Label-Free Detection of DNA Hybridization Using Pyrene-Functionalized Single-Walled Carbon Nanotubes: Effect of Chemical Structures of Pyrene Molecules on DNA Sensing Performance

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We investigate the effect of functional groups of pyrene molecules on the electrical sensing performance of single-walled carbon nanotubes (SWNTs) based DNA biosensor, in which pyrenes with three different functional groups of carboxylic acid (Py-COOH), aldehyde (Py-CHO) and amine (Py-NH₂) are used as linker molecules to immobilize DNA on the SWNT films. UV/Visible absorption spectra results show that all of the pyrene molecules are successfully immobilized on the SWNT surface via π–π stacking interaction. Based on fluorescence analysis, we show that the amide bonding of amine terminated DNA via pyrene containing carboxylic groups is the most efficient to immobilize DNA on the nanotube film. The electrical detection results show that the conductance of Py-COOH modified SWNT film is increased upon DNA immobilization, followed by further increase after hybridization of target DNAs. It indicates that the pyrene molecules with carboxylic acid groups play an important role to achieve highly efficient label-free detection by nondestructive and specific immobilization of DNAs.

Keywords: Single-Walled Carbon Nanotube, Pyrene, Label-Free Detection, DNA Hybridization.

1. INTRODUCTION

Single wall carbon nanotubes (SWNTs) have attracted much interest for electrical devices due to superior electronic and structural properties.¹,² Introduction of guest species to the nanotube is normally accompanied by considerable changes in electrical signals, which is attributed to changes in electrical transport properties of the nanotube.³ In particular, the flexible surface chemistry of nanotubes provides potential to immobilize various biological elements.⁴ Accordingly, these excellent properties are allowing the application to label-free biosensor.⁵

For the detection of biological entities with SWNTs, biomolecules have generally immobilized on the nanotubes via noncovalent functionalization.⁵,⁶ For examples, single stranded DNA molecules can be adsorbed on the graphitic surface of SWNT via the π–π interaction, enabling to decorate DNA molecules on SWNT surface via easy and simple methods.⁷ However, it is well known that physical adsorption of DNAs brings about weak and randomly oriented binding of molecules, which give rise to decrease of subsequent reaction yield.⁸ Furthermore, nonspecifically adsorbed single strand DNAs cannot be easily hybridized with their complementary DNA.⁹ On the contrary, covalent binding method provides generally more stable and site-specific linkages between biomolecules and SWNTs, resulting in high immobilization efficiency.¹⁰,¹¹ However, the covalent binding method leads to degradation of electronic properties of carbon nanotubes.⁸ Thus, the use of bifunctional material of biomolecules to graphitic materials has been suggested to enhance their binding stability of guest molecules and electrical sensing performance.

Recently, it has been reported that pyrene derivatives can be used as interlinkers of guest species to graphitic materials. The pyrene has polycyclic aromatic hydrocarbon consisting of four fused benzene rings, which enables to bind...
the surface of SWNT via π–π stacking interaction.\textsuperscript{12–14} Thus, the pyrene functionalization is possible to decorate biomolecules without any structural deformation of SWNT. Especially, by changing the pyrene derivatives, various functional groups can be functionalized on the surface of carbon nanotubes, which enable to employ various chemical linkages of biomolecules. Therefore, functionalization of pyrene derivatives is essential to realizing the SWNT based biosensor.

In this study, we introduced pyrene molecules with three different functional groups of carboxylic acid, aldehyde and amine groups on the SWNT films to investigate DNA sensing performance of pyrene derivative-modified SWNT. UV/Visible absorption spectra and fluorescence results showed that probe DNAs were covalently attached to the pyrene modified SWNT film via coupling agent, dehydroxylation and UV irradiation, followed by hybridization of target DNAs. In particular, the pyrene containing carboxylic groups via amide bonding of DNA is the most efficient to immobilize DNA on the nanotube film. Based on the results, we investigated the detection of DNA hybridization on pyrene modified SWNT sensor which was covalently bonded with DNAs. The resulting $I–V$ plots after DNA hybridization on the pyrene-assisted SWNT films showed remarkable selectivity for complementary DNA hybridization in comparison with noncomplementary DNA.

2. EXPERIMENTAL DETAILS

2.1. Materials

The Single-walled carbon nanotubes (SWNTs) containing raw soot (85 wt% purity, prepared by a high-pressure carbon monoxide process) were purchased from Carbon Nanotechnologies Inc., USA. N, N-dimethylformamide (DMF, 99.5%), sodium hydroxide (NaOH, 97%), hydrogen peroxide (H$_2$O$_2$, 35%), sulfuric acid (H$_2$SO$_4$, 95%), hydrochloric acid (HCl, 35%) and N-methyl-2-pyrrolidone (NMP, 99%) were obtained from Junsei Chemical Co. Ltd. 1-pyrene butanoic acid, 1-pyrene carboxaldehyde and 1-pyrene methyl amine were obtained from Aldrich. DNA oligonucleotides (Table I) were purchased from GenoTech Co. (Daejeon, Korea). 4-morpholineethanesulphonic acid (MES), N-hydroxy succinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma.

2.2. Preparation of SWNT Networks Based Devices

The SWNTs were purified and shortened as previously reported.\textsuperscript{15} The procedure is briefly explained as follows; the nanotubes were treated by dry oxidization at 365 °C for 90 min in a flow of dry air (0.1 SLM) and acid oxidation in HCl solution. The purified nanotubes were shortened by treatment with piranha solution (H$_2$SO$_4$:H$_2$O$_2$ = 4:1 in volume) for 4 h at room temperature. The shortened SWNTs were diluted and dispersed in DMF (1 mg/L) by ultrasonication for several hours. The solution was centrifuged at 15000 g for 30 min to separate the cut and isolated SWNTs from nanotube bundles, and the supernatant solution was collected. Some amount of SWNT ropes in the suspension was settled on a porous alumina membrane filter (200 nm pore size; Whatman) by vacuum filtration.\textsuperscript{16} For the fabrication of electrical device based on SWNT film, as-prepared SWNT films were covered with a photoresist polymer, usually by spin coating. Light emanating from a photomask provides a spatial pattern of the photochemical reaction that defines which photoresist areas will be removed or preserved when the photoresist is developed. After the photoresist is developed, a portion of the nanotube population is exposed on the substrate according to the photomask pattern. O$_2$ plasma now obliterates only the exposed nanotube portions. Note that portions of nanotubes underneath the resist pattern remain protected from etching and damage. After exposure to O$_2$ plasma, the remaining resist patterns were removed. To accomplish final construction of the device, the patterned SWNT film were corporate by four Cr/Au (10/100 nm thick) electrodes via metal evaporation.

2.3. Functionalization of Pyrene Molecules on SWNT Films for DNA Immobilization

As-fabricated SWNT networks were incubated in 5 mM pyrene derivative in NMP (10 ml) for 12 h at room temperature and then rinsed thoroughly with NMP to remove unbound pyrene molecules, followed by air-drying. For the immobilization of DNAs on the SWNT films functionalized by pyrene carboxylic acid (Py-COOH), the nanotube films were treated with MES buffer (25 mM, pH 6.5) containing 15 mM NHS and 75 mM EDC at 30 °C for 30 min in order to activate the carboxylic acid groups of pyrene-modified SWNT network. Fifty μM probe DNA, which have amine residues at 3’-termini, were incubated with the chips at 30 °C for 8 h. After incubation, the SWNT films was washed with 25 mM MES buffer containing 0.1% (w/v) SDS to remove unbound DNA and air-dried. To immobilize the DNAs on SWNT chips treated with 1-pyrene carboxaldehyde (Py-CHO), the probe DNAs modified with amine groups at their 3’-termini were suspended in 3× SSC solution (0.45 M NaCl, 15 mM C$_6$H$_5$Na$_3$O$_7$, 0.015 M Na$_2$HPO$_4$).
pH 7.0) at a final concentration of 50 μM. After spotting probe DNAs on the chips, the chips were air-dried at room temperature for 12 h for DNA immobilization via Schiff’s base reaction. For the stable bond formation, the SWNT films were treated with sodium borohydride (NaBH₄, Sigma-Aldrich Inc., Louis, MO, USA) after washing the nanotube film coated CHO-pyrene with 0.2% (w/v) SDS for 5 min. In the case of the SWNT film decorated by 1-pyrene methylamine (Py-NH₂), the unmodified 50 μM probe DNA in 3 × SSC solution were spotted on the surface of nanotube chips. The films were treated by ultra-violet (UV) cross-linking and then were incubated at room temperature for 12 h for DNA immobilization. To remove the unbound probe DNAs, the films were washed with 2 × SSC buffer containing 0.1% (w/v) SDS for 5 min. The DNA immobilized on the SWNT networks were then hybridized with the 0.5 μM of target DNA (Table I) in 5 × SSC buffer (0.75 M NaCl, 25 mM C₆H₅Na₃O₇, pH 7.0) at 42 °C for 8 h, followed by washing with 2 × SSC buffer containing 0.1% (w/v) SDS.

2.4. Measurements of Optical and Electrical Signals

The optical absorbance of resulting film was measured by a V-570 UV/VIS/NIR spectrophotometer (JASCO) and a quartz sheet was used for the reference. Fluorescence signal data were obtained by ArrayWorx biochip scanner (Applied Precision Inc., Issaquah, WA) and Imagene (BioDiscovery, Inc., El Segundo, CA) at excitation wavelength 635 nm. Electrical measurement was conducted by measuring current versus voltage (I–V) characteristics of the SWNT based devices through manual probe station (Cascade SUMMIT (12000)) with semiconducting characterization system (Keithley 4200-SCS/F). The out current values from the devices were taken with voltage sweep condition from −1 V to 1 V.

Fig. 1. Schematic illustration of the procedure for the immobilization of probe DNAs and the hybridization of target DNA on the pyrene-functionalized SWNTs and followed process for hybridization of target DNAs.
Fig. 2. UV/Visible absorption spectra of (a) (left) as-prepared SWNT film and 1-pyrene butanoic acid (Py-COOH) functionalized SWNT films, (right) Py-COOH solution and adsorbed Py-COOH (b) (left) as-prepared SWNT film and 1-pyrene carboxaldehyde (Py-CHO) functionalized SWNT films, (right) Py-CHO solution and adsorbed Py-COOH (c) (left) as-prepared SWNT film and 1-pyrene methyl amine (Py-NH₂) functionalized SWNT films, (right) Py-NH₂ solution and adsorbed Py-NH₂. Absorption spectra of adsorbed pyrene derivatives were obtained by subtracting absorbance of SWNT film from one of pyrene modified SWNT film. Asterisks (*) indicate characteristic absorption peaks of each pyrene derivative functionalized on the SWNT surface.
3. RESULTS AND DISCUSSION

Figure 1 shows the overall process involved in immobilization of probe DNA molecules and hybridization of target DNAs on pyrene-functionalized SWNT film. Pyrene molecules with three kinds of functional groups (carboxylic, aldehyde and amine group) are functionalized on the SWNT film. According to the functional groups generated on the SWNT film, different immobilization methods are respectively employed as shown in Figure 1. After immobilization of probe DNAs, target DNA molecules are hybridized on the resulting SWNT network. Fluorescence and electrical detection are carried out for the detection of immobilization and hybridization of DNA.

To confirm the decoration of various functional groups, simple immersion of SWNT film substrates in a pyrene solution for 12 h was conducted and confirmed by UV/Vis absorption spectra. As shown in Figure 2, each pyrene derivative was successfully functionalized on the SWNT film. Referring to the original absorption peaks for pyrene derivatives solution, all spectra of adsorbed pyrene derivatives on SWNT film show respectively similar absorption peaks (Fig. 2), which represent the existence of pyrene molecules on SWNT. Notably, the absorption peaks of the pyrene derivatives were slightly shifted after the attachment of the pyrene molecules on the SWNT films, indicating each pyrene derivative was adsorbed on the surface of nanotube via $\pi-\pi$ interaction of pyrenyl groups and six-membered carbon rings of the nanotubes.17

Prior to hybridization of target DNA, the pyrene-modified SWNT films were immobilized by different probe DNAs having complementary sequences (A1) and noncomplementary ones (A2) to target DNAs (Table I). The amine modified probe DNAs were covalently immobilized on the Py-COOH functionalized SWNT films by using amide coupling reagent consisting EDC and NHS. On the other hand, the DNAs immobilized on the Py-CHO functionalized SWNT films by dehydration involving Schiff’s base reaction.18 In the case of the Py-NH$_2$ modified SWNT films, the phosphate backbones of DNAs interact with positively charged amine groups of the pyrene by electrostatic interaction. The interaction between DNA molecules and the amine groups are somewhat weak and thus additional UV cross-linking was carried out to covalently connect thymidine residues in the DNA with positively charged amine groups.19

To investigate the effect of the pyrenes with three different functional groups on the sequence-specific DNA hybridization, target DNAs labeled with Cy5 dye were hybridized with probe DNAs immobilized films. Figure 3 shows the fluorescence intensities after hybridization of target DNAs onto the pyrene-modified SWNT films attached with probe DNAs by three different immobilization methods. It was found that Py-COOH modified SWNT films on which probe DNAs linked via amide bond showed the highest fluorescence intensity, compared to...
the SWNT film region and the area of clean substrate (Fig. 4(c)).

For the electrical detection of DNA hybridization, the patterned SWNT films were functionalized with Py-COOH, which exhibited the highest discriminatory power in sequence-specific hybridization, followed by immobilization and hybridization of unlabeled target DNAs. Figure 5 shows $I–V$ graphs of the SWNT networks (black line), Py-COOH functionalized SWNTs (red line), Py-COOH functionalized SWNTs after immobilization (green line) and Py-COOH functionalized SWNTs after hybridization (violet and blue line). The devices exhibited metallic behavior, resulting from the SWNTs containing metallic and semiconducting nanotubes. The conductance of the SWNT film was decreased after Py-COOH adsorption, which might be attributed to the $\pi–\pi$ coupling of the pyrenyl groups on the SWNT surface. The adsorption of aromatic molecules of the pyrene on the nanotube surface can induce the scattering to electrical transport of SWNTs. After immobilization of probe DNAs with complementary sequences, the slope of $I–V$ plots was increased. Upon the addition of the target DNA, the slope was further increased in the device immobilized with complementary probe DNAs. As expected, no significant changes were observed in the devices with noncomplementary probe DNAs. Such increases in the slopes might be due to the fact that DNA molecules provide the current path for electrons, in which electron transfer through DNA takes place through the overlap between $\pi$-orbitals in adjacent base pairs.

The increase of charge transfer after the DNA functionalization implies that electron transport occurs through hopping or band conduction between guanine (G) bases, once electrons are injected, onto the SWNT films delocalized by $\pi$-conduction electron with the aromatic molecule over nanotubes. Thus, the Py-COOH/SWNT films display a good selectivity in detecting complementary DNA hybridization.

4. CONCLUSIONS

We investigated the effect of the functional groups of pyrene molecules, as inter-linker molecules of SWNT films and DNA, on the DNA sensing performance. Three different pyrene derivatives were successfully decorated on the nanotube film via $\pi–\pi$ stacking interaction, which was confirmed by UV/Visible absorption spectra. Fluorescence analysis revealed that the DNA immobilization method on Py-COOH functionalized SWNT film showed the highest efficiency in the detection of sequence-specific DNAs. The resulting Py-COOH modified SWNT sensor exhibited the discriminative changes of $I–V$ plots after DNA immobilization and hybridization, which provides the potential to serve as a label-free biosensor.

SUPPORTING INFORMATION

Fig. 5. Representative $I–V$ plots for (a) as-fabricated SWNT devices (black line), Py-COOH-functionalized SWNT devices (red line), the SWNT devices immobilized with probe DNAs having complementary sequences (green line) and subsequently hybridized with target DNAs (violet line), (b) as-prepared SWNT devices (black line), Py-COOH-functionalized SWNT devices (red line), the nanotube based circuits immobilized with probe DNAs having noncomplementary sequences (green line) and the devices subsequently hybridized with target DNAs (blue line).

Fig. S1. Fluorescence intensities of three different pyrene derivatives (Py-COOH, Py-CHO and Py-NH$_2$) functionalized SWNT films after immobilization of Cy5 labeled probe DNAs (Cy5-TGTGCGACC ATCAACCTGTG-amine).
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References and Notes

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