences of probe will be defined. In soybean numerous numbers of tolerances could be found as ampicilin, vitamin and herbicide tolerances…. In this work, the inserted herbicide tolerance gene was selected as DNA target sequence. The sequence of the selected strand was followed 35S-5'-CGAGGATGTTTACGGTAGT-3'. As the result DNA probe was interpreted as *35S-5'-GCT CCT ACA AAT GCC ATCA-3'*.

3. DNA immobilization

Theoretically, DNA strand can bind directly to the surface of sensor. But it is found that this combination is too weak and the DNA strand can be removed immediately when sensor is immersed in measuring cell. To overcome this problem, we prepared a conducting polymer that is considered as a media junction to conjugate DNA probe and substrate. This material enhances the electric mobility then in turn, improves the sensitivity of DNA sensor. The polymer film used in this DNA sensor has many different kinds such as polypyrrole, polyaniline, polythiophen. Detail of using such polymers for DNA sensor application will be reported in another paper. In this work, we used APTS film as a conducting polymer deposited onto microelectrodes surface by using dip-coating technique. The micro-electrodes was dipped in APTS/Ethanol (v/v = 7:3) solution in 5 minutes then pulled up with 1mm/minute velocity by using COMTEN system. After that the deposited membrane was dried in air for 10 minutes.

Before using, the DNA probe strand was purified in ethanol. The concentration of DNA strands was determined by measurements of absorbance at 260 nm on a UV-Vis spectrophotometer UDV-3500.

DNA immobilization is based on reaction between amine group of the polymer membrane and phosphate group of DNA probe sequence. N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) $1.5x10^{-2}$ M and N-methyl-imidazole (MIA) were used to activate the phosphate group, via the formation of intermediate labile ester. After immobilization, the DNA sensor was kept in DI water at T = 37°C for 18 hours.

4. Measurements

The signal measurement relied on hybridizing of two DNA sequences which change electric density at near surface of DNA sensor then will be traced by the sensor.

The reference signal of alternative current had frequency of 10 KHz and amplitude of 100mV, taken out from generator of the Lock-in Amplifier SR830, was applied on two identical micro-electrodes of DNA sensor. The output signal was acquired by measuring the voltage dropped on two 1 KΩ resistances by the channels A and B of the Lock-in Amplifier, which was set on automatically differential measurement mode.

III - RESULTS AND DISCUSSION

1. The characterizations of the DNA sensor

As mentioned previously, the hybridization releases the change in conductance at near surface of DNA sensor. If this event is not detected, it can be said that no hybridization between DNA strands due to unchanged conductance is observed and interpreted by the horizontal line in figure 1. The contrary, the hybridization is explained by vertical line where the conductance at near surface of DNA sensor is modified. The response time of DNA sensor is very fast, less than 1 second which can be applicable fast detection of the real sample.

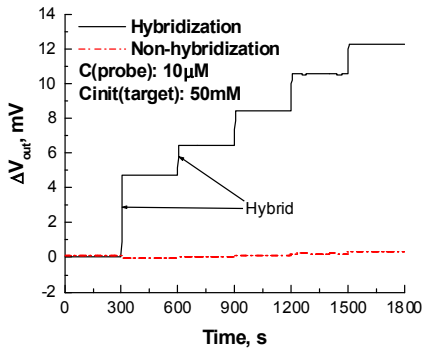

Figure 1: The timing characteristic of the DNA sensor

2. The DNA concentration dependence of DNA sensor

As previously described, the DNA sensor expresses an excellent response to hybridization detection of DNA strands. Figure 2 illustrated DNA concentrations is a function of conductance.

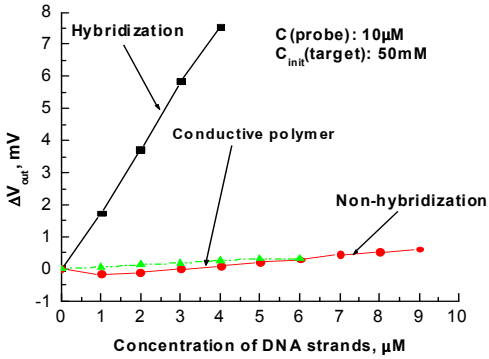

Figure 2: The concentration characteristic of the DNA sensor

For 100% matching hybridization of DNA strand, the sensitivity of DNA sensor is found at 2 mV/µM. In case of no–hybridization, the conductance change was not observed.

3. The life – time of DNA sensor

Unlike enzyme based biosensors that the enzyme activity is decreased dramatically due to environmental parameters such as temperature, buffer [7], the repeatability of DNA sensor was maintained in much longer term. As illustrated in figure 3, the activity of DNA sensor almost unchanged was observed after 9 months stored in DI water. In addition, the DNA storage does not require specific conditions (just DI water as compared to buffer in case of enzyme).

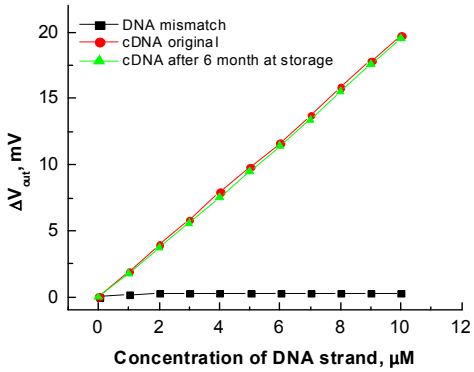

Figure 3: The lifetime of the DNA sensor

4. Detection of the transgenic soybean by using microelectrodes-based DNA sensor and compared with PCR method

In this work, transgenic soybean was detected by DNA sensor and then the results were compared with polymerase chain reaction (PCR) method that is traditional detection method for genetic modified organism (GMO). The samples of soybean that non-transgenic and transgenic were provided by the IBT-VAST.

In this first generation, the hybridization is fully matched (100%). In the later generation, F1b, F2, F2b, and F5 (sequence not shown) the matching levels were decreased as illustrated in table 1. For other samples named N1 ÷ N5 (provided by IBT) the matching level is around 40%. Theoretically, this value should be zero. However, the genomes of soybean are very large (over 3000 nucleic acids), the hybridization can be randomly occurred between DNA probe and similar DNA sequences.

IV - CONCLUSIONS

In this work, the microelectrodes were designed and fabricated by using microelectronic technology at ITIMS with the 70 µm×30 µm dimension. The DNA probe was designed to hybridize with the CaMV 35S

Table 1: Detection of the transgenic of soybean by DNA sensor and PCR method

Sample	Matching level in PCR method, %	Matching level in DNA sensor, %	Sample	Matching level in PCR method, %	Matching level in DNA sensor, %
F1	100	100	N1	40	40.7
F1b	100	96.5	N2	41	40.7
F2	78	76.4	N3	38	39.2
F2b	78	78.9	N4	38	38.2
F5	–	-	N5	35	39.7

promoter, which is inserted in RR soybean genome (herbicide tolerance). The APTS conducting polymer was used for immobilize DNA sequence on the surface of sensor.

The response time of DNA sensor is smaller than 1 second, and sensitivity is 2 mV/μM for the 100% matching level. After 9 months of storage at -4°C in DI water, the activity of DNA sensor remains unchanged.

As mentioned from beginning, this work is just a first step to verify the feasibility of this method for DNA detection. In the next phase, the temperature changes which influence the reliability and the analysis results will be investigated. The target and probe sequences will be also lengthened to enhance the specific of the sensor.

Acknowledgements: *This work was supported by the project KHCB-4050-06. The authors would like to thank kindly helps from MSc. Nguyen Dang Ton, Applied DNA Technology Lab Institute of Biotechnology, Vietnamese Academy of Science and Technology.*

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