Direct Measurements of Interactions between Polypeptides and Carbon Nanotubes

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The interactions of various polypeptides with individual carbon nanotubes (CNTs), both multiwall (MW) and single wall (SW), were investigated by atomic force microscopy (AFM). While adhesion forces arising from electrostatic attraction interactions between the protonated amine groups of polylysine and carboxylic groups on the acid-oxidized multi-wall carbon nanotubes (Ox-MWCNTs) dominate the interaction at a low pH, weaker adhesion forces via the hydrogen bonding between the neutral $-NH_2$ groups of polylysine and $-COO^-$ groups of the Ox-MWCNTs were detected at a high pH. The adhesion force was further found to increase with the oxidation time for Ox-MWCNTs and to be negligible for oxidized single-wall carbon nanotubes (Ox-SWCNTs) because carboxylate groups were only attached onto the nanotube tips in the latter whereas onto both the nanotube tips and sidewall in the former. Furthermore, it was demonstrated that proteins containing aromatic moieties, such as polytryptophan, showed a stronger adhesion force with Ox-MWCNTs than that of polylysine because of the additional $\pi-\pi$ stacking interaction between the polytryptophan chains and CNTs.

Introduction

Due to their unique electronic, physical, and chemical properties, carbon nanotubes (CNTs) are attractive for many potential applications, including molecular electronics and sensors.[1] With the rapid development in nanoscience and nanotechnology, large-scale production of carbon nanotubes has recently attracted a great deal of attention and the environmental impact of CNTs, especially on humans and other biological systems, is critical to ascertain.[1] Although CNTs appear to be nontoxic to cells at a low level, they may cause a health hazard at higher levels of exposure.[2] A few investigations on the biological effects of CNTs have been recently reported. For instance, certain peptide-functionalized CNTs have been introduced into a cell nucleus through the mammalian plasma membrane[3] to elicit an antibody response in vivo.[4] CNT-mediated delivery of a plasmid DNA was also demonstrated to cause a much stronger gene expression than that of naked DNA alone.[5,6] Therefore, CNTs could be promising new materials for use in future gene therapy and drug delivery systems. Consequently, it is indispensable to study the interactions between CNTs and the constituent components of cells, especially proteins. Although some amphiphilic peptides have been used to assist CNT dispersion by wrapping CNTs with their nonpolar surfaces and having their polar surfaces dangling into the aqueous medium,[7,8] no direct measurement has been made for the interaction between any peptide and CNTs as far as we are aware. Here, we report the first measurement of interactions between various polypeptides (e.g. polylysine, polytryptophan) and carbon nanotubes (both pristine and oxidized nanotubes).

Experimental Section

Materials. Buffer solutions of pH = 3.0, 5.0, and 7.0 were prepared by mixing sodium hydroxide and phosphorous acid (H_3PO_4). Buffers of pH = 10 and 12 were made from NH_4Cl and ammonium hydroxide (NH_3H_2O, 28.0% (w/w)). An aqueous solution of pH = 1 was prepared by dilution of HCl in water. All buffer solutions were diluted to give a final ionic strength of 0.01 M. Muscovite mica sheets were purchased from Electro Microscope and freshly cleaved prior to use. Multiwall CNTs (MWCNTs) were prepared by prolysis of FePc according to the published procedure,[9] whereas single-wall CNTs (SWCNTs) were purchased from CNI@Rice. SWCNTs grown on dispersed catalyst particles on a silicon substrate were produced by the published method.[10]

CNT Sample Preparation. Oxidation of CNTs was carried out by adding a predetermined amount of pristine CNTs into a concentrated acid solution of H_2SO_4:HNO_3 = 3:1 under sonication for 3 h unless otherwise stated,[12] followed by purification with Milli-Q water through filtration. The resultant carboxylated CNTs were then dissolved into Milli-Q water for subsequent use. For AFM measurements, an aqueous solution of the sonication-dispersed pristine CNTs was directly deposited on a freshly cleaved mica, while the carboxylated CNTs were deposited onto an APS ((3-aminopropyl)triethoxysilane, Sigma)-modified mica surface with the positively charged APS serving as a link reagent between the mica surface and negatively charged CNTs. In the latter case, interactions between the atomic force microscopy (AFM) tip and the top-half nanotube surface covered with carboxylic groups were measured, while the COOH groups on the bottom half of the nanotube surface interacted with the APS surface. The total amount of COOH groups for a particular oxidized carbon nanotube depends on the type of nanotube (e.g. MWCNT, SWCNT) and the oxidation degree.

Modification of AFM Tips. To attach polypeptide chains to AFM probes for force measurements, silicon nitride AFM probes (spring constant = 0.06 N/m, Veeco Co.) were immersed in an aqueous solution of polylysine (MW = 55 800, Sigma)

and a solution of polytryptophan (MW = 3900, Sigma) in dimethylformamide (DMF), respectively, at 1 mg/mL for 30 s at 20 °C. We controlled the amount of polypeptides attached onto the AFM tips by controlling the solution concentration and adsorption time, followed by thoroughly washing with pure water. Although the amount of polypeptide attached on an AFM tip is not necessarily the same for each peptide, this simple procedure allowed us to deposit a fairly constant amount of a particular polypeptide onto different AFM tips, as indicated by reproducible adhesion forces from independent measurements. The gold coatings of the AFM tips were performed on a commercially available sputter-coater (Denton Vacuum LLC.) for grafting thiol-end-functionalized peptides. Force measurements with the freshly modified AFM tips were performed on a Topometrix Explorer AFM with an opened liquid cell at 20 °C. AFM images were performed on a Dimension 3100 Nanoscope IIIa controller system. The interactions between the polypeptide and an individual CNT were measured by point focusing the polypeptide-modified AFM tip onto the nanotube surface after imaging. The consistency for all of the calculated force data reported in this paper was checked by at least five independent measurements, and their average value was used.

Results and Discussion

Interactions between Polylysine and Carbon Nanotubes. The interactions between an AFM tip and pristine CNTs were investigated via force−distance curves. The attractive force in the retracted part of the force−distance curves reflects the adhesion between the AFM tip and sample. As a control, we measured the interaction between an unmodified AFM tip and the pristine MWCNTs in a phosphate-buffered saline (PBS) buffer. No obvious attractive force was observed in this particular case. The interactions between the polylysine-modified tips and pristine CNTs in buffer solutions were also investigated. In this case, we first carried out the force−distance measurements with various indention distances to check if polypeptide chains transfer from the AFM tip to CNTs when the tip was strongly or repeatedly pressed against the nanotube surface. The measured adhesion force, if any, remained unchanged and was almost zero with different indention distances, indicating no transfer of polypeptide chains between the AFM tip and nanotube had occurred due to the strong multisite adsorption of individual polypeptide chains onto the AFM tip.[1]

Adhesion between Polylysine-Modified AFM Tips and Carboxylated MWCNTs at Different pH Values. Having established the interaction between the polylysine-modifed AFM tip and pristine CNTs, we proceeded to investigate the adhesion between carboxylated CNTs and polylysine-modified tips at different pH values. As shown in Figure 1, there was almost no detectable interaction between the polylysine-modified tips and pristine CNTs within the entire pH range of 1−12. When pH increases from 1 to 12, the adhesion between polylysine-modified tips and APS mica increases from about 0.1 to 1.7 nN. However, adhesions between the polylysine-modified tips and carboxylated nanotubes decrease from about 1.1 nm to 0.4 nN with increasing pH.

The variation of adhesion as a function of pH may result from protonation/deprotonation of −COOH groups on the oxidized CNTs and the −NH$_2$ groups of polylysine and APS. At low pH values (Figure 2a), the protonated −NH$_3^+$ groups of polylysine show a relatively strong adhesive force with the −COOH (−COO$^-$ + H$^+$) groups on the oxidized carbon nanotubes, while the repulsive electrostatic force between the
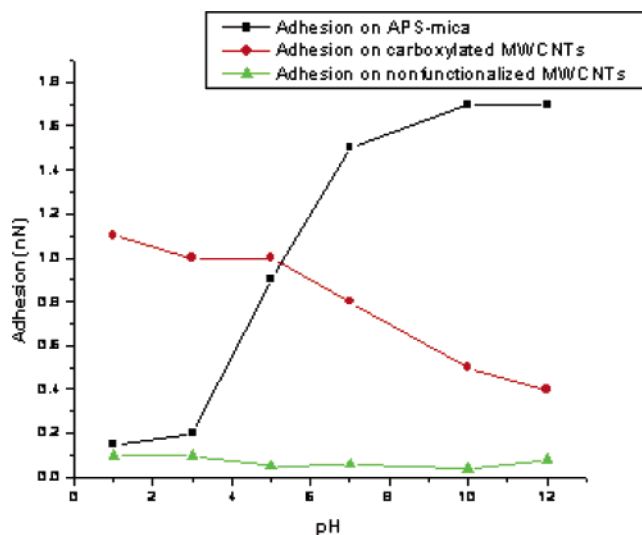

Figure 1. Adhesion forces of polylysine with APS-modified mica, carboxylated MWCNTs adsorbed on the APS-modified mica, and the pristine MWCNTs adsorbed on a freshly cleaved mica measured as a function of pH values.
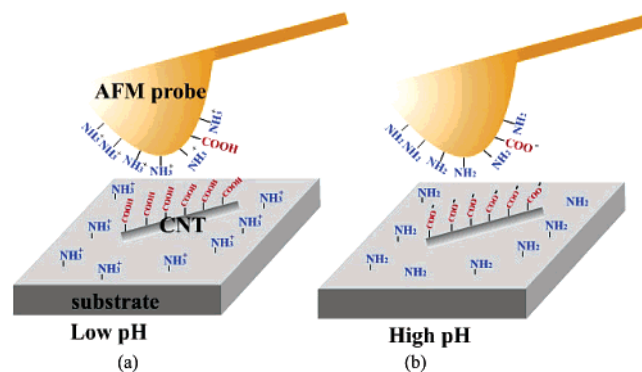

Figure 2. Schematic representation of interactions between polylysine and carboxylated CNTs at different pH values.

protonated −NH$_3^+$ groups of polylysine and the APS mica surface effectively minimize the adhesive force between the polylysine-modified AFM tip and the APS mica surface. At high-pH values (Figure 2b), however, the −NH$_3^+$ and −COOH groups are deprotonated into −NH$_2$ and −COONH$_4$ (−COO$^-$ + Na$^+$; with the Na$^+$ from the buffer solution) moieties to significantly reduce the adhesion force between them. In contrast, adhesion forces between the polylysine-modified AFM tip and the APS mica surface dramatically increase with increasing pH due to the hydrogen bonding between the oxygen moieties of the polylysine-modified AFM tip and neutralized −NH$_2$ groups on the APS-modified mica surface. These observations are consistent with the previously reported results from the measurements on pH-dependent adhesions between functionalized AFM tips and self-assembled monolayers (SAMs) with terminal amino/carboxylic groups.[11] Because of the lower density of functional groups on oxidized CNTs than that of SAMs, relatively weaker adhesion forces were observed in the present study with respect to the previously reported results.[11]

Adhesion between Polylysine-Modified AFM Tips and Carboxylated MWCNTs as a Function of Oxidation Times. The degree of carboxylation for the oxidized CNTs depends on the oxidation time.[12] The longer the oxidation time, the higher degree of carboxylation for the CNTs. Parts a, c, and e of Figure 3 clearly show a significant increase in the O 1s X-ray photoelectron spectroscopy (XPS) peak intensity over 531.6−
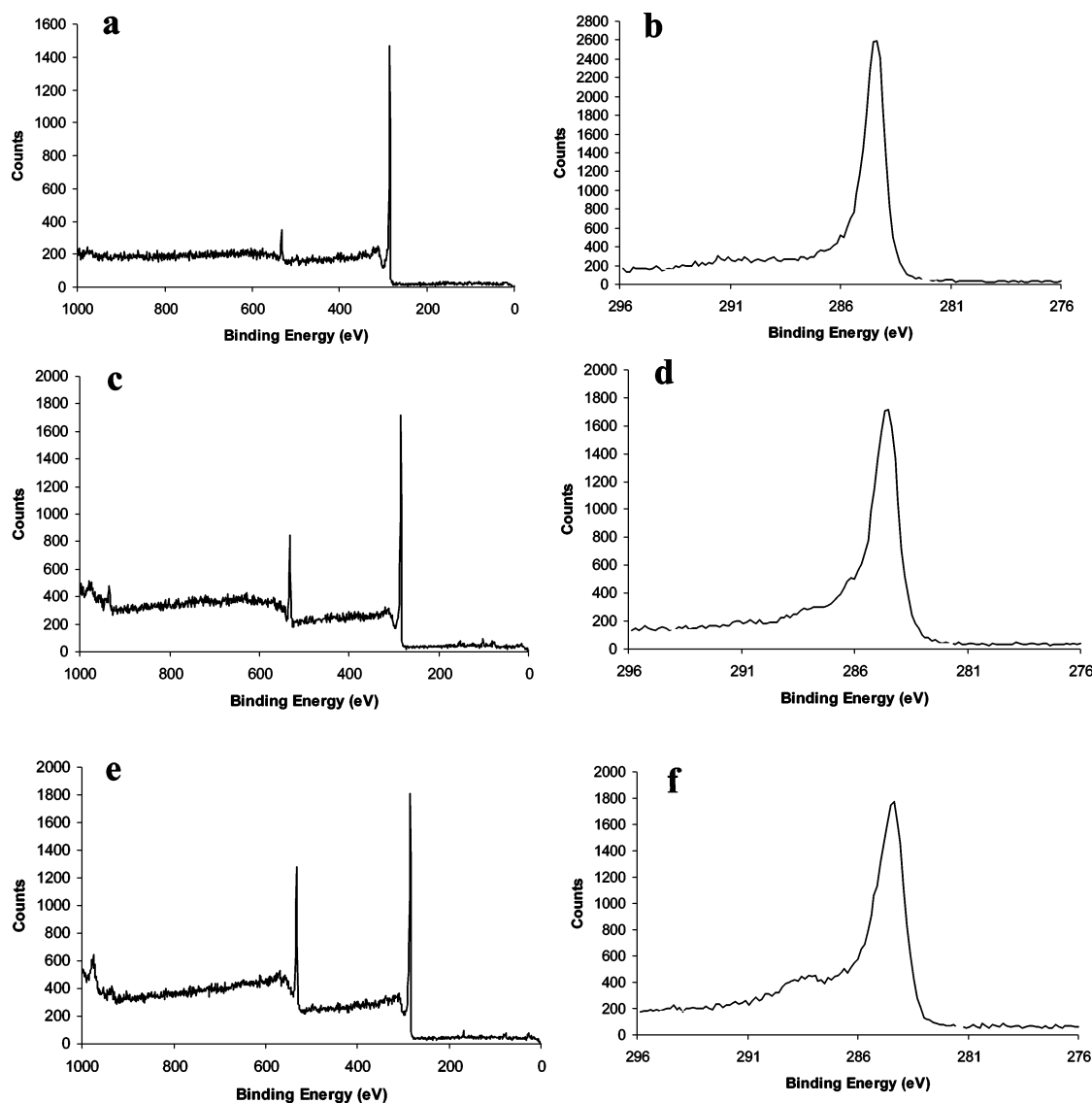
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Figure 3. XPS spectra of carboxylated MWCNTs as a function of oxidation time. Oxidation times are 1 (a, b), 3 (c, d), and 6 h (e, f), respectively. Panels a, c, and e are the survey spectra of the functionalized MWCNTs, and b, d, and f are the corresponding high-resolution C 1s spectra.

533.8 eV [13] with increasing acid-oxidation time. The corresponding XPS C 1s spectra in Figure 3b,d,f show a continuous increase of the peak intensity for −COOH groups in the range of 286.5−291.6 eV.[13]

Figure 4 shows AFM images of the oxidized MWCNTs (Ox-MWCNTs) of different oxidation degrees, along with the corresponding force−distance profiles for individual CNTs measured with a polylysine-modified AFM tip.

Figure 4A(a) shows that the slightly oxidized MWCNTs still exist as large bundles after being oxidized for about 1 h, leading to adhesion forces that are difficult to detect (Figure 4B(a)). After about 3 h of oxidation, however, the ∼1.5 μm long Ox-MWCNTs disperse well on the substrate (Figure 4A(b)) with an increased adhesion (1.2 nN) against the polylysine-modified AFM tip (Figure 4B(b)). After oxidation for ∼6 h, carbon nanotubes are "cut" into short fragments that are ∼0.5 μm long (Figure 4A(c)) with an increase of adhesion force from 1.2 to 2.1 nN (Figure 4B(c)). Figure 4C clearly shows a continuous increase in the adhesion force with oxidation time.

Adhesion between Oxidized MWCNTs and Polytryptophan. Polytryptophan represents a class of polypeptides with aromatic structures.[14] Therefore, it should be interesting to investigate adhesion between polytryptophan and CNTs as

recent publications have already established that nanotubes are viable substrates for noncovalently anchoring aromatic molecules.[15] As expected, the adhesion forces between the polytryptophan-modified AFM tips and Ox-MWCNTs are significantly stronger than those between polylysine and Ox-MWCNTs under the same conditions due to $\pi-\pi$ stacking interactions in addition to hydrogen bonding between the amine on polytryptophan and the carboxylate groups, though it is not necessary for each residue of the polypeptide chain doing both.

Adhesions between Oxidized SWCNTs and Polylysine or Polytryptophan. Adhesion forces were also measured for oxidized SWCNTs (Ox-SWCNTs). There is almost no detectable adhesion force between the polylysine-modified AFM tip and SWCNTs before and after oxidation. The difference in the adhesion forces between the Ox-SWCNTs and Ox-MWCNTs can be attributed to the fact that SWCNTs can only be oxidized at ends, whereas MWCNTs can be carboxylated not only at ends but also at the side walls.

Adhesion measurements were also performed on carboxylated/non-carboxylated CNTs by polytryptophan-modified AFM probes. It turns out to be 3.5−4.5 nN for both carboxylated/non-carboxylated SWCNTs and MWCNTs, suggesting a dominant role of the $\pi-\pi$ interaction especially at a low
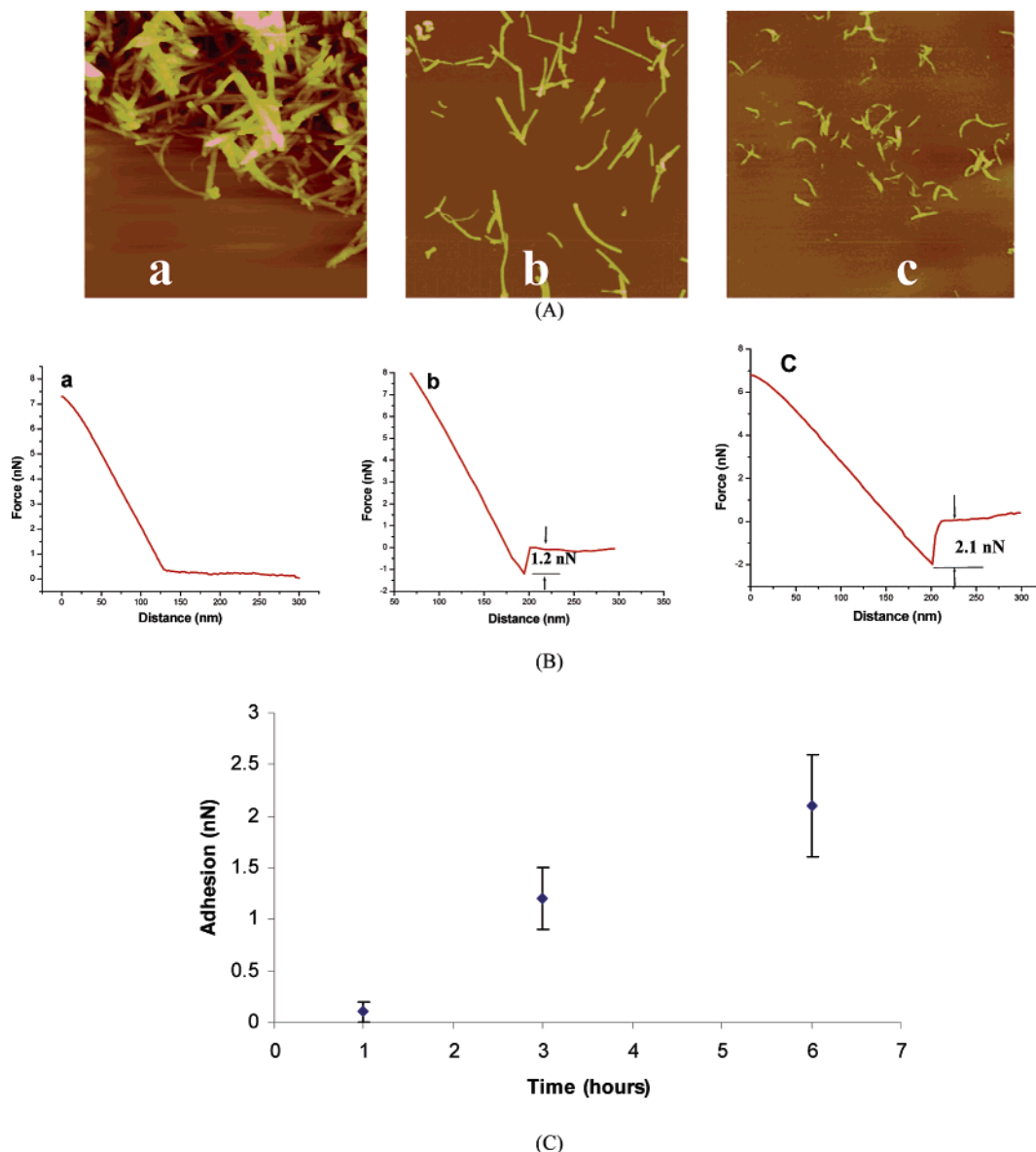
Figure 4. (A) AFM images of carboxylated MWCNTs of different oxidation times on APS mica. Oxidation for (a) 1, (b) 3, and (c) 6 h. Scan area: 5 μm × 5 μm. (B) Force–distance curves measured in a pH 5.0 buffer solution for a polylysine-modified AFM tip against carboxylated MWCNTs oxidized for (a) 1, (b) 3, and (c) 6 h. (C) Adhesion force vs the oxidation time.

carboxylic content. As reported previously,[16] bio-panning with phage display libraries identified that peptides with a high aromatic content bind specifically to SWCNTs produced by the HiPco method. The focus peptide of that study was modified with four glycines and one cysteine residue at the C-terminus (HSSYWYAFNNKTGGGGC). This peptide was grafted onto gold-coated AFM probes through thiol–gold linkages to form a self-assembled monolayer (SAM). Force measurements were carried out between the peptides and SWCNTs grown on dispersed catalyst particles on a silicon substrate, as shown in Figure 5.[10]

The adhesion is around 1.8–3.0 nN, a little smaller than that for polytryptophan. Additionally, if the SAM is densely packed on the surface, interactions between the peptide and SWCNTs could be frustrated as the peptides may be sterically restricted from adopting the best conformation to interact with the surface of the nanotube. For carboxylated SWCNTs, the measured adhesions are quite similar to pure SWCNTs. These results indicate, once again, the carboxylation degree for SWCNTs is very low due to the end oxidation only.
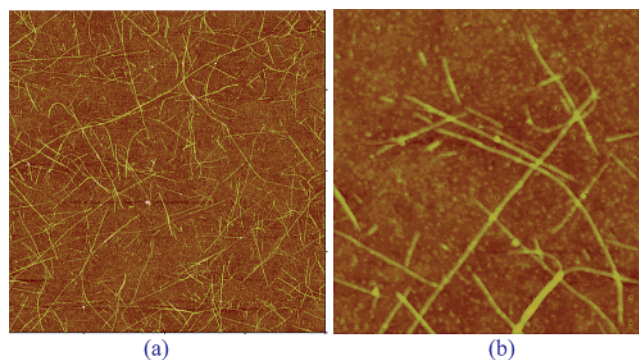

Figure 5. AFM image of SWCNTs grown on the dispersed catalyst particles. Scan area: (a) 5 μm × 5 μm and (b) 0.5 μm × 0.5 μm.

Conclusions

In summary, we have used atomic force microscopy to investigate the interactions of various polypeptides with individual carbon nanotubes. This approach enabled us to demonstrate the effects of pH and the oxidation degree on adhesion

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forces between different polypeptides (e.g. polylysine, poly-tryptophan) and carbon nanotubes. It was found that the adhesion force between polylysine and acid-oxidized MWCNTs increased with decreasing pH value and increasing oxidation time. The correspondence pH and oxidation-time dependences for SWCNTs were found to be much weaker than those of MWCNTs as carboxylate groups were only attached onto the nanotube tips in the former, whereas onto both the nanotube tips and sidewall in the latter. Peptides containing aromatic moieties, including polytryptophan, showed a stronger, non-nanotube-specific adhesion force than that of polylysine because of a predominant role of the $\pi-\pi$ stacking interaction between those aromatic moieties in the peptide chains and CNTs. These results should have important implications for understanding and designing CNT surfaces of biological significance.

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