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Monitoring the antioxidant effects of catechin using single-walled carbon nanotubes: Comparative analysis by near-infrared absorption and near-infrared photoluminescence

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ABSTRACT

We measured the optical responses of single-walled carbon nanotubes (SWNTs) after adding Japanese green tea or catechin. SWNTs were covered with DNA in aqueous solution, and tea or catechin solution was added to the DNA–SWNT suspension. The antioxidant effects of tea and catechin were detected as changes in the near-infrared (NIR) absorption (ABS) and NIR-photoluminescence (PL) spectra of the SWNTs. Commercial Japanese tea, diluted 100 times and containing 15 μg/mL catechin, was sufficient for recovering NIR-ABS and NIR-PL spectra when the DNA–SWNT suspension was pre-treated with 0.03% hydrogen peroxide(H_2O_2). Similar results were obtained with 15 μg/mL of pure catechin solution. SWNTs with specific chirality were sensitive to the NIR-ABS and NIR-PL changes. The (10, 5)/(8, 7) and (9, 4) SWNTs showed the highest recovery in NIR-ABS and NIR-PL, respectively. NIR-PL recovery was higher than that of NIR-ABS for (10, 5)/(8, 7) and (9, 4). Spectral changes could be monitored thoroughly at pH 8.0, contrary to pH 6.0 and 7.3. However, the most dynamic recovery of NIR-ABS and NIR-PL was observed at pH 6.0. Furthermore, time-lapse measurements revealed that recovery was faster with tea or catechin addition than H_2O_2-induced oxidation.

1. Introduction

Various biological applications, such as biological sensing, [1,2] DNA detection, [3,4] and drug delivery, [5,6] using unique optical responses have been proposed. For these applications, SWNT surfaces are typically covered with biomolecules or organic molecules to solubilize them [7–9]. Hybrids of DNA and SWNTs (DNA-SWNT), in particular, are commonly used for such applications, [10,11] and are synthesized by mixing a single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) solution with SWNT powder, and sonicating the mixture under suitable conditions [8].

DNA-SWNT hybrids show different responses to radiation of different wavelengths. When irradiated with near-infrared (NIR) radiation, it is absorbed only by the SWNT, and not by DNA, because DNA absorbs light at approximately 260 nm [12]. The absorption of NIR radiation (NIR-ABS) is one of the unique responses of SWNTs. However, when DNA-SWNTs are irradiated with VIS light, they absorb the light, and exhibit photoluminescence (PL) in the NIR wavelength range (NIR-PL) [12,13].

The NIR-ABS and NIR-PL phenomena have been applied in the characterization of DNA-SWNT hybrids [8,14]. For example, Kam et al. characterized the adsorption of DNA functionalized on SWNT surfaces at various concentrations of SWNTs by analyzing NIR-ABS [2]. Lee et al. observed that the NIR-PL of DNA-SWNT hybrids increased upon DTT addition [15]. This directly demonstrated that the NIR-PL of SWNTs was enhanced when SWNT concentration was reduced. Several researchers have studied both the NIR-ABS and NIR-PL of DNA-SWNT hybrids [16,17]. In a recent study, Yamamoto et al. employed thymine 20-mers (T20) and adenine 20-mers (A20) to cover SWNT surfaces [17]. They observed that the NIR-ABS and NIR-PL of T20-SWNT, A20-SWNT, and A20-T20-SWNT were distinguishable. In the abovementioned studies, various types of DNA-SWNT hybrids were prepared and compared.

Recently, several research groups have begun demonstrating specific applications for biological or chemical sensors. Xu et al. analyzed the NIR-ABS of DNA-SWNT hybrids with hydrogen peroxide (H_2O_2), glucose, and glucose oxidase (GOx) [1]. They observed that the NIR-ABS spectra gradually changed as a function of time. A related study was conducted using SWNTs dispersed with sodium dodecyl sulfate [18]. Zheng et al. studied changes in NIR-ABS with strong oxidants, such as K_2IrCl_6 [19]. Tu et al. also reported changes in NIR-ABS with H_2O_2 addition and pH regulation [20].

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Zhao et al. demonstrated a novel approach to the use of DNA-SWNT hybrids for biosensing [21]. They synthesized only one type of DNA-SWNT hybrids. They examined the redox reactions of SWNTs caused by the injection of H_2O_2, coffee, caffeine, and vitamin C utilizing NIR absorption (NIR-ABS). Specifically, when a small quantity of H_2O_2 was added to a DNA-SWNT suspension, the NIR-ABS of the DNA-SWNT hybrids decreased with peaks at 1130, 1197, and 1278 nm. These three peaks corresponded to the first interband transitions (E11) of (9, 4), (12, 1), and (10, 5) semiconducting SWNTs. They observed that the peak at 1278 nm was sensitive to oxidation. Therefore, when coffee, caffeine, or vitamin C was added to the DNA-SWNT suspension containing H_2O_2, NIR-ABS was restored to its original intensity. Probably, the study of the effects of DTT on the NIR-PL of the same DNA-SWNT hybrid by Lee et al., and the characterization of aged DNA-SWNT hybrids by Cathcart et al. provided suggestions for this approach [22,23]. Kurnosov et al. analyzed the NIR-PL of DNA-SWNT hybrids in the presence of cysteine [24,25]. PL intensity was observed to be affected by cysteine concentrations. Kruss et al. covered SWNTs with polymers, including DNA. The NIR-PL of the polymer-covered SWNTs was altered by adding various chemicals, such as dopamine [26]. They also applied their method for NIR imaging [27]. From the same research group, Polo et al. utilized various types of polymers to cover SWNTs for comparison [28]. They discovered that the polymer charges affected the NIR-PL spectra of the SWNTs.

In this study, we utilized both NIR-ABS and NIR-PL to simultaneously detect the oxidation/reduction reactions of DNA-SWNT hybrids caused by the addition of Japanese green tea and catechin. The oxidation of DNA-SWNT hybrids by H_2O_2 injection and their reduction by the injection of commercially available Japanese tea, and/or catechin were monitored using NIR-ABS and NIR-PL. NIR-ABS and NIR-PL were separately employed to monitor the same phenomenon. This is the first study to directly compare the changes in NIR-ABS and NIR-PL due to the antioxidant activity of tea and catechin using one type of DNA-SWNT hybrids. Furthermore, we examined the effects of pH on the sensitivity of oxidation/reduction detection.

2. Materials and methods

SWNTs produced by high-pressure CO conversion (HiPco) synthesis were purchased from Unidym Inc. (Menlo Park, CA, USA). dsDNA (deoxyribonucleic acid sodium salt from salmon testes, D1626) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Hydrogen peroxide (approximately 30%, 084-07441) and epigallocatechin gallate (553–74471, catechin) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Healthya Green Tea^{TR} (Japanese tea) was purchased from Kao Corporation (Tokyo, Japan). The commercially available Japanese tea included 1.5 mg/mL of catechin based on the data sheet from the manufacture.

DNA was dissolved in 10 mM phosphate buffer (pH 6.0), 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer solution (pH 7.3), and 10 mM Tris-HCl buffer (pH 8.0). DNA concentration was 1 mg/mL. Then, the DNA solution was sonicated using an ultrasonic bath (80 W) for 90 min on ice. Finally, the DNA solution was gently agitated for 3 h. The lengths of DNA were verified by agarose gel electrophoresis. The predominant lengths of DNA were 80–300 mers, although longer molecules were also present, based on agarose gel electrophoresis. Catechin was dissolved in pure water (1.5 mg/mL), and stored after gentle agitation.

For synthesizing the DNA-SWNT hybrids, 0.5 mg of SWNT powder and 1 mL of the DNA stock solutions (pH 6.0, 7.3, and 8.0) were mixed, and then, sonicated for 2 h using a probe-type sonicator (5 W) on ice. The supernatant was stored as the DNA-SWNT suspension after centrifugation at 15,000 rpm (17,360*g*) for 3 h at 8 °C.

An NIR spectrometer (SolidSpec-3700DUV, Shimadzu Corp., Kyoto, Japan) was used for NIR spectral measurements. For PL measurements, a Ti:sapphire laser was used for excitation (690–850 nm), and an InGaAs array detector was used for measuring the PL spectra. PL maps were obtained by varying the excitation wavelengths (700–840 nm). The laser spot was 1–2 μm in diameter, and the laser power was 1.6 mW. The sample preparation and measurement procedures were the same for both the NIR and PL measurements. A volume of 100 μL of the DNA-SWNT solution (pH 6.0, 7.3, and 8.0) and 880 μL of the buffer (pH 6.0, 7.3, and 8.0) were mixed in cuvettes, and the initial spectra of the samples were measured. Then, 10 μL of H_2O_2 diluted with pure water was added to the samples (final concentration of 0.03%), and incubated for 10 min at 21 °C. The spectra of each sample were measured again. Finally, 10 μL of the Japanese green tea or catechin solution was added to the samples, and the spectra were measured again after incubation for 10 min at 21 °C. In both of tea and pure catechin samples, the final concentration of catechin during measurements was 15 μg/mL. Each experiment was repeated thrice for reproducibility. The obtained NIR spectra were normalized to an absorption wavelength of 731.50–735.25 nm (E22 of (9, 4)).

3. Results and discussion

Fig. 1 shows the NIR-ABS spectra of the dsDNA-SWNT hybrids incubated with and without catechin (1000–1330 nm). The dsDNA-SWNT hybrids were prepared at three different pH values (6.0, 7.3, and 8.0). The samples were designated samples at stage 1 (red lines). Then, H_2O_2 (final concentration of 0.03%) was added to the dsDNA-SWNT suspension. If the dsDNA-SWNT hybrids were oxidized by H_2O_2, the NIR-ABS would change [21]. The NIR-ABS spectra were measured after incubation for 10 min (stage 2, sky-blue lines) and 30 min (stage 3, blue lines) after adding H_2O_2. Finally, catechin was added to the samples (stage 4, dark gold lines). The reduction of the dsDNA-SWNT hybrids by this treatment was detectable through changes in the NIR-ABS spectra. Similar results with Japanese tea treatment are shown in Fig. S1. Although the exact content of the Japanese tea components was not be determined specifically, since it is a commercial beverage, it should contain 1.5 mg/mL of catechin based on the information provided by the manufacturer. When we added tea to the dsDNA-SWNT suspension, final catechin concentration in the diluted tea was 15 μg/mL. Thus, we adjusted the final catechin concentration to 15 μg/mL when we used pure catechin solution for comparison.

The spectra were normalized to absorbance at 731.50–735.25 nm E22 of (9, 4), since the absorbance of this peak was minimally affected by the addition of H_2O_2, tea, and catechin. Minute responses of small peaks appearing in the non-normalized spectra were lost in the normalized data. However, when the experiments were repeated, the biggest source of data fluctuation was sonication process. The fluctuation due to sonication agreed with the minute responses in the non-normalized data. Therefore, we concluded that the use of normalized data was suitable, in this study. Discussion about the minute responses and improvements of sonication will be considered in future studies.

Three clear peaks were detected in each spectrum in Fig. 1. Based on previous studies, we speculated that peaks 1, 2, and 3 in Fig. 1 originated from (9, 4), (12, 1)/(8, 6), and (10, 5)/(8, 7), respectively [29,30]. Regarding peak 2, because the absorbance wavelengths of (12, 1) and (8, 6) were very close, it was difficult to determine a single chirality value from the NIR-ABS. Peaks 3 also exhibited the overlap of two chirality values ((10, 5) and (8, 7)).

The NIR-ABS spectra of the initial samples (stage 1) are indicated by red lines in Fig. 1. The wavelengths of the three peaks were 1133, 1193, and 1265 nm at pH 8.0 (Fig. 1(c)). The spectra of the samples incubated with 0.03% H_2O_2 for 10 min (stage 2) and 30 min