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Atomic Force Microscopy of DNA-wrapped Single-walled Carbon Nanotubes in Aqueous Solution

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ABSTRACT

We evaluated hybrids of DNA and single-walled carbon nanotubes (SWNTs) in aqueous solution and in air using atomic force microscopy (AFM). Although intensive AFM observations of these hybrids were previously carried out for samples in air, this is the first report on AFM observations of these hybrids in solution. As expected, diameters of DNA-SWNT hybrids dramatically increased in tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid (TE) buffer solution. The data suggest that DNA molecules maintain their structures even on the SWNT surfaces. Furthermore, we simultaneously observed single DNA-SWNT hybrids using three different AFM modes in air and in the TE buffer solution. Height value of the hybrids was largest in the solution, and lowest for the mode that repulsive force is expected in air. For the bare SWNT molecules, height differences among the three AFM modes were much lower than those of the DNA-SWNT hybrids. DNA molecules adsorbed on SWNT surfaces flexibly changed their morphology as well as DNA molecules on flat surfaces such as mica. This is hopeful results for biological applications of DNA-SWNT hybrids. In addition, our results revealed the importance of the single-molecule approach to evaluate DNA structures on SWNT surfaces.

1. Introduction

Single-walled carbon nanotubes (SWNTs) can be coated with DNA molecules by physical adsorption through sonication in aqueous solution [1–7]. These DNA-coated SWNTs, so-called DNA-SWNT hybrids, are attractive nanodevices because they can potentially be used in medicine and biology, including drug delivery and gene detection [8–13]. It is essential to precisely determine the structures of DNA molecules adsorbed onto SWNTs to fully realize the potential of such applications. Several theoretical calculations concerning the mechanism of DNA adsorption onto the SWNT surfaces have been reported thus far [14–20]. From an experimental viewpoint, structures of DNA-SWNT hybrids have been investigated by various microscopic techniques. In particular, detailed structural characterization of DNA-SWNT hybrids by atomic force microscopy (AFM) has been widely performed [21–30]. Surface morphologies and diameters of DNA-SWNT hybrids were extensively discussed in these previous reports. However, to our knowledge, all of the AFM observations of DNA-SWNT hybrids were performed in air or in gases. On the other hand, there is an abundance of AFM studies of DNA molecules on a flat surface without SWNTs both in air and in liquids. In these studies, AFM images reveal a much larger morphology of DNA molecules in aqueous solution in contrast to the dried state [31–36]. Rich information has been given by comparison of wet structures and dried structures of DNA molecules. We think that analysis of the structures of DNA-SWNTs in solution is a more relevant approach for investigating the biomedical applications of DNA-SWNT hybrids.

In this study, we demonstrate AFM observations of DNA-SWNT hybrids both in air and in aqueous solution for the first time. Although comparison of wet structures and dried structures of DNA molecules has been intensively studied for DNA molecules on flat surfaces, our study is the first example for DNA molecules on SWNTs. Our research interest is evaluation of DNA-SWNT hybrids not native DNA molecules. Furthermore, we obtained AFM images with three different force conditions to evaluate the effects of interactions between DNA-SWNT hybrids and AFM probes. For this approach, the same DNA-SWNT hybrids were directly compared by simultaneous AFM observation under different force conditions in air and in liquid. There is no previous report of direct comparison of DNA-SWNT structures by AFM observation with different force conditions. This experiments gave helpful information about flexibility of structures of DNA-SWNT hybrids.

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2. Materials and Methods

2.1. Preparation of DNA-SWNT hybrids

Single-stranded DNA (ssDNA), consisting of 30-mers of thymine (T30), was obtained from Life Technologies Japan Ltd. (Tokyo, Japan) and double-stranded DNA (dsDNA) from salmon testes (D1626) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). SWNTs grown using a high-pressure carbon monoxide decomposition process (Super Purified HiPCO Single-wall Carbon Nanotubes) were purchased from Unidym, Inc. (Sunnyvale, CA, USA). Using these DNA samples and SWNTs, we prepared the ssDNA- and dsDNA-SWNT hybrids as follows. DNA (0.5 mg) dissolved in tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid (TE)-buffer (1.0 mL, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with SWNT powder (0.5 mg) [26]. The mixture was sonicated (VCX 130, Sonics & Materials, Inc., Newtown, CT, USA) on ice for 90 min at 3 W using a probe-type sonicator equipped with a tip of 2 mm in diameter. The sonicated mixture was diluted 100-fold, and then sonicated again for 30 min using a bath-type ultrasonic cleaner (LEO-80, Steady Ultrasonic Sdn. Bhd., Selangor, Malaysia) to obtain individually dispersed DNA-SWNTs. The twice-sonicated sample was then centrifuged at 18500 × *g* for 6 h (himac CR15D, Hitachi Koki Co., Ltd., Tokyo, Japan), after which 0.8 mL of the supernatant was collected. The supernatant was diluted three-fold to optimize the subsequent AFM observations. The diluted supernatant (10 μL) was deposited onto a mica substrate that had been treated with 3-aminopropyltriethoxysilane (APTES) [26]. The sample was incubated for 10 min, rinsed with ultrapure water, and finally dried in a desiccator. For the preparation of APTES-modified mica, freshly cleaved mica substrates were immersed in a 0.01% aqueous solution for 1 h, and then rinsed with water and ethanol. Then, the substrate was annealed at 90 °C for 1 h followed by rinsing with water and ethanol. Finally, the treated mica substrates were dried at 60 °C for 30 min.

2.2. Preparation of the SWNT dispersion without DNA

SWNT powder (0.3 mg) was added to 10 mL of 1,2-dichloroethane (DCE), and then the mixture was sonicated for 90 min in the LEO-80 ultrasonic bath. Then, 1.0-mL aliquots of sonicated dispersion were centrifuged at 17360 × *g* for 6 h (MX-150, Tomy Seiko Co. Ltd., Tokyo, Japan), after which 0.5 mL of the supernatant was collected. The supernatant (3 μL) was deposited onto the APTES-modified mica, and then dried in air. Subsequently, 20 μL of TE buffer was dropped onto the sample. The sample was incubated for 10 min, rinsed three times with 1 mL of ultrapure water, and finally dried in a desiccator.

2.3. AFM for measuring height distribution of multiple samples

A JSPM-5200 Scanning Probe Microscope (JEOL Ltd., Tokyo, Japan) was employed to obtain the height distributions of ssDNA-SWNT and dsDNA-SWNT hybrids in air and in solution in an AC-AFM mode with a Si cantilever (RTESP, typical spring constant ≈ 40 N/m, Veeco Instruments Inc., Plainview, NY, USA) under nitrogen-injected air and with a Si_3N_4 cantilever (HWMS-06AU, typical spring constant ≈ 0.5 N/m, Park Scientific Instruments, Sunnyvale, CA, USA) in the presence of TE buffer. The height distribution of the hybrids was established from cross-sections of AFM images. One hundred objects were randomly measured. The Z-scale of the AFM was calibrated with colloidal gold particles (G1402 Gold colloid 5 nm, Sigma–Aldrich). The colloidal gold suspension was appropriately diluted with water, and then an aliquot of the suspension was dropped on an APTES-mica surface. The sample was rinsed with water, and dried.

2.4. AFM with the three different force conditions

An MFP-3D-SA (Asylum Research, Goleta, CA, USA) with amplitude modulation (AM)-AFM with three modes was used for direct comparison of the same molecules in air and in TE buffer. We defined the three types of imaging methods by "target percent", "free amplitude" values and environmental conditions. First, a sample was observed with low free amplitude (0.2 to 0.3 V) in air. The target percent values were set to +15%. "+" means the drive frequency is larger (right-shifted) than its resonance frequency. "15%" means the drive frequency is set to the value which have oscillation amplitude decreased by 15% against the amplitude at resonance frequency in the resonance curve of cantilever. Second, the same sample was observed with high free amplitude (1.9 to 2.0 V) in air. Target percent was −5%. "-" means the drive frequency is smaller (left-shifted) than its resonance frequency. Finally, the same sample was observed with low free amplitude (0.2 to 0.3 V) in the TE buffer solution. Target percent was −5%. Following names were used in this text to explain the three AFM modes: low amplitude and plus (+, right-shifted) mode (LP mode) in air, high amplitude and minus (-, left-shifted) mode (HM mode) in air, and low amplitude and minus (-, left-shifted) mode (LM mode) in liquid. A Si cantilever with a typical spring constant ≈2 N/m (OMCL-AC240TS-R3, Olympus Corporation, Tokyo, Japan) and a Si_3N_4 cantilever with a typical spring constant ≈ 0.1 N/m (BL-AC40TS-C2, Olympus Corporation) were used for the AFM observations in air and TE buffer, respectively. For the observations of the same area in air and TE buffer, the undersides of sample substrates were scratched in a cross shape to make a landmark using sharp tweezers before being fixed on the AFM sample holder. The areas were identified by rough adjustment based on the cross-shaped scratch and following wide-ranging AFM imaging. In a series of AFM observations, the samples were firstly observed in air. Subsequently, the cantilever oscillation was re-tuned, and then the same areas observed in air were imaged in TE buffer. After the AFM observations performed in air, 100 μL of TE buffer was dropped onto the sample substrate, and then the AFM in solution was conducted on the same areas as AFM in air.

3. Results and Discussion

In the initial experiments, we observed hundreds of DNA-SWNT hybrids in the air and TE buffer solution to statistically compare the diameters of the hybrids. Fig. 1 shows AFM images of ssDNA-SWNT and dsDNA-SWNT hybrids in TE buffer solution. To our knowledge, this is the first demonstration of AFM of DNA-SWNTs in solution. In both cases, rod-like features were clearly observable. Numerous DNA-SWNTs displayed homogeneous heights, while some displayed non-uniform heights. This non-uniformity might have been caused by the aggregation of DNA molecules on the SWNTs or the bundling of the DNA-SWNTs. AFM images of DNA-SWNT hybrids obtained in air are shown in Supplementary Fig. SP1 in the online version at DOI: 10.1016/j.colsurfb.2016.03.068. There were no significant differences in morphology between the images obtained in air and in solution, or between ssDNA and dsDNA.

Fig. 2 shows histograms of height distributions of the DNA-SWNTs obtained from cross-section analysis of AFM images (n = 100). In the height analysis, we excluded visibly non-uniform DNA-SWNTs from the measurements because it was difficult to determine the location at which such DNA-SWNTs should be measured. The height distributions in wet conditions were apparently right-shifted and broadened compared to those in dry conditions. The average diameters of the ssDNA-SWNTs were 0.94 ± 0.19 nm