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Functionalization of Single-walled Carbon Nanotubes with Ribonucleic Acids

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The optical properties of single-walled carbon nanotubes (SWCNTs) dispersed in aqueous solutions of ribonucleic acids (RNA) purified from *Escherichia coli* were studied using photoluminescence (PL), Raman, and absorption spectroscopy. SWCNT-RNA complexes, down to a single isolated nanotube level, were successfully synthesized. SWCNT signatures in Raman, PL, and absorption spectroscopy were observed from the SWCNT-RNA complexes. Observation of two distinct PL peaks, one at 1.248 eV and the other at 1.392 eV, confirmed the existence of isolated (6,5) and (6,4) SWCNTs, respectively. Atomic force microscope images and height profiles also showed evidence of isolated SWCNT-RNA complex.

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I. INTRODUCTION

For the past several years, single-walled carbon nanotubes (SWCNTs) have been intensively studied for biological applications [1–7]. Due to their sizes of a few micrometers in length and a few nanometers in diameter, SWCNTs tend to interact easily with various biomolecules. Moreover, as all atoms of SWCNTs are located on the surface, they have very high chance of interacting with biomolecules [8]. Since the first report of SWCNT dispersion with DNA using the - stacking process [9], DNA has been extensively used in functionalizing SWCNTs, and chirality-selective sorting of SWCNTs using DNA was recently reported [10]. Furthermore, as semiconducting SWCNTs have bandgaps in the near-infrared region, where the biological tissues are transparent, they have great potential for biological applications [5–7]. Recently, SWCNT-based optical biosensors have been reported by many research groups [11–16]. These include optical biosensors capable of glucose detection by functionalizing SWCNTs with K3Fe(CN)6 [11], highly selective and sensitive optical biosensors based on SWCNTs functionalized with multicolor Raman labels for protein detection [12], and avidin-detecting optical biosensors based on functionalized SWCNTs with dye-ligand conjugates [13]. In addition, SWCNTs wrapped in dye-labeled single-stranded DNA have been used to make sensing platforms for probing bio-molecular interactions [14]. Hybridization of DNA and SWCNTs was detected through the band gap modulation of semiconducting SWCNTs, where the solvatochromic shift of SWCNTs strongly depends on the surface coverage of SWCNTs with DNA [15]. For in vivo use of SWCNT-based biosensors, SWCNTs functionalized with Rituxan antibody have been shown to be able to selectively detect the B-cell lymphoma with CD20 proteins on its surface [16].

RNA has intrinsic properties to form dynamic structures to function as catalysts [17,18], antisense repressors [19,20], and ribosensors [21,22], and these properties provide many merits for development of SWCNT-RNA complexes for biological applications. CNTs have recently been studied as innovative nanocarriers for delivery of small interfering RNA (siRNA), capable of expressing or silencing a specific gene, into cells without damage [23]. Both covalently functionalized [24–30] and non-covalently functionalized [31–34] CNTs used for siRNA delivery have been reported. Especially, Bartholomeusz *et al.* reported synthesis, by using direct non-covalent

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functionalization, of functionalized SWCNTs with a specific siRNA, targeting HIF-1 for gene silencing [35]. However, the focus of these papers was mainly on delivery of siRNA rather than studying the physical properties of the SWCNT-RNA complexes.

There are several reports on the functionalization of SWCNTs by using RNA [36–40]. HiPCO SWCNTs were purified using dispersion in total cellular RNA from yeast, followed by treatment with ribonuclease A [36]. HiPCO-SWCNT-RNA nanocomposites for self-assembled thin films were synthesized using poly(A), poly(C), and poly(G) [37]. Raman measurements were performed on fraction of HiPCO SWCNTs dispersed with RNA from torula yeast [38]. HiPCO SWCNTs were functionalized using poly(rA) and poly(rU) [39]. Transparent conductive films were made using functionalized SWCNTs with RNA from torula yeast [40]. However, dispersion of SWCNTs with total RNA to an isolated single nanotube level and detailed physical properties of the SWCNT-RNA complexes have not been reported as yet. In this work, SWCNT dispersion with total RNA purified from *Escherichia coli (E. coli)* to an isolated-single-SWCNT level was studied, and the physical properties of the synthesized SWCNT-RNA complexes were investigated using Raman, photoluminescence, absorption spectroscopy, and atomic force microscopy (AFM) measurements.

II. EXPERIMENTS AND DISCUSSION

CoMoCat SWCNTs (SouthWest NanoTechnologies Inc.) were sonicated in aqueous solutions of RNA, purified from *E-coli*, of random sequence by using a tip-type ultrasonicator (VCX750, Sonics and Materials). The purified RNAs from *E-coli* were obtained using the phenol-extraction and ethanol-precipitation method, and such as-purified total RNA dominantly consisted of 2,900 and 1,400 base-pairs, corresponding to 990 and 480 nm in length, respectively [41]. SWCNTs (20 mg)and RNA (1.4 mg) were sonicated at 158 W in RNase-free water. The solution temperature was maintained at ∼26 °C during sonication by using a water-cooling system. After a 3-hour sonication, the as-sonicated solution remained unperturbed for 24 hours under ambient condition so that large bundles of SWCNTs sank to the bottom part of the as-sonicated solution. Then, the top part of the solution was collected, and it was ultracentrifuged at 50,000 G for 1 hour. The top part of the ultracentrifuged solution was collected and put into a quartz cuvette for optical measurements. In order to compare the optical properties of RNA-dispersed SWCNTs with those of surfactant-dispersed SWCNTs, we also prepared a SWCNT suspension dispersed in an aqueous solution of deoxycholate sodium salts (DOC) through sonication at 540 W for 30 minutes, followed by ultracentrifuging at 100,000 G for 2 hours. RNA molecules in the as-ultracentrifuged samples were analyzed in an 8% polyacrylamide gel containing 8-M urea, and the size of RNA molecules was estimated to be ∼50 nucleotides

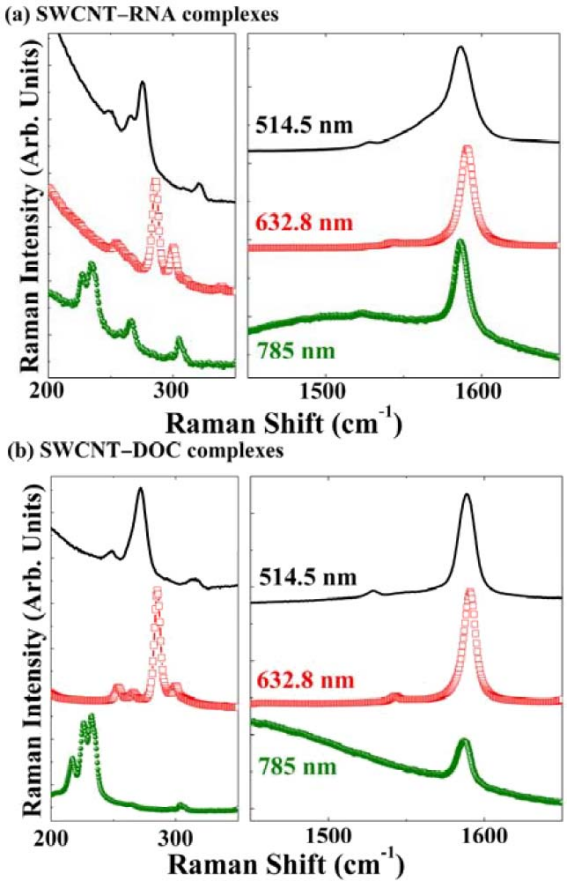

Fig. 1. (Color online) Radial breathing mode (left panels) and G-band (right panels) Raman spectra of (a) SWCNT-RNA complexes and (b) SWCNT-DOC complexes measured with three different excitation laser lines: 514.5 nm (black), 632.8 nm (red), and 785.0 nm (green).

Raman and photoluminescence (PL) spectra were measured at room temperature by using three different excitation sources: the 514.5-nm (2.410-eV) line from an Ar^+ laser, the 632.8-nm (1.959-eV) line from a He-Ne laser, and the 785.0-nm (1.579-eV) line from a diode laser. Scattered light from the samples was analyzed through a single-grating monochromator with a focal length of 50 cm and was detected with a liquid-nitrogen-cooled CCD detector. Absorption spectra were measured using tunable monochromatic light from the exit slit of a single-grating monochromator whose input slit was open to white light from a tungsten-halogen lamp as incident light. Transmitted light through the samples was detected with a silicon photodiode detector. AFM (Park Systems, Korea) images were obtained in non-contact mode. The resolution of our AFM image is 256 × 256 pixels.

The radial breathing mode (RBM) and the G-band Raman spectra of the SWCNT-RNA complexes mea-

sured with three different excitation laser lines (514.5, 632.8, and 785.0 nm) are shown in Fig. 1(a). In the low-frequency spectral range, where RBM features are displayed, signatures of metallic SWCNTs whose chiralities are (7,7), (8,5), (9,3) and (8,2) were observed with 514.5-nm excitation, but those of semiconducting SWCNTs whose chiralities are (10,3), (7,5) and (8,3) were observed with 632.8-nm excitation [42]. For the 785.0-nm excitation, the features of semiconducting SWCNTs were observed at 227, 235, 266, and 305 cm^{-1}. The RBM features at 227, 266, and 305 cm^{-1} can be assigned to (10,5), (10,2), and (9,1) SWCNTs, respectively. The chirality of the Raman peak at 235 cm^{-1} is not unambiguously identified in the literature, where it is assigned to (11,3), (12,1) or (13,0) [43,44].

For comparison, we have also measured the Raman spectra of the SWCNT-DOC complexes under the same experimental conditions as shown in Fig. 1(b). Most of the RBM features from SWCNT-DOC complexes are more or less the same as those from SWCNT-RNA, except the RBM feature at 266 cm^{-1} with 785.0-nm excitation. In the high-frequency spectral range, where G-band Raman features are shown, SWCNT-RNA and SWCNT-DOC exhibit almost the same spectral features, except for the distinct shoulder-like feature on the low-frequency side of the asymmetric G-band for SWCNT-RNA with 514.5-nm excitation. This shoulder-like feature originates from metallic SWCNTs, which is consistent with the corresponding RBM spectrum of SWCNT-RNA.

PL spectra of SWCNT-RNA complexes measured with three different excitation laser lines (514.5, 632.8, and 785.0 nm) are shown in Fig. 2(a). They all exhibit two distinct PL signatures of (6,5) and (6,4) SWCNTs at 1.248 eV and 1.392 eV, respectively. The PL spectrum of the SWCNT-free RNA solution was also measured, but it does not show any PL feature in the spectral range shown in Fig. 2. The observed relative PL intensity between (6,5) and (6,4) SWCNTs changes with different excitation laser wavelengths, which can be attributed to the fact that the relative absorption coefficient between (6,5) and (6,4) SWCNTs is not the same for different excitation laser wavelengths. In contrast, the PL spectra of SWCNT-DOC complexes, shown in Fig. 2(b), exhibit four distinct PL signatures of (6,5), (8,3), (9,1), and (6,4) SWCNTs at 1.260, 1.283, 1.341, and 1.402 eV, respectively, and the PL intensity of the (6,4) SWCNTs is substantially suppressed for all excitation laser wavelengths as compared to the case of SWCNT-RNA. Thus, RNA shows much stronger affinity to (6,4) SWCNTs than DOC does.

We should also notice that the observed PL signature of (6,5) SWCNT-RNA at 1.248 eV exhibits a large red-shift of 12 meV with respect to that of (6,5) SWCNT-DOC at 1.260 eV. A similar red-shift of 10 meV was also observed for (6,4) SWCNTs. The observed red-shift, known as the solvatochromic shift, is attributed to the difference in the effective dielectric constants of the aqueous solutions of RNA and DOC.

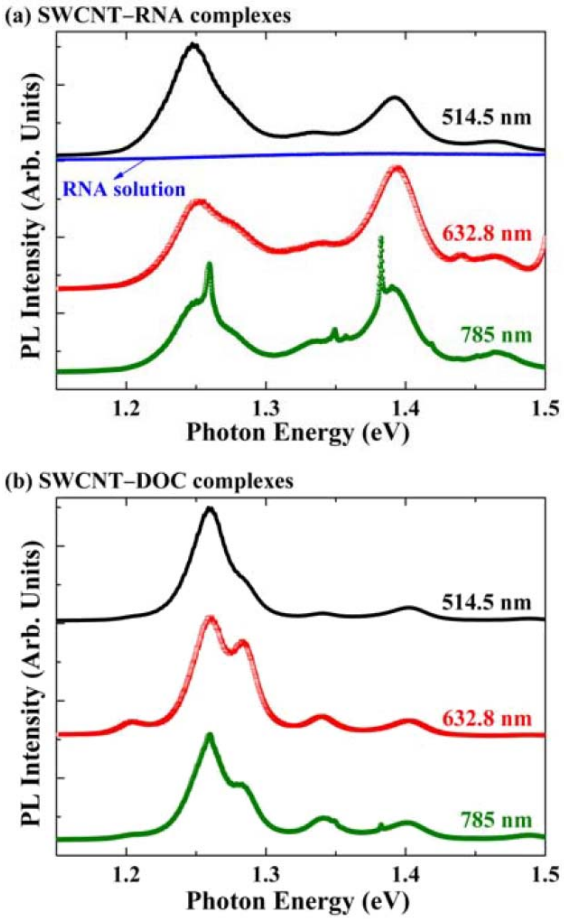

Fig. 2. (Color online) PL spectra of (a) SWCNT-RNA complexes and (b) SWCNT-DOC complexes, measured with three different excitation laser lines: 514.5 nm (black), 632.8 nm (red), and 785.0 nm (green). The dashed lines are guides for the eye. The PL spectrum (blue) without any spectral feature from the SWCNT-free RNA solution is also shown. The sharp peaks in the PL spectrum of SWCNT-RNA with 785.0-nm excitation are Raman features.

Neither the E_{11} transitions of metallic SWCNTs nor the E_{22} transitions of semiconducting ones can be observed in the PL spectra because the observed PL features originate only from the E_{11} transitions of semiconducting ones. In order to investigate the existence of metallic SWCNTs and the E_{22} transitions of semiconducting ones, we measured the absorption spectra. Several E_{11} and E_{22} transitions from semiconducting SWCNTs and the E_{11} transition from a metallic (6,6) SWCNT are shown in Fig. 3 in the spectral range from 400 nm to 1100 nm [45,46]. Absorption features in the spectral range of E_{11} transitions of semiconducting SWCNTs are consistent with corresponding emission features shown in Fig. 2 for both SWCNT-RNA and SWCNT-DOC complexes. Similar solvatochromic shifts, as manifested in the PL spectra, were also observed in the absorption spectra shown in Fig. 3. The SWCNT-RNA suspension was dropped on a gold-coated SiO_2 substrate for

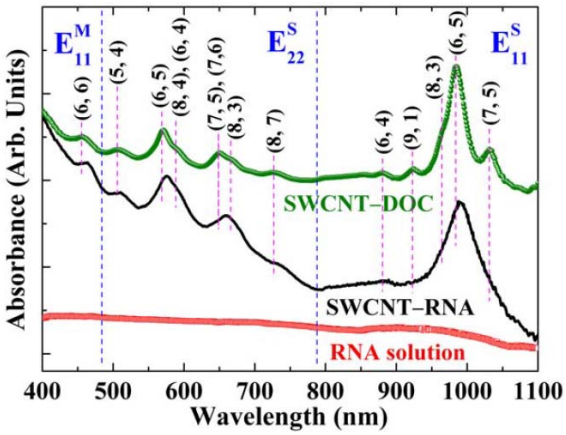

Fig. 3. (Color online) Absorption spectra of SWCNT-DOC complexes (green), SWCNT-RNA complexes (black), and SWCNT-free RNA solution (red). The dashed lines are guides for the eye.

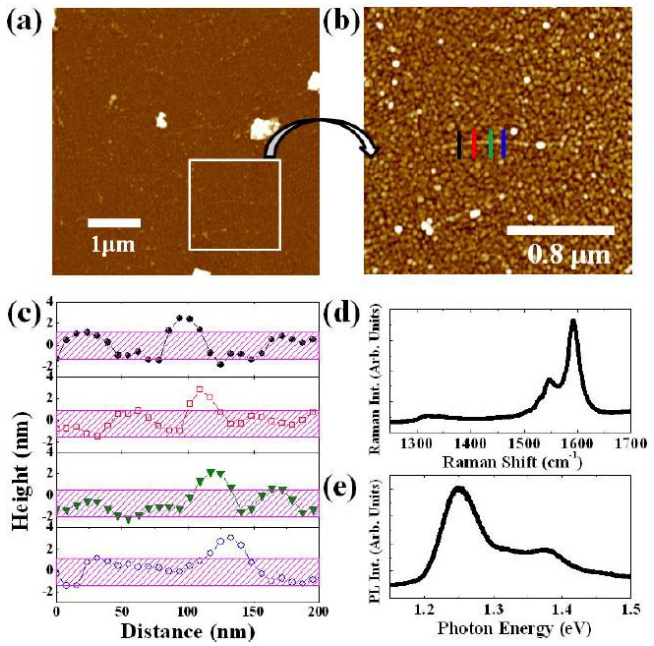

Fig. 4. (Color online) (a) AFM image of SWCNT-RNA complexes on a gold-coated SiO_2 substrate in an area of 5 m 5 m. (b) Enlarged AFM image of the boxed area in (a). (c) Height profiles along the black, red, green, and blue lines shown in (b). The shaded part in each height profile is a guide for the eye and represents the surface roughness of the gold-coated SiO2 substrate. (d) and (e) Raman and PL spectra from the sample shown in (a), respectively.

the AFM measurements.

An AFM image measured in non-contact mode is shown Fig. 4(a), where several line-like features can be attributed to isolated SWCNT-RNA complexes. When the boxed area in Fig. 1(a) is enlarged, an isolated SWCNT-RNA can be unambiguously identified, as shown in Fig. 4 (b). Height profiles along the black, red, green, and blue lines shown in Fig. 4(b) are plotted in Fig. 4(c), where the shaded height-band represents the surface roughness of the gold-coated SiO_2 substrate. In all height profiles shown in Fig. 4(c), the peak height above the shaded band is approximately 1.7 ± 0.2 nm. When the diameters of our SWCNTs, ∼0.8 nm, and the thickness of RNA, ∼0.5 nm are consiered [47,48], the observed height profiles confirm that the line-like feature in Fig. 4(b) is, indeed, an isolated SWCNT-RNA complex. Micro-Raman and micro-PL spectra, measured on the same sample, are shown in Fig. 4(d) and 4(e), respectively, where SWCNT signatures are clearly observed, indicating that those features in the AFM image are really correlated to the SWCNTs.

III. CONCLUSION

We have synthesized a SWCNT-RNA suspension by using ultrasonication of SWCNTs in an aqueous RNA solution followed by ultracentrifugation. Results from PL, Raman, and absorption measurements indicated that the SWCNT-RNA complexes, down to a single isolated nanotube level, were successfully synthesized. Observation of two distinct PL peaks, one at 1.248 eV and the other at 1.392 eV, confirmed the existence of isolated (6,5) and (6,4) SWCNTs, respectively. Atomic force microscope images and height profiles also showed evidence of isolated SWCNT-RNA complex. Finally, it is worthwhile to note that the SWCNT-RNA complexes can be utilized for gene delivery, gene recognition, drug delivery, and biosensing via integration with various biomolecules.

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