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Enzyme-catalyzed Oxidation Facilitates the Return of Fluorescence for Single-Walled Carbon Nanotubes

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Abstract

In this work, we studied enzyme-catalyzed oxidation of single-walled carbon nanotubes (SWCNTs) produced by the high-pressure carbon monoxide (HiPco) method. While oxidation *via* strong acids introduced defects sites on SWCNTs and suppressed their near-infrared (NIR) fluorescence, our results indicated that the fluorescence of SWCNTs was restored upon enzymatic oxidation, which provided new evidence that the reaction catalyzed by horseradish peroxidase (HRP) in the presence of H₂O₂ is mainly a defect-consuming step. These results were further supported by both UV-vis-NIR and Raman spectroscopy. Therefore, employing acid oxidation followed by HRP-catalyzed enzyme oxidation, shortened (< 300 nm in length) and NIR-fluorescent SWCNTs were produced. In contrast, when treated with myeloperoxidase (MPO), H₂O₂, and NaCl, the oxidized HiPco SWCNTs underwent complete oxidation (*i.e.* degradation). The shortened, NIR-fluorescent SWCNTs resulting from HRP-catalyzed oxidation of acid cut HiPco SWCNTs may find applications in cellular NIR imaging and drug delivery systems.

Keywords

carbon nanotube; fluorescence; enzyme; drug delivery

INTRODUCTION

Because of the unique properties of carbon nanotubes (CNTs) such as small size, large surface area, high strength, the ability to transport electrons, and inert chemical nature, this carbon-based nanomaterial has been incorporated in a wide array of applications including building composites, electronics, and medical therapeutics.^{1–3} The full implementation of CNTs in consumer goods, however, can be hindered as a result of emerging evidence that this nanomaterial can induce cytotoxic effects such as inflammation, epithelioid granulomas, fibrosis, and oxidation stresses that stem from both the chemical/electronic properties and the fibrous, anisotropic geometry of CNTs.^{4,5} Moreover, CNTs that are engulfed by cells during endocytosis may rupture the cell membrane due to their length;⁵ therefore, there is a direct correlation between cytotoxic response and CNT length. A recent study by Ali-

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SUPPORTING INFORMATION

Experimental procedure (Scheme S1), Lorentzian fitted Raman RBM section of the pristine SWCNTs, extra DFT calculations for defective CNTs, AFM characterization, and spectroscopic data for control experiments (Figures S1–S8). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Boucetta et al. has also demonstrated that the toxicity of CNTs is dependent on both lengths of and surface functionalities imparted on this nanomaterial.⁶ For this reason and as a result of better circulation,^{7,8} shortened CNTs, which are generally fabricated through chemical oxidation, have been employed for *in vitro* and *in vivo* drug delivery applications.^{9,10} The primary shortcoming to employing harsh chemical oxidation methods to shorten (*i.e.* “cut”) the length of semiconducting single-walled carbon nanotubes (s-SWCNTs) entails damaging of the sp^2 lattice and creation of oxygen functionalities both of which result in the loss of the nanotubes’ intrinsic near-infrared (NIR) fluorescence.^{11,12}

Recently, we and others have demonstrated that peroxidases such as the plant derived enzyme, horseradish peroxidase (HRP),^{13–18} and myeloperoxidase (MPO),^{18–21} an enzyme expressed by inflammatory cells in humans, can oxidatively degrade CNTs and graphene oxide. HRP is obtained from the root of the horseradish plant and contains 308 amino acid residues and an iron (III) protoporphyrin IX heme active site. In the presence of hydrogen peroxide (H_2O_2), this enzyme demonstrated the ability to oxidize organic substrates^{22,23} and carbon based nanomaterials^{13–18}, including SWCNTs. After reacting with H_2O_2 , the oxidation state of the iron atom in the heme group is increased from (III) to (IV), and a porphyrin-based cation radical is formed. The iron (IV) porphyrin cation radical state of the enzyme, known as Compound I, oxidizes a substrate by two sequential one-electron oxidation steps, whereby Compound I is reduced to Compound II and subsequently back to the native iron (III) form of HRP; this is referred to as the peroxidase cycle.²⁴ The presence of oxygen functionalities on the carbon nanomaterial may also influence the ability of HRP to oxidize the substrate. For example, it has been demonstrated that attractive electrostatic interactions between the negatively charged carboxyl functional groups on the surface of a SWCNT and the positively charged arginine residues of HRP may decrease the distance between the SWCNT and the reactive heme site of HRP thereby permitting oxygen-functionalized SWCNTs but not pristine SWCNTs, which do not possess these negatively charged functional groups, to undergo further oxidation.¹⁴ MPO, a 144 kDa protein dimer that also contains two heme groups, acts in the same way as HRP, except when in the presence of chloride (Cl^-); MPO is known to react with H_2O_2 and Cl^- to yield hypochlorite (ClO^-), which is the major oxidant responsible for the degradation of nanomaterials.^{19,20}

In this work, we extend our study of enzymatic oxidation to SWCNTs synthesized by the high-pressure carbon monoxide (HiPco) method because the small diameter of these nanotubes enables one to probe the material’s electronic properties and chirality distribution using photoluminescence (PL) mapping.²⁵ While oxidation *via* strong acids introduces defects sites on SWCNTs and suppresses their NIR fluorescence, our results indicated that the NIR fluorescence of certain types of SWCNTs is restored upon enzymatic oxidation, which provides new evidence that the oxidation reaction catalyzed by HRP in the presence of H_2O_2 occurs at the defect sites. Employing acid oxidation followed by HRP-catalyzed enzyme oxidation, shortened (< 300 nm in length) and NIR-fluorescent SWCNTs were produced. The resulting products could find applications in NIR imaging and drug delivery systems.

RESULTS

Figure 1 presents the photoluminescence map of SWCNTs at various stages of oxidation. The typical characteristic band-gap luminescence was assigned to SWCNTs chirality according to the literature²⁵. The emissions are disturbed upon the acid oxidation (Figure 1a and 1b),^{11,12} upon HRP/ H_2O_2 treatment, however, the fluorescence returned to the SWCNTs with certain chirality (*i.e.* (9,4), (10,2) and (7,6), Figure 1c). This observation was not detected for the MPO/ H_2O_2 /NaCl system (Figure 1d).

For pristine SWCNTs, four major peaks were determined in the radial breathing mode (RBM) section of the Raman spectra (*i.e.* at 196, 217, 258 and 283 cm^{-1}). Upon acid oxidization, the 283 cm^{-1} peak is fully suppressed (Figure 2a). A similar result was also reported by Yang *et al.* in which they attributed the loss of signal to the destruction of smaller diameter SWCNTs during the acid oxidation.²⁶ The D/G ratio decreases from 0.51 for acid oxidized SWCNTs to 0.24 for acid oxidized SWCNTs treated with HRP/ H_2O_2 . Likewise, a decrease in D/G ratio is observed for the MPO/ H_2O_2 /NaCl treated SWCNTs (Figure 2b); this decrease to 0.27, however, was likely the result of nanotube degradation. Further evidence of degradation is substantiated by the decrease in the RBM and G band signals, which possibly stemmed not only from the loss of resonance as the sp^2 surface is damaged but also from a high fluorescence background arising from organic fragments.^{17,27}

UV-vis-NIR absorption spectra of the samples before oxidation, after acid oxidation, and after enzymatic treatment are presented in Figure 3. In Figure 3a, the S_{11} (900 – 1600 nm) and S_{22} (600 – 900 nm) optical transitions for the semiconducting nanotubes, which are clearly present before oxidation, disappeared after acid oxidation, and reappeared after HRP/ H_2O_2 treatment, which is all in agreement with the PL results. On the other hand, for the MPO/ H_2O_2 /NaCl system, the overall absorbance of the SWCNTs decreased by ~50%; such a loss in absorbance can be attributed to the loss of carbon content (Figure 3b).¹⁹

The spectroscopy results were further supported by transmission electron microscopy (TEM) images of the SWCNTs samples. Figure 4 indicates that the SWCNT bundles were shortened by 35% during the acid treatment (Figure 4b). High resolution TEM (HRTEM, Figure 4c), which was performed on oxidized SWCNTs, revealed both significant bundling of SWCNTs and a rough/defective sidewall structure. For the MPO/ H_2O_2 /NaCl treated oxidized SWCNTs (Figure 4d), only carbonaceous fragments were observed under the TEM, which are the typical products of the degradation process.^{13,16,17,19,29} As tubular objects were not present in the image, a histogram for this sample was not recorded. In contrast, after HRP/ H_2O_2 treatment (Figure 4e and f), a 30% reduction in bundle length was observed. The average length of the HRP/ H_2O_2 treated SWCNT bundles is 215 ± 125 nm. Under HRTEM, this sample appeared to be less bundled and demonstrated more defined CNT sidewall structure.

DISCUSSION

The enzyme-catalyzed oxidation of acid treated SWCNTs revealed some interesting chiral selectivity as demonstrated in Figure 1c. To identify the chiralities of these nanotubes, PL results were cross-examined with the Raman RBM data. From our PL maps (Figure 1c), the (10,2), (7,6) and (9,4) nanotubes were identified, but the Raman spectra demonstrated an absence of the (10,2) species (Figure 2). Therefore, we hypothesize that these seemingly contradictory results stemmed from the S_{22} absorbance bands of (10,2) s-SWCNTs, which have corresponding wavelengths of 733 nm, being out of resonance with the excitation laser (*i.e.* 633 nm) thereby resulting in the absence of their Raman signal.²⁸ From the Raman RBM spectrum of the pristine SWCNTs, four major peaks were located at ~196, 217, 258 and 283 cm^{-1} (Figure 2, back dotted). Further fitting of the RBM data utilizing the Lorentzian function revealed that the four peaks were comprised mainly of (13,4), (13,1), (11,1) and (7,5) SWCNTs respectively, which the former two are metallic and lack photoluminescence properties, whereas the latter two are semiconducting.³⁰ It is also interesting to note that the RBM for the metallic SWCNTs were up-shifted by ~10 cm^{-1} upon acid oxidation; such an up-shift, however, was not observed for the semiconducting SWCNTs at 258 cm^{-1} . While the possible causes of this up-shift are still debatable, with reasons ranging from bundling-induced red-shifts of transition energies,^{31,32} to charge transfer from SWCNTs to $-\text{COOH}$ groups³³, the fact that metallic SWCNTs shift more than

their semi-conducting counterparts agrees with a previous report that metallic tubes are more reactive than semi-conducting SWCNTs in the 0.9 to 1.1 nm diameter range.²⁶ The observed up-shift is reversible upon the HRP/H₂O₂ treatment, which is similar to what has been reported for oxidized SWCNTs upon annealing.³⁴

Meanwhile, the Raman signal for the (7,5) SWCNTs were absent after the acid treatment, and their fluorescence did not return after incubation with HRP/H₂O₂ (Figure 1c), suggesting that the (7,5) nanotubes were destroyed during the acid treatment process.²⁶

The observed decrease in the Raman D/G ratios appeared similar to the data obtained for enzyme-catalyzed oxidation of MWCNTs.¹⁶ While a decrease in the D/G ratio can be interpreted as a decrease in defect density as demonstrated by Strano and coworkers,³⁵ this change would result also from the decrease in both the D and G bands at high degrees of functionalization due to the loss of resonance enhancement. Therefore, a decrease of D/G ratio alone is not a conclusive way to show a decrease in defect density.³⁶ As a result, UV-vis-NIR and photoluminescence spectroscopy were also utilized to provide insight into the defect density of oxidized SWCNTs. Such a degradation-induced decrease in the D/G ratio was exhibited by the oxidized SWCNTs upon MPO/H₂O₂/NaCl treatment and will be discussed later.

Functionalized SWCNTs are known to exhibit a flattened UV-vis-NIR absorbance spectra³⁷ and no NIR fluorescence³⁰ as the introduced defect sites disrupt the electronic structure of the nanotubes. Such behavior was demonstrated by acid treated SWCNTs (Figure 1b and 3). The opposite trend, however, was observed upon enzymatic oxidation *via* HRP/H₂O₂, where the UV-vis-NIR signal and NIR fluorescence were restored, and these results are similar to those reported for Ar annealing (Figure 1c and 3a).³⁸ Our PL map for the acid treated SWCNT sample after the HRP/H₂O₂ reaction revealed the presence of nanotubes that are (10,2), (7,6) and (9,4); their corresponding absorption bands were also present on the UV-vis-NIR absorption spectrum (Figure 3a, blue). Combining our Raman and optical results, we concluded that the HRP/H₂O₂ reaction was specifically attacking defect sites and restoring the sp² lattice.

In Figure 1c, the emission (λ_{em}) for oxidized (7,6) SWCNTs that were treated with HRP/H₂O₂ was blue-shifted by ~14 nm (*i.e.* from ~1122 nm to ~1108 nm). In contrast, when a duplicate experiment was performed, acid oxidized SWCNTs that were treated with HRP/H₂O₂ did not demonstrate any blue shift (Figure S2). We attributed these apparently contradictory results to differences in the local environments.³⁹ Also, for both sets of data, the same restoration of emission was observed for (9,4), (8,6) and (7,6) chiralities with the removal of (6,5), (7,5) and (8,4) species (Figure S2), which is consistent with previous studies that show that acid treatment removes SWCNTs with diameters smaller than 0.88 nm (*i.e.* (8,6) SWCNTs have a diameter of 0.966 nm).^{26,28} Finally, the presence of (8,6) was more intense in the duplicate experiment.

We attribute these results to the removal of defect sites (most likely oxygen-containing functional groups) from the surfaces of SWCNTs. The data presented herein indicated that HRP/H₂O₂ oxidized the functionalized SWCNTs through reactive intermediates of the peroxidase cycle thereby removing oxygen groups and restoring the sp² lattice possibly through decarboxylation.^{14,16,17,40} As suggested by Kane and colleagues, removal of carboxyl groups have only one intact bond that is connected to the CNT backbone; removal of hydroxyl, epoxide and peroxides groups, on the other hand, require breaking three backbone bonds.⁴¹ Therefore, the decarboxylation would be more energetically favorable than the oxidation and eventual removal of other oxygen-containing functional groups.⁴¹ Our work, however, does not suggest any further oxidation of graphitic lattice of the

nanotube after the enzymatic reaction. Instead, our Raman D/G ratio, UV-vis-NIR absorbance and PL map all indicate a reduction in the defect sites and a restoration of sp^2 lattice.

To exclude the possibility that the return in PL emission resulted from the reduction of oxygen functional groups via the oxidase cycle of HRP,²⁴ a control experiment was performed, where oxidized SWCNTs were incubated in the absence of H_2O_2 for 35 days. Under our experimental conditions, no PL emission, which is characteristic of SWCNTs, was observed (Figure S3) thereby indicating that oxidation via the peroxidase cycle and not reduction via the oxidase cycle is the likely mechanism for the return of fluorescence.

Density Functional Theory (DFT) calculations were performed to estimate the overall energy change of decarboxylation for a SWCNT that contained both a carboxyl and an adjacent ketone group (Figure 5). To estimate the overall energy of decarboxylation for a (14,0) SWCNT functionalized with both a carboxyl and a ketone group, the minimum energy path using the nudged elastic band was determined for the following reaction:

The reactants correspond to a $-COOH$ group and the oxygen of the ketone group both adsorbed on the neighboring sites of a sidewall ring are shown schematically in the inset image of Figure 5. The functionalization disrupts locally the π -orbital network of the tube. The products correspond to complete decarboxylation of the oxidized SWCNT resulting in the formation of a hydroxyl group on the nanotube and carbon dioxide in the gas phase.¹⁴ As can be seen in Figure 5b, the decarboxylation process proceeds with a small energy barrier of 0.15 eV. In the transition state shown as an inset in Figure 5b, the hydrogen of $-COOH$ group rotates from the optimum position towards the oxygen of the ketone group. It is also noteworthy that the decarboxylation process is exothermic and thermodynamically very favorable with an energy gain of ~ 2.9 eV per desorbed CO_2 molecule in the gas phase.

Although the employed model of the SWCNT is very idealistic as it ignores the presence of defects, it nevertheless reveals that the decarboxylation is thermodynamically very favorable under experimental conditions. To show that this is also the case even in the presence of CNT defects, we examined the energetics of potential initial and final structures in the decarboxylation process. This is shown in Figure 5(c) and (e) for a (14,0) SWCNTs with a carbon vacancy that is functionalized with $-COOH$ and two ketone groups. As in the case of the pristine SWCNT, the decarboxylation process for the defected CNT is also exothermic by ~ 1.75 eV for each desorbed CO_2 molecule in the gas phase. Configuration (d) is a potential transition state for this process and is only 0.25 eV higher in energy than the initial structure. In the presence of a solvent as is the case in the experiments, it would be expected that this transition state would be lower in energy as the proton can be transferred from solution to passivate the dangling bond of the CNT that is left by the desorbed CO_2 molecule.

The decarboxylation process was also evidenced in a control experiment, where pristine HiPco SWCNTs were subjected to HRP/ H_2O_2 oxidation and demonstrated an improved UV-vis-NIR and NIR fluorescence signal as well as a decrease in Raman D/G ratio (Figure S4). Recent research has suggested that defect sites are present in pristine HiPco SWCNTs, which constrain the fluorescence to $\sim 40\%$ of their maximum brightness.⁴² Therefore, HRP/ H_2O_2 oxidation may be removing defect sites on the pristine SWCNTs just as this enzymatic system removes defect sites on the oxidized samples.

The MPO/H₂O₂/NaCl reaction is known to produce hypochlorite (ClO⁻), which is the strongest oxidant of the MPO system.^{19,20} ClO⁻ oxidizes the sidewall of SWCNTs, which in turn, damages the sp² network and results in loss of the resonance condition for the Raman response. In comparison to the HRP/H₂O₂ reaction, the MPO/H₂O₂/NaCl system demonstrated no return of fluorescence, which suggested a different oxidation mechanism that does not involve the restoration of sp² surfaces. Therefore, the acid treated SWCNTs were likely degraded by the MPO/H₂O₂/NaCl system as evidenced by the fragment-induced fluorescence in the Raman spectrum (Figure 2b),^{17,27} the loss of overall UV-vis-NIR absorption (Figure 3b),^{19,20} and the fragmented by-products visualized by TEM (Figure 4d).

Pristine SWCNTs were also subjected to the MPO/H₂O₂/NaCl system. Given the same time scale, no degradation was observed for the pristine SWCNTs (Figure S5); we believe that this observation was a consequence of insufficient ClO⁻ being produced under the current experiment setup. For a different control experiment (Figure S6), pristine SWCNTs were incubated with 0.20 M of NaClO; the SWCNTs were destroyed within 1 day thereby providing evidence that high concentrations of ClO⁻ can degrade even pristine SWCNTs.

In comparison to earlier work on HRP/H₂O₂ oxidation of larger diameter SWCNTs,^{13,14} the smaller diameter HiPco SWCNTs degraded at a slower rate. The complete degradation of carbon nanotubes by HRP/H₂O₂ system was reported after approximately 30 days at room temperature.^{14,16,17} Since the oxygen-containing defects were known to be essential for this enzymatic oxidation,^{14,16,17} one might expect the defect density to be higher for the large diameter SWCNTs than the SWCNTs used in this work, where the lower defect density would be responsible for the slower reaction rate. To test this hypothesis the defect density was determined by acid titration method following a published procedure.^{16,43} The oxygen-containing defect density for the SWCNTs used in the work was calculated to be 7.8 ± 0.5 $\mu\text{mol/mg}$ versus 3.4 ± 0.2 $\mu\text{mol/mg}$ from the large diameter SWCNTs used before. This affirms that defect density was not a reason for the difference in the degradation rate as SWCNTs with smaller diameter have both a higher defect density and a slower rate than their larger diameter counterparts. Though this defect density value of 7.8 ± 0.5 $\mu\text{mol/mg}$ is an average number from SWCNTs of all chiralities in the sample, and it is likely that some SWCNTs (*e.g.* metallic tubes) are more defective than others as reported by the literature.²⁶ The difference in our results cannot be solely explained by defect density.

Selectivity by the redox potential of SWCNTs has been suggested and utilized by O'Connell *et al.*⁴⁴ A similar reasoning was constructed by us for the oxidation of HRP, MPO and HOCl.¹⁸ Recently, the redox potential of SWCNTs with different chiralities and diameters were modeled⁴⁵ and experimentally measured⁴⁶ *via* electrochemistry coupled with spectroscopy. In Figure 6, the electrochemical potentials of ized SWCNTs were incubated in the ae plotted against the oxidation potentials of HRP, MPO and HOCl (black dashed line).^{18,44} The electrochemical potentials of the SWCNTs valence band have been shown to increase as the tube diameter decreases (Figure 6 V1s line). In this model, electron transfer takes place from the top of the valence band to the oxidizing species. With HRP/H₂O₂, the redox potential is ~0.95 V, which is higher than the V1s bands of the large diameter SWCNTs (pink region), but similar to the small diameter SWCNTs (green region). As a result of this difference in the redox potential, large diameter SWCNTs are more favorably oxidized relative to smaller diameter SWCNTs, which is in agreement with the shortened degradation time that we observed for large SWCNTs.^{14,16} For the MPO/H₂O₂/NaCl system, HOCl is produced and demonstrates a redox potential of 1.48 V,¹⁸ which represents a much higher oxidizing capacity than HRP. At this potential, HOCl oxidizes even small diameter SWCNTs, which have significant potential difference, as evidenced by the degradation of acid treated HiPco SWCNTs *via* the MPO/H₂O₂/NaCl system. Furthermore, the fact that small diameter pristine SWCNTs, which cannot be degraded by HRP/H₂O₂

(Figure S4), were degraded by hypochlorite ions (Figure S5) also hold up for the electrochemical potential argument.

While this model works well for pristine SWCNTs, this carbon-based nanomaterial is often p-doped by acid treatment,³³ which further increases their redox potential and theoretically lowers their reactivity compared to the pristine SWCNTs by drawing their valence band closer to the oxidation potential of reactive species. This may not be the case, however, as pristine SWCNTs were shown not to be degradable by enzymatic oxidation.^{14,16} Therefore, certain functionalities are required to undergo the peroxidase cycle. Carboxyl and hydroxyl (phenol) groups have labile hydrogen atoms that assist the peroxidase cycle, in which Compound I oxidizes a substrate by two sequential, one electron oxidation steps to form water. In pristine SWCNTs that have neither functional groups nor labile hydrogen atoms, the biodegradation of pristine SWCNTs cannot be initiated.

From our results, the HRP/H₂O₂ oxidation has resulted in a fluorescence restoration of SWCNTs indicating a chemical pathway in which functional groups have to be eliminated in order to restore their sp² lattices. Such pathway was not observed in the MPO/H₂O₂/NaCl or the NaOCl system. From the degradation of pristine SWCNTs by NaOCl, it appears that the hypochlorite ions directly attack the sidewalls of the SWCNTs.

CONCLUSION

HRP/H₂O₂ is capable of oxidizing highly defective, carboxylated SWCNTs and restoring their optical properties. To this end, SWCNTs that were oxidized by a strong acid mixture and demonstrated weak absorption bands with no luminescence were further oxidized using HRP/H₂O₂ to yield shorter, less defective CNTs with well-defined absorption bands and strong luminescent signal. HRP may be capable of oxidizing only carboxylic acid groups, which would be removed as CO₂ from the CNT lattice.^{14,16,17} It is possible that other oxygen containing functional groups such as hydroxyl, epoxide and peroxides groups are not as easily removed from the lattice as carboxylic groups, due to the number of bonds between the carbon in the oxygen functional group and the lattice.⁴¹ In contrast, when treated with MPO/H₂O₂/NaCl, the oxidized HiPco SWCNTs underwent complete oxidation (*i.e.* degradation) as a result of the highly oxidative reagent, ClO⁻. The shortened, fluorescent SWCNTs produced by HRP and H₂O₂ may find applications as NIR imaging agents/nanocarriers for use in medical diagnostics and therapeutics.

MATERIALS AND METHODS

Chemical Oxidation

Purified high-pressure carbon monoxide (HiPco) SWCNTs^{38,48} were purchased from NanoIntegris, Inc. (Skokie, IL). 10 mg of the SWCNTs were sonicated in sulfuric acid (Fisher Scientific)/nitric acid (J.T. Baker) solution (15 mL H₂SO₄, 5 mL HNO₃) for 3 hours 50 minutes at 40 °C. The oxidized SWCNT sample was diluted with deionized (DI) water, vacuum filtered, washed with DI water several times, and collected on separate polytetrafluoroethylene (PTFE) filter papers to form thin films known in the literature as buckypaper.⁴⁹ Pieces of the buckypaper were cut and redispersed in DI water by sonication for 40 minutes at room temperature. The resulting suspensions were centrifuged (3,500 RPM, 20 minutes), and the supernatant was collected for enzymatic oxidation.

Enzymatic Oxidation of SWCNTs Using Horseradish Peroxidase (HRP)

The procedure for the enzymatic oxidation of SWCNT with HRP/H₂O₂ is described elsewhere.^{13,14,16} Briefly, 8 mL of the supernatant that contained oxidized SWCNTs were combined with 1.5 mg of HRP (Sigma Aldrich). The sample was incubated for 24 hours at

room temperature on a shaker in the absence of light. After the incubation period, 4 μL of hydrogen peroxide (0.089 M, Sigma Aldrich) was added daily to the oxidized SWCNT sample for 35 days. The sample was incubated with continuous shaking during this period. In a separate control experiment, non-oxidized SWCNTs were treated with 1.5 mg of HRP/ H_2O_2 employing the same method. Both samples were characterized after 35 days using UV-vis-NIR spectroscopy, transmission electron microscopy (TEM), Raman spectroscopy, and PL mapping.

Enzymatic Oxidation of SWCNTs Using Myeloperoxidase (MPO)

The protocol for enzymatic oxidation catalyzed by MPO was adopted from Kagan *et al.* with the total volume scaled for spectrometer requirements.¹⁹ Lyophilized purified native human myeloperoxidase (MPO) was purchased from Athens Research and Technology, Inc. (Athens, GA, USA) and was reconstituted with 350 μL of nanopure water for a final concentration of 2.0 μM . Sodium chloride, diethylene triamine pentaacetic acid (DTPA) and 0.1 M phosphate buffer were purchased from Sigma Aldrich. 100 μL of the oxidized SWCNTs supernatant solution were mixed with 21 μL of 5 M NaCl, 45 μL of 5 mM DTPA, 12 μL of MPO solution, 4 μL of 18.75 mM H_2O_2 and 568 μL of 0.1 M phosphate buffer. Every hour, 4 μL of 18.75 mM H_2O_2 were added for a total of 7 additions a day for 5 days. The MPO solution was added to the mixture at a rate of 12 μL per day to compensate the loss of enzyme activity in the incubation system. The sample was characterized after 5 days using UV-vis-NIR spectroscopy, TEM, Raman spectroscopy, and PL mapping. In a separate control experiment, non-oxidized SWCNTs were treated with MPO/ H_2O_2 /NaCl under the same conditions. In a different control experiment, pristine SWCNTs were incubated with 0.20 M of NaClO for 1 day to explore the effect of high NaClO concentrations on SWCNTs degradation.

Raman Spectroscopy

SWCNTs were excited with a 633 nm laser, and measurements were performed using the Renishaw InVia Raman microscope (Wotton-under-Edge, UK). Aliquots (~ 0.1 mL) of suspended SWCNTs were drop-casted on a glass slide and allowed to dry in ambient conditions overnight thereby forming aggregates of SWCNTs. Scans were carried out at 1.7 mW laser power with 10 second accumulation time over the range of 100 to 3200 cm^{-1} . Spectra were acquired from multiple locations on the SWCNT aggregates, and the results were normalized to the most intense peak and averaged in accordance with published procedures.⁵⁰

Transmission Electron Microscopy (TEM) and Histogram Determination

A suspension of dispersed SWCNTs (6 μL) was diluted by a factor of 100 and drop-casted onto lacey carbon coated copper grids (Pacific-Grid Tech, San Francisco, CA) and allowed to dry overnight. Analysis was performed using a FEI Morgagni TEM (Hillsboro, OR) with an 80 keV electron beam. The mean SWCNT length distributions were derived from the TEM micrographs for approximately 100 nanotubes per sample.

High Resolution Transmission Electron Microscopy (HRTEM)

High resolution TEM images were obtained with a JEOL 2100F microscope at an accelerating voltage of 200 kV. TEM samples were evenly dispersed in aqueous solution. 10 μL of each sample were drop-casted on an ultrathin carbon film/copper TEM grids (Ted Pella) and dried at room temperature overnight before imaging.

UV-vis-NIR Spectroscopy and Photoluminescence Mapping

Sodium cholate was added to the SWCNT suspensions (pristine SWCNTs, acid oxidized SWCNTs, HRP treated SWCNT and MPO treated SWCNTs respectively) at a concentration of 1% w/v, and the suspensions were sonicated for 2 hours. UV-vis-NIR measurements of dispersed SWCNTs were acquired using a Perkin Elmer Lambda 900 spectrophotometer over the wavelength range 200 to 1300 nm. Photoluminescence maps were attained employing a JY Horiba Fluorolog-322 (Kyoto, Japan) spectrofluorometer equipped with an Electro-Optical Systems, Inc. (Phoenixville, PA) DSS-IGA020 L detector. The excitation wavelength was scanned from 580 to 800 nm in 5 nm increments, and the emission was detected from 900 to 1300 nm in 2 nm increments. Spectra were corrected for variation in lamp intensity and monochromator response.

Density Functional Theory (DFT) Calculations

The quantum chemical calculations of equilibrium geometries were carried out using the self-consistent charge-density-functional-based tight-binding (SCC-DFTB) method.⁵¹ The SCC-DFTB method has been successfully applied to a wide class of systems of interest including carbon nanotubes.⁵² As a validity check on our calculations, we find that the adsorption energy of COOH on (11,0) CNTs is 1.47 eV, which is in good agreement with 1.42 eV obtained previously using standard DFT calculations. The minimum energy paths are determined with the nudged elastic band (NEB) method.⁵³ Calculations were carried out using a periodic supercell approach with three repeat units of a (14,0) SWCNT sampled at the Γ -point of the Brillouin zone.

Modified Boehm's Titration

A modified Boehm's Titration procedure was utilized to determine the oxygen-containing functional group density on the surface of the acid oxidized SWCNTs.^{16,43} Approximately 3 mg of oxidized SWCNTs were dispersed in 6 mL NaOH (Mallinckrodt Chemical, Inc. MO) at 10 mM concentration. The sample was sealed with a septum stoppers and sonicated and degassed for 1.5 minutes under vacuum. The samples were incubated for 72 hours. After the incubation process, the solutions were filtered through a 0.22 μ m Teflon membrane. A small amount of the filtrate (0.25 mL) was transfer to a separate vial and added with 10 μ L of indicator, 0.1% Bromocresol Green and Methyl Red (Sigma Aldrich) aqueous solution. The solution was titrated with 0.0082 M HCl (Fisher) using a pipette (Eppendorf, Germany). A blank control consisting of only NaOH was also treated under the same procedure to determine the concentration of NaOH. All titrations were repeated for at least three times. The defect density was calculated from the NaOH uptake by the SWCNTs over the mass (in the unit of μ mol/mg). SWCNTs with larger diameters ($\sim$ 1.4 nm) were purchased from Carbon Solutions, Inc. CA and were oxidized for 4 hours using the acid oxidation method. The sample was also characterized for defect density.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Photoluminescence (PL) maps of SWCNTs (a) before oxidation and (b) after acid oxidation. PL maps of the acid oxidized SWCNT sample (c) after treatment with HRP/H₂O₂ and (d) after treatment with MPO/H₂O₂/NaCl. Prior to PL mapping 1%wt of sodium cholate was added, and the samples were sonicated for 2 hr. Literature values were employed to assign SWCNT chirality.²⁵

Figure 2.

Enlarged radial breathing mode (RBM) section and the full Raman spectra of (a) HRP/H₂O₂ and (b) MPO/H₂O₂/NaCl treated SWCNTs at various experimental stages (*i.e.* before oxidation (black, dotted), after oxidation (red), and after enzymatic reaction (blue)). SWCNT chirality was assigned according to the literature.²⁸

Figure 3. UV-nvis-NIR absorption spectra of (a) HRP/H₂O₂ and (b) MPO/H₂O₂/NaCl treated SWCNTs at various experimental stages (*i.e.* before oxidation (black, dotted), after oxidation (red), and after enzymatic reaction (blue)).

Figure 4.

Transmission electron microscopy (TEM) micrographs of SWCNT bundles (a) before and (b) after acid oxidation. (c) High Resolution TEM (HRTEM) of the acid oxidized SWCNTs bundles. TEM micrographs of the acid oxidized SWCNT bundles (d) after treatment with MPO/H₂O₂/NaCl and (e) after treatment with HRP/H₂O₂. (f) HRTEM micrograph of the HRP/H₂O₂ treated sample. Insets depict histograms of the length distributions for the SWCNT bundles at various experimental stages.

Figure 5.

(a) Top view of the COOH functionalized (14,0) SWCNT. (b) Reaction energy profile for decarboxylation of a (14,0) pristine SWCNT functionalized with a carboxyl and a ketone group. Side views of selected parts of the SWCNT are also shown for the initial, transition and final configurations during the decarboxylation process. The potential energy surface near the final state is very flat due to small energy barrier for the rotation of -OH group around the O-C bond, as can be seen by inspecting the inset images. (c) and (e) show initial and final configurations for the decarboxylation process of a defective (14,0) CNT functionalized with -COOH and two ketone groups. This process is exothermic with 1.75 eV per CO₂ molecule. (d) shows the structure of a possible intermediate transition state where the hydrogen of -COOH group is transferred to the ketone group forming an -OH group with the release of CO₂ in the gas phase. Carbon atoms are turquoise, oxygen is red, and hydrogen is white.

Figure 6.

Electrochemical potentials of SWCNTs with different diameters plotted against the oxidation potentials of HRP, MPO and HOCl (black dashed line). The dependence of electrochemical potentials of SWCNTs on their diameter were modeled in Ref 45 with experimental data adapted from Ref 46 (squares). The green region indicates the diameter range of SWCNTs studied in this work (HiPco). The pink region indicates the larger diameter SWCNTs utilized in earlier work.^{13,14} HRP, MPO and HOCl potentials were adapted from Ref 18. Structures of HRP and MPO possible binding positions with carboxylated SWCNTs (right) were adapted from Ref 14 and Ref 19, respectively. The density of states diagrams (left) were adapted from Ref 47, indicating the valence and conduction band of the semiconducting and metallic SWCNTs.