Notes

Carbon Nanotubes as a Scaffold to Display Paired Sugars in Solution

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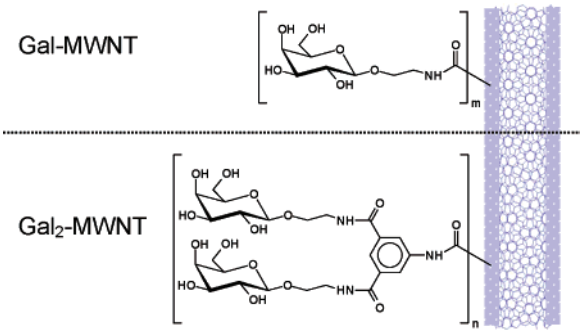
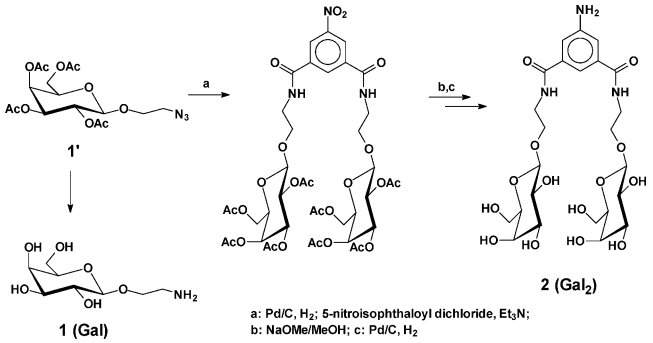
Single-walled (SWNT) and multiple-walled (MWNT) carbon nanotubes have been studied extensively for their interesting and often unique properties.[1] Recently, their potential biological and biomedical applications have been attracting increasing attention.[2] For example, Kam et al. found that protein-SWNT conjugates could be internalized in various types of mammalian cells,[3] and according to Hasegawa et al., schizophyllan (a natural β-1,3-glucan) could noncovalently wrap around SWNTs, with the pendant lactoside functional groups on schizophyllan still available for specific lectin recognition.[4] It has also been shown that the one-dimensional nanostructure of a nanotube could be used for displaying multiple copies of bioactive species for their specific interactions with bacterial cells,[5,6] such as the use of the bovine serum albumin-functionalized SWNTs in conjugation with *Escherichia coli*-specific antibody for capturing the pathogen in physiological solution.[5] In another example, Gu et al. solubilized SWNTs via covalent functionalization with the derivatized galactose 2′-aminoethyl-β-D-galactopyranoside (**1**) in likely the amidation of nanotube-bound carboxylic acids.[6] These galactose-functionalized nanotubes (Gal-SWNT), each displaying multiple copies of the sugar, were found to have adhesion to *E. coli* O157:H7 to result in significant cell agglutination.[6] It is also desirable to use MWNTs for the same purpose, not only for their more economical production and generally better purity but also for their unique properties and related opportunities, such as their larger inner tubular cavities allowing easier access and encapsulation of various species. However, the functionalization of MWNTs with **1** (Scheme 1) resulted in the Gal-MWNT sample of poor aqueous solubility, hardly useful to the cell adhesion and other biological applications in solution. An obvious difference between MWNT and SWNT is the former being heavier, thus requiring larger and/or more extended functional groups in the solubilization. In fact, the necessary aqueous solubility could be achieved by functionalizing MWNTs with the compound containing a galactose pair, β-aminophthaloyl-*N*,*N*′-bis[11-*O*-(β-D-galactopyranosyl)-ethyl]-diamide (**2**, Scheme 1).

The synthesis of **1** was already reported in the literature.[7] Its acetyl-protected precursor **1′** was used as starting material for the synthesis of **2** (Scheme 2). Briefly, to a dispersion of Pd/C (10%, 208 mg) in methylene chloride (30 mL) was added **1′** (2.085 g, 5 mmol), and the mixture was purged with hydrogen gas and stirred for 4 h. Upon the filtration to remove Pd/C,

Scheme 1



Scheme 2



triethylamine (4.5 mL) and 5-nitroisophthaloyl dichloride (0.6 g, 2.4 mmol) were added at 0 °C, and the resulting mixture was stirred under nitrogen atmosphere first at 0 °C for 2 h and then at room temperature for an additional 8 h. It was washed with aqueous HCl (1 M, 30 mL), saturated $NaHCO_3$ (30 mL), and then water (30 mL), and the organic layer was collected, dried with $MgSO_4$, and concentrated for silica gel column separation. The separated compound (ethyl acetate as eluent) was the acetyl-protected Gal_2 with nitro headgroup (89% yield). It was hydrolyzed in sodium methoxide/methanol solution (0.05 M) and then reduced with catalytic hydrogenation to obtain **2** as a colorless liquid (>95% yield).[8]

It is well-established that carbon nanotubes contain defect-derived carboxylic acids after oxidative acid treatment (as a part of sample purification). These nanotube-bound acid groups were targeted in amidation reactions with **1** and **2**.[9] In a typical experiment, a purified MWNT sample (20 mg) was suspended in KH_2PO_4 buffer (pH = 7.4, 20 mL), and to the suspension was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 180 mg, 0.94 mmol). After sonication for 1 h, **2** (496 mg, 0.84 mmol) was added, followed by sonication for another 2 h. The mixture was stirred for 36 h and then centrifuged (1380 *g*) for 30 min. The supernatant was transferred to a membrane tubing (molecular weight cutoff ∼12 000) for dialysis against deionized water for 3 days. The solution from the dialysis tubing contained only a small amount of solid residue, which was removed via centrifuging at 1380 *g* for 30 min. The resulting colored but transparent supernatant was the solution of Gal_2-MWNT (Scheme 1).

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Figure 1. Visual comparison of the supernatants from centrifuging the aqueous dispersions of Gal_2-MWNT (left) and Gal-MWNT (right).

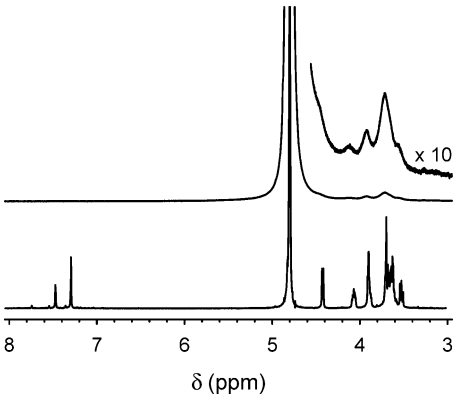

Figure 2. ^1H NMR spectra of Gal_2-MWNT (top) in comparison with that of Gal_2 (bottom), both in room-temperature D_2O.

The same reaction conditions were applied to the functionalization of MWNTs with **1**, but the resulting Gal-MWNT could only form an unstable aqueous suspension. Shown in Figure 1 is a visual comparison of Gal_2-MWNT and Gal-MWNT for their different solubilities. For the comparison, the two samples in similar amounts were dispersed in water, followed by the same centrifugation. The supernatant obtained from the Gal-MWNT dispersion is essentially colorless (Figure 1), suggesting that the sample is hardly soluble. For Gal_2-MWNT, on the other hand, the aqueous solubility was estimated gravimetrically (by drying a fix volume of the solution) to be ~0.3 mg/mL, which is lower than those of other water-soluble functionalized carbon nanotubes.[2a,10]

The solution of Gal_2-MWNT in D_2O was used for ^1H NMR measurement. The spectrum is compared with that of Gal_2 in Figure 2. The proton signals in the nanotube-bound Gal_2 are obviously much broader, consistent with effects associated with the low mobility of the nanotubes.[9b] In addition, the signals of aromatic protons in Gal_2 are suppressed in the spectrum of Gal_2-MWNT, which is likely due to the aromatic moiety being close to the nanotube surface, as already observed and discussed in the literature on other functionalized carbon nanotubes.[11,12]

The Gal_2-MWNT sample was characterized by various microscopy techniques, including scanning (SEM) and transmission electron microscopy (TEM) and atomic force microscopy (AFM). As shown in Figure 3, there are apparently abundant MWNTs of different lengths, well-dispersed to individual

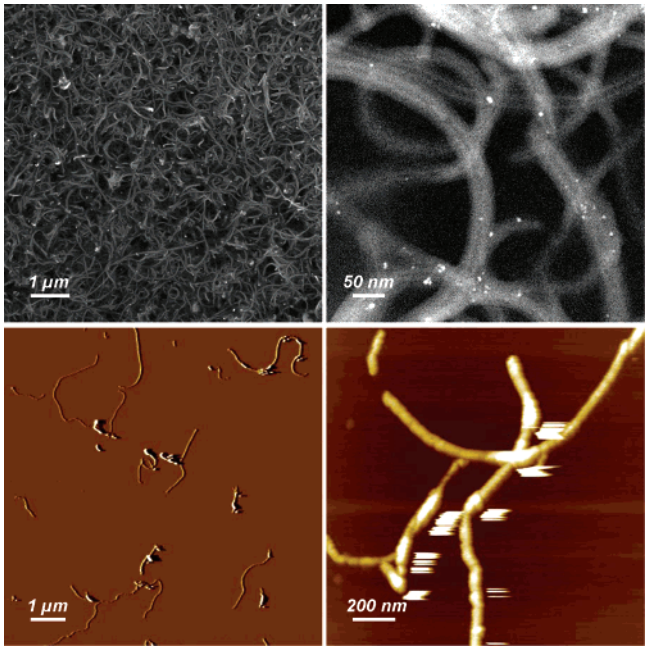

Figure 3. Representative SEM (top left), TEM (dark-field, top right), and AFM (amplitude: bottom left, and topography: bottom right) images of the Gal_2-MWNT sample. The SEM specimen was from evaporation of a concentrated Gal_2-MWNT solution, whereas the AFM specimen was from spraying a more dilute sample solution onto a heated mica substrate to preserve nanotube dispersion.

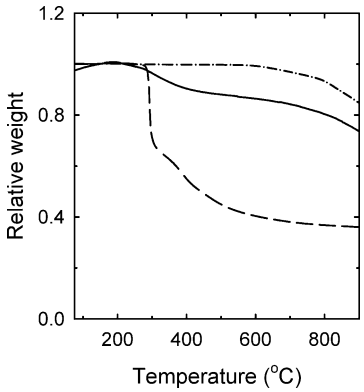

Figure 4. TGA traces (N_2, 10 °C/min) of Gal_2-MWNT (—), the stable solid precursor of Gal_2 (with nitro headgroup instead of amine, – – –), and the MWNT sample (-·-).

nanotubes. In TEM and AFM at a high magnification, the coating on the nanotube surface could be observed, which might logically be attributed to the Gal_2 functionalities.

To determine the nanotube content in the Gal_2-MWNT sample, thermogravimetric analysis (TGA) with inert gas (nitrogen) was tried to selectively remove the sugar functionalities from the nanotube surface (or thermal defunctionalization[9b]). However, it was found that, unlike Gal,[6] Gal_2 carbonized at high temperatures thus could not be evaporated under TGA conditions (Figure 4). There was no evidence suggesting that the carbonization of Gal_2 remained quantitatively the same before and after the attachment to nanotubes. Therefore, the TGA results (Figure 4) could not be used to provide a reasonable estimate of the nanotube content. On the other hand, the solubility of Gal_2-MWNT allowed the classical sugar analysis in aqueous solution, which is based on spectrophotometry with anthrone as the coloring agent.[13] In the analysis, a small aliquot (50 μL) of the Gal_2-MWNT solution (0.3 mg/mL) was mixed with deionized water (550 μL), HCl (1 mL), formic acid (0.1

mL), and 80% anthrone/H_2SO_4 solution (8 mL). The mixture was kept at 100 °C for 12 min and then rapidly cooled in an ice-bath for 30 min. The corrected absorption spectrum of the mixture was obtained, and the absorbance at 625.5 nm was used against a standard curve (prepared separately under the same experimental conditions) to determine the sugar content. According to the analysis, the sample solution contained 0.12 mg/mL of galactose (or 0.2 mg/mL Gal_2). Thus, the Gal_2 content in the solid-state Gal_2-MWNT sample was 67 wt % (the remaining 33 wt % being MWNTs).

In the solubilization of carbon nanotubes via chemical functionalization, the size of the functional group relative to that of the nanotube should be a critical factor. The results presented here are rather striking, providing a useful piece of evidence for the expected effect. The Gal-SWNT is soluble,[6] but not Gal-MWNT because MWNTs are considerably larger than SWNTs. It requires a larger functional group like Gal_2 to "drag" the nanotube into solution. The solubilization is obviously important to the purpose of these materials. The reported work shows that, similar to SWNTs, MWNTs can also be used to display multiple copies of sugars in physiological solution, which thus makes it possible to exploit those properties only available to sugar-functionalized MWNTs. In addition, since Gal-SWNT is known to exhibit significant cell adhesion,[6] the galactose pairs displayed on the nanotube scaffold may prove to be more efficient in the binding with cells. A comparative biological evaluation of these materials is in progress.

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(8) ^{1}H NMR (500 MHz, D_2O) δ 7.47 (s, 1H), 7.29 (s, 2H), 4.41 (d, J = 8.25 Hz, 2H), 4.09−4.04 (m, 2H), 3.92−3.87 (m, 4H), 3.73−3.62 (m, 12H,), 3.54−3.50 (m, 2H); ^{13}C NMR (500 MHz, D_2O): 170.33, 147.31, 135.34, 117.49, 116.08, 103.06, 75.10, 72.66, 70.74, 68.55, 68.42, 60.86, 39.96. MALDI: 614.79 $(M+Na)^+$
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