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DNA COVALENT ATTACHMENT ON CONDUCTOMETRIC BIOSENSOR FOR MODIFIED GENETIC SOYBEAN DETECTION

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Abstract. *This paper described the covalent attachment method to immobilize Deoxyribonucleic acid (DNA) sequences on surface of DNA sensor which was used to determine herbicide tolerance transgenic of soybean. The probe sequence was (5'GCCATCGTTGAAGATGCCTCTGCC-3') which can hybridize with the CaMV 35S promoter of Roundup Ready soybean that was attached onto the surface of sensor by means of 3-AminoPropyl Triethoxy-Silane (APTS). The DNA sequence bindings were identified by the FTIR spectra. The morphology of attached – DNA sequences onto that was investigated by atomic force microscopy (AFM). The sensitivity of sensor was found, in this work, 4.28mV/μM. Temperature effect on the hybridization process was also described.*

I. INTRODUCTION

Genetic testing requires the development of simple construction, ease of use, fair cost, miniaturized analytical and fast detect methods. Traditional methods for detecting DNA hybridization such as PCR, or electrophoresis are slow and labor intensive. The DNA biosensor offers a promising alternative for faster, cheaper, and simpler nucleic acid assays. The DNA hybridization commonly relies on immobilization of probe DNA onto a transducer surface to recognize its complementary target sequence. The binding of probe attached onto surface and 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. Those biosensors have been reported based on electrochemical [2-4], optical [5-6], and micro gravimetric detection methods [7-8]. Among them, DNA electrochemical biosensors have attracted considerable attention to the detection of DNA hybridization. The high sensitivity, compatibility with modern micro fabrication technologies, inexpensive, portability, label – free make them excellent candidates for wide variety applications in areas such as medical diagnostics [1,9], drug screening [10-13], food safety [14-16], and many other fields.

To prepare DNA biosensor, the probe immobilization step plays a crucial role. The achievement of high sensitivity and selectivity requires maintaining its activity, maximization of the efficient hybridization and minimization of non-specific adsorption oligonucleotide [1]. Therefore, it is very important to control the density of probe on the sensor surface to assure high reactivity, orientation as well as avoiding non-specific adsorption/binding events. Moreover, it is essential that the probe should be designed to not

denature on the surface. Up to now, number of the approaches such as covalent attachment [17-21], cross – linked [22], electrostatic interaction [23], self-assembly mono-layer (SAMs) [24-28] have been developed to produce sensor surface- immobilized DNA.

Recently, the covalent attachment used to immobilize oligonucleotides to a functioned surface because that can be achieved in a short time, less chemicals consumption and adapt to different transducers. In addition, this strategy allows reducing non-specific adsorption and prevents the loss of probe oligonucleotides from the surface.

In this paper, we describe the covalent attachment of oligonucleotides to surface of sensor based on conductometric biosensor using APTES conducting polymer and EDC, MIA – activated DNA sequences. Results were verified by Fourier Transform IR spectroscopy and atomic force microscopy. The characteristic of DNA sensor and influence matching temperature also studied.

II. EXPERIMENT

II.1. Activation and silanization of sensor surface procedures

The surface of sensor can be contaminated by different kinds of compounds. Such contaminants are bound onto the surface by weak electrostatic forces or by Van der Waals forces. So the surface pre-treatment is necessary to get a contamination-free transducer surface. It was dipped in boiled- 65% HNO_3 acid followed by rinsing in deionized water and nitrogen dry.

The surface of sensor was then activated by saturated KCr_2O_7 in H_2SO_4 98% at room temperature for 15 minutes, rinsed with deionized water and dried by nitrogen gas. This treatment enriches the number of hydroxyl groups on the surface on which chemical bindings of the functional organiosilane were formed.

The silanization of the surface was accomplished for an hour in APTS/Ethanol (3:7 V/V) at room temperature and then was rinsed with de-ionized water and dried under nitrogen.

II.2. DNA immobilization

DNA probe (*5'-GGCCATCGTTGAAGATGCCTCTGCC-3'*), itself, can not couple directly to the amino groups of APTES, it needs to be activated by using N'-(3-dimethylaminopropyl)-N-ethylcarbo-diimide hydrochloride (EDC) 1.5×10^{-2} M and N-methyl-imidazole (MIA). Product of this process is an intermediate labile ester which is easy to bond with APTES film. To stabilize such complex, the DNA sensor was annealed in DI water at T=37°C for 18 hours.

II.3. Measurements

Differential measurement was realized to determine the changes of conductance of DNA sensor. A 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 microelectrodes of DNA sensor. The output signal was acquired by measuring the voltage drop on two resistances of 1 KΩ by the channels A and B of the Lock-in Amplifier.

III. RESULTS AND DISCUSSION

FTIR spectra of DNA – APTS binding

In this work, we used the FTIR spectroscopy to make sure the existence of conducting polymer (APTS) and DNA sequence onto the microelectrode surface. The infrared spectrum of the DNA-APTS complexes was performed on Nicolet 6700 FT-IR spectrometer. The IR spectra was illustrated in Fig. 1 in which 1647 –1559 cm^{-1} vibration plane implied G-C pairs and A-T base pairs while the backbone phosphate group at 1128 - 1263cm^{-1} were perturbed upon APTS interaction [29]. The presence of NH_2 group of conducting polymer (APTS) can be seen by a strong absorption at 1508cm^{-1} (data not shown). These results are similar M.Yamaura's [30].

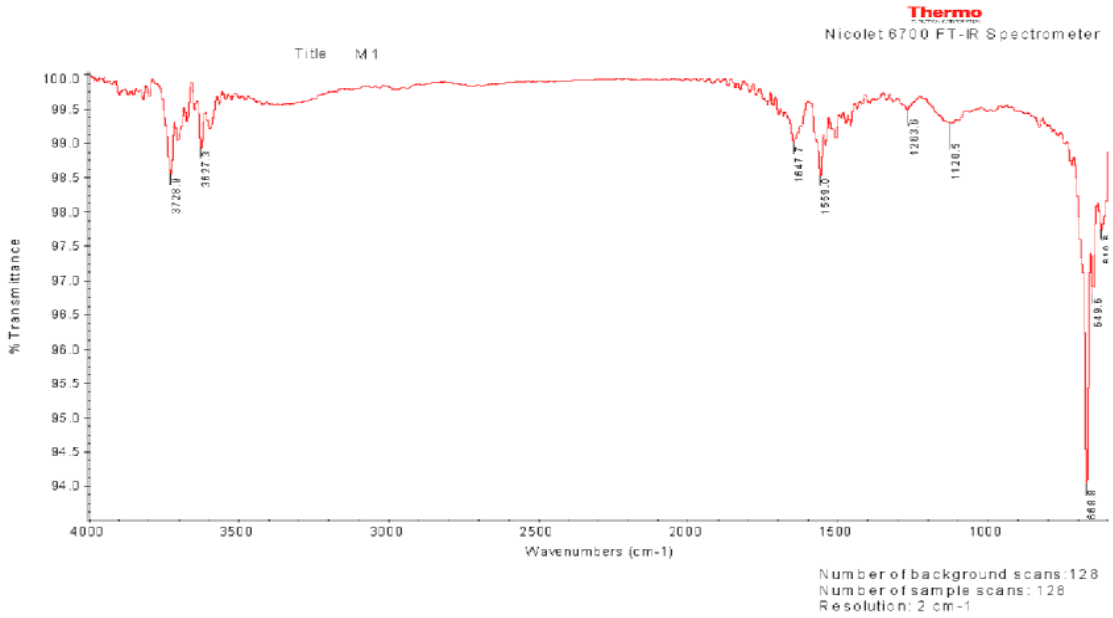


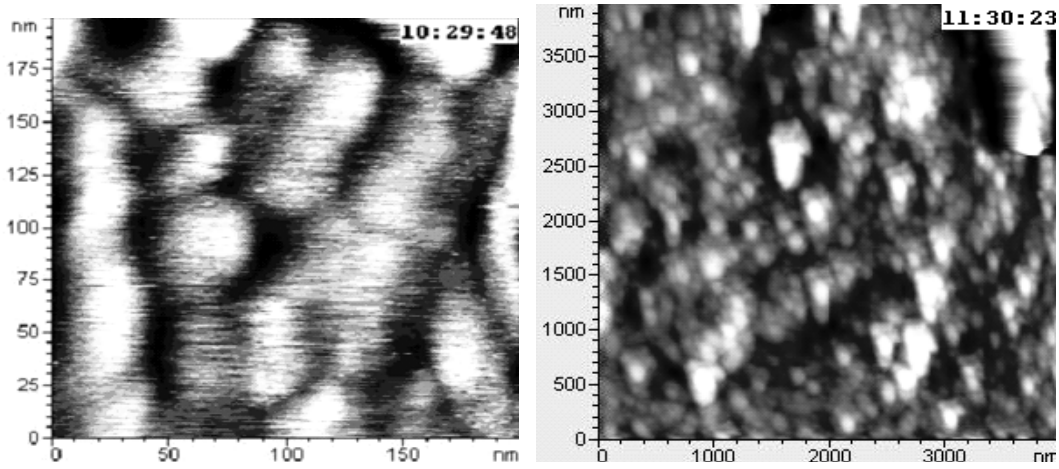
Fig. 1. FTIR spectra of APTS and DNA strand

AFM characterization of immobilized oligonucleotides

The mobility and the orientation of oligo strand onto sensor surface can be given by AFM morphology through which non-specific adsorption can be avoided; the uniform distribution of the oligo inside the polymeric matrix can also be improved leading to a better sensitivity of the DNA sensor.

Fig. 2a shows the AFM image of sensor surface after the DNA immobilization. The attachment is not so uniform over the surface; the molecules formed small clusters and superposed preventing the oligos from the binding onto ATPS/conductive polymer substrate.

In Fig. 2b, in small scale, the DNA distribution was very consistent. However, the oligo density was, not as expected, still scattered; they left the blank holes, corresponding to dark areas in the image, which promote the non- specific adsorption of DNA sequence from solution onto the membrane. This issue would be improved in future work.



(a) (b)

Fig. 2. (a) AFM topographical images of immobilized. (b) DNA film on the sensor surface

The characterizations of the DNA sensor

As above-mentioned, the probe-attached electrode is commonly soaked into solution of target DNA strand. A DNA helix sequence was formed on surface of electrode when target/immobilized DNA matching occurred. Such the event of hybridization is commonly detected by changes in the conductance of the conductive membrane on the surface of sensors.

The hybridization of DNA strands change in conductance at next to surface of DNA sensor leading to the change in output signal of the system. This event described in Fig. 3 where the hybridization was explained by linear that output signal is a function of DNA target strands concentration. In case of matching hybridization between DNA probe strands and DNA target strands are 100 percents, the sensitivity of DNA sensor is 4.28mV/μM. The fast response time of DNA sensor, as illustrated in smaller window, was less than 1 minute which is one of strong point of such kind of DNA sensor for the feasibly of in field/on site detect.

Influence of matching temperature

The detection of oligonucleotide often depends on many factors including the DNA matching temperature, the double strand length [31], and concentration of surrounding buffer [32]. Among them, matching temperature is considered as one of the factors that influence the hybridization of DNA sequence. To optimize the matching temperature, it is

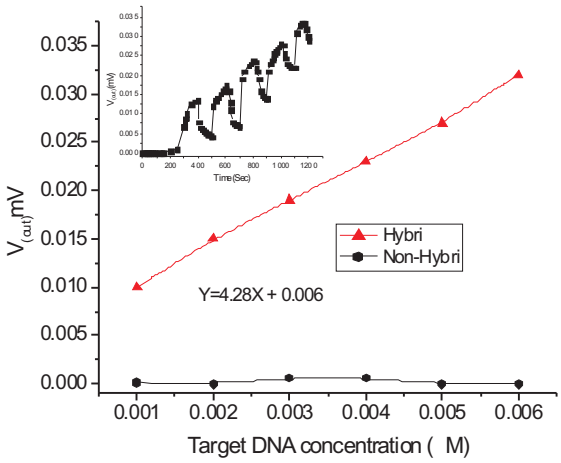


Fig. 3. The characterizations of the DNA sensor

necessary to define the melting temperature (T_m) of DNA at which the duplex is unfolded into paired and other unpaired sequence [33].

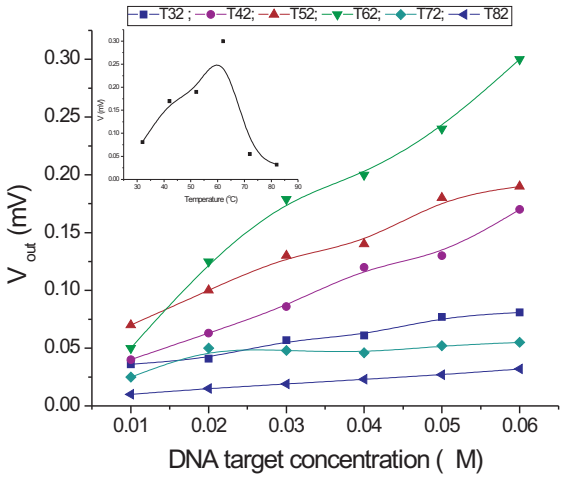


Fig. 4. Impact of oligonucleotide matching temperature

The T_m indicates the transition from double helix to random coil formation and determined by DNA G-C base pairs in the sequence [34]. It is also proven that the closer matching point to T_m the better it is. T_m can be predicted by using the thermodynamic nearest–neighbor model through its entropy and enthalpy [31] or simpler equations [33]. T_m depends upon on each DNA sequence including the species and its length.

As presented in Fig. 4, from 32^0C to 62^0C, the output signal increases proportionally to the change in temperature. It begins to decrease at 62^0C which is considered as T_m of

this sequence. This result matches well with calculation given by Marmur–Schildkraut–Doty formulas [33]. And, the matching temperature should not excess the T_m (62^0C) for better analysis.

IV. CONCLUSIONS

This work report the DNA (5'GCCATCGTTGAAGATGCCTCTGCC-3') covalent attachment onto the surface of DNA sensor to determine herbicide tolerance transgenic of soybean. These sequences were successfully immobilized on the surface of sensor by means of APTS conducting polymer using covalent attachment method. The attachment was verified by FTIR spectra and the morphology of DNA film was characterized by mode AFM images.

The hybridization occurred in aqueous solution shows that the DNA sensor limit detection is 4.28mV/μM, the influence of hybridization temperature on output signal of the DNA sensor was also investigated. The range between 30^0C and 50^0C can be considered optimal matching temperature to detect hybridization of this DNA sequence.

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