Amino acid functionalization of double-wall carbon nanotubes studied by Raman spectroscopy

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Double-wall carbon nanotubes (DWNT) have been functionalized with lysine after a strong oxidation with MnO_4^- in acid solution which, as suggested by the Raman spectra, attacked the external nanotube of the DWNT.

Nanotubes functionalization is important for technological applications of carbon nanotubes (NT)[1] since it allows to make nanotubes compatible with different environments such as solutions, polymer matrices, and surfaces.[2] Furthermore it can also give them specific functions to address biological targets.[2] However when extended functionalization is needed, NT can lose their properties due to the large number of defects introduced by the new functional groups. It was recently shown, by STM measurements, that shortened oxidized single-wall NT (SWNT) are functionalized on the tips but also on the walls.[3] To solve this problem one can cover SWNT with a protection layer on which a functionalization can be operated. The natural choice, using carbon nanotubes, is another nanotube as protecting layer, namely to use double-wall nanotubes (DWNT). DWNT have been recently synthesized with very good yield and purity in particular by catalytic CVD.[4] Synthetic methods for the production of DWNT have been extensively evaluated,[5] whereas only fluorination[6] of DWNT has been reported as functionalization. A very recent study in the exohedral doping of DWNT shows the interest for this type of carbon nanostructures.[7]

We present the functionalization of DWNT with an amino acid, lysine. Raman spectra suggest that the internal nanotubes were protected by the external nanotubes and that we have functionalized the external wall of the DWNT.

We treated unpurified DWNT (CNI lot n. DW0923) in air at 400 °C for 2 h to purify them from residual carbon and we observed a reduction of about 10% of the original weight. Fig. 1 reports the Raman spectrum of the purified NT excited at 632.8 nm (Invia Renishaw microspectrometer). A low intensity D band at 1330 cm^{-1} indicates a low number of C sp^3 arising from defects on the NT. Furthermore one finds a G′ band at 2630 cm^{-1} with two components and a larger intensity of that at lower frequency. This is an indication[8] of a sample with a low content of SWNT, which are always present together with DWNT.[4] Below, we show that the functionalization process purifies the sample from SWNT.

The first step of the functionalization was a strong oxidation of DWNT with MnO_4^-, according to a modified approach used by Zhang *et al.*,[9] which produces carboxylic groups on the DWNT. An amount of 400 mg of DWNT were suspended by sonication in

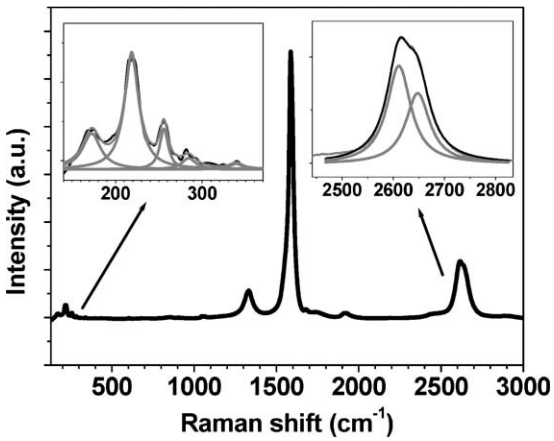

Fig. 1 Raman spectrum of pristine DWNT exciting at 632.8 nm.

80 ml of CH_2Cl_2. To this solution 100 ml of water, 640 mg of methyltrioctylammonium bromide and 3.08 g of $KMnO_4$ were added. The reaction mixture was ultrasonicated (power: 100 W) for 5 min and, after 10 min, for another 5 min. Then 8 ml of concentrated H_2SO_4 was carefully added to the solution which was sonicated for 8 h always using the same procedure. The solution was then filtered on a Teflon filter (0.2 μm pores) and thoroughly washed. The solid was dried under high vacuum and then suspended in 40 ml of water and 0.3 ml of tetrabutylammonium hydroxide 1.4 M, which produced more soluble carboxylates. The solution was ultrasonicated for 30 min and then centrifuged at 5000 *g* for 5 min. The solution was separated and the nanotubes (soluble fraction) were precipitated with 1 M HCl and dried under vacuum. The yield of the overall treatment was between 15 and 20% of the starting material. Fig. 2 shows TEM images of the original and of the oxidized DWNT where one can see significant aggregation of NT probably due to hydrogen bondings related to the new carboxylic functions.

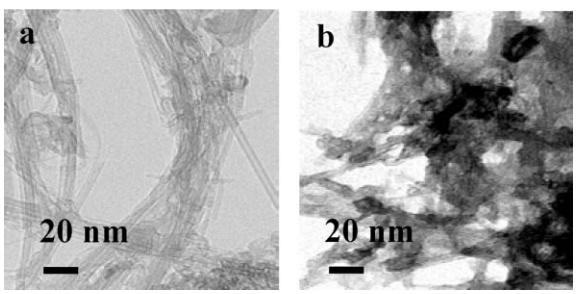

Fig. 2 TEM images of original (a) and oxidized (b) DWNT.

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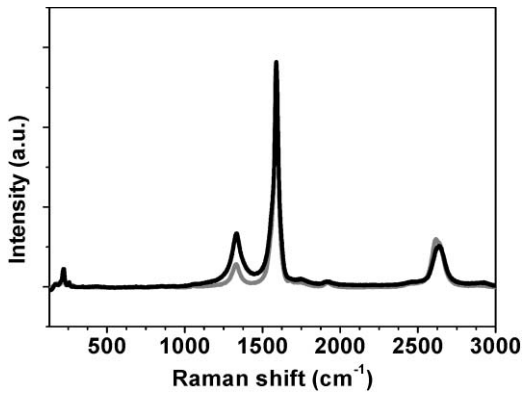

Fig. 3 Raman spectra (632.8 nm exciting line) of purified (gray line) and oxidized (black line) DWNT. The spectra have been normalized on the G band at 1586 cm^{-1}.

Comparison of the Raman spectra of the pristine DWNT and of the soluble fraction (see Fig. 3) shows a more intense D band at 1333 cm^{-1} after the oxidation, confirming the presence of more defects introduced on the NT.

Fig. 4 reports the spectral region of the radial breathing mode (RBM) below 400 cm^{-1} exciting at 632.8 and at 488 nm. The two spectra show the presence of different nanotubes because the exciting lines are in resonance with nanotubes of different chiralities and, for example, one finds that in the spectrum excited at 632.8 nm the RBM of metallic nanotubes are observed below 230 cm^{-1} whereas in that excited at 488 nm they are observed above 240 cm^{-1}.[10] Moreover, the frequency of the RBM is inversely proportional to the diameter d of the NT according to $\omega_{RBM} = (A/d) + B$, where A and B have been suggested to be 234 and 10 cm^{-1} respectively.[4b] One can easily find that bands above 205–210 cm^{-1} have to be assigned to internal nanotubes. In fact, if they would refer to external ones, one finds that their diameters have to be below 1.2 nm and, therefore, since the diameters of the internal nanotubes is 0.7 nm smaller then the external one,[5c] the diameters of the internal nanotubes should be below 0.5 nm, which is too small for usually synthesized nanotubes.

To understand the variation of the RBM intensities, we also oxidized HiPco SWNT (CNI) under the same conditions used for oxidizing DWNT. We found that SWNT were destroyed by the strong oxidizing conditions since the Raman spectrum (see Fig. 5) shows a huge D band, characteristic of amorphous carbon, and does not show RBM bands, which, in the pristine sample, are present from 180 to 340 cm^{-1} (exciting line: 632.8 nm) and from 200 to 310 cm^{-1} (exciting line: 488 nm). This result suggested to us that the small intensity RBM at 340 and 281 cm^{-1} (see Fig. 4(a)) or that at 289 cm^{-1} (see Fig. 4(b)) of the pristine DWNT can be related to SWNT which are destroyed by the strong oxidation conditions. However, contrary to what happens for SWNT of similar diameters, the strong band at 219 cm^{-1} of DWNT (see Fig. 4(a)), which can be assigned to internal nanotubes, is present in the spectrum of the oxidized sample and shows, therefore, that internal nanotubes are protected by the external ones during the

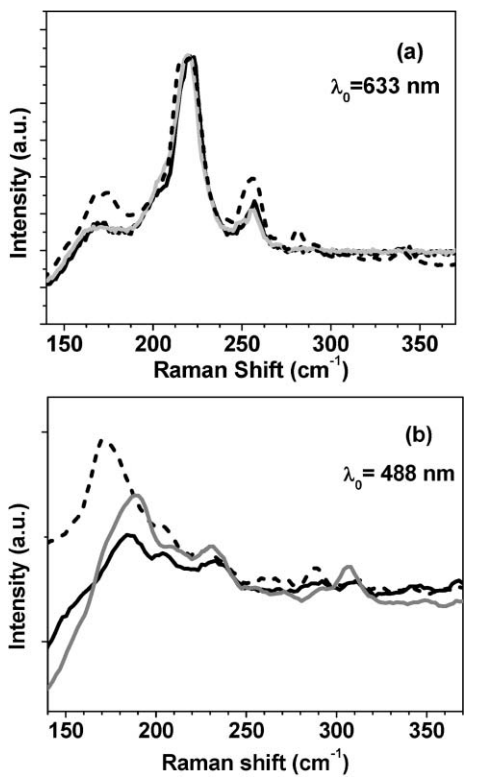

Fig. 4 Raman spectra of the purified DWNT (dashed line), soluble fraction of the oxidized DWNT (black line) and functionalized DWNT with lysine (gray line). Exciting line at 632.8 nm (a) and 488 nm (b).

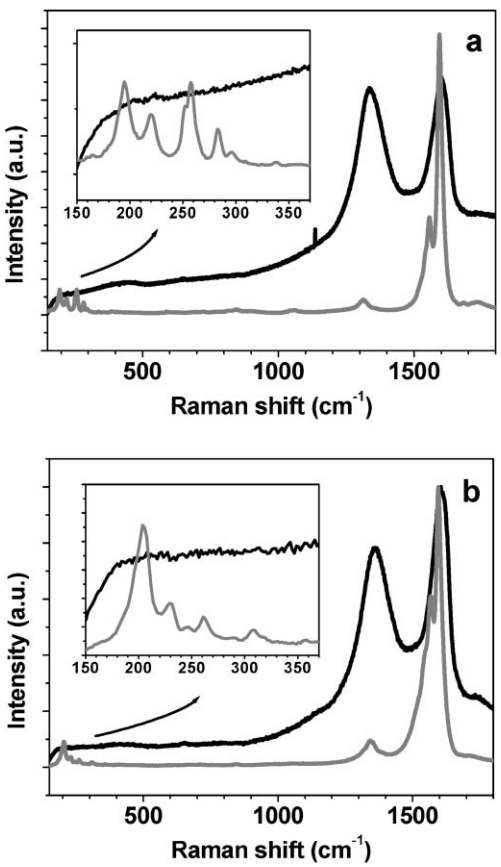

Fig. 5 Raman spectra (632.8 nm (a) and 488 nm (b) exciting line) of original HiPco SWNT (grey line) and of HiPco (black line) oxidized with the same protocol used for oxidizing DWNT.

oxidation with MnO_4^-. Other bands with smaller intensity, but frequency above 205 cm^{-1} and therefore related to internal nanotubes, like that at 257 cm^{-1} in Fig. 4(a) and at 233 cm^{-1} in Fig. 4(b) survive the strong oxidation.

Decreasing intensity of the bands at about 170 cm^{-1} in Fig. 4(b) shows, on the other hand, that external nanotubes were functionalized since a modification of their walls changes their electronic structure and therefore the resonance condition which determines the RBM intensities. Confirmation of this result also comes from the Raman spectrum excited at 488 nm (see Fig. 4(b)) where a band at 172 cm^{-1} is strongly reduced with respect to other bands above 205 cm^{-1}. This also shows that both metallic (RBM below 210 cm^{-1} in Fig. 4(a)) and semiconducting (RBM below 230 cm^{-1} in Fig. 4(b)) external NT are affected by oxidation. Therefore, the Raman data strongly suggest that the oxidation with MnO_4^- occurs on the external walls of DWNT, and that this does not depend on the electronic characteristic of the nanotubes, as in other cases it was observed for SWNT.[11]

The second step of the functionalization was the formation of an amide bond between the α-amino group of the lysine derivative H–L-Lys(Boc)–OCH_3 and the carboxylic functions on the DWNT. Amino acid functionalization allow us to use a standard peptide synthesis test for the quantitative determination of amino acids and, therefore, of the carboxylic groups generated by the oxidation. Furthermore, the side-chain amino function of lysine can be available for interaction with biological targets.[12] An amount of 10 mg of soluble DWNT were solubilized in anhydrous DMF by ultrasonication for 30 min and 20 μl of triethylamine and 10 mg of HBTU[13] were added to the solution and sonicated for another 10 min. Then, 30 mg di H–Lys(N^{ε}Boc)–OCH_3·HCl and 30 μl of triethylamine in 1 ml of DMF were added to this solution. The solution was ultrasonicated for a further 30 min and, then, diluted with 20 ml of deionized water and filtered on a regenerated cellulose membrane (0.2 μm pores). The solid was thoroughly washed and then dried under high vacuum. The Raman spectrum does not show, as expected, any significant variation with respect to that of the soluble DWNT (see Fig. 4).

We used the Kaiser test[14] for a quantitative evaluation of the presence of lysine groups after deprotecting their amine groups with trifluoroacetic acid. We found that the concentration of amino acids is one lysine group every 613 carbon atoms considering the amount of material used for the analysis and the concentration of the lysine groups found with the Kaiser test. Considering that we mainly functionalised the external wall of DWNT and that, roughly, the atoms on the external nanotube are 2/3 of the total number of carbon atoms, the concentration of lysine groups is about one per 409 carbon atoms of the external wall. We have also confirmed the content of lysine by an amino acid analysis after acid hydrolysis[15] of the functionalized DWNT. We found, using the above method, a concentration of one lysine group every 390 carbon atoms, and, considering only the average number of external carbon atoms, one lysine group every 260 carbon atoms. Considering that the above tests have less then 10% error, we think that the difference between the figures obtained by the two methods derives from the lysine groups included in bundles which are less available to the reaction required by the Kaiser method.

In conclusion we have shown that the internal nanotube of a DWNT is protected by its external wall on which carboxylic functions can be produced by oxidation with MnO_4^- and that an amidation reaction allowed to link lysine groups to the acid functions.

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