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전극 기반의 전하 주입을 통한 DNA 전하수송 특성 측정

(Probe-based Charge Injection Study of DNA Charge Transfer for Applications to Molecular Electro-optic Switching)

류 호 정*, 김 희 영**, 김 동 현***

(Hojeong Ryu, Heeyoung Kim, and Donghyun Kim)

요 약

본 논문에서는 DNA 올리고뉴클리오타이드(oligonucleotide)를 통한 전하 이동을 기반으로 하는 분자성 전자광학 스위칭 소자를 제시한다. DNA 올리고머(oligomer)가 흡착되어 있는 금전극에 전자들이 주입되어 전극으로부터 DNA 올리고머로 전하가 흘러가게 하고 이 전하의 이동도를 광학적 스위칭으로 확인할 수 있도록 제안되었다. DNA 올리고머의 흡착량이 증가함에 따라 DNA를 통한 전하의 이동성과 전극 표면에서의 전하전달 제한성으로 인해 전리전류는 감소하였다. DNA의 끝단에 합성된 Cy3 형광 분자의 점멸도를 전극 기반의 전하 주입법을 이용하여 확인하였다. 이러한 결과들은 DNA 올리고머를 이용한 새로운 분자성 전자광학 스위칭 소자에 이용될 수 있다.

Abstract

Charge transfer through DNA oligonucleotides has been investigated for potential applications of DNA into molecular electrooptic switching devices. Electrons were injected using gold electrode probes where DNA oligomers were adsorbed that are separated in medium. The results show that increased adsorption of DNA reduces the ionization current due to the combined effect of charge transfer through DNA and surface-limited charge transport. The probe-based charge injection was extended to examine the capability of extinguishing fluorescence of Cy3 dye molecules attached to DNA. It is expected that the results may be employed to implementing a novel electrooptic switching device based on DNA molecules.

Keywords : Charge transfer, DNA, Charge injection, Molecular electrooptic switching

Ⅰ. Introduction

Over the past decades, nanoscale electronic devices have drawn tremendous interests for the potential of intensive device integration. To this goal, charge transport in molecular complexes including photorefractive devices, various organic and inorganic systems, and biomaterials has received significant attention[1~5]. Particularly, DNA-mediated charge transport has been increasingly studied to apply DNA as a material for making molecular electronic devices[6~7]. DNA-mediated charge transport in solution exhibits exquisite sensitivity to perturbations in the intervening base stack or surrounding medium. A well-ordered DNA facilitates charge transport through DNA molecules over long distances, but that disruption of the base pair stack such as by mismatched bases or bending of the duplex by mediums attenuates charge transport[8~10].

* 정회원, 연세대학교 나노메디컬 국가핵심연구센터
(Nanomedical National Core Research Center, Yonsei University)
** 정회원, 연세대학교 화학과
(Department of Chemistry, Yonsei University)
*** 정회원-교신저자, 연세대학교 전기전자공학부
(School of Electrical and Electronic Engineering, Yonsei University)
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This relationship between DNA structure and function has been investigated as various conductivity measurements on a single or a few DNA strands in recent years. It was shown that the order and type of nucleobase pairs alter electrical and optical properties of DNA charge transport systems, for instance, by photoinduction[11~12], electrochemical atomic force microscopy (AFM)[13], scanning tunneling microscopy (STM)[14], and direct conductivity studies[15].

In general, photo-induced charge transport reactions have provided a useful tool for elucidating dynamics in charge transport through DNA on a picosecond scale. However, photo-induction tends to be restricted in the sense that it provides only rates for analyzing the ability of DNA to transport electrons and understanding the nature of the conduction mechanisms through DNA.

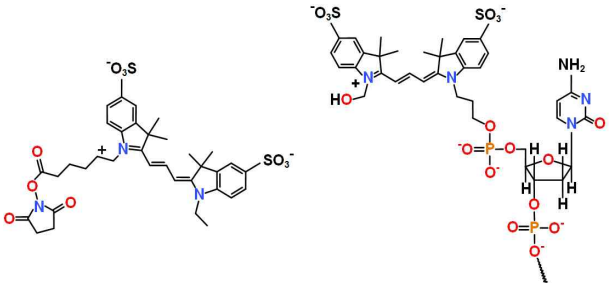
To address these issues, direct measurement of electrical transport using electric detection methods has emerged in DNA charge transport studies. These methods have proven difficult to control the interaction of DNA with substrates and contacts between molecules and electrodes. To overcome these difficulties, Cohen *et al.* measured current passing through double-stranded DNA (dsDNA) molecules by electrochemical AFM using a metal-covered AFM tip, while the molecules are chemically connected to a metal substrate at one end and to a gold nanoparticle at the opposite end[13]. Ceres *et al.* have investigated thiol-modified DNA films on gold surfaces using in situ STM to probe the electronic properties of DNA in a metal-molecule-metal assembly under physiological conditions[14]. Typically, these experiments are difficult to measure with short DNA sequences. Porath *et al.* measured electrical transport of a DNA molecule that was directly positioned between nanoelectrodes by electrostatic trapping from a dilute aqueous buffer containing about one molecule per $(100\ nm)^3$ [15]. Guo *et al.* proposed a general method to integrate DNA strands between single-walled carbon nanotube electrodes and to measure their electrical properties[9].

In this work, we present a simple scheme that may supplement previous electric detection methods for understanding electrical properties of DNA sequences. The probe-based charge injection allows direct supplies of charges to DNA bases without any photo sensitizer that is required in photoinduction. Furthermore, the probe-based charge injection was used to investigate the influence of charge transfer on the fluorescence of Cyanine 3 (Cy3) that is widely used to modulate energy states in DNA[16]. Understanding the influence of charge transfer is important because it may help enlighten the pathways of charge transport for applications of DNA as molecular electrooptic devices based on fluorescence. The pathway studies have been largely limited to theoretical aspects due to experimental difficulties[17]. The probe-based charge injection here was used to provide a tool that can help evaluate the influence of charge transfer when its pathways through DNA are changed by Cy3 and eventually to lay a basis for an alternative to conventional biosensing techniques.

Ⅱ. Methods and Materials

1. DNA oligomer preparation

PAGE-purified DNA oligonucleotides and their Cy3 modified derivatives were purchased from Bioneer Corporation (Daejeon, Korea). The DNA oligomers were 24-mer sequences designed in formats for control and Cy3 modified single-stranded DNA (ssDNA) (C-ssDNA for Control ssDNA; 5′ - GAG AGA GAG AGA GAG AGA GAG AGG - 3′ - thiol, and Cy3-ssDNA for Cy3 modified ssDNA; Cy3 - 5′ - GAG AGA GAG AGA GAG AGA GAG AGG - 3′ - thiol). Here, 3′ - thiol represents a thiol molecule attached to the 3′ terminal of ssDNA. Similarly, Cy3 - 5′ is a Cy3 molecule attached covalently to the 5′ terminal of ssDNA (see Fig. 1). Cy3 modified ssDNA oligomers were used to understand the effect of charge transfer on Cy3.



(a) Cy3 (b) Cy3 - 5′

Fig. 1. 형광 물질의 화학 구조 : (a) Cy3 와 (b) Cy3-5′. (b) 에서의 물결선은 ssDNA 올리고뉴클리오타이드 내에서 다른 염기와의 결합을 나타낸다

Fig. 1. Chemical structures of (a) Cy3 and (b) Cy3 - 5′ DNA. The wavy line in (b) refers to binding to other bases in the ssDNA oligonucleotide.

2. Probe preparation

The probe was a gold electrode adsorbed with C-ssDNA or Cy3-ssDNA, which was prepared in multiple steps. For electron injection, DNA oligonucleotides were adsorbed through covalent bonds between thiol and the surface of the gold wire electrode (dia. = 0.125 mm, Goodfellow, Huntingdon, UK). The junction allows electrons to go over from the electrode to DNA bases and Cy3. For spatially selective adsorption of DNA molecules, gold electrodes were coated with polyester and the tip was exposed through piranha treatment by 1 cm. Details of DNA adsorption protocol are as follows. A commercial gold wire used as an electrode was processed by sonication in acetone for 5 min. and in ethanol for another 5 min. Subsequently, the gold wire underwent surface treatment for increased attaching efficiency of ssDNA molecules to the gold surface using a plasma cleaner (Harrick Scientific Products, Pleasantville, NY, USA) for 5 min. For immobilization, the tip of gold wire electrodes was incubated in 1 μM ssDNA with immobilization solution of 1 M KH_2PO_4 at 4 °C. After incubation, the probe samples were washed with distilled water for 5 min. For C-ssDNA, a probe electrode was incubated for 4 hours before each time it was measured, while it was 8 hours for Cy3-ssDNA.

3. Electrical characterization

For characterization of charge transport through DNA, electric current was measured with a picoammeter (#6485, Keithley, Cleveland, OH, USA) while the distance between gold probes was kept at 1 cm under 1 V potential difference. Distilled water was used as buffer to reduce ionic leakage.

4. Optical set-up

For the measurement of fluorescent intensity from Cy3, a fluorescence detection system was set up as illustrated in Fig. 2. Light from a DPSS green laser (λ = 532 nm, SDL-532, Shanghai Lasers Tech, Shanghai, China) was used for excitation of Cy3 on the gold probe. Cy3 emission was detected by a photomultiplier tube (PMT, #77360. Oriel, Newport, Irvine, CA) and recorded by a LabView^{TM}-enabled optical power meter (#70310, Oriel). The optical system was temperature-controlled in an incubator at 35 °C. Preliminary tests were performed to confirm the independence of the results from humidity that may be caused by the evaporation of buffer solution. Also, identical measurements were conducted multiple times and statistically averaged.

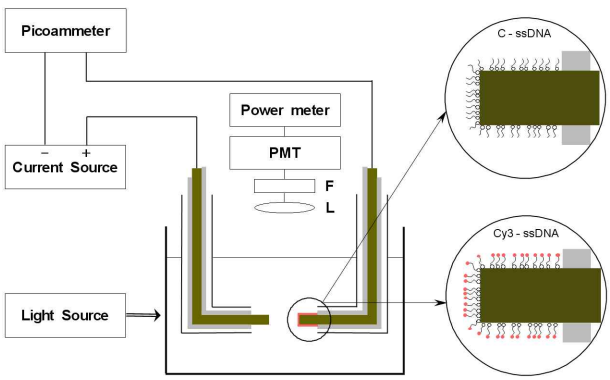


Fig. 2. 광학 실험 장치의 모식도. (F: 필터, L: 콜렉션 렌즈, ~: ssDNA, ●: Cy3, ○: 사이올기)

Fig. 2. Schematic of optical set-up (F: filter, L: collection lens, ~: ssDNA, ●: Cy3, ○: thiol)

Ⅲ. Results and Discussion

Steady-state electrical properties of charge transfer through C-ssDNA were first measured before optical

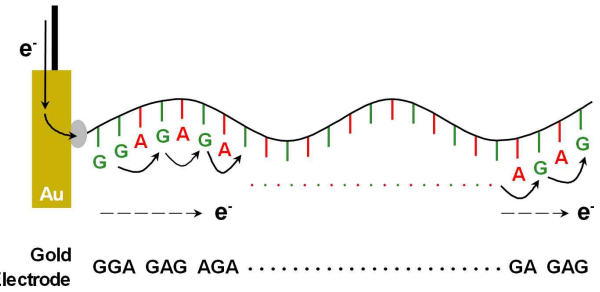


Fig. 3. 전극 기반의 전하 주입을 통한 DNA 전하수송의 모식도

Fig. 3. Schematic illustration of DNA charge transfer through probe-based charge injection.

characterization of Cy3-ssDNA using probe-based charge injection to confirm electronic conduction through DNA. The steady-state electrical measurement of C-ssDNA was performed in distilled water as a function of time and current. Fig. 3 illustrates electrons directly injected into C-ssDNAs through thiol junction, which move toward ssDNA and migrate subsequently to the nearest G bases. The reduction potential of guanine is lower than that of adenine. This can lead to irreversible charge transfer from thiol to the end of C-ssDNA. Charge transfer process through specific DNA sequences in this device can also be of importance in terms of elucidation of charge transfer mechanism. In this work, however, we are interested in modulation of optical properties of the dye molecule caused by charge transfer processes in the medium.

The measured current for control probes without DNA is presented in Fig. 4. It takes approximately three hours for the current to be stabilized. This is the time for the current through DNA and probe surface to reach equilibrium with the current associated with spontaneous ionization of water molecules under bias. For this reason, actual measurement of DNA charge transfer was conducted after sufficient current stabilization for at least three hours. Note also that the control data for a probe without DNA in Fig. 4 show that the measured current converges at the same value, which ensures the experimental reproducibility.

Fig. 5 presents the current measured by electron injection using DNA-adsorbed probes. DNA(1) and DNA(2) denote the number of adsorption steps, i.e., only a single step of DNA adsorption was performed for DNA(1) and twice for DNA(2). In other words, a much larger number of DNA molecules are presumed to be adsorbed in DNA(2) than in DNA(1). Obviously the degree of DNA adsorption affects the charge transfer through DNA bases and the overall current through the medium quite strongly. More specifically, it is observed that increased adsorption of C-ssDNA lowers the current flowing between probes.

Also notice that the current significantly reduced after the first adsorption step, while it is less so after the second adsorption. This may suggest that the current between the probes is not the direct result of charge transfer *per se*; rather, the measured current

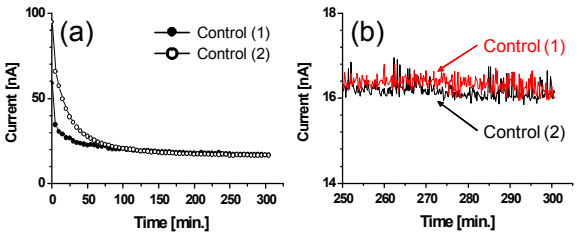


Fig. 4. 순수 전극(DNA가 흡착되기 전)에 대한 전류 측정 : (a) 측정시작(t = 0)~300 분, (b) 안정화 이후

Fig. 4. Measured current for control probes without DNA: (a) results from start (0^{th}) to 300th minutes and (b) after stabilization.

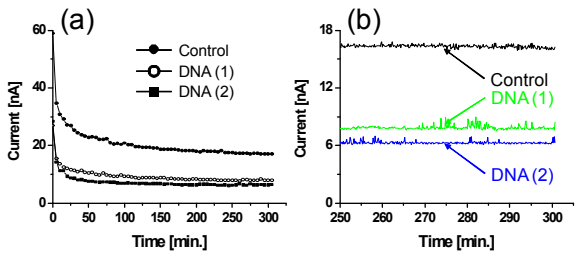


Fig. 5. DNA 흡착 전극으로의 전하 주입에 의한 전류 측정(서로 다른 시간 축). DNA(1) 과 DNA(2) 는 각 전극에 대한 DNA 흡착 횟수를 나타낸다

Fig. 5. Current measured by electron injection using DNA-adsorbed probes for different time scales. DNA(1) and DNA(2) represent the number of DNA adsorption steps for each type of probes.

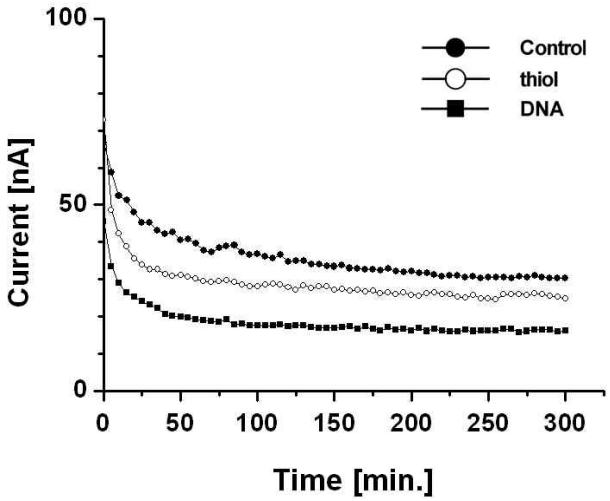


Fig. 6. DNA 흡착 전극으로의 전하 주입에 의한 전류 측정(DNA)과 디옥시리보뉴클리아제에 의해 DNA 분자가 끊어진 상태에서의 전류 측정 (thiol).

Fig. 6. Current measured by electron injection using DNA-adsorbed probes (DNA) and when DNA molecules have been enzymatically cleaved by deoxyribonuclease (thiol).

is more of a result from the blocking or resistance associated with thiolated surface. We stress that the ionization current is modulated by the DNA adsorption on the probe, the mechanism of which is closely related to DNA charge transfer processes.

For clarification, we have measured the change of current after we used deoxyribonuclease to cleave out DNA molecules from the probe surface. Once DNA is cleaved, DNA charge transfer cannot exist. The results presented in Fig. 6 suggest that current loss by charge transfer disappears after cleavage of DNA, thus an increase of measured current, which opens the possibility of DNA charge transfer by probe-based charge injection. The current after cleavage did not recover to that of control because of surface modification by remaining thiol.

The influence of charge transfer on the fluorescence of Cy3 was measured using an optical set-up illustrated in Fig. 2. Recently charge transfer was used to extinguish fluorescence excitation of tetramethylrhodamine dye molecules by way of intramolecular activities[10]. A control experiment without electric potential applied to Cy3-ssDNA has shown stable emission of fluorescence. In the case of Cy3, experimental results tend to attest that application of electric potential does not affect the fluorescence emission. Because we did not see significant photobleaching or disruption of fluorescence with Cy3, the results indicate that the intramolecular activities of Cy3 are not strongly dependent on charge transfer.

This result has important significance on the implementation of electrooptic devices: fluorescence emission of fluorescent dyes may show an extreme sensitivity towards perturbation and interruptions of the medium such as base stacking that are caused by base mismatches or DNA lesions. Thus, studies based on electrooptic devices may be suitable to obtain a highly sensitive electrochemical readout on molecular electronics. Using this methodology, DNA lesions may be detected by implementing a DNA biosensor with specific base sequence.

Ⅳ. Concluding Remarks

In summary, we have investigated charge transfer through DNA oligomers for potential implementation of molecular electrooptic switching devices. Gold electrode probes adsorbed with DNA oligomers and separated under a potential were used to inject electrons for DNA charge transfer. We have observed that the ionization current through medium was reduced as a result of increased DNA adsorption on the probe surface, attributed both to the charge transfer through DNA and to surface-limited charge transport. Furthermore, the capability of turning off fluorescence of Cy3-ssDNA was examined by the probe-based charge injection. The results are expected to be useful to implementing a new type of DNA-based electrooptic switching device.

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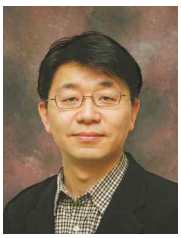
저 자 소 개

류 호 정(정회원)
2009년 연세대학교 전자공학과 학사 졸업.
2011년 연세대학교 전자공학과 석사 졸업.
<주관심분야 : DNA 전하 이동, 바이오 센서 >



김 동 현(정회원)-교신저자
1993년 서울대학교 전자공학과 학사 졸업.
1995년 서울대학교 전자공학과 석사 졸업.
2001년 Massachusetts Institute of Technology 박사 졸업.
<주관심분야 : 광학의료영상, 바이오 센서 >

김 희 영(정회원)
2004년 경희대학교 화학공학과 학사 졸업.
2006년 연세대학교 화학과 석사 졸업.
2010년 연세대학교 화학과 박사 졸업.
<주관심분야 : 양자물리화학, DNA 전하 이동>