NON- SPECIFIC ADSORPTION OF BIOMOLECULES ON SINGLE WALLED CARBON NANOTUBES

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Carbon Nanotubes, CNTs, exhibit a unique combination of excellent mechanical, electrical and electrochemical properties, which has stimulated increasing interest in the application of CNT as components in bio-sensors. Nevertheless, the control of non-specific protein adsorption is important for the use of CNTs in specific protein-binding or biorecognition.. There are several molecules reported in the literature that has been used to prevent the protein adsorption. Among them, polyethylene glycol, PEG, bonded no covalently to CNTs has been reported to be an effective way to achieve the protein resistance [1]. The protein resistant coating usually requires amphiphilic molecules with a hydrophobic backbone that interactions hydrophobically with the CNT and a hydrophilic segment extended in the aqueous solution. In this work we have explored the existence of non specific protein adsorption on single wall nanotubes, SWNTs, using the biotin- streptavidin system. We have modified the hydrophobic character of the SWNTs by surface functionalization with carboxylic groups, amine groups and PEG that renders the SWNTs surface more hydrophilic. The streptavidine adsorption on these modified SWNTs has been determined and compared with the biotin-streptavidine interaction on SWNTs covalently functionalized with biotin.

The specificity of streptavidin adsorption on SWNTs have been determined using a colorimetric method based on the horseradish peroxidase-catalyzed oxidation of 3,3',5,5'-tetramethylbenzidine[2]. The nanotubes were dispersed in PBS buffer by sonication and streptavidina-HRP solution is added. After 30 minutes of reaction time, the solution is filtered and washed with PBST buffer. SWNTs were sonicated, filtered again and dried under vacuum at room temperature. Substrate HRP buffer was then added and blue colour was observed in the presence of streptavidin. The reaction was stopped after 30 minutes by addition of H_2SO_4 4N the colour turning to yellow. For quantitative study, UV detection can be used measuring the absorbance at 450 nm of supernatant.

Single-walled nanotubes were produced by arc discharge method (100A, 20V), using Ni and Y as catalysers (4:1) under 660mb of helium [3,4]. The as-produced SWNTs is a multicomponent material consisting of entangled SWNT bundles associated with metal nanoparticles and carbon phases more or less graphitized. (Figure 1)

Oxidation of as-produced SWNTs was achieved by adding an aqueous solution of nitric acid 1'5M and refluxing during 2 hours. The resulting suspension was then centrifuged, filtered, washed with milliQ water and dried under vacuum.[5,6,7]

To obtain aminated SWNTs, carboxylated carbon nanotubes were first acylated, by addition of thionyl chloride and dimethylformamide and stirred at 120ºC during 24 hours. After filtration and washing with tetrahydrofuran to remove the $SOCl_2$ excess, the SWNT material was dried under vacuum. Afterwards, it was mixed with ethylenediamine and stirred at 60ºC during 4 days. The material was then filtered, washed repeatedly with ethanol and dried under vacuum to obtain the final aminated nanotubes.[6,7]

The aminated nanotubes were then dispersed in dimethylformamide and reacted overnight with N-hydroxysuccinimidyl biotin at room temperature to form biotinated SWNTs. The dispersion was then filtered and dried under vacuum. A scheme of the reaction is shown in Figure 2.

To obtain SWNTs functionalized with polyethylene glycol, Figure 3, the acylated nanotubes were mixed with PEG and pyridine in dimethylformamide, and stirred at 100ºC during 5 days. After filtration, the product was washed with water and dried under vacuum.

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Figures:

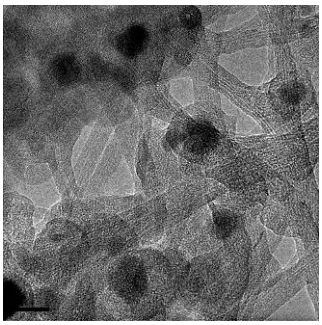

Figure 1. TEM image of as-produced SWNTs.

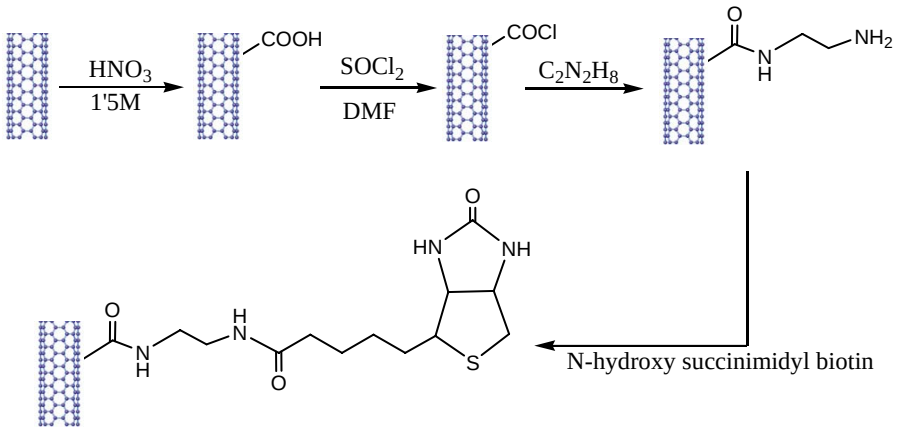

Figure 2. Scheme of biotin functionalization of SWNTs

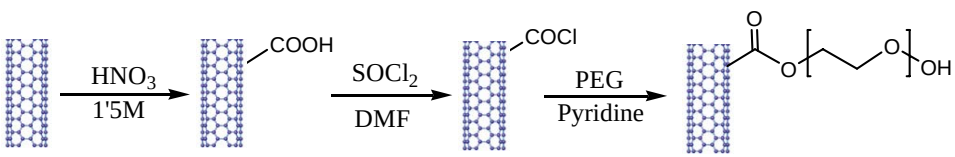

Figure 3. Scheme of PEG functionalization of SWNTs