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Carbohydrate conjugation through microwave-assisted functionalization of single-walled carbon nanotubes using perfluorophenyl azides

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ABSTRACT

Carbohydrate–functionalized single-walled carbon nanotubes (SWNTs) were synthesized using microwave-assisted reaction of perfluorophenyl azide with the nanotubes. The results showed that microwave radiation provides a rapid and effective means to covalently attach carbohydrates to SWNTs, producing carbohydrate–SWNT conjugates for biorecognition. The carbohydrate–functionalized SWNTs were furthermore shown to interact specifically with cognate carbohydrate-specific proteins (lectins), resulting in predicted recognition patterns. The carbohydrate-presenting SWNTs constitute a new platform for sensitive protein- or cell recognition, which pave the way for glycoconjugated carbon nanomaterials in biorecognition applications.

1. Introduction

Single-walled carbon nanotubes have attracted much attention from a fundamental physics/chemistry perspective, as well as for applied fields such as nanoelectronics and nanomedicine. This is in part due to their unusual properties resulting from the pseudo-one-dimensional nanosize and the unique structure. However, several disadvantages limit their biological applications, such as poor water solubility, lack of reactive functionalities, and potentially high cytotoxicity.[1,2] An effective way to overcome these limitations is to introduce an organic coating on the carbon materials, for example, by using polar structures such as carbohydrates.[3–7] Functionalization of carbon nanotubes (CNTs) with carbohydrates not only increases the biocompatibility and solubility, but also introduces molecular recognition features, which can impact cellular interactions and uptake of CNTs. These features of carbohydrate-functionalized carbon nanomaterials have been demonstrated in biomedical applications such as biosensing and drug delivery.[3–5] For example, Zhang et al. wrapped SWNTs with boronic acid-derivatized phenoxy dextran. The resulting complex demonstrated a concentration-dependent riboflavin recognition resulting from the redshift of emission upon addition of riboflavin, which could be reversed by the addition of a riboflavin-binding protein.[8] Torres and co-workers covalently functionalized fewer-layer graphene and SWNTs with α-D-mannosyl dendrons, and used the resulting glyco-SWNTs for the selective interaction with lectin.[9] In the work of Sun and coworkers, monosaccharide-modified SWNTs were used to effectively bind *B. anthracis* spores in the presence of Ca^{2+}.[10,11]

For CNTs and graphene, the most common way to achieve covalent functionalization is to use the oxidized forms. Oxidation generates oxygen-containing functional groups at the material surfaces, such as epoxides and carboxylic acids, which can subsequently be used to conjugate various entities such as amine-functionalized carbohydrates.[10–13] Oxidation, however, may lead to extensive structural damage of the carbon materials, impacting the lattices and intrinsic properties. Milder conjugation methods are therefore desired. A number of alternative reactions have been developed for the chemical functionalization of CNTs, including radical additions via diazonium salts, alkyl or aryl peroxides, carbene or nitrene cycloadditions, as well as electrophilic and nucleophilic addition reactions.[14,15] However, owing to the relatively low reactivities of the pristine carbon materials, these reaction often require lengthy reaction times at elevated temperatures.

In this context, microwave radiation has become a standard technique in organic synthesis, inducing molecular collisions with

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direct impact on the reagents of the reaction. The efficient heating and milder reaction conditions generally result in faster kinetics, higher reaction yields, and lower side products as compared to the traditional heating protocol.[16,17] Microwave radiation has also been successfully used in the process for the chemical modification of CNTs and fullerenes.[9,18–20]

Organic azides provide a particularly attractive functionalization method related to carbon nanomaterials.[21–26] Azides may thus undergo cycloaddition reactions with reactive alkenes, eventually resulting in aziridines.[27] Of particular interest is the fact that thermal or light activation of organic azides leads to nitrogen extrusion, generating nitrene intermediates that add to the sp^2 carbons of the carbon material, forming aziridine adducts. Perfluorophenyl azides (PFPAs) are especially useful, being among the most reactive aryl azides,[28] and resulting in singlet perfluorophenyl nitrenes that have longer lifetime than other aryl nitrenes, thus promoting efficient covalent adduct formation.[29,30] We have, for example, shown that pristine graphene can be effectively functionalized with PFPA either photochemically or thermally.[21–23]In addition, Fréchet and co-workers used a PFPA structure bearing an ATRP initiator entity to functionalize SWNT forests, and then generated polymer brushes by in situ polymerization.[31] Besides light and heat, PFPAs can also be activated by other energy sources such as electrons and X-rays.[28,32]

In this work, we investigated the potential for microwave-assisted functionalization of SWNTs by PFPA derivatives, in order to generate functional nanoplatforms for carbohydrate presentation. Following conjugation, the biorecognition properties of the resulting glyconanomaterials were evaluated from analysis of the cognate lectin binding.

2. Results and discussion

As-prepared SWNTs are usually contaminated with a variety of impurities including residual metal catalysts and carbonaceous impurities such as amorphous carbon and carbon nanoparticles.[33] The degree of contamination and the percent impurity vary depending on the preparation method and the manufacturers. Of the three common synthesis methods used to produce SWNTs, that is, chemical vapor deposition (CVD), laser ablation and arc discharge, the lower temperature CVD technique has become a preferred production method owing to the better control over nanotube alignment, size, purity, and density.[34] The HiPco SWNT sample used in this study was produced by CVD with 85 wt % purity, and the purity was further inspected by TEM before use. The TEM image shows large ropes which were bundled nanotubes, as well as dark particles that were mostly residual catalysts coated with amorphous carbon (Fig. 1A).[33,35] To remove these impurities, chemical oxidation and physical purification methods are employed to purify as-prepared CNTs. The chemical oxidation selectively oxidizes carbonaceous impurities, however, the method often leads to structure damage by the oxidizing agent. In order to avoid damage of the pristine SWNTs, a mild, non-oxidative purification method was preferred. Thus, a differential centrifugation protocol was adopted to remove the metal catalysts, carbonaceous impurities, or heavy bundles based on their weight difference. This process resulted in improved sample purity and smaller nanotube bundles (Fig. 1B).

To functionalize SWNTs, the reaction was first carried out by heating a suspension of SWNTs and PFPA-NHS in 1,2-dichlorobenzene (DCB) at 130 °C in an oil bath. The azide functionality, showing a characteristic IR absorption band at ~2130 cm^{-1}, was used as a convenient way to monitor the reaction by FTIR (indicated by arrows in Fig. 2A). The reaction was in this case relatively sluggish, where the PFPA was converted in 3 days (Fig. 2A). The reaction was subsequently carried out in a microwave reactor under the same conditions, resulting in almost complete conversion in 6 h. Upon increasing the temperature to 150 °C, the reaction proceeded considerably faster, resulting in only trace amounts of PFPA after 1 h radiation (Fig. 2B). No other changes could be detected, indicating that the components were not further affected under the reaction conditions. These microwave reaction conditions (150 °C, 1 h) were thus chosen for the subsequent studies.

Further evidence for the covalent functionalization was provided by Raman spectroscopy. Conversion of sp^2 carbons to sp^3 carbon atoms is expected to lead to an increase in the intensity of the disorder (D) band (1250–1450 cm^{-1}) along with a decrease in the graphite (G) band (1500–1605 cm^{-1}). The intensity ratio of the D and G bands, I_D/I_G, is thus commonly used to evaluate the degree of functionalization; the larger the value, the higher the degree of functionalization.[36] In addition, covalent functionalization of SWNTs would reduce the intensity of the radial breathing mode (RBM, between 100–300 cm^{-1}), and this band would completely disappear for SWNTs having high degrees of functionalization.[11] In the present study, the Raman spectra were recorded for SWNTs before and after functionalization with PFPA-NHS (Fig. 3). The I_D/I_G values were calculated to be 0.15 and 0.43 for purified SWNTs and PFPA-NHS-functionalized SWNTs, respectively. The increase in I_D/I_G value observed for the modified SWNTs compared to that of pristine SWNTs is consistent with the covalent functionalization of the nanotube lattice.[37] SWNTs functionalized by the conventional thermal reaction and the microwave-assisted

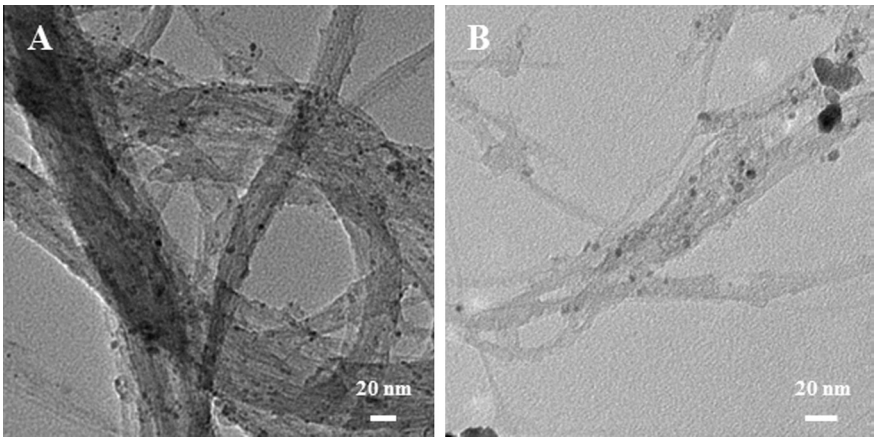

Figure 1. TEM images of (A) purchased SWNTs without purification, and (B) SWNTs after purification.