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Protective roles of single-walled carbon nanotubes in ultrasonication-induced DNA base damage

Elijah J. Petersen[1], Xiaomin Tu[2], Miral Dizdaroglu[1], Ming Zheng[2], Bryant C. Nelson[1*]*

[1]Biochemical Science Division and [2]Polymers Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

[*] Dr. Bryant C. Nelson
Biochemical Science Division, National Institute of Standards and Technology
Gaithersburg, MD 20899 (United States)
E-mail: Bryant.nelson@nist.gov
Dr. Ming Zheng
Polymers Division, National Institute of Standards and Technology
Gaithersburg, MD 20899 (United States)
E-mail: Ming.zheng@nist.gov

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Engineered nanomaterials are a promising new set of materials with exciting properties that promise a broad range of applications. However, concerns about their potential toxic effects are one of the primary factors slowing down their use and application in biomedicine [1]. Perhaps the foremost of proposed mechanisms for how nanomaterials may induce harmful effects is by oxidative damage to biomolecules [2, 3], yet studies in this area generally demonstrate a lack of molecular level understanding. In particular this has been the case for single-wall carbon nanotubes (SWCNTs), a family of prototypical nanomaterials with well-defined molecular structures, that have been one of the most extensively studied nanomaterials with regard to their toxic effects. The potential toxic effects of SWCNTs have been a topic of substantial interest and many contradictory observations have been reported [4-7], yet there is often a perception that SWCNTs are highly toxic. We attempt to establish well-

controlled *in vitro* systems to quantitatively examine the effect of SWCNT on biomolecules. Ultrasonication is a process that has been shown to damage DNA bases [8-10] through the production of hydroxyl radicals ($^{\bullet}OH$), hydrogen atoms ($H^{\bullet}$), hydrogen peroxide (H_2O_2), and superoxide radicals ($O_2^{\bullet-}$) [8, 11]. During ultrasonication, the driven cavitation, expansion and implosion of bubbles, can generate extremely high temperatures and can split water molecules into $^{\bullet}OH$ and $H^{\bullet}$. Hydroxyl radicals can react with $H^{\bullet}$ to form H_2O_2, or directly interact with other molecules. This process is similar to oxidative stress-induced damage in cells, albeit at substantially more intense conditions. Ultrasonication is also used to suspend carbon nanotubes (CNTs) in the presence of single-stranded DNA (ssDNA) oligomers [12, 13]. However, the extent to which this process damages the DNA bases and thus could impact the non-covalent interactions of ssDNA sequences with CNTs is unknown; ultrasonication has also been shown to damage and shorten the SWCNTs themselves [14-16]. An understanding of how SWCNTs impact oxidatively induced DNA damage under the highly reactive conditions of ultrasonication may yield insights into the redox properties of SWCNTs, how they may interact in cellular environments with electron pathways critical to healthy cell functioning, and the extent to which SWCNT potentiate oxidative stress. In this work, we have used high-sensitivity mass spectrometry analysis to quantify ultrasonication-induced DNA damage in $(ATT)_{14}$ and $(GT)_{20}$ oligomers in the presence and absence of SWCNTs. We have quantified a range of DNA base lesions derived from reductive [2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] and oxidative [(8-hydroxyguanine (8-OH-Gua) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd)] transformation of the initial OH-adduct radicals. We find that the overall level of accumulated DNA damage is reduced in the presence of SWCNTs, suggesting a protective effect of the SWCNTs. We cannot exclude the possibility of generation of the aforementioned radical species and H_2O_2 by SWCNTs, but we maintain that the overall effect of SWCNT

must be scavenging rather than generation of these species in order to be consistent with the observed decrease in DNA lesions in the presence of SWCNTs. As shown by by others in the literature [17-18], SWCNTs do not generate detectable levels of $^{\bullet}OH$, $H^{\bullet}$, $O_2^{\bullet-}$ and H_2O_2 unless under the condition of UV light irradiation. Our data also show that DNA base lesions formed by the reductive pathway from the initial OH-adduct radicals are preferentially decreased as compared to those formed by the oxidative pathway. Lesion formation under these conditions has also been quantified in the presence and absence of dimethyl sulfoxide (DMSO), a well-known scavenger of $^{\bullet}OH$, to elucidate the molecular basis for the SWCNT effects.

Ultrasonication of the $(ATT)_{14}$ and $(GT)_{20}$ oligomers for 1 h produced substantial, and statistically significant, increased levels for all of the lesions tested (see **Figure 1**), in accordance with previous findings that ultrasonication substantially damages DNA [8, 9]. The lesion levels typically increased nearly an order of magnitude from the un-sonicated control samples. The addition of DMSO significantly decreased the lesion levels produced during ultrasonication. The decrease in lesion levels after DMSO addition was observed in preliminary experiments to follow a dose-dependent trend with 1.0 % DMSO causing a greater decrease than 0.1 % (data not shown). The presence of SWCNTs also significantly decreased the lesion levels. The combined use of DMSO and SWCNTs had an additive effect, reducing the lesion levels more significantly than either treatment alone. The combination treatment decreased the lesions levels such that they were not significantly greater than those of the un-sonicated controls.

Importantly, the lesions levels measured here were not high enough to substantially impact the wrapping of DNA oligomers onto SWCNTs. In agreement with this finding, the presence of DMSO during the sonication process to prepare ssDNA-wrapped SWCNTs had negligible impact on nanotubes' fluorescence quantum yield as compared to SWCNTs sonicated with DNA but without DMSO (data not shown). One important trend observed is that the presence

of SWCNTs caused a more significant decrease in the levels of the lesions formed by one-electron reduction (FapyAde and FapyGua) of the intermediate OH-adduct radicals (see Scheme 1 for proposed mechanism for the $(GT)_{20}$ oligomers) as compared to one-electron oxidation (8-OH-Gua) and reaction with oxygen followed by ring-reduction (5-OH-5-MeHyd). This protective behavior of SWCNTs toward the oligomers differed from that observed for DMSO for which there did not appear to be a difference in the impacts on reductive and oxidative pathways for $(ATT)_{14}$ oligomers (**Table 1**) although there did appear to be a difference for $(GT)_{20}$ oligomers (**Table 2**). When comparing the relative effects of SWCNTs and DMSO, it is important to note that both were present at similar nominal concentrations of 0.1% on a mass fraction basis in the test samples. SWCNTs were shown to decrease all of the measured lesions, a result that likely stems from the capacity of the SWCNTs to scavenge $^{\bullet}OH$, in combination with their unique effects on reductive reaction pathways (**Scheme 1**). Together, our results suggest that SWCNTs behave as $^{\bullet}OH$ scavengers and/or as electron sinks. Our explanation for the mechanism of formation of products formed by one-electron oxidation (8-OH-Gua) and one-electron reduction (FapyGua) of the OH-adduct radical is given in **Scheme 1**. We propose that SWCNTs effectively act as electron scavengers, and thereby decrease the chance of DNA lesion formation through the one-electron reduction of the OH-adduct radical. In contrast, DMSO at the concentration used reacts with $^{\bullet}OH$ with a diffusion-controlled reaction rate, before $^{\bullet}OH$ can react with a DNA base, preventing the formation of the OH-adduct radical and thus product formation.

The fact that SWCNTs caused a decrease in oxidatively-induced DNA base damage suggests that SWCNTs may actually mitigate oxidative damage to cellular macromolecules. One related recent study on SWCNT toxicity with *E. coli* bacteria showed that SWCNTs cause a depletion of antioxidant glutathione in the organisms in a time-dependent manner, and that metallic SWCNTs had a greater impact than semiconducting SWCNTs likely as a result

of their enhanced electrical conductivity properties [19]. It was not possible in the current experiment to differentiate the impacts of the semiconducting and metallic SWCNTs on the formation of the DNA base lesions. Overall, the SWCNTs showed a remarkable potential to decrease the formation of oxidatively-induced lesions during the aggressive conditions produced by ultrasonication. It remains to be determined to what extent the SWCNTs would have similar effects in environments where the concentrations of free radicals are substantially smaller such as within cells, and how SWCNTs may interact with various cellular pathways or mitigate harmful oxidative stresses. Once inside a cell though, SWCNTs may become a redox mediator and conserve the reductive power of the cell. While it is clear that SWCNTs may mitigate the impacts of certain oxidative stresses in cells, their overall impact may be nuanced and dependent on the cellular environment and the extent to which the cell is under various stresses.

Experimental Section

We chose a well characterized SWCNT material, CoMoCAT, for this study. The chirality distribution in CoMoCAT [20], optical spectroscopy characterization of these SWCNTs, and their non-covalent interaction with ssDNA have been well-documented [21, 22]. Two representative DNA oligomers, $(GT)_{20}$ and $(ATT)_{14}$, were used. To prepare sonicated oligomer samples, 1 mg of an oligomer was dissolved in 1 mL 0.1 mol/L NaCl solution followed by ultrasonication. To obtain sonicated oligomers in the presence of SWCNTs, 1 mg of an oligomer in 1 mL 0.1 mol/L NaCl solution was first mixed with 1 mg of SWCNT and ultrasonicated under identical conditions. To separate the oligomers from the SWCNTs, 10 µL of 10 % (mass fraction) sodium deoxycholate solution was added to the oligomer-SWCNT suspension and incubated for a few hours [12]. To collect the oligomer for analysis, the sample was repeatedly ultracentrifuged until all SWCNTs were removed; samples without SWCNTs were similarly centrifuged. The remaining clear supernatant was filtered with a

MWCO 10 000 cellulose membrane filter to remove the salt and the surfactant by collecting the oligomer on the membrane. To examine the effect of an •OH scavenger, 0.1 % (mass fraction) DMSO was added into the DNA samples before ultrasonication with and without SWCNTs. For the control experiments, DNA samples without ultrasonication were used.

Gas chromatography/mass spectrometry (GC/MS) with isotope-dilution was used to determine the levels of different oxidatively modified DNA bases in treated and untreated $(GT)_{20}$ and $(ATT)_{14}$ oligomers [23-25]. Five independent samples were prepared for every treatment. After additional washing steps, oligomer aliquots of 50 µg were prepared from each sample and stable isotope-labeled analogues of the base lesions (8-OH-Gua-$^{15}N_5$, FapyGua-^{13}C,$^{15}N_2$, 5-OH-5-MeHyd-^{13}C,$^{15}N_2$, and thymine glycol- d_4 for $(GT)_{20}$ oligomers, and FapyAde-^{13}C,$^{15}N_2$, 5-OH-5-MeHyd-^{13}C, $^{15}N_2$, and thymine glycol-d_4 for $(ATT)_{14}$ oligomers) were added to each sample. These samples were then dried under vacuum and stored at 4 °C prior to enzymatic digestion.

Two enzymes, *E. coli* Fpg and endonuclease III were used for digestion of oligomers. Hydrolysis using these enzymes prevents artifactual formation of DNA lesions because it only releases modified bases; consequently, there is no intact DNA base present during the trimethylsilylation step (see below).[24] Digested samples were solubilized, lyophilized, and then trimethylsilylated. GC/MS measurements were performed as previously described [21]. Trimethylsilyl derivatives of DNA lesions and their stable isotope-labeled analogues were detected using electron ionization mass spectrometry in selected-ion-monitoring mode. Quantification of DNA base lesions was determined using the signal area ratios from the modified base of interest and its labeled analogue in conjunction with the known amount of labeled analogue added to each sample. We identified and quantified FapyGua, 8-OH-Gua, and 5-OH-5-MeHyd in the $(GT)_{20}$ oligomers, and FapyAde and 5-OH-5-MeHyd in the $(ATT)_{14}$ oligomers. Thymine glycol could not be accurately quantified.

[1] Kostarelos, K.; Bianco, A.; Prato, M., *Nat. Nanotech.* **2009,** *4* (10), 627-633. DOI 10.1038/nnano.2009.241.

[2] Stone, V.; Donaldson, K., *Nat. Nanotech.* **2006,** *1* (1), 23-24. DOI 10.1038/nnano.2006.69.

[3] Nel, A.; Xia, T.; Madler, L.; Li, N., *Science* **2006,** *311* (5761), 622-627.

[4] Bai, Y. H.; Zhang, Y.; Zhang, J. P.; Mu, Q. X.; Zhang, W. D.; Butch, E. R.; Snyder, S. E.; Yan, B., *Nat. Nanotech.* **2010,** *5* (9), 683-689. DOI 10.1038/nnano.2010.153.

[5] Schipper, M. L.; Nakayama-Ratchford, N.; Davis, C. R.; Kam, N. W. S.; Chu, P.; Liu, Z.; Sun, X. M.; Dai, H. J.; Gambhir, S. S., *Nat. Nanotech.* **2008,** *3* (4), 216-221.

[6] Petersen, E. J.; Zhang, L. W.; Mattison, N. T.; O'Carroll, D. M.; Whelton, A. J.; Uddin, N.; Nguyen, T.; Huang, Q. G.; Henry, T. B.; Holbrook, R. D.; Chen, K. L., *Environ. Sci. Technol.* **2011,** *45* (23), 9837-9856. DOI 10.1021/es201579y.

[7] Aschberger, K.; Johnston, H. J.; Stone, V.; Aitken, R. J.; Hankin, S. M.; Peters, S. A. K.; Tran, C. L.; Christensen, F. M., *Crit. Rev. Toxicol.* **2010,** *40* (9), 759-790. DOI 10.3109/10408444.2010.506638.

[8] Riesz, P.; Kondo, T., *Free Radical Biol. Med.* **1992,** *13* (3), 247-270. DOI 10.1016/0891-5849(92)90021-8.

[9] Elsner, H. I.; Lindblad, E. B., *DNA* **1989,** *8* (10), 697-701. DOI 10.1089/dna.1989.8.697.

[10] Milowska, K.; Gabryelak, T., *Biomol. Eng.* **2007,** *24* (2), 263-267. DOI 10.1016/j.bioeng.2007.02.001.

[11] Edmonds, P. D.; Sancier, K. M., *Ultrasound Med. Biol.* **1983,** *9* (6), 635-639. DOI 10.1016/0301-5629(83)90009-1.

[12] Roxbury, D.; Tu, X. M.; Zheng, M.; Jagota, A., *Langmuir* **2011,** *27* (13), 8282-8293. DOI 10.1021/la2007793.

[13] Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; McLean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G., *Nat. Mater.* **2003,** *2* (5), 338-342.

[14] Heller, D. A.; Barone, P. W.; Strano, M. S., *Carbon* **2005,** *43* (3), 651-653.

[15] Chen, C. Y.; Jafvert, C. T., *Carbon* **2011,** *49* (15), 5099-5106. DOI 10.1016/j.carbon.2011.07.029.

[16] Chen, C. Y.; Jafvert, C. T., *Environ. Sci. Technol.* **2010,** *44* (17), 6674-6679. DOI 10.1021/es101073p.

[17] Huang, X.; McLean, R. S.; Zheng, M., *Anal. Chem.* **2005,** *77* (19), 6225-6228. DOI 10.1021/ac0508954.

[18] Hennrich, F.; Krupke, R.; Arnold, K.; Rojas Stütz, J. A.; Lebedkin, S.; Koch, T.; Schimmel, T.; Kappes, M. M., *J. Phys. Chem. B* **2007,** *111* (8), 1932-1937. DOI 10.1021/jp065262n.

[19] Vecitis, C. D.; Zodrow, K. R.; Kang, S.; Elimelech, M., *ACS Nano* **2010,** *4* (9), 5471-5479. DOI 10.1021/nn101558x.

[20] Jorio, A.; Santos, A. P.; Ribeiro, H. B.; Fantini, C.; Souza, M.; Vieira, J. P. M.; Furtado, C. A.; Jiang, J.; Saito, R.; Balzano, L.; Resasco, D. E.; Pimenta, M. A., *Phys. Rev. B: Condens. Matter* **2005,** *72* (7). DOI 10.1103/PhysRevB.72.075207.

[21] Bachilo, S. M.; Balzano, L.; Herrera, J. E.; Pompeo, F.; Resasco, D. E.; Weisman, R. B., *J. Am. Chem. Soc.* **2003,** *125* (37), 11186-11187. DOI 10.1021/ja036622c.

[22] Zheng, M.; Diner, B. A., *J. Am. Chem. Soc.* **2004,** *126* (47), 15490-15494. DOI 10.1021/ja0457967.

[23] Dizdaroglu, M., *Anal. Biochem.* **1985,** *144* (2), 593-603. DOI 10.1016/0003-2697(85)90158-7.

[24] Jaruga, P.; Kirkali, G.; Dizdaroglu, M., *Free Radical Biol. Med.* **2008,** *45* (12), 1601-1609. DOI 10.1016/j.freeradbiomed.2008.09.019.

[25] Atha, D. H.; Wang, H. H.; Petersen, E. J.; Cleveland, D.; Holbrook, R. D.; Jaruga, P.; Dizdaroglu, M.; Xing, B. S.; Nelson, B. C., *Environ. Sci. Technol.* **2012,** *46* (3), 1819-1827. DOI 10.1021/es202660k.

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Scheme 1

Figure 1. GC/MS DNA damage evaluation of oligomers sonicated in the presence or absence of 0.1% DMSO and SWCNTs. Sonication time was 60 min. Two different oligomers were used: $(ATT)_{14}$ (upper) or $(GT)_{20}$ (lower). The ratio of DNA lesions/10^6 DNA bases represents the mean from five independent samples except for the DMSO only condition for $(GT)_{20}$ oligomer for which four samples were analyzed. The uncertainties represent standard deviations. Statistical analyses based on one-way ANOVA with posthoc Dunnett's multiple comparison test: * p value < 0.05; ** p value < 0.01; *** p value < 0.001.

The table of contents entry should be fifty to sixty words long, written in the present tense, and refer to the chosen figure.

Table of contents entry: The overall level of ultrasonication-induced DNA damage is reduced in the presence of single-walled carbon nanotubes (SWCNTs), particularly for DNA lesions formed by one-electron reduction of intermediate radicals. The protective role of SWCNTs observed in this work suggests a contrary view to the general idea that carbon nanotubes have damaging effects on biomolecules.

TOC Keyword: SWCNT

Title : Protective roles of single-wall carbon nanotubes in ultrasonication-induced DNA base damage.

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