Journal of Chemistry, Vol. 46 (1), P. 127 - 132, 2008

ELECTROCHEMICAL DNA SENSOR FOR HERPES VIRUS DETECTION

Received 23 July 2007

PHUONG DINH TAM[1,2], MAI ANH TUAN[1,2], TRAN QUANG HUY[4], NGUYEN DUC CHIEN[2,3]

[1]*Hanoi Advanced School of Science and Technology*

[2]*International Training Institute for Materials Science (ITIMS)*

[3]*Institute of Engineering Physics, Hanoi University of Technology*

[4]*National Institute of Hygiene and Epidemiology*

SUMMARY

This paper reports the development of electrochemical DNA sensor for biomedical application. The micro-sensor was fabricated at Hanoi University of Technology clean room by microelectronic technology. Two pairs of electrodes of which optimized dimension is 70 μm x 30 μm (the width of electrode x inter-distance between fingers), were created on a chip for differential measurements. The DNA of herpes simplex virus 1 (HSV-1) with the DNA probe sequence is 5□□AT CAC CGA CCC GGA GAG GGA C□3□ was immobilized onto the surface of the sensors by means of 3-aminopropyltriethoxysilance (APTS). The results showed that the DNA sensor can determine the DNA target of HSV-1 in the sample at the concentration as low as 0.5 nM. The response time of the DNA sensor is less than a minute. Temperature effect was also investigated in this work.

Keywords: *DNA sensor, electrochemical detection, APTS, virus, hybridization.*

I - INTRODUCTION

In recent years, many works have been devoted to DNA sensor development thanks to simplicity, specificity, exceptional sensitivity and selectivity for the detection of specific genes [1 - 3]. This technology has opened a perspective to overcome most of disadvantages of the conventional methods for applications in food, agriculture, environment, clinic and drug etc [3 - 12]. The detection of specific sequences in clinical samples collected from patients diseases is very important in DNA pre-diagnostics which allows us a time long enough to find out the solution for that [8 - 12]. With the ability of amplification from tiny amounts of DNA into readable quantities, the conventional methods such as polymerase chain reaction (PCR) and Real-time PCR have been applied mostly in the fields of biomedicine. However, these techniques require time, sample preparation, well-equipped apparatus and well-educated operators [3, 9].

DNA sensor is considered as a promising tool in pre-diagnostics, and prevention and control of the infectious diseases which supposed to be able for real-time and on site analysis [2, 3, 9].

High sensitive and selective DNA sensors are of great importance in the diagnosis of genetic diseases, the detection of infectious agents, and identification in forensic and environmental cases [1 - 12]. There are various

types of DNA sensors which developed over the years. Methods used for DNA detection in those sensors have been reported to be based on radiochemical, enzymatic, fluorescent, electrochemical, optical, and acoustic wave techniques [13]. These days, optical DNA sensors are used predominantly and promising results have been reported. The disadvantage of these optical sensors, however, is the requirement of a separate labeling process, equipment to stimulate the transducer, high complexity, and thus, a higher cost in order to conduct an analysis [14].

Electrochemical methods of hybridization detection present a good alternative in comparison with well-developed fluorescent detection. Over the past decade, a large progress has been made towards the development of electrochemical DNA sensors. Considerable advantage has been described these devices due to their promise for obtaining specific information in a faster, simpler and less expensive way. In addition, they have a high potential for automation and miniaturization since only basic electrochemical equipment is required [2, 12, 15 - 17].

We report in this paper the development of DNA sensor for detection of herpes simplex viruses 1 (HSV-1). The herpes simplex virus (HSV) is an enveloped double-stranded DNA virus. There are two distinct forms of HSV, serotype 1 and serotype 2 (HSV-1 and HSV-2). HSV-2 is the most common cause of genital herpes, whereas HSV-1 is the most common cause of facial herpes or cold scores. HSV-1 is transmitted through contact with oral secretions. More information about this kind of virus and diagnostic tests can be found in [18].

II - EXPERIMENT

1. Chemical reagents

DNA probe, with a specific sequence to HSV-1 of 5'—AT CAC CGA CCC GGA GAG GGA C—3' and complementary DNA target sequence for HSV-1 of 3'-TA GTG GCT GGG CCT CTC CCT G-5' was supplied by Invitrogen Life Technologies Company.

Other chemicals including nitric acid; hydrogen fluoride; hydrochloric acid; acetone; methanol; alcohol 100%; H_2SO_4; KCr_2O_7; 3-aminopropyl-triethoxy-silance (APTS), 1-ethyl-3-(dimethyl-aminopropyl)carbodiimide (EDC); 1-methylimidazole (MIA); KCl; NaCl; Na_2HPO_4; KH_2PO_4; nuclease-free water are of analytical grade.

2. Sensor fabrication

The DNA sensor based on microelectrode with various configurations were designed and fabricated at ITIMS, Hanoi University of Technology. The sensor consists of a pairs of microelectrodes on the surface of silicon substrate, one of which acts as working sensor and the other as a reference electrode. The optimal dimension was 70 μm × 30 μm. The detailed fabrication process was discussed in [19]. A SEM image of the microstructure is shown in figure 1.

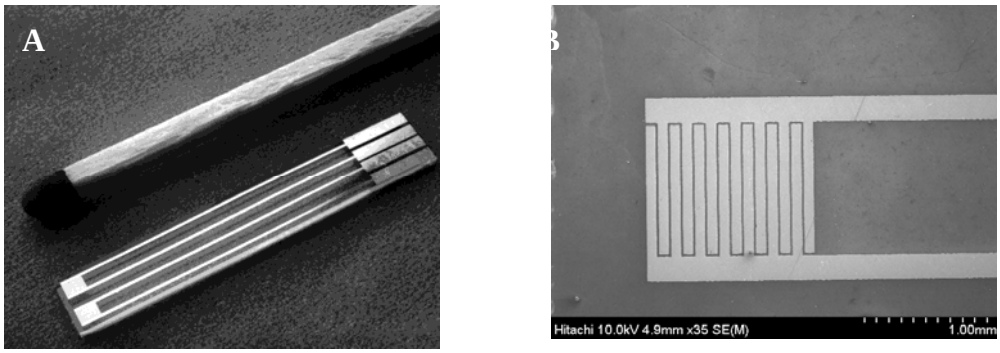
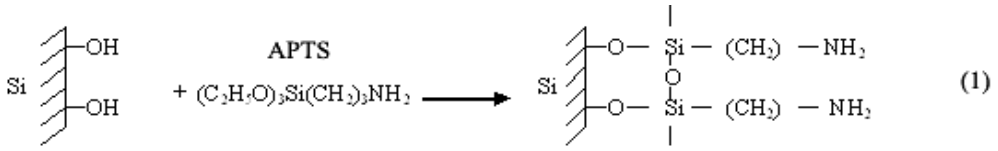


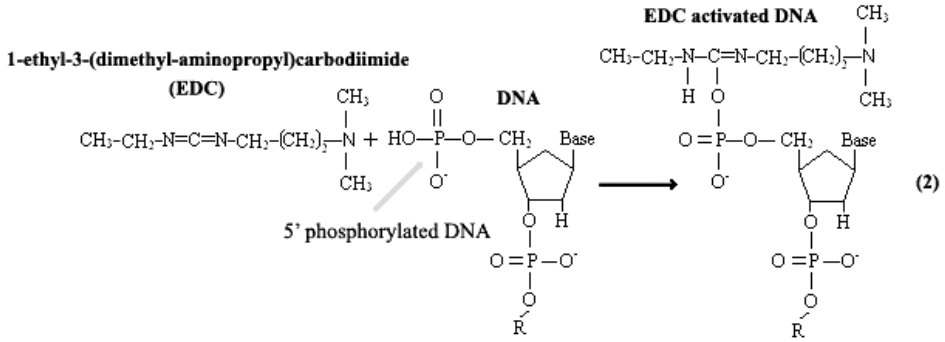
Figure 1: SEM image of an Au micro-electrode
A) The electrode based sensor; B) The electrode sensing area as mixed comb structure

3. DNA immobilization

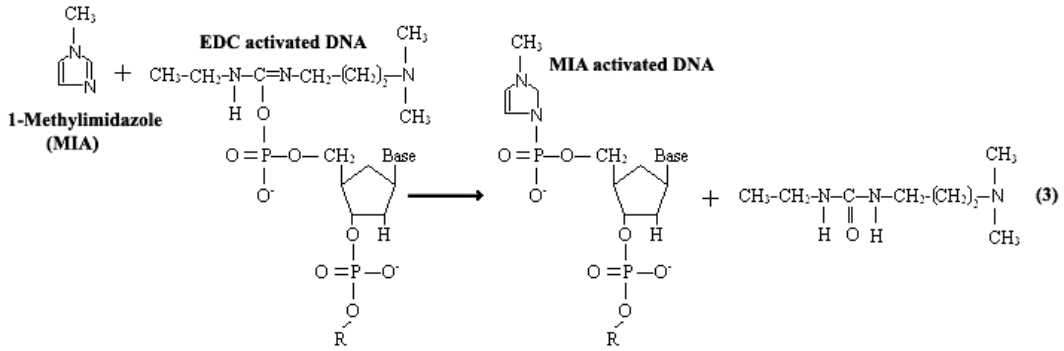
Several methods available are used to label single—stranded DNA probes to sensing surface of electrodes [20, 21, 22] by using conducting polymer. In this work, the covalent method was used to attach DNA sequences onto the surface of sensor. This immobilization was based on the reaction between amino group of the APTS conducting polymer and phosphate group of DNA sequence. The sensor, after the surface clean process to remove contaminations and activate the hydroxyl groups, was immersed in APTS: ethanol mixture (3:7 v/v) for an hour (eq. 1):



The oxygen atom in phosphate group of DNA (5' terminal) was used as interface media to bind DNA probe with amino group of APTS. These atoms were, first, activated for covalent bonds with amino group by means of EDC $1.5x10^{-2}$ M.



In this step, MIA was added to stabilize the activated EDC molecules which were labile in solution. The reduction of MIA and EDC activated DNA was described in eq. (3).

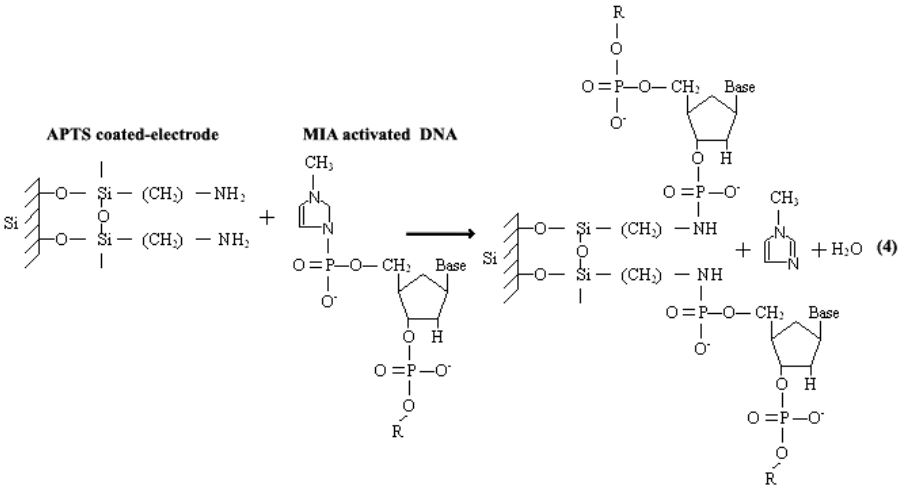


The MIA activated DNA probes were immobilized on electrode surface that was shown in eq. (4). The DNA sensor was then annealed in DI water at t = 37°C for 18 hours.

4. Measurements

Differential measurements were realized to determine the changes in conductance of DNA membrane. AC reference signal (10 KHz, 100 mV sine wave) generated by the generator of Lock-in Amplifier SR830, was applied on two identical micro-electrodes of DNA sensor. The output signal was acquired by measuring the voltage drop on two 1 K□ resistances by the A and B channels of the Lock-in Amplifier. The DNA probe concentration of 100 mM was

immobilized onto APTS coated-electrode in our experiments. The different concentrations of complementary DNA targets varying from 0.5 to 3nM were dropped into DNA sensor contained solution. All measurements were performed at room temperature. The results of output signals were recognized by Lock-in Amplifier SR830. In this experiment, five DNA sensors were used to test the hybridization of DNA sequences.



III - RESULTS AND DISCUSSION

1. FTIR spectrum measurements

To verify the existence of DNA sequence onto the microelectrode surface, the infrared spectrum of the DNA-APTS complexes performed on Nicolet 6700 FT-IR spectrometer machine (Thermo USA) in the effective range 4000 - 600 cm^{-1} was used to evaluate the bonds between phosphate group of DNA strains and amine group of APTS. The result shows a good matching with known data base of the FTIR library. As seen in Fig. 2, the IR spectra was illustrated 1750 - 1600 cm^{-1} vibration plane implied G-C pairs and A-T base pairs while the backbone phosphate group at 1085 cm^{-1} were perturbed upon APTS interaction (data not shown). The presence of NH_2 group of conducting polymer (APTS) can be seen by a strong absorption at 1526 cm^{-1}.

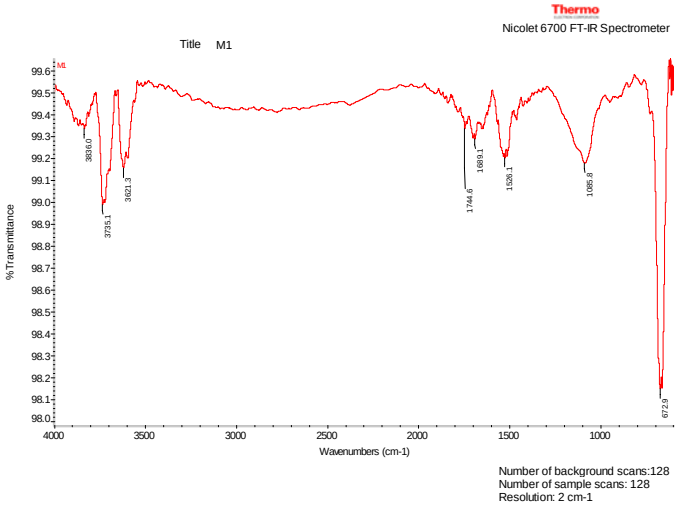


Fig. 2: FTIR spectra (swept range 4000 - 600 cm^{-1}) of the bonds DNA- APTS

2. DNA sensor for sequence detection

Figure 3 presents the change in conductance of the DNA membrane versus concentration by the DNA target. The probe/target DNA sequence mismatch in the sample was explained the dash line where the curve was nearly unchanged while the hybridization between the DNA probes and complementary DNA targets was described as the solid black line in which the signal was linearly proportional to concentration of the DNA target. The figure shows that DNA sensor can detect the complementary DNA targets as low as 0.5 nM of in less than 1 minute (data not shown) at room temperature.

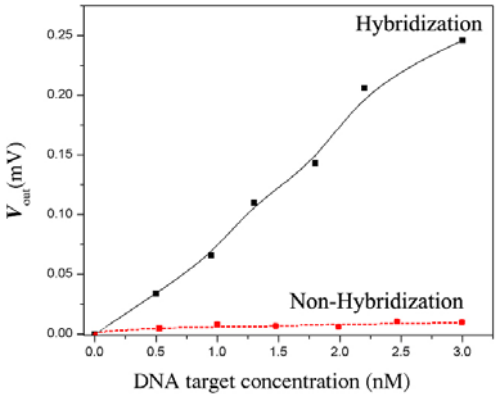


Fig. 3: The hybridization between probes and targets of HSV-1 at room temperature

Figure 4 illustrates the temperature effect on coupling ability of the DNA sequence. We performed several experiments from 37 to 85ºC. As seen in the figure, the matching rate between probe and target sequence was increased at elevated temperature.

With specifically designed (5'—AT CAC CGA CCC GGA GAG GGA C—3' for probe and 3'-TA GTG GCT GGG CCT CTC CCT G-5'for complementary DNA target sequence of HSV-1) 70ºC was considered as melting point at which the signal was began to decrease which understood by unfolding the double helix. The optimal temperature was found at 60 - 65ºC range.

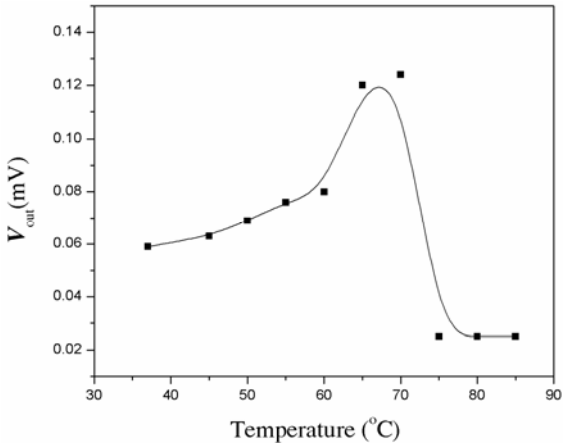


Fig. 4: Temperature effect on hybridization of the HSV-1 DNA sequence.
The melting temperature was found at 70□C

IV - CONCLUSION

This paper reports the potential ability of DNA sensor HSV-1 virus detection. The optimal temperature range found for such kind of DNA was from 60 to 65^{0}C. The DNA sensor can determine the DNA target of HSV-1 in the sample at the concentration as low as 0.5 nM. Such concentration is very important in enzyme based biosensors application for detection of toxic compound in water but it is still a quite big one in DNA application for virus detection. Such characteristic was believed to be improved by increasing the resolution of the fingers of sensor and needs further work.

Acknowledgement: *This work is financed by VLIR□HUT program, Project No. AP07/PRj03/Nr.02.*

REFERENCES

1. Joseph Wang. Analytica Chimica Acta, 469, 63 - 71 (2002).
2. T Gregory Drummond et al. Nature Biotechnology, Vol. 21 (23) (Oct. 2003), 1192 - 1199.
3. Bansi D. Malhotra et al. Current applied physics, 5, 92 - 97 (2005).

4. A. Lermo, S. Campoy et al. Biosensors and Bioelectronics, 22, 2010 - 2017 (2007).
5. D. Bystricka, O. Lenz et al. Journal of Virological Methods, 128, 176 - 182 (2005).
6. Sara Rodriguez-Mozaz et al. Talanta, 65, 291 - 297 (2005).
7. J. Justin Gooding et al. Analytica Chimica Acta, 559, 137 - 151 (2006).
8. Olivier Lazcka., F. Javier Del Campo et al. Biosensors and Bioelectronics, 22, 1205 - 1217 (2007).
9. Carla dos Santos Riccardi et al. Talanta, 70, 637 - 643 (2006).
10. Y.K. Ye., J.H. Zhao. Biosensors and Bioelectronics, 18, 1501 - 1508 (2003).
11. R. Gonzalez, B. Masquelier et al. Journal of clinical microbiology (July 2004), 2907 - 2912.
12. Pinar Kara a, Burcu Meric et al. Analytica Chimica Acta, 518, 69 - 76 (2004).
13. J. E. Pearson et al. Ann. Clin. Biochem, 37, 119 - 145 (2000).
14. R.Gabl et al. Biosens. Bioelectron, 19, 615 - 620 (2004).
15. C. Fan, K W. Plaxco, A. J. Heeger. Proc. Natl. Acad. Sci. U.S.A. 100(16), 9134 - 9137 (2003).
16. J. Wang, G. Liu, A. Merkoci. J.Am.Chem. Soc., 125(11), 3214 - 3215 (2003).
17. J. Wang, R. Polsky, A. Merkoci, K. Turner. Langmuir 19, 989 - 991 (2003).
18. Michael Costello M.T et al. Clinical Microbiology Newsletter 28: 24, Vol. 28, No. 24; 185 - 192 (2006).
19. Thanh P. D, Tuan M. A, Chien N. D, CHOVELON J-M. The 7th Vietnamese - German Seminar on Physics and Engineering, Halong - Vietnam, March 28 - April 5, 2004, 158.
20. Marie K. Walsh., Xinwen Wang et al. J. Biochem. Biophys. Methods, 47, 221 - 231 (2001).
21. Tarusshee Ahuja et al. Biomaterials, 28, 791 - 805 (2007).
22. Bansi D. Malhotra. Analytica Chimica Acta, 578, 59 - 74 (2006).