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Immobilization of DNA molecules on the metal surface through alkyl amine for biosensor applications

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Abstract

Efforts to immobilize DNA molecules directly onto the metal surface have been carried out by many researchers. At present, deposition of DNA onto the metal surface of gold is usually carried out through alkylthiol molecules. In this work, DNA molecules were succesfully deposited on the metal surface of Indium Tin Oxide (ITO) through alkylamine of octadecylamine (denoted as ODA). The optical properties characterization was carried out using UV-Vis spectrometer for DNAs and DNAs mixed with Octadecylamine (denoted as DNAs-ODA) in liquid form. Film depositions of DNAs-ODA on the ITO substrate was also characterized. Based on the observation, it was found that the structure of metal-amine-DNA was potential to be used for DNA sensor applications.In addition, electrical properties of ODA and DNA300-ODA were characterized by applying various voltages to the sample and measuring its electrical current flow. It was found that the electrical resistance of ODA decreased due to the presence of the DNA molecules.

Keywords: *immobilization of DNA molecules – biosensor applications*

Introduction

Direct immobilization of DNA molecules on the metal surface is not possible to be carried out yet. For biosensor applications, immobilization technique plays an important role (Lvov *et al.*, 1993; Kung et al., 1990; Yevdokimov *et al.*, 1995). Most works reported in the area of DNA biosensors were done by immobilizing DNA molecules through covalently bonding with the sensitive surface of transducer (Shukorukov *et al.*, 1996). This work had limitations on the possibilities of the use of different types of materials as transducer surfaces because the surface should contact chemical groups that able to link the fragment of DNA.

The chemical reactions between transducer surfaces and DNA lead to damage of the DNA structure in the binding places. In addition, the chemical treatment may also affect some physical properties of transducers which creates undesirable changes. Therefore, the problem to develop methods of immobilization of DNA on transducer surface while saving the nucleic acid structure is neccesary. The immobilization of DNA on a solid substrate by means of electrostatic interactions has clear advantages compared to chemical bonding. These

are not only allow the simplicity and accessibility of the immobilization technique, but also the possibility of using solid substrates with any optical, electrical, magnetic, mechanical and other properties.

Some works were carried out by depositing alkylthiole molecules in between metal surface of gold and DNA. Other researchers have deposited material on a thin film form of octadecylamine reacted with DNA of sturgeon sperm helical structure on the solid substrates of quartz and fluoride slides but not the metal surface (Shukorukov *et al.*, 1996). In this paper we report our results showing that DNA was succesfully immobilized on the metal Indium Tin Oxide (ITO) through octadecylamine (ODA) molecules by dropping the solution on the metal surface. The interesting feature of this structure is simple preparation and use of low cost transparent conductor ITO. By using the transparent substrate of ITO, it is possible to characterize the immobilization process based on optical and electrical properties as well. Current-voltage behavior of ODA and DNA300-ODA materials are also presented.

Materials and Methods

For this experiment, double stranded DNA of 100 bp linear (denoted as DNA100) as a PCR product, 300 bp linear (denoted as DNA300) and 2700 bp circular (denoted as DNA2700) extracted from bacteria were used and dissolved in methanol (Analar, Merck). Meanwhile octadecylamine, $CH_3(CH_2)_{17}NH_2$ (denoted as ODA) (Analar, Sigma) was dissolved in chloroform (Analar, Merck). The optical properties of DNAs and ODA were characterized, particularly in the UV region (200 – 300 nm) at a scan speed of 500 nm/minute using a UV-Vis Spectrometer (Beckman DU – 65, with accuracy of wavelength and absorption reading of ± 2 nm and 0.05 A, respectively). This characterization was also done for a substrate of commercialized ITO film deposited on the glass slide (for film thickness of ~ 1 µm). The samples were prepareed by dropping the solution of ODA on the ITO surface and then followed by DNAs at appropriate quantities. Each treatment was incubated for ~ 15 minutes at room temperature, thereafter thin film of DNA mixed with ODA (DNA-ODA) compound was formed on the substrate. Prior to be used, ITO substrate was cleaned using chloroform. After characterization, the samples were kept in the desicator to avoid any oxidation which may occur. For current - voltage characterization, ODA and DNA300-ODA have been deposited on silver paste electrodes with a gap of ~ 10 µm. The low current measurement was carried out using the electrometer (Keithley 6517A).

Results and Discussion

Two kinds of DNAs prepared (linear and circular forms) could not be deposited directly onto the ITO substrate. To overcome this problem, the DNAs were mixed with ODA (denoted as DNA-ODA). Figure 1 shows the absorption spectra observed from the characterization of the DNA mixtures in liquid form and also for ODA. This figure clearly shows that wavelength of absorption peak position observed for the DNA300-ODA and the DNA100-ODA are similar, but the first one is relatively higher than the latter. The differences were due to the different concentration of solution. Two peaks appear at wavelength of 268 and 275 nm, indicating the presence of ODA material. Meanwhile, peak at wavelength of 260 nm is assumed originated from the DNA molecules

(Sambrook *et al.*, 1989). However, for the DNA2700-ODA, those three peaks were not found. It is assumed that the molecular orientation of circular form of DNA experienced a different structure when reacted with ODA.This phenomenon, however, was not observed when the linear DNA was used.

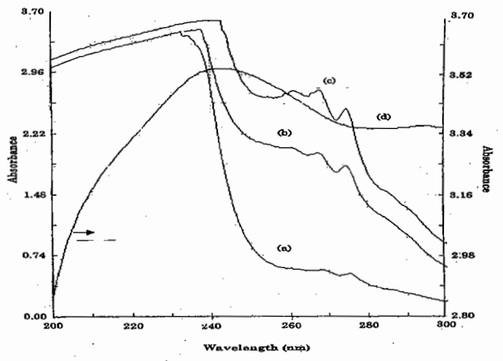

Figure 1. UV spectra for (a) ODA, (b) DNA100-ODA, (c) DNA300-ODA, and (d) DNA2700-ODA

During the reaction, NH_2 of the alkylamine is transformed to NH_3^+. The presence of this positively charged group allows the ODA binds the negatively charged PO_3^- group of DNA molecules. It was assumed that linear form of DNA and NH_3^+ groups of alkyl amines formed a hydrophilic cannal together as shown schematically in Figure 2(a). The amino groups of alkyl amine interact electrostatically with phosphate groups. This result is analogous to the research work done by Shukorukov *et al* (1996). Hydrophilic group of alkylamine molecules were assumed to attach to the metal surface through van der Waals force. In the case of circular molecules, it was assumed that some alkyl amine tails have made contact with the metal surface and the rest of them are considered randomly floating (Figure 2b).

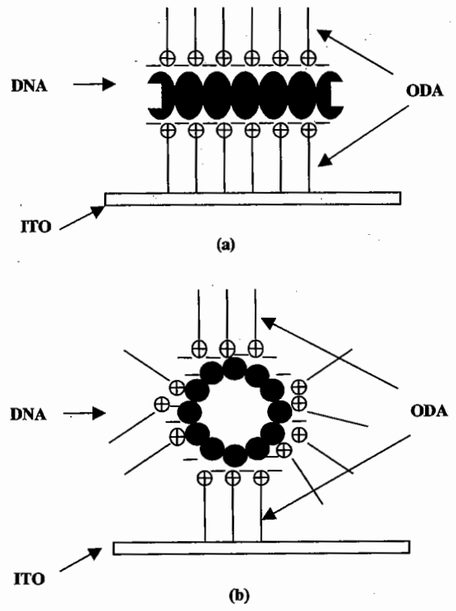

Figure 2. A schematized diagram of an ideal molecular orientation proposed for (a) DNA100-ODA and DNA300-ODA and (b) DNA2700-ODA, deposited on the ITO. Positively charged group NH_3^+ (symbolized with +) of ODA chain with negatively charged group PO_3^- (symbolized with ¯) of DNAs molecules.

The absorption spectra of methanol, DNA100 (linear) and DNA-2700 (circular) were shown in Figure 3. The ordinate of absorbance for DNA2700 is at the right side. Peaks observed for DNA100 is not sharp enough at wavelength of ~ 280 nm, probably due to less concentration of sample solution. The peak of DNA2700, however, is observed at ~ 260 nm. These results support the evidence that the reaction between ODA and DNA molecules as explained above occurred, as demonstrated by the spectra that are not the result of combination between ODA and DNA spectra (Figure 1). The curve (Figure 3), demonstrates that a combination of G-C contents in DNA material will only contribute to a (accumulative) single peak absorption, despite the fact

that every single base molecules of adenine (A), cyanine (C), guanine (G), and thymine (T) has different absorption.

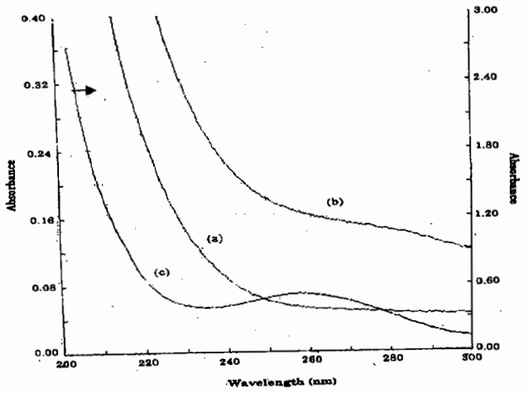

Figure 3. UV spectra for (a) methanol, (b) DNA100, and (c) DNA2700

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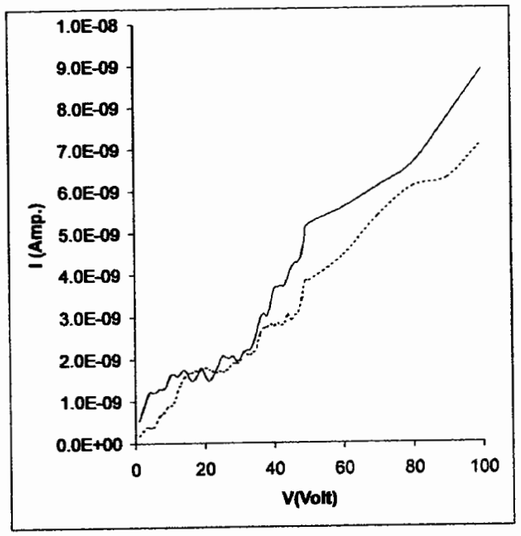

Figure 4. Current-voltage characterization of ODA (dash line) and DNA300-ODA materials (bold line) .

DNA300-ODA and the results were taken as the average values of raw data as shown in Figure 4. This was done by applying various electrical voltages and the electrical properties characterization was carried out to ODA and measuring electrical current flow through the sample. From the experiment, it was observed that the current increases slowly in the region of about 15 to 30 V, while the rapid increase occurred beyond that region. The electrical current is assumed to flow more easily in the DNA300-ODA material rather than in the ODA as deduced from the curve. Electrical resistance of ODA is found to be 11GW for voltages applied from 35 to 100 V. This value is higher compared to that of the DNA300-ODA which was found to be 13 GW. The electrical resistance difference is ~ 11 %. This phenomenon is very interesting as electrical resistivity of ODA material decreases by the presence of the DNA molecules.

The optical properties of the DNAs-ODA deposited on the ITO were characterized in the UV region as shown in Figure 5. Unfortunately, the curve obtained is similar to the pattern obtained from the ITO substrate,

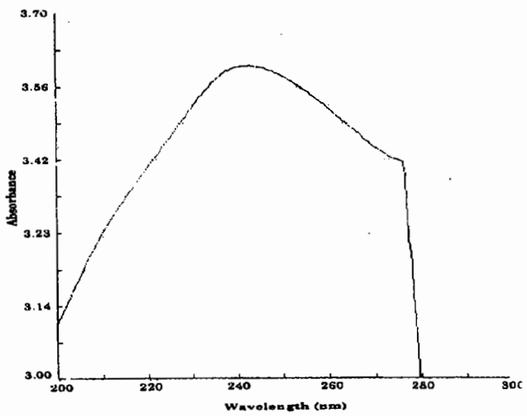

Figure 5. UV spectrum of the DNAs-ODA deposition on the ITO substrate

which resulted in the loss of peak absorption of DNAs, ODA, or DNAs-ODA. This may due to the relatively higher absorption of the ITO substrate than that of DNAs within the range of 200 to 280 nm. However, physically (by naked eyes) it is believed that the DNAs-ODA have been clearly deposited on the ITO surface as evidenced by the milky white color appearance.

Conclusion

A helical form of various DNA molecules have been succesfully deposited on the metal surface of ITO through octadecylamine molecules. This work has some advantages such as simplifying DNA probe preparation without using many chemicals. Molecular orientation of DNAs-ODA is assumed to occur in random due to the treatment that was done by dropping the molecules on the substrates. From current-voltage characterization of ODA and DNA-ODA materials, an interesting phenomenon was observed in which the electrical current underwent a blocking and increased the insulating property at a region of 15 to 30 V. For further work, it is suggested that the deposition of molecules should be carried out using a Langmuir-Blodgett (LB) technique to obtain a good order orientation, instead of random orientation. The use of an X-ray diffractometer is also recommended for material structure characterization.

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