

Covalently-linked Adducts of Single-walled Nanotubes with Biomolecules: Synthesis, Hybridization, and Biologically-Directed Surface Assembly

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ABSTRACT

Covalently-linked adducts of single-walled carbon nanotubes (SWNTs) with biomolecules have been fabricated. Results are presented here for DNA-SWNT and for biotin-SWNT adducts. DNA-SWNT adducts are shown to be biochemically accessible and to exhibit high selectivity, favoring hybridization with complementary vs. non-complementary DNA sequences. Biotin-SWNT adducts were also prepared and used to direct the assembly of nanotubes onto a biotinylated glass surface.

INTRODUCTION

Covalently-linked adducts of nanotubes with biomolecules have great potential in biosensing and as a possible means of implementing nanoscale assembly, using the selectivity of biomolecular interactions to control assembly of nanometer-sized objects [1,2] Previous studies have focused primarily on the use of non-covalent interaction.[3-6] However, the use of covalently-linked adducts has a number of potential advantages. For example, covalent attachment disrupts the CNTs structure only at the attachment site, whereas noncovalent functionalization typically involves coating the nanotube with various large molecules/polymers. Indeed, a recent report showed that oxidation of “defect-free” HiPCO nanotubes (Carbon Nanotechnologies, Inc.) retained the van Hove features, thereby indicating that the electronic properties are relatively unperturbed by formation of oxidized surface sites. Additionally, covalently-linked adducts are expected to be more thermally and chemically stable. While DNA hybridization involves a large number of very weak interactions (hydrogen bonds), the interaction of biotin with avidin has one of the largest known binding constants of 10^{15} Molar⁻¹. This large binding constant makes the biotin-avidin interaction potentially useful for the fabrication of robust nanoscale structures.

Here, we report recent results aimed at preparing and characterizing covalently-linked adducts of single-walled carbon nanotubes with biomolecules.[7,8] We show that covalently-linked adducts of DNA with single-walled nanotubes retain the high accessibility and selectivity associated with DNA hybridization.[7] We also extend these methods to prepare biotin-modified nanotubes,[8] and we show that the biotin-avidin interaction can also be used to as a means of assembling nanotubes onto selected locations on a surface.

EXPERIMENTAL

Experiments were performed using two different sources of single-walled carbon nanotubes. Single-wall nanotubes (Carboxlex, Lexington, Ky) were first purified by refluxing the as-received nanotubes in 3 M nitric acid for 24 hours and then washing the SWNTs with water using a 0.6 micron polycarbonate membrane filter (Millipore). HipCO Tubes were also prepared by oxidation in 9:1 H_2SO_4 :30% H_2O_2 solution.[9] To functionalize the nanotubes with amine groups, the purified, oxidized material (~60% of initial weight of SWNTs) was dried under vacuum and then suspended in 1 ml of anhydrous dimethylformamide (DMF) in an ultrasonic bath. This dispersion was immediately added to 20 ml thionyl chloride (Aldrich) and heated under reflux for 24 hours to convert the carboxylic acids to acyl chlorides. These nanotubes were rinsed over a 0.2 micron PTFE membrane (Millipore) with anhydrous THF to remove excess SOCl_2 , and were then added to ethylene diamine (neat, Aldrich) and stirred for 3-5 days in order to form the amine-terminated product depicted in Fig. 1c.

The amine-terminated nanotubes (Fig. 1c) provide a versatile starting point for further modification. To prepared DNA-modified SWNT's, the tubes are reacted with the heterobifunctional cross-linker succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, (SMCC), leaving the surface terminated with maleimide groups (Fig. 1d) which can then be reacted with thiol-terminated DNA to produce DNA-modified SWNT's (Fig. 1e). Alternative, the amine-terminated SWNT's can be reacted with N-hydroxy succinimidyl biotin (Vector Labs), producing SWNT's covalently linked to biotin as depicted in Fig. 1f.

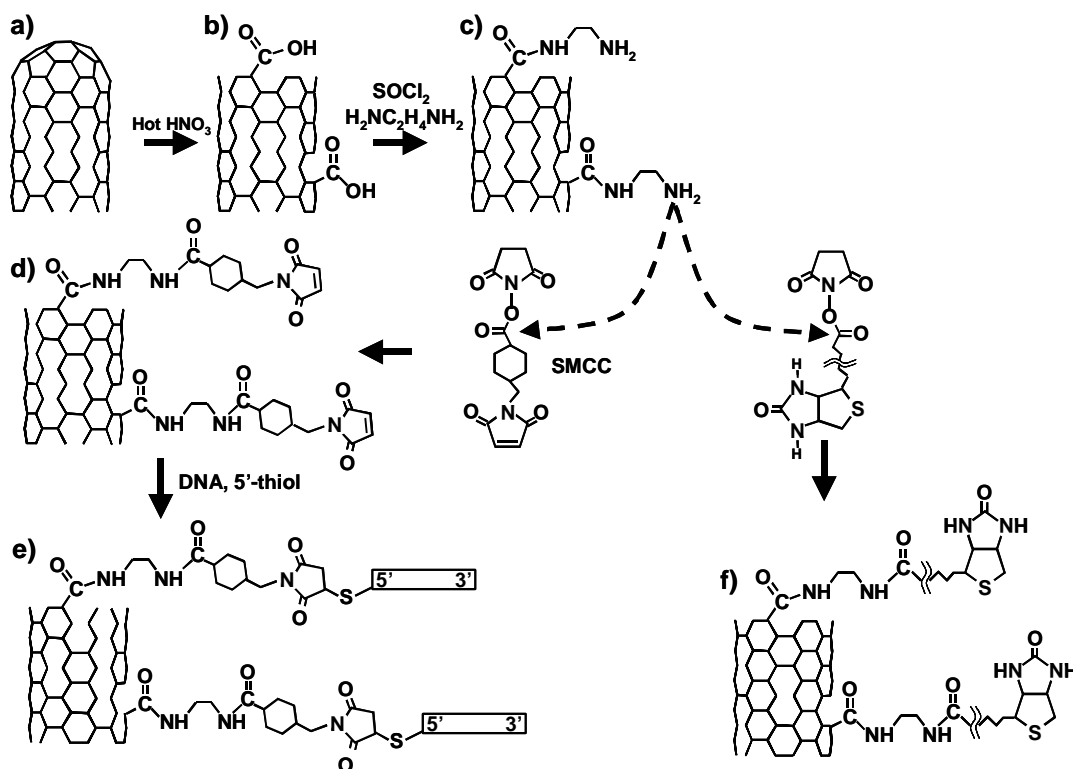


Figure 1: Schematic illustration of chemical scheme for producing covalently-modified adducts of SWNT's with DNA (1e) and with biotin (1f).

RESULTS AND DISCUSSION

DNA-modified SWNTs:

Several different DNA oligonucleotides were used in these experiments. To optimize the DNA-SWNT linkage chemistry, a 32-base oligonucleotide (5'-HS-C₆H₁₂-T₁₅GC TTA ACG AGC AAT CGT FAM-3') ("S1") was used. This oligonucleotide was modified at the 5' end using the reagent 5'-thiol modifier C6 (Glen Research) to give a thiol group for attachment to the maleimide group on the nanotubes (Fig. 1d), and was modified at the 3' end using 6-FAM amidite (Applied Biosystems) to attach a fluorescein group.

Tests to verify the formation and stability of the covalent linkage between the nanotubes and the DNA were performed by directly linking DNA molecules with a fluorescent tag. These tests showed that the DNA-SWNT adducts are quite stable even in the presence of hot surfactant-containing solutions that would normally denature physically-adsorbed molecules. This, together with detailed chemical information presented elsewhere,^[7] establishes that the DNA molecules are indeed covalently linked to the SWNTs.

Since the above experiment proved that the DNA-SWNT adducts are stable, we conducted further experiments to test whether the DNA molecules that are tethered to the SWNTs remain biochemically accessible to hybridization, and whether the attachment to the nanotubes significantly impacts the selectivity for hybridization with complementary vs. non-complementary sequences. For these experiments, we linked DNA without a fluorescent tag to the nanotubes, and then investigated the hybridization of these DNA-SWNT adducts with fluorescently-tagged complementary and non-complementary sequences of DNA in solution. These experiments were conducted using the oligonucleotide "S2", with the sequence (5'-HS-C₆H₁₂-T₁₅GC TTA ACG AGC AAT CG -3'), linked to the nanotubes. After immobilization onto the SWNTs following the procedures above, the resulting DNA-nanotube adduct was then portioned into two aliquots, and each was immersed in a 5 micromolar solution of DNA oligonucleotides that were labeled at the 5' end with fluorescein. The first sequence, "S3", (5'- FAM- CG ATT GCT CGT TAA GC -3'), has sixteen bases complementary to S2. The second sequence, "S4", consists of the 16-base sequence (5'-FAM- CG TTT GCA CGT TTA CC -3') that has four-base mismatch to S2. Each sample was hybridized for 2 hours at 37 °C with shaking, washed using a 0.2 micron polycarbonate membrane with SDS/2xSSPE buffer, and then placed in a 96 well microtiter plate in buffer. Figure 2 shows the resulting fluorescence image of this experiment. The top row shows the fluorescence images (black=high intensity) for hybridization of S2-SWNT with its complement, S3 (left) and with the 4-base mismatch, S4 (middle). The image at right shows the background from an empty titerplate well. Measurement of the fluorescence intensity within each well yields a median value of 1287 I.U. for the perfect match (left), 680 I.U. for the mismatch (middle) and 427 I.U. for the background. Since there is a much higher intensity from the perfect-matched pair (S2-SWNT + S3) than the mismatched pair (S2-SWNT + S4), we conclude that hybridization of the DNA-SWNT adducts with solution-phase oligonucleotides is highly specific.

The reversibility of hybridization was tested by denaturing with 8.3 M urea solution, and then re-hybridizing to a different sequence. After denaturing, the

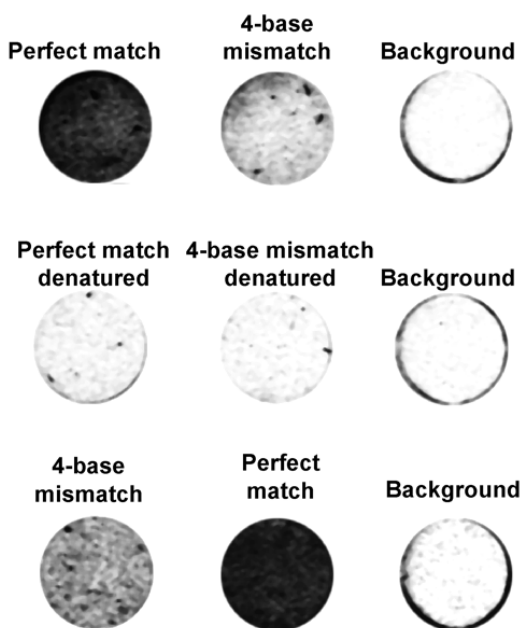


Figure 2 Fluorescence images (black=high intensity) of DNA-SWNT adducts that were hybridized with complementary and 4-base mismatched sequences, as described in text. The top row shows the initial hybridization. The second row shows the same samples after denaturing in urea, and the bottom row shows the same samples after hybridizing a second time with a different sequence, as described in text.

fluorescence images (Fig. 2, middle row) show only low levels of fluorescence from the two samples (intensity = 304 I.U. from perfect match, 267 I.U. from 4-base mismatch) comparable to the background level (intensity = 238 I.U.). These denatured samples were then hybridized a second time. In this second hybridization, the sample that was previously hybridized with a perfect match was now hybridized with a mismatched sequence, and vice versa. The images in the bottom row of Fig. 2 show that again, the fluorescence intensity of the 4-base mismatched pair S2-SWNT + S4 (bottom left, intensity=441 I.U.) is close to that of the background (bottom right, 257 I.U.), while the relative intensity of the perfect mach S2-SWNT + S3 (bottom middle, intensity = 1073 I.U.) is much higher than either. Again, the hybridization appears to be quite specific.

The above results strongly point to the successful synthesis of covalently-linked DNA-SWNT adducts. These experiments show that the DNA-SWNT adducts are biochemically accessible and exhibit a high degree of selectivity in hybridization experiments. This high degree of selectivity can be potentially useful in a number of applications, such as as fabrication of nanoscale chemical sensors and in the use of biological molecules to direct the assembly of nanotubes and other nanoscale objects.

Biotin-modified SWNTs and surface assembly:

While DNA hybridization involves weak interactions, the interaction between biotin (a small vitamin) and avidin (a small protein) is one of the strongest biomolecular

interactions known, with a formation constant of 10^{15} M^{-1} . This very high stability implies that the biotin-avidin interaction can be used to assist in the assembly of nanoscale supramolecular architectures by making use of the fact that avidin has four sites that can bind to biotin molecules. We used the biotin-avidin interaction to selectively link biotin-modified SWNTs to biotin-modified surfaces, using avidin as a kind of glue to bind the assembly together. This experiment involves multiple steps, as shown schematically in Fig. 3.

Biotin-modified SWNTs were produced using chemistry very similar to that used for preparing DNA-modified nanotubes. The procedure involves fabrication of amine-terminated SWNTs and then reacting these with a small molecule containing a biotin group and an N-hydroxy succinimide group, which forms a covalent link to the amine groups to produce a covalently-linked SWNT-biotin adduct like that shown in Fig. 1f.

Because proteins such as avidin are often sensitive and easily subject to denaturation or other degradation processes, avidin was linked to the surfaces via a two-step procedure in which surfaces of silicon, glassy carbon, or glass were first modified to provide accessible primary amine groups. These amine-terminated surfaces were then reacted with a modified N-hydroxy-succinimide (NHS) ester of biotin, yielding the covalent biotin-SWNT adduct depicted in Fig. 1f. We investigated silicon, glassy carbon, and glass as substrate surfaces because they can all be modified via similar chemistry to amine groups as described previously,[10] while having significantly different optical and electrical properties. Data presented here was obtained on amine-terminated glass surfaces that were purchased commercially (Corning GAPS-II). The second step, linking biotin to the amine-terminated surfaces, can also be performed using several different reagents. Our experiments used Sulfo-Succinimidyl-6-(biotinamido) hexanoate from Pierce Endogen. However, a number of compounds are available commercially with NHS esters linked to biotin; these compounds differ slightly but would be expected to provide similar functionality. Details of this linkage have been eliminated from Fig. 1f to improve the clarity.

Figure 3 shows the procedure, along with the fluorescence data. Corning GAPS-II amine-terminated glass surfaces were modified with biotin. Avidin that was fluorescently labeled with rhodamine dye was then bonded to the surface, thereby producing an avidin-terminated surface that fluoresced in the red region of the spectrum. Carbon nanotubes were covalently linked to biotin as in Fig. 1f, and were simultaneously linked to the green fluorescent dye fluorescein using an NHS-ester of fluorescein from Molecular Probes. Covalently linking the nanotubes simultaneously to biotin and fluorescein provides a way of directly imaging the nanotubes via fluorescence in the green region of the spectrum. The avidin-modified glass surfaces were then briefly dipped into a dilute solution of nanotubes (modified with biotin and fluorescein, as described above) and then rinsed with a standard buffer solution.

Figure 3 shows the resulting images of fluorescence intensity, measured at two different wavelengths, along with a control experiment from an avidin-modified sample that was not exposed to nanotubes. In fig. 3, the “red” images show the fluorescence intensity obtained using a 605 nm long pass filter, representing fluorescence from the rhodamine-labeled avidin molecules covalently linked to the glass surface. The “green” images show the fluorescence intensity measured using a 512 nm band pass filter, which represents fluorescence from the fluorescein groups covalently linked to the nanotubes.

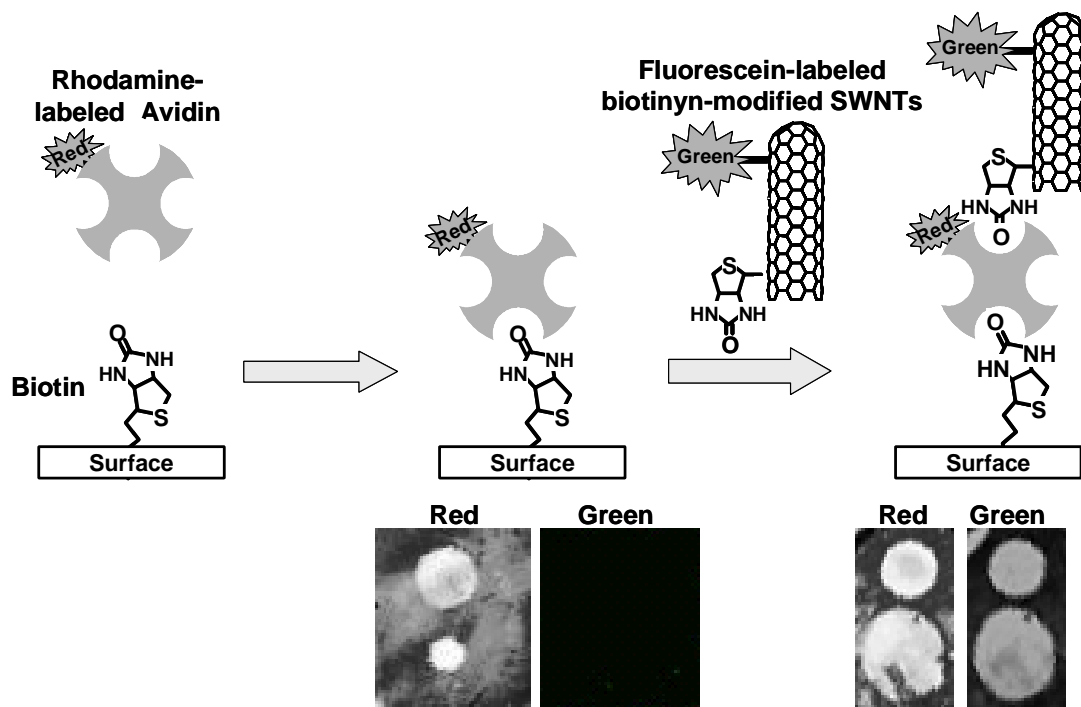


Figure 3: Biologically-directed assembly on SWNTs on a surface. The “red” and “green” images show fluorescence intensity using a 605-nm long-pass filter and a 512-nm bandpass filter, respectively. Two samples were used; one glass surface (center images) was modified only with biotin and rhodamine-labeled avidin, while the second (right images) was modified with to biotin, then rhodamine-labeled avidin, and then immersed in a solution of biotin-modified nanotubes that were also labeled with green fluorescein dye. Each sample was modified with biotin in two circular regions. The red and green images are obtained simultaneously for each sample.

A control experiment (center) shows that the avidin-modified surface fluoresces in the red, but no fluorescence is observed in the green if the avidin-modified surface before being exposed to the nanotubes. After being exposed to biotin, the fluorescence images at right show fluorescence both in the red (from the avidin) and in the green (from the nanotubes). It is important to note that the fluorescence from the rhodamine-labeled avidin and the fluorescein-labeled nanotubes is only observed in the surface regions that were modified with biotin (two spots). Other regions of the surface do not show significant fluorescence intensity.

These images therefore show that biotin-modified SWNTs will link specifically to surface regions that have been modified with avidin. This experiment establishes that it is possible to use the biotin-avidin interaction as a means of controlling the assembly of nanotubes onto a surface. The use of biomolecular interactions (such as protein-substrate interactions, antibody-antigen interactions, or DNA hybridization) between a surface-bound biomolecule and a biologically-modified nanotube is expected to be a general method that can be used to achieve biomolecularly-assisted assembly of nanotubes.

CONCLUSIONS

The integration of nanotubes with biological molecules provides a wealth of opportunities in nanoscale assembly, by using the highly selective nature of biochemical interactions to control the behavior of nanoscale objects. Our results show that it is possible to prepare covalently-linked adducts of single-walled nanotubes with DNA and with biotin. The use of DNA hybridization provides a potential pathway for controlling complex objects by taking advantage of the high degree of selectivity and reversibility, and the ability to readily design, synthesize, and link different DNA sequences to a variety of surfaces and nanoscale objects. The use of biotin and avidin provides complementary qualities, since the very high binding constant of avidin-biotin leads to nearly irreversible binding. Overall, the use of covalently-linked adducts of nanotubes with biomolecules to control nanoscale assembly and for sensing remains a very fruitful area of research.

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