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Single-walled carbon nanotubes/DNA hybrids in water are highly stable

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ABSTRACT

Unbound double-stranded DNA (dsDNA) was removed from an aqueous solution (solution 1) of dsDNA-solubilized single-walled carbon nanotubes (SWNTs) using size-exclusion chromatography (SEC). The SEC chromatogram of solution 1 showed two separated peaks, and the earlier eluent fraction was separated into four size-separated fractions, fr1–fr4, in which individually solubilized SWNTs were found to exist. The stability of the DNA/SWNT hybrids was evaluated by the re-injection of fr1–fr4. It was found that the chromatograms of fr1–fr4 were identical to those of the original ones even after storage for one month, indicating the high stability of the dsDNA/SWNT hybrids in water.

1. Introduction

Since the discovery that double-strand DNA (dsDNA) [1] and single-strand DNA (ssDNA) [2] solubilize single-walled carbon nanotubes (SWNTs) in water, many groups [3–15] have endeavored to understand the fundamental properties of the SWNTs/DNA nanobio hybrids and their applications in nano- and nanobio-related materials science. However, one must pay attention to the fact that large amounts of unbound (free) DNA molecules exist in SWNTs/DNA aqueous solutions. Are the hybrids containing no free DNAs in the bulk stable enough for use in many experiments? Do we need to consider the desorption of the bound DNA molecules from the hybrids to the bulk solutions to reach thermodynamic equilibrium? To the best of our knowledge, no clear-cut answers have yet been published on these questions. We undertake this fundamental issue in the present study. For this purpose, we first completely separated free dsDNA from an aqueous solution of the DNA/SWNT hybrids using size selective chromatography (SEC), and then examined the stability of the bulk DNA-free SWNTs/DNA hybrids using SEC. We have found that the bulk DNA-free SWNTs/DNA solutions are stable for more than one month. This observation indicates that we virtually need not consider the desorption of bound DNA from the nanohybrids in water, which is of great advantage to the utilization of the hybrids in wide areas of science.

2. Experimental

The dsDNA (salmon testes, 100–200 bp by electrophoresis) and as-produced SWNTs, so-called HiPco, were obtained from Nichiro Co. and Carbon Nanotechnologies Inc., respectively, and were used as received. Solubilization of the SWNTs using the DNA was conducted following the procedures described in Ref. [1]. Typically, ~1.0 mg of the SWNTs were added to an aqueous solution of DNA (5 mL, 0.68 mg/ml, Tris–EDTA buffer pH 8.0) and sonicated by a bath-type ultrasonic cleaner (Branson 2210) at temperatures below 5 °C for 1 h, followed by centrifugation (Sigma, 3K30C) at 60000*g* for 1 h. The UV–vis–near IR spectra were measured at room temperature using a spectrophotometer (JASCO, V-570). Size-exclusion chromatography columns (CNT SEC-2000, CNT SEC-1000, and CNT SEC-300, each 4.6 (inner diameter) × 250 mm (length), Sepax Technologies, Inc.) were connected to an HPLC system (Shimadzu). Tris HCl buffer (1 mM, pH 8.0) containing 0.1 mM EDTA was eluted at a flow rate of 0.25 mL/min. The elution was collected at 0.25 ml/fraction. Atomic force microscope (AFM, tapping mode) measurements were performed using a Veeco NanoScope® IIIa. The sample solution was deposited onto a freshly cleaved mica substrate, rinsed with water, and then dried before the measurement.

3. Results and discussion

Fig. 1 shows SEC chromatograms of the dsDNA-solubilized SWNT aqueous solution and that of dsDNA aqueous solution after the sonication. A peak of the chromatogram of the DNA solution appeared at 30.9 min, as shown in the figure. The dsDNA-solubilized SWNT solution gave two peaks (bold line in Fig. 1); the first fraction can be identified as the dsDNA/SWNTs hybrid and the second as unbound (free) dsDNA. The obtained elution profile for the dsDNA-solubilized SWNTs resembles the SEC chromatogram for solutions of ssDNA-solubilized SWNTs reported in Ref. [16].

The first peak portion in the chromatogram of the dsDNA-solubilized SWNT solution was distinctly fractionated into four samples, fr1–fr4. As shown in Fig. 2, these fractions had pale gray

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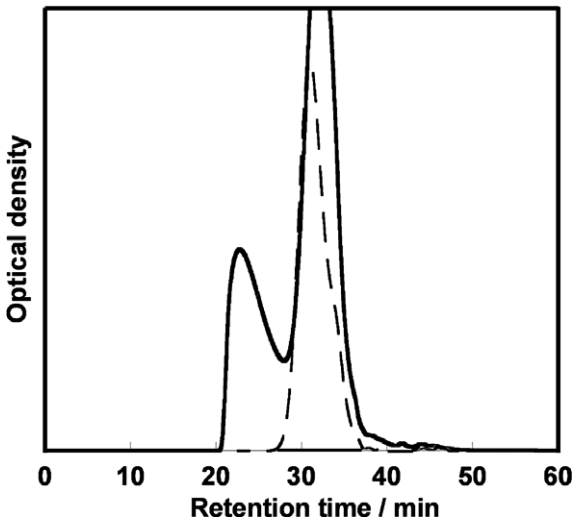

Fig. 1. SEC chromatograms of a dsDNA-solubilized SWNT aqueous solution (solid line) and that of a dsDNA solution (not containing the SWNTs) after sonication (broken line). The chromatograms were measured at 260 nm by an HPLC flow cell.

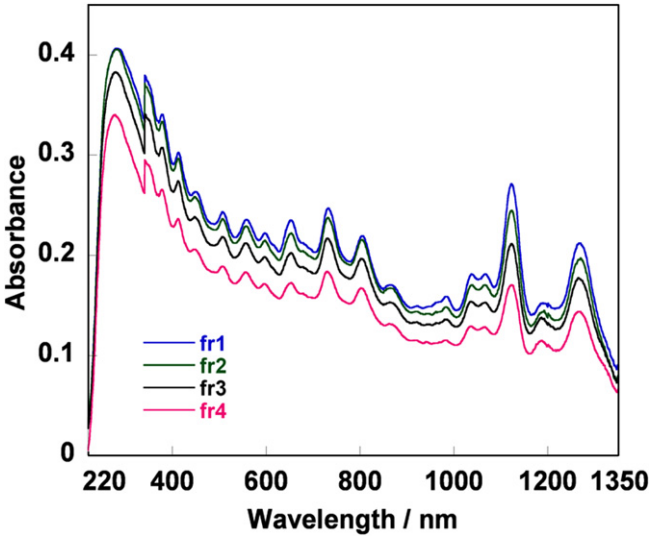

Fig. 3. UV–vis–NIR spectra of SEC-fractionated samples fr1–fr4.

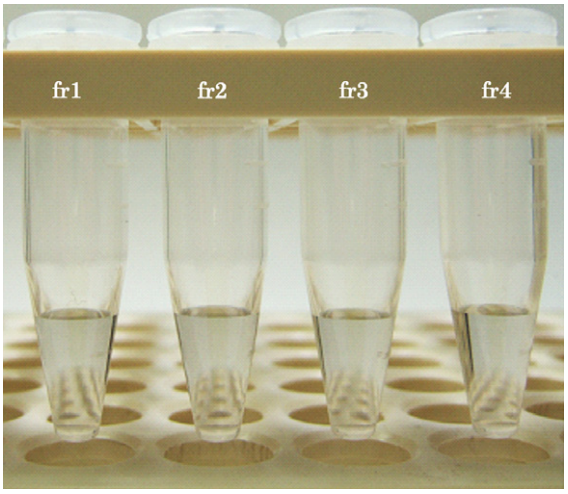

Fig. 2. Photographs of SEC-fractionated samples fr1–fr4.

color and were transparent. The UV–vis–near IR spectra of these solutions, displayed in Fig. 3, show characteristic absorption bands to be assigned to the interband transition of SWNTs, indicating that the fractions contain SWNTs and that the SWNTs are individually solubilized in these solutions. However, we were unable to determine the amount of the DNAs in each fraction from the absorption spectra, because both DNA and SWNTs have absorption at 260 nm. The retention times of the four samples are discussed later in relation to Fig. 4 and Table 1.

An AFM study was carried out using the fractionated samples. We observed individual dissolution of the nanotubes from the height profiles of the AFM images. The length distributions for the randomly-selected 50 tubes in fr1, fr2, fr3 and fr4 obtained from their AFM images gave average lengths of 447, 270, 227 and 205 nm, respectively. Earlier fractions evidently contain longer dsDNA/SWNTs hybrids, which agrees with the SEC separation principle.

The stabilities of the dsDNA/SWNT were evaluated by re-injection of these fractionated samples after storage in a refrigerator (4 °C) for 1 day, 1 week and 1 month; the results are presented in Fig. 4. The chromatograms after 2 days and 1 week are virtually identical to those injected immediately after the collection of these fractions. No peak was observed around the retention time of 31 min, which corresponds to that of the DNA solution not containing SWNTs. Even after the storage for 1 month, the chromatograms for fr1–fr4 were virtually identical with the original, although very small peaks were observed around a retention time of 34.5 min, which is somewhat longer that that of the free DNA. The observed small peaks might be ascribed to free DNAs with smaller molecular weights. This would be due to the instability of DNA molecules. We carried out the experiment by the same procedures twice (runs 1 and 2), as shown in Table 1, for both runs, the retention times in the chromatograms for fr1–fr4 that were stored for 2 days, 1 week or 1 month were essentially identical to those of the corresponding original samples, indicating that the desorption of the bound DNA from the DNA/SWNTs hybrids during these periods, for all fractionated samples is almost negligible. This finding indicates that the affinity between DNA and SWNTs in water is very strong.

4. Concluding remarks

We have removed the unwrapped dsDNA from the dsDNA-solubilized SWNT aqueous solutions to obtain size-separated DNA/SWNT hybrids (fr1–fr4). By using the re-injection procedure of the SEC-HPLC, we have discovered that, at the SEC chromatograph level, the fractionated dsDNA/SWNT hybrids are highly stable without desorption from the hybrids to the unbound dsDNA for at least 1 month. The interaction of ssDNA and SWNTs is expected to be stronger than that of dsDNA and SWNTs. Therefore, isolated ssDNA/SWNTs hybrids should have similar stability. The present finding is important not only in the fundamental analysis of dsDNA/SWNT hybrids, but also for their applications especially in biological areas.

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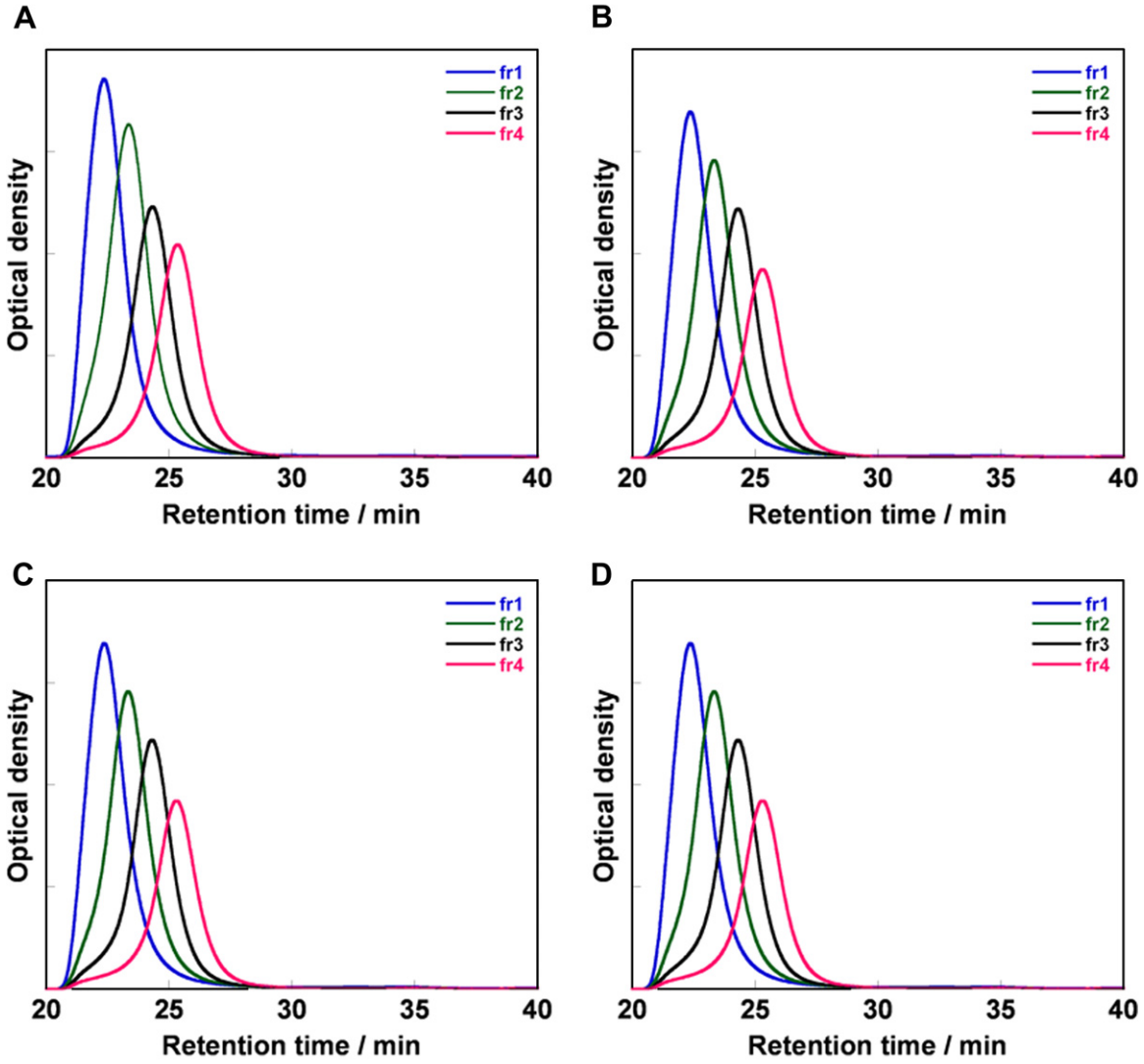

Fig. 4. SEC chromatograms of fr1–fr4 injected immediately after the separation (A) and those for the samples after storage at 4 °C for 2 days (B), 1 week (C) and 1 month (D). The chromatograms were measured at 260 nm by the HPLC flow cell.

Table 1
Retention times of the SEC chromatographs for samples fr1–fr4 obtained by two experiments, run1 and run2

		Retention time (min)			
		fr1	fr2	fr3	fr4
Immediately after the separation	Run1	22.34	23.35	24.33	25.35
	Run2	22.38	23.34	24.31	25.36
After 2-day storageat 4 °C	Run1	22.36	23.33	24.30	25.29
	Run2	22.37	23.38	24.39	25.38
After 1-week storage at 4 °C	Run1	22.29	23.27	24.29	25.33
	Run2	22.36	23.38	24.36	25.37
After 1-month storage at 4 °C	Run1	22.25	23.24	24.24	25.26
	Run2	22.41	23.29	24.32	25.33

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