Research Article

Interactions of Secondary DNA and Initial DNA on Single-Walled Carbon Nanotube Surfaces Studied by Photoluminescence, Atomic Force Microscopy, and Electrophoresis

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We examined the interactions of initial single-stranded DNA (ssDNA) and secondary ssDNA molecules on single-walled carbon nanotubes (SWNTs). Thymine 30-mers (T30) and 30-mers from a partial sequence of φx174 DNA (φ30) were used to prepare the DNA-SWNT hybrids. First, the hybrids were annealed at various temperatures without secondary DNA to evaluate the stability of the hybrids. As a result, aggregates of SWNTs were formed in the T30-SWNT hybrids, even at 54°C, although the φ30-SWNT hybrids were stable up to 84°C. Second, we added secondary DNA molecules during the annealing procedure. We reacted adenine 30-mers (A30) with the T30-SWNT hybrids and characterized the samples by combining agarose gel electrophoresis with/without ethidium bromide and atomic force microscopy (AFM) as well as near-infrared photoluminescence (PL) spectroscopy. Cross-links appeared to form among the SWNTs because of nonspecific hybridization of T30 and A30. PL measurements revealed clear shifts in the PL emission wavelength of SWNTs. However, when complementary φ30 DNA (cφ30) was reacted with φ30-SWNT hybrids, there was no significant difference in the PL spectra after the reaction, although electrophoresis suggested the hybridization of the cφ30 and φ30 DNA molecules. Our results suggest that the hybridization manner of DNA molecules with unnatural sequences greatly differs from that of natural DNA molecules.

1. Introduction

The surfaces of single-walled carbon nanotubes (SWNTs) can be wrapped with DNA molecules (DNA-SWNT hybrids) [1, 2]. These structures show huge potential for use in applications such as nanobiosensors and carriers for drug delivery [3–8]. Evaluating DNA molecules is important for developing biological applications. Many authors have reported the physical and chemical characterizations of DNA-SWNT hybrids using microscopy [9–11] and spectroscopy [12–17]. Furthermore, theoretical models of the hybrid structures have been proposed by several authors [18–22].

Our research group has published several papers on the biological evaluation of DNA-SWNT hybrids [23–31]. Nii et al. demonstrated that selective binding of single-stranded DNA-binding proteins can be used to distinguish hybrids of single-stranded DNA (ssDNA) and SWNT from hybrids of double-stranded DNA (dsDNA) and SWNT [26]. Oura et al. found that RecA proteins can bind to ssDNA-SWNT surfaces, although the proteins are not adsorbed onto hybrids of carboxymethyl cellulose and SWNT [27]. These results reveal the conditions required for the adsorption of DNA molecules onto the surfaces of SWNTs.

Recently, several authors have reported that the hybridization of DNA with SWNTs can be detected by fluorescence quenching [32, 33]. When fluorescently labeled DNA is adsorbed onto the surface of SWNTs, the fluorescent dyes are quenched, even when the samples are appropriately excited. DNA hybridization can be detected by measuring the quenching ratio. The thermodynamics of DNA molecules adsorbed onto SWNT surfaces have been intensively studied by several authors [34–36].
The stability of DNA-SWNT hybrids was also widely examined. Chen and Zhang reported detachment of ssDNA molecules from SWNT surfaces by replacement with fluorescent dyes and DNA hybridization [37]. Karachevtsev et al. prepared DNA-SWNT hybrids with polynucleotides and studied aggregate formation of the hybrids [38, 39]. Jung et al. also demonstrated detachment of DNA molecules from SWNT surfaces through DNA hybridization [40]. In the present study, we examined the interactions of initial ssDNA and secondary ssDNA molecules on SWNTs using near-infrared photoluminescence (PL) from SWNTs, atomic force microscopy, and agarose gel electrophoresis without ethidium bromide (EtBr) staining. Initially, we wrapped two types of ssDNA molecules around the SWNT surfaces: one type comprised a 30-mer of thymine (T30), while the other was a 30-mer containing the partial sequence of bacteriophage φx174 DNA (φ30). Next, we added 30-mers of T30, 30-mers of adenine (A30), φ30, and complementary φ30 (cφ30), which were not labeled with fluorescent dyes, to the T30-SWNT or φ30-SWNT samples and incubated the samples for hybridization. We also investigated the effects of annealing temperature on the stability of T30-SWNTs and φ30-SWNTs. We also combined EtBr-stained and unstrained electrophoresis with PL spectrum evaluation. Our results suggest that a new definition of “Tm values” between DNA and SWNTs may be useful for controlling DNA hybridization and other biological reactions on SWNT surfaces.

2. Materials and Methods

2.1. Materials. The DNA molecules were purchased from Life Technologies (Carlsbad, CA, USA). The sequences of DNA molecules were as follows: (1) 30-mers of thymine (T30), (2) 30-mers of adenine (A30), (3) a partial sequence of φx174 single-stranded DNA (GGCGAAAAATGAGAAAA TTCGACCTATCCTT (φ30)), and (4) a complementary sequence of φ30 (AAGGATAGGTCGAATTTTCTCATT TTCCGC (cφ30)). SWNT powder was synthesized by the high-pressure carbon monoxide method and was purchased from Unidym (Menlo Park, CA, USA).

2.2. Preparation of ssDNA-SWNT Hybrids. The DNA-SWNT hybrids were prepared from 0.5 mg of SWNT mixed with 500 μL of DNA solution (1 mg/mL, 200 mM Tris-HCl buffer solution, pH 8.0). The mixture was sonicated for 90 min at 3 W (VCX 130; Sonics & Materials Inc., Newton, CT, USA) on ice and then centrifuged for 3 h at 15,000 rpm (17,360 xg, MX-150; Tomy Seiko, Tokyo, Japan) to remove aggregates. After centrifugation, 350 μL of the supernatant was collected and filtered ten times using an Amicon Ultra centrifugal filter (Merck Millipore, Darmstadt, Germany) to remove excess ssDNA molecules. The obtained ssDNA-SWNT suspensions were stored as the initial solutions in a refrigerator.

2.3. Annealing of the ssDNA-SWNT Hybrids. To evaluate the stability of the hybrids, 10 μL of the ssDNA-SWNT suspension was annealed at 54°C, 64°C, 74°C, 84°C, and 94°C for 30 s at each temperature, then cooled to 24°C (cooling by 2°C every 30 s) using a thermal cycler (2720, Applied Biosystems, Foster City, CA, USA).

2.4. Annealing of the ssDNA to ssDNA-SWNT Hybrids. To examine hybridization, 10 μL of an ssDNA solution prepared from DNA molecules (1)–(4) in Tris-HCl buffer and 10 μL of the ssDNA-SWNT suspension were mixed in a sample tube, and the mixture was annealed for 30 s using the thermal cycler. The annealing temperatures were 56°C for the T30-SWNT hybrids with A30 and T30 and 67°C for the φ30-SWNT hybrids with φ30 and cφ30. The temperatures were determined based on the Tm value of each DNA molecule and agarose gel electrophoresis. After annealing, the samples were cooled to 24°C (cooling by 2°C every 30 s). The samples were prepared simultaneously to ensure the uniformity of the experimental conditions. PL measurements were conducted immediately after sample preparation. AFM was performed within 2 days of sample preparation. All experiments were repeated at least three times.

2.5. Agarose Gel Electrophoresis Analysis. Agarose gel electrophoresis (Mupid-2plus, Advance Co. Ltd., Tokyo, Japan) was carried out using a 0.8% gel (Agarose S, Wako Pure Chemical Industries Ltd., Osaka, Japan). A volume of 10 μL of the mixture of single-stranded DNA-binding proteins, ssDNA/dsDNA, and SWNT was mixed with 2 μL of glycerol, after which 10 μL of the sample was loaded. A volume of 1 μL of Coomasie Brilliant Blue solution (0.25% CBB R-250, 5% methanol, and 7.5% acetic acid) was also added to the sample mixture when the protein bands were monitored. Electrophoresis was carried out at 50 V for 30 min in TAE buffer solution. Electrophoresis examination was repeated more than three times to verify reproducibility. To observe the locations of DNA molecules, the gel was stained with 1.0 μg/mL EtBr for 30 min and then observed using a UV transilluminator (Mupid-Scope WD, Advance Co. Ltd.). DNA locations were appeared as white bands. For monitoring the SWNT locations, the same gel was observed under visible light (5000K, Light Viewer 5700, Hakuba Photo Industry Co. Ltd., Tokyo, Japan). SWNT locations were visualized as black bands.

2.6. AFM Analysis. AFM was carried out on an MFP-3D instrument (Asylum Research, Santa Barbara, CA, USA) using the tapping mode, which employed a NANOSENSORS PPP-NCSTR-W silicon cantilever probe (NanoWorld AG, Neuchâtel, Switzerland) in air. The annealed suspension (10 μL) was dropped onto a mica surface that had been pre-treated with 0.01% 3-aminopropyl triethoxysilane. After incubating for 10 min at ~25°C, the samples were rinsed with 1 mL pure water and dried overnight in a desiccator. Calibration of AFM was carried out using gold nanoparticles (diameter: 5 nm) (Sigma-Aldrich, St. Louis, MO, USA), present as a stabilized suspension in 0.1 mM phosphate. A commercially available digital camera was used to capture the observations.
2.7. Photoluminescence (PL) Analysis. PL measurements were obtained using a titanium-sapphire laser for excitation (690–850 nm) and an InGaAs array detector. The laser spot was 1–2 μm in diameter, and the laser power was 1.6 mW. The annealed mixtures were investigated directly in the sample tubes without using a cuvette at room temperature. PL maps (excitation wavelength: 700–850 nm, emission wavelength: 1000–1500 nm) were obtained. Numerical analysis was carried out by exporting the data to an Excel file.

3. Results and Discussion

Figure 1 is a schematic representation of the present study. First, we prepared suspensions of T30/φ30 and SWNT hybrids. We then annealed the hybrids with other oligo-DNA molecules. Four types of oligo-DNA, T30, A30, φ30, and cp30, were used as secondary DNA molecules.

Figure 2 shows the results of agarose gel electrophoresis for the T30-SWNT hybrids (Figure 2(a)) and φ30-SWNT hybrids (Figure 2(b)). The gels were not stained. Therefore, the black bands indicate the locations of the SWNTs rather than those of the DNA. The samples were annealed at 24°C, 54°C, 74°C, 84°C, and 94°C for 30 s before electrophoresis. When the samples were annealed at 64°C, some SWNTs remained at the starting point for the T30-SWNT (as indicated by the arrow). When the annealing temperature was increased, the quantity of remaining hybrids increased, and the smear band completely disappeared when the sample was annealed at 94°C. In contrast, for the φ30-SWNT hybrids, most SWNTs migrated, even when the sample had been annealed at 84°C. Only the SWNTs in the sample annealed at 94°C failed to migrate. We repeated the experiments at least three times to confirm the reproducibility of the results.

At least two major factors should be considered to understand the electrophoresis results. First, the Tm values of DNA molecules. Tm values of T30 and φ30 were 61.1°C and 72.4°C, respectively. The value is defined as the transition temperature between dsDNA and ssDNA rather than between DNA and SWNTs. Another factor is the difference in DNA affinity against SWNT surfaces. It is known that thymine and guanine have higher affinities for SWNTs compared to adenine and cytosine [2, 43]. Thus, the affinity of T30 should be higher than that of φ30. However, φ30-SWNT hybrids were more stable than T30-SWNTs in our annealing experiments. The results may suggest the overlapped effects of Tm values and DNA affinity against SWNTs. A new concept such as “Tm values between DNA and SWNTs” may be useful for establishing DNA-SWNT nanotechnology. Second, we examined the reactions between the T30-SWNT/φ30-SWNT hybrids and additional DNA molecules (T30, A30, φ30, and cp30). Secondary DNA was added to the T30-SWNT or φ30-SWNT samples, and the samples were incubated for 30 s. The annealing temperatures were 56°C and 67°C for the T30-SWNT and φ30-SWNT hybrids, respectively, based on the Tm values of T30 and φ30.

When the T30-SWNT hybrids were annealed with A30, most SWNTs remained at the top of the gel (Figure 3, lane 1 without EtBr staining). There are two possible explanations for this. One is detachment of DNA molecules from the SWNT surfaces. The other is cross-linking of SWNTs with A30 molecules. When the T30-SWNT hybrids were annealed with T30, most SWNTs migrated. When φ30 or cp30 were reacted with the φ30-SWNT hybrids, the SWNTs also migrated. In contrast, we verified the same samples by EtBr staining to determine DNA locations. In the mixture of T30-SWNT and A30, bright DNA bands were observed in the middle of the gels. It is well known that the affinity of EtBr for dsDNA is much higher than that for ssDNA. Thus, our data clearly revealed DNA hybridization. When T30 was added to the T30-SWNTs, we did not observe the added T30 in the electrophoresis photograph.

When cp30 was reacted with the φ30-SWNTs, the migration of φ30-SWNT was confirmed by electrophoresis (Figure 3, lane 5 without EtBr staining). The results suggest that φ30 was not detached from the SWNT surfaces. However, DNA hybridization between cp30 and φ30 was also confirmed (Figure 3, lane 5 with EtBr staining).

Additionally, when cp30 was reacted with the φ30-SWNT hybrids, we observed obscure bands attributable to the added φ30 with the SWNTs. This suggests that some of the φ30 molecules were adsorbed onto the φ30-SWNT hybrids.

Figure 4 shows the AFM images for the T30-SWNT and φ30-SWNT hybrids with or without additional DNA. The average heights were 0.85 ± 0.05, 0.96 ± 0.04, 0.98 ± 0.06, 1.21 ± 0.06, and 0.90 ± 0.05 nm for T30-SWNT, T30-SWNT+T30, φ30-SWNT, φ30-SWNT+φ30, and φ30-SWNT+cp30, respectively. When the T30-SWNT hybrids were incubated with A30 (Figure 4(b)), we observed numerous aggregates, although the rod-like structures of the SWNTs were also visible. Cross-sectional analysis of the observed heights of the hybrids in Figure 4(b) was difficult. In fact, various shapes of aggregates were detected. In the case of φ30-SWNT+cp30, the thickness of the hybrid was increased (>1.5 nm in height) (Figure 4(f)), although aggregates were not observed. T30 and A30 can hybridize in various manners because A and T can bind at any location on the DNA molecule. Thus, cross-linked structures are expected to result from annealing. In the case of hybridization of φ30 and cp30, only one structure was expected, and thus, cross-linking of the hybrid would have been impossible. As expected, the T30-SWNT and φ30-SWNT hybrids showed some differences. We observed no increases in height for the other combinations.

Figure 5 shows the PL maps of similar samples to those investigated by AFM. The PL spots of the T30-SWNT and φ30-SWNT hybrids are represented by black dots at the initial position of each spot in each map. When the PL maps for T30-SWNT+A30 and T30-SWNT+T30 were compared, a clear shift in the emission wavelength of the PL spots was observed as indicated by (9,4) in T30-SWNT+A30 (as indicated by the arrow in Figure 5(a)). This suggests that the A30 molecules were adsorbed on the T30-SWNT hybrids, although there are many possible hybridization structures. There was no significant peak shift in the other three samples (Figures 5(b)–5(d)).
Figure 6 shows the PL spectra obtained at an excitation wavelength of 730 nm. The main peak (indicated by the arrow in Figure 6(a)) is likely attributable to the SWNTs indicated by (9,4) [44, 45]. Comparison of the T30-SWNT and T30-SWNT+A30 spectra revealed a wavelength shift of 7.85 nm. We did not detect a shift in the cφ30 spectra. There are several possible explanations for the PL shift. One is the addition of adenine on the SWNT surfaces because of DNA hybridization. It is known that PL wavelengths of SWNTs are affected by the types of DNA bases wrapping SWNTssDNA(T30, φ30)

Sonication

SSDNA-SWNT (T30-SWNT or φ30-SWNT)

Anneal (> melting temperature)

and

SSDNA-SWNT (T30-SWNT or φ30-SWNT)

Anneal (< melting temperature)

(b)

Figure 2: Agarose gel electrophoresis of (a) T30-SWNT- and (b) φ30-SWNT-annealed hybrids. The annealing temperatures were as follows: lane 1: 24°C, lane 2: 54°C, lane 3: 64°C, lane 4: 74°C, lane 5: 84°C, and lane 6: 94°C. The gel was not stained.

Figure 3: Agarose gel electrophoresis of T30-SWNT and φ30-SWNT hybrids. Lane 1: T30-SWNT, lane 2: T30-SWNT+A30, lane 3: T30-SWNT+T30, lane 4: φ30-SWNT, lane 5: φ30-SWNT +cφ30, and lane 6: φ30-SWNT+φ30. (a) Without staining. (b) With ethidium bromide (EtBr) staining.

Figure 1: Schematic representation of the present research.

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the SWNT surfaces. For example, Ito et al. reported that A30-SWNT hybrids showed longer PL wavelengths compared to T30-SWNT hybrids [44]. Another possibility is the formation of cross-linked aggregates according to nonspecific hybridization between A30 and T30. The effects of SWNT aggregates including bundles on PL spectra have been reported by several authors [46, 47].

Comparison of the AFM, PL, and electrophoresis results led to the following predictions. DNA hybridization likely occurred in both the A30/T30-SWNT and φ30-φ30-SWNT hybrids based on the electrophoresis results. The AFM results suggested that large numbers of A30 molecules bound to T30-SWNT via nonspecific hybridization between A30 and T30 and then formed aggregates. Based on these observations, the PL shift in the case of A30/T30-SWNT may be explained by the overlap of two factors. One is change in the physicochemical properties of SWNT surfaces caused by hybridization of A30 [44]. The other is cross-linked aggregate formation.

In contrast, φ30-φ30 were hybridized by one-to-one matching. The hybridization was confirmed by electrophoresis. Although there is a possibility that PL can detect DNA

Figure 4: Atomic force microscopy (AFM) images of annealed T30-SWNT and φ30-SWNT hybrids: (a) T30-SWNT hybrids, (b) T30-SWNT hybrids+A30, (c) T30-SWNT hybrids+T30, (d) φ30-SWNT hybrids, (e) φ30-SWNT hybrids+φ30, and (f) φ30-SWNT hybrids+φ30. The scan size was 1000 × 1000 nm. Histograms of height distribution were obtained by 250 cross sections from 50 hybrids.
hybridization without fluorescently labeling the DNA in principle, a further breakthrough is required to detect the hybridization of natural DNA molecules such as cφ30/φ30-SWNT. Our results revealed that the hybridization manner of DNA molecules with the A-T combination on SWNT surfaces significantly differed from that of natural complementary DNAs.

4. Conclusions

We investigated the reactions of complementary and non-complementary oligo-DNA molecules on DNA-SWNT hybrids. Our data suggest that the DNA-SWNT hybrids were affected by annealing when the annealing temperature was higher than the Tm values of each DNA. With additional secondary DNA molecules, the hybridization of DNA/SWNTs likely occurred on the SWNT surfaces. AFM imaging, PL measurements, and electrophoresis suggested that the T30-SWNT hybrids cross-linked with the A30 molecules. However, for the combination of φ30 and cφ30, which have natural complementary sequences, there were no changes in the PL spectra, although electrophoresis suggested the hybridization of cφx30 with the φ30-SWNT hybrids. Our results provided fundamental information regarding interactions between DNA molecules on SWNT surfaces.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
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