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MORPHOLOGY OF DNA/SINGLE WALLED NANOTUBES COMPLEXES

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In order to get a better understanding of the association mechanism that leads to the formation of DNA/single-walled carbon nanotube (SWNT) complexes, we solubilised and dispersed SWNTs in aqueous medium using single-stranded DNA with a poly $(GT)_{15}$ sequence. Optical and microscopic characterizations of the complexes permits to confirm that the SWNTs were individualized or embedded in small bundles. The presence of these bundles excludes that helical wrapping may be the unique possible association mechanism.

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1. Introduction

The formation of bio(macro)molecule/single-walled carbon nanotube (SWNTs) complexes may open a broad range of applications at the interface between the physics, the chemistry and the biology such as the biosensor field for example [1]. Among them, the DNA/SWNT complexes (where DNA stands for deoxyribonucleic acid) may be used for example for DNA hybridization detection [2], biocompatibility [3], targeting [4]… Alternatively, associating DNA to SWNTs allows also to sort the SWNTs [5] or to organize them [6].

There are two different approaches to realize DNA/SWNTs complexes. A first one consists in covalently grafting the DNA macromolecule to the SWNT backbone [7]. It requires first to create an anchor point on the SWNT surface (for example a -COOH group which is introduced by treatment with very strong acids) and then to substitute functionalised DNA to this point. While this method is very efficient, it presents the disadvantage to modify chemically the nanotubes, thus altering some of their properties. The second method consists in a direct association between DNA and SWNTs, as demonstrated by M. Zheng *et al*. [5, 8]. According to their work, a π stacking interaction stabilizes wrapping of single strands DNA of poly(T), poly(G) or poly$(GT)_{10\text{-}45}$ sequences around the SWNTs. Indeed, wrapping can only occur if the SWNTs are individualised. The advantage of this route is that it preserves the properties of the nanotubes. The resulting DNA/SWNTs complex can thus be used as an optical biosensor for example.

In this paper, we characterize DNA/SWNTs complexes prepared according to the Zheng's procedure. We show that helical wrapping of DNA around the SWNT may not be the unique possible association mechanism.

2. Experimental part

Single-stranded DNA (ssDNA) with a poly$(GT)_{15}$ sequence were synthesized by Sigma-Proligo. HiPCo SWNTs were purchased from Carbon Nanotechnologies, Inc. (Houston, TX), sodium chloride (NaCl) and heavy water (D_2O) from Sigma-Aldrich. The SWNTs bundles are first dispersed into heavy water using the method described by Zheng *et al*.[5, 8]. Briefly, SWNTs are suspended with the 30-base sequence of ssDNA in

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a 1:1 mass ratio in 0.1 M NaCl in heavy water and ice-water bath sonicated for 2 hours. The mixture is first centrifuged for 3 hours at 7500 g to remove insoluble materials and then for 90 minutes at 15000 g. The supernatant is examined using UV-Vis-NIR optical spectroscopy, Fourier-Transform Raman Spectroscopy (FT-Raman), High-Resolution Transmission Electron Microscopy (HRTEM) and Atomic Force Microscopy (AFM). Optical absorption is obtained on solutions with a CARY 5G UV-Vis-NIR spectrometer, FT Raman scattering is performed with a Bruker spectrometer and a Nd-Yag laser working at 1064 nm. Samples for AFM measurements are prepared by incubating 10 µl of the supernatant on top of a freshly cleaved mica surface for 10 minutes before rinsing with deionised water and drying under a stream of dry air. Images are recorded in Tapping-Mode using a commercial AFM (Nanoscope IIIa, Digital Instruments, Santa Barbara, CA) equipped with a 120-µm scanner. The Transmission Electron Microscope (TEM) used in this study is a Hitachi HF-2000 equipped with a field emission gun operating at 200 kV. The samples are prepared by dipping a perforated carbon-coated copper grid into 10 µl of the supernatant.

3. Results

As produced SWNTs are embedded into bundles. In order to form complexes, the nanotubes have thus to be individualised. One way to probe if the bundles are broken or not is to investigate the optical properties of the sample [9]. For isolated nanotubes, sharp and well defined peaks are observed in the optical absorption spectrum. These peaks are due to the different possible and allowed optical transitions E_{ii} between the Van Hove singularity pairs of the SWNT density of states. When the nanotubes are embedded in bundles, these peaks are broaden and redshifted, at least for i=1 [9]. Fig. 1 presents the absorption spectra of SWNTs dispersed in ethanol and of the DNA/SWNTs complex solution in the visible and near infra-red range. The spectra were obtained by removing the π plasmon contribution . The features observed above 900 nm and in the 500-900 nm range are due to E_{11} respectively E_{22} transitions of semiconducting nanotubes. The spectrum of the complex (full line) is composed of much narrower lines than the spectrum of the raw tubes It can also be seen that the E_{11} transitions (presumably the E_{22} as well) of raw SWNTs are redshifted by comparison with the complex lines. It suggests that the nanotubes in DNA/SWNTs complexes are much more isolated than the as-grown tubes.

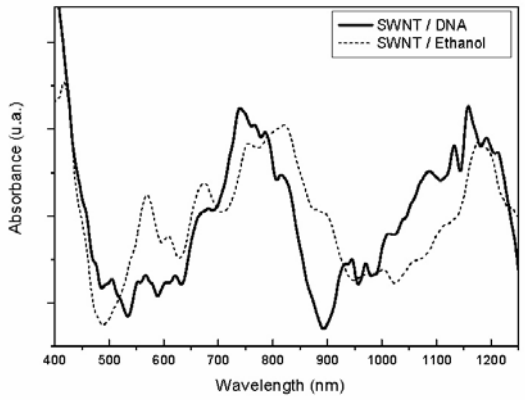

Fig. 1. Absorption spectra of SWNTs dispersed in ethanol (continuous line) and DNA/SWNTs complex dispersed in heavy water (dotted line). The absorption spectra have been corrected of the π plasmon baseline contribution.

This is confirmed by the emission spectra shown in Fig. 2, which displays the Raman/emission spectra of the raw tubes (spectrum 3), of the complex solution after the first centrifugation step (spectrum 2) and of the complex supernatant solution obtained after the second centrifugation step (spectrum 1) respectively. These spectra have been obtained with an excitation line at 1064 nm. This excitation line corresponds to allowed optical E_{11} transitions of semiconducting tubes as seen in Figure 1. The Raman peaks are labelled with stars on Fig. 2. The two main peaks are due to the radial breathing mode (at 268 cm^{-1} for the pristine sample) and to the tangential G modes close to 1600 cm^{-1} (at 1590 cm^{-1} for the pristine sample). The peak at 2551 cm^{-1} is the second order G' mode. There is no significative modification of the Raman line position within our experimental accuracy. More interestingly, it can be noticed that two broad lines found around 500 cm^{-1} and

1500 cm^{-1} (below the Raman G lines) are growing from bottom to top (indicated by arrows on Figure 3). These lines are fluorescence lines associated to the NIR emission of semiconducting SWNTs. Since the pioneering work of O'Connell *et al.* [9], a lot of work is devoted to NIR fluorescence of SWNTs. The NIR emission is observed for isolated semiconducting SWNTs. When the nanotubes are embedded in bundles, the emission is quenched as soon as the bundle contains metallic nanotubes. The fluorescence comes from the radiative decay of the excitons formed after the absorption of the E_{11} photon. Each kind of SWNTs should thus give a specific fluorescence line. Here, the lines at 558 cm^{-1} and 1556 cm^{-1} may be attributed to (9,2) and (11,1) SWNTs according to S. M. Bachilo *et al.* [10]. It is obvious that the fluorescence intensity is higher in trace 1 than in trace 2. It suggests that the second centrifugation step is actually improving the quality of the SWNTs dispersion. However, some SWNTs have already been dispersed after the first step as deduced from trace 2. Actually, a similar spectrum (not shown) with small but observable fluorescence lines is already obtained without any centrifugation. It means that the DNA/SWNT interaction is competitive with the nanotube-nanotube Van der Waals interaction which stabilizes the bundles [11].

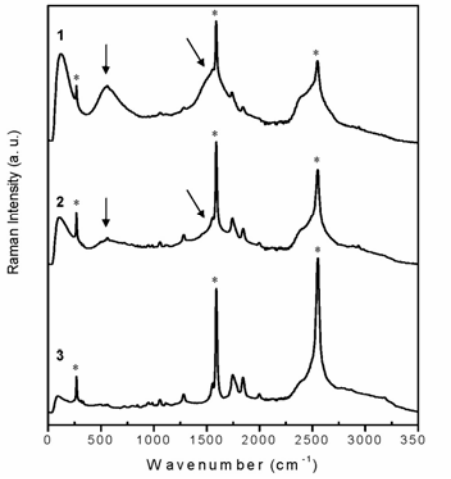

Fig. 2. FT Raman/emission spectra of (1) DNA/SWNTs complex solution after the two centrifugation steps (2) DNA/SWNTs complex solution after the first centrifugation step (3) raw SWNTs dispersed in ethanol. The spectra have been obtained with an excitation line at 1064 nm. The stars refer to Raman peaks. The arrows indicate NIR fluorescence lines.

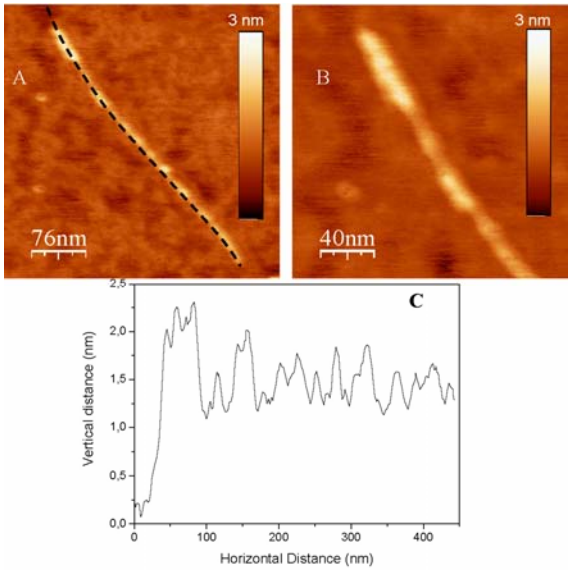

Fig. 3. Tapping mode AFM images of a DNA/SWNT complex . Figure 3A is a large scan image. Figure 3B is a zoom. Figure 3C gives the height profile along the dotted line of Fig. 3A.

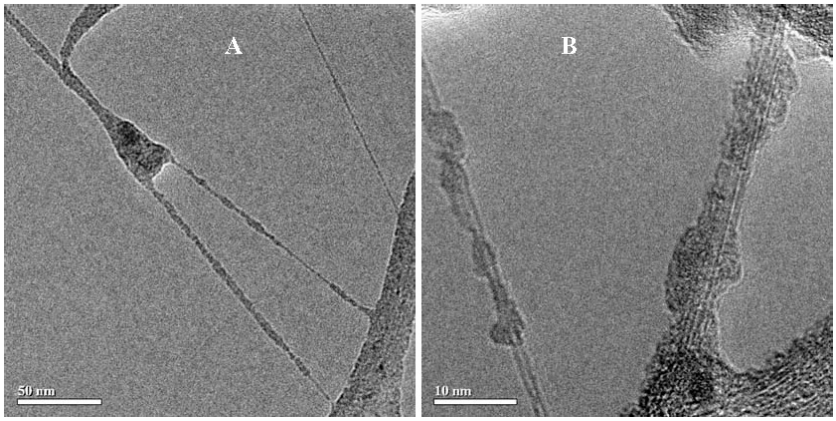

Fig. 4. TEM images of DNA/SWNT complex. Fig. 4A shows that the SWNTs are not uniformly covered by DNA. Figure 4B is a higher magnification image where both an isolated tube and a small bundle are observed with different DNA coverage morphology.

The morphology of the complex can also be studied by means of microscopic tools. Fig. 3 shows tapping mode AFM images of a complex deposited on a mica substrate. Part B of the figure is a physical zoom of image A obtained by focusing the xy-piezo displacements on the top left part of image A. Part C is a height profile obtained along the dashed line drawn in Figure 3A. An isolated tubular structure is easily identified. The height of the tube is between 1 nm and 3 nm (white spots). It is consistent with a ~1 nm thick unique tube covered by an organic layer. When looking more carefully (see the height profile of Figure 3-C), it seems that the organic layer is designing 0.5 - 1.5 nm high bubbles periodically along the tube (with a typical periodicity around 15-20 nm). The same kind of observation has been done by Zheng *et al*. [8]. It suggests that ssDNA is actually wrapping the tube with such a periodicity. However, the ssDNA does not seem to fully cover the nanotubes. This is confirmed by high resolution transmission electron microscopy (TEM). Two representative micrographs are shown in Fig. 4. Different nanotubes can be identified on Figure 4A where it can be seen that they are not uniformly covered all over their length. Fig. 4B, which is obtained with a higher magnification, is clearly showing that the covering layer is not continuous along the whole nanotube. On the right side of Figure 4B, it can also be seen that small bundles of SWNTs survive in this sample and they are covered by a much thicker organic layer. The coexistence of individualized SWNTs and small bundles was observed by Zhao *et al*. [13] too when they disperse SWNTs with long (more than 1000 oligonucleotides) ssDNA molecules.

4. Discussion

The optical properties of our dispersions show that poly$(GT)_{15}$ ssDNA macromolecules are able to interact efficiently with isolated nanotubes and to stabilize a dispersion. It means that the hydrophobic surface of the SWNTs is protected from the water molecule. In its single strand form, the poly(GT) DNA molecule presents on one side aromatic bases and on the other side sugar-phosphate groups which are hydrophilic. It is thus quite reasonable to assume that the aromatic base units of DNA faces the graphitic surface while the sugar-phosphate part faces water. Calculations [12] have already shown that the interaction of thymine or guanine base with the nanotube is favourable. Indeed such an interaction may induce a wrapping of the DNA around the nanotube. Since the DNA strand is only a 30-mer, its length is typically of ~ 10 nm, which means that it is not possible to cover the whole length of a SWNT, in agreement with our AFM or TEM observations.

However, as already noticed, small bundles are also observed. It means that either there is no wrapping of ssDNA or that the conformation of the DNA is not the same around these small bundles. It can be noticed that the coverage of the bundle seen in Fig. 4 seems to be much thicker than for isolated nanotubes. It suggests that the DNA morphology is different and that the DNA strands are not isolated which discards somewhere a wrapping supramolecular organisation. Isolated or small bundles of SWNTs can emit NIR photons as long as there is no neighbouring metallic tubes. It explains the observed optical properties.

5. Conclusion

Poly$(GT)_{15}$ ss-DNA/SWNTs complexes have been prepared in aqueous solution. Stable dispersions are obtained after two centrifugation steps. Optical characterizations and microscopic observations suggest that the DNA strand wraps around isolated nanotubes and can stabilize the SWNTs in solution. However, small bundles covered with DNA are also observed which suggests that a non helical wrapping interaction may also occur. Recent calculations from the literature have shown that thymine or guanine bases are happily interacting with the graphitic surface. It may mean that the DNA strand which wraps around the isolated tube may also adopt a different configuration on the surface of small bundles. Successive centrifugations allow to discriminate between the small bundles and the isolated tubes.

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