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Single-Walled Carbon Nanotube Dispersion in Tryptic Soy Broth

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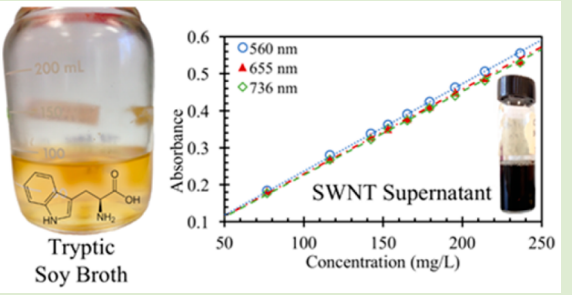
Supporting Information

ABSTRACT: There has been little research on the dispersion of carbon nanotubes in dispersions of standard microbiological media. We report that tryptic soy broth (TSB) containing casein digest disperses single-walled carbon nanotubes (SWNT) at concentrations similar to those achieved in lysozyme (LSZ), one of the best known biomolecular SWNT dispersants. Similar to LSZ, the proposed mechanism for SWNT dispersion in TSB is favorable $\pi-\pi$ stacking interactions with L-tryptophan. This is supported by similar SWNT concentrations in both LSZ and TSB supernatants, and the absence of appreciable dispersion in TSB that does not contain a source of L-tryptophan. Since L-tryptophan alone is insufficient to enable dispersion, it was previously hypothesized that LSZ's macromolecular structure created steric hindrance that was critical for SWNT dispersion. These new results show that intermediately sized L-tryptophan containing species can also enable dispersion. In addition, since TSB is a commonly used growth medium for microbiological research, its dispersive ability presents new research avenues for studying the effect of SWNT on prokaryotic cells without the need to oxidize SWNT or add dispersants that may induce microbial stress.



Dispersion of carbon nanotubes (CNT) in aqueous media is a prerequisite for research into their potential applications as biosensors, drug delivery devices, cancer therapeutics, or antimicrobial materials. Due to their hydrophobicity and the strong van der Waals attractive forces between CNT, achieving stable aqueous dispersions typically requires oxidative functionalization of ends and defects, covalent functionalization with hydrophilic ligands, or the use of water-soluble polymers or synthetic surfactants.[1−4] Each of these can affect the intrinsic properties of carbon nanotubes and their interaction with biomolecules. While there are well established schemes for covalently functionalizing CNT with biomolecules, these methods result in damage to the CNTs' sp^2 hybridized carbon structure and a deterioration of electrical and mechanical properties.[2,5] In contrast, adsorption of biomolecules on SWNT avoids CNT property deterioration. There have been numerous recent advances in understanding the noncovalent interactions between carbon nanotubes and proteins such as lysozyme (LSZ) or β-lactoglobulin, large synthetic polypeptides,[6−12] and nucleotide sequences.[13,14] These macromolecules can strongly adsorb onto SWNT through $\pi-\pi$ stacking interactions between their hydrophobic moieties and the sp^2 hybridized walls of the SWNT.[7,10,11,15] For example, significant research has shown that LSZ, a naturally occurring antimicrobial enzyme found in hen egg white, has particularly favorable interactions with carbon nanotubes and can be used to produce antimicrobial materials.[16−23] Both computational[11,24] and experimental research[10] have attributed this to interactions between CNT and LSZ's L-tryptophan residues. These and other advances in understanding the interactions of individual biomolecules with CNT have enabled a range of biosensors, cancer diagnostics, drug delivery vehicles, and antimicrobial materials.[25−28] However, little attention has been paid to understanding SWNT dispersion in complex nutrient media frequently employed in microbiological research such as tryptic soy broth (TSB). As a result, much of the research into potential antimicrobial applications of SWNT has relied on the use of oxidized nanotubes, which have a different surface chemistry than pristine SWNT, or dispersions stabilized by surfactants such as sodium dodecyl sulfate (SDS), which can damage lipid bilayers.[29−32] Biologically derived dispersants like LSZ are also not ideal for this purpose, due to antimicrobial activity or other intrinsic properties.[16]

We report that TSB containing casein digest can stably disperse individual and small bundles of carbon nanotubes at concentrations comparable to LSZ for at least one month. TSB is an undefined growth medium in microbiological research, useful for the culture of a broad range of bacteria; it is specified by its available nutrient content and not the structure of those nutrients. There are numerous variations of TSB both commercially available and prepared by individual microbiological laboratories.[33] In many cases, the primary source of its protein content is an enzymatic digest of casein, an L-tryptophan-containing family of four proteins that comprise the majority of suspended solids in milk.[34−38]

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The dispersive ability of TSB was probed through ultraviolet−visible spectroscopy (UV−vis), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and atomic force microscopy (AFM). The UV−vis absorbance spectra of TSB-SWNT supernatants following centrifugation is shown in Figure 1. The spectra exhibit the local absorbance

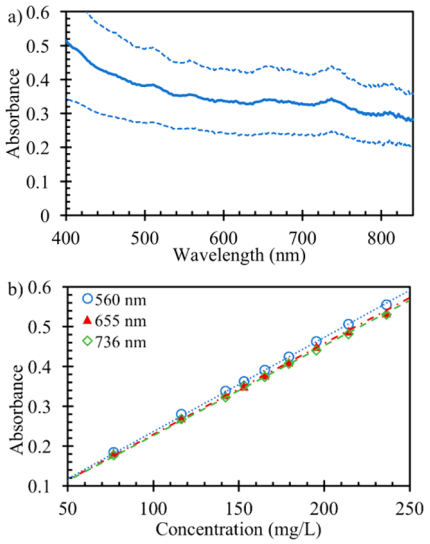

Figure 1. (a) Average UV−vis spectra of SWNT dispersed in TSB after centrifugation (dashed lines represent standard deviation of 7 samples) and (b) Beer−Lambert Law calibration curves for absorbance measurements at 560, 655, and 736 nm. The extinction coefficients are 0.0024, 0.0023, and 0.0023 L/mg mm, respectively; $R^2 > 0.98$.

maxima typical of individualized SWNT at approximately 515, 560, 655, and 736 nm due to Van Hove singularities in the density of states. These singularities arise from the 1-D nature of the SWNT; their presence in the absorbance spectra is a necessary but not sufficient indicator of individualized SWNT, as they may also be exhibited by small bundles.[39] SWNT concentrations in the supernatants were determined using the Beer−Lambert law. Based on this result, TSB can disperse SWNT at 153 ± 40 mg/L, similar to LSZ (108 mg/L) and SDS (500 mg/L).[10,21,40] The precise determination of the size and composition of the polypeptide chains in TSB is difficult to assess due to nonspecific protein digestion.[41−43] Liquid chromatography/mass spectrometry (LC/MS) of several TSB varieties revealed that that the average molecular weight of the fragments was in the range of 400−500 Da, with maximum weights of ∼1300 Da in casein-containing media. Based on the amino acid content of raw bovine casein, the largest fragments are expected to contain 10−15 amino acids.[38] This makes TSB's ability to disperse SWNT surprising as the peptide sequences in the protein fragments comprising TSB are significantly smaller than other biological macromolecules that are typically used to disperse SWNT.

Varieties of TSB that did not contain casein digest did not show any ability to disperse SWNT. Mass spectrometry results show that there is little difference in fragment size between casein and noncasein TSB preparations, highlighting the role of casein digests in SWNT dispersion (see Supporting Information). Undigested caseins are insoluble in water and unable to disperse SWNT. Both the undigested and enzymatically digested casein contain the aromatic amino acid L-tryptophan which has previously been shown to be the origin of the favorable interactions between carbon nanotubes in LSZ.[10,11] However, previous research indicated that L-tryptophan alone is insufficient to disperse SWNT and led to the belief that both L-tryptophan and a macromolecular structure were needed to achieve dispersion. Therefore, it is surprising that TSB, which consists of casein digests on the order of 15 peptides in length is able to disperse SWNT. To contextualize this size difference, a LSZ molecule (126 peptides) would be ∼45 nm in length if fully extended, while a 20 peptide long fragment would only be ∼7 nm in length on average.[44] To date, relatively little attention has been paid to the question of how large these proteins must be to facilitate SWNT dispersion. These results suggest that while L-tryptophan, which has a molecular size of approximately 0.46 nm, is insufficient to disperse SWNT, which are approximately 1 nm in diameter and 500 nm long, adsorption of a protein digest can induce sufficient repulsion to overcome van der Waals attraction. This finding indicates that media such as TSB can provide a useful platform for studying the interaction of CNT with prokaryotic cells, serving as a common tool between materials and microbiological researchers.

AFM of dried samples was used to confirm the presence of individualized SWNT suggested by the absorbance spectra. Since drying samples can result in aggregation or bundling, but not SWNT individualization, the presence of individuals supports the UV−vis spectral data. The AFM micrographs show numerous rod like objects and aggregates that appear irregularly studded with small globules of protein on a scattered field of unbound protein fragments (see Supporting Information). A diameter of less than 1.4 nm was used as the threshold for individuals; this is a conservative value based on the SWNT used in this work. Numerous rods were between 0.68 and 1.1 nm, confirming that they are individualized SWNT, though there are larger rods and aggregates present. The irregular appearance of the covering and the numerous scattered protein molecules on the surface suggest that numerous polypeptide fragments bind to the SWNT while many more do not bind and remain in solution.

To further probe the interactions of TSB and SWNT, ATR-FTIR was performed on dried samples of TSB as is shown in Figure 2. Spectra show far more peaks compared to complete individual proteins like LSZ or casein due to the complexity of the media but contain similar features.[10] Comparison of the spectra to that of undigested casein confirms the presence of the amide I (∼1650 cm^{-1}) and amide II (∼1520 cm^{-1}) peaks expected from proteins, as well as other less prominent peaks in casein spectra. Both the uncentrifuged mixtures and supernatants show a steady increase in absorbance from approximately 1700 to 700 cm^{-1}. These results are in good agreement with the behavior of LSZ examined in Horn et al., suggesting that polypeptides that are present on the SWNT are not simply a byproduct of the drying process, but are in fact bound to the SWNT.[10]

The identification of TSB, a commonly used microbiological medium, as a SWNT dispersant opens new research avenues for studying the effect of SWNT on prokaryotic cells without the use of additional dispersants which can affect microbiological growth. These results also suggest that the importance of extended macromolecular structure present in other peptide-based dispersants may have been overestimated. Furthermore, it provides additional evidence of L-tryptophan's ability to disperse SWNT.

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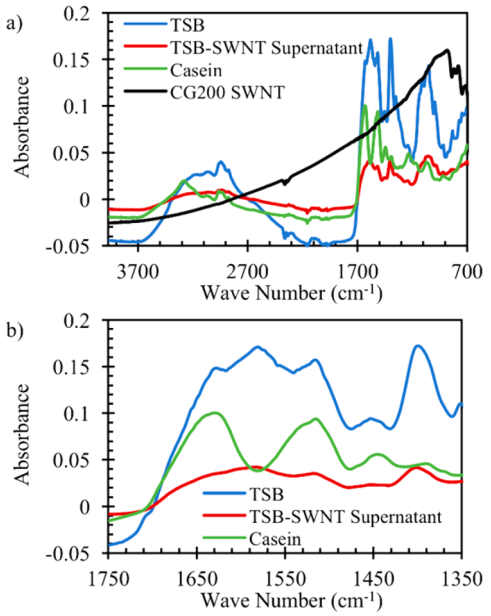

Figure 2. (a) ATR-FTIR absorbance spectra over the entire frequency range and (b) enlarged section showing the amide I and II peaks of tryptophan containing samples.

EXPERIMENTAL SECTION

Materials. This study utilized CG200 CoMoCAT SWNT, lots 4, 14, and 17, provided by Southwest NanoTechnologies (Norman, OK, now Chasm Advanced Materials), which were used as received. The carbon content of bulk samples was 95%. The D/G ratio was below 0.09 for all examined lots. Commercially available TSB from EMD Millipore (Billerica, MA) was prepared to specification and used as received. The recipe was composed of 17.0 g/L casein peptone, 3.0 g/L soy peptone, 2.5 g/L glucose, 5.0 g/L NaCl, and 2.5 g/L K_2HPO_4 dissolved in ultrapure water from a Milli-Q filtration system. After mixing, the TSB was autoclaved at 121 °C for 15 min. The TSB variations tested for the quantification of the role of casein were: an in house replicate of the commercial TSB recipe, a recipe which exchanged casein peptone for bacteriological peptone, and a recipe containing only casein peptone in the same buffer conditions as TSB. A solution of 0.025 wt % L-tryptophan in water was also tested.

Sample Preparation. Samples were prepared by weighing media and SWNT for a target SWNT concentration of 0.1 wt %. Samples were bath sonicated for approximately 1 min to reduce foaming and then tip sonicated for 30 min at 50 W in an ice bath. Sonication was pulsed in intervals of 5 s on, 2 s off. Samples were subjected to centrifugation at 17000g for 1 h to remove aggregates and large CNT bundles.[10] The top 85% of the volume from each sample was collected as a supernatant. Longer centrifugation times were found to have negligible effects on supernatant properties. All reported values are for supernatant samples.

Sample Characterization. A Thermo Scientific (Waltham, MA) NanoDrop 2000c UV−visible spectrophotometer was used to measure the absorbance of samples. Scans were done over wavelengths of in 190−840 nm with a 1 nm resolution. All scans were carried out at room temperature with a 1 mm path length. Absorbance measurements were used to estimate the concentration of SWNT in supernatant samples using the Beer−Lambert Law:

$$A = \varepsilon l c$$

where A is the absorbance, ε is the extinction coefficient, l is the path length of the light beam, and c is the concentration of the samples. The extinction coefficient of the uncentrifuged mixtures was determined through serial dilution of the mixtures with deionized water. The extinction coefficient was then used to estimate supernatant sample concentrations from absorbance measurements. A Nicolet (Waltham, MA) iS10 Fourier-Transform-Infrared (FTIR) spectrometer was used to examine the interactions between SWNT and TSB. All measurements consisted of 64 scans from 4000 cm^{-1} to 650 cm^{-1} on a germanium crystal. To eliminate interference from residual water, all samples were dried for 24 h at 60 °C and stored with desiccant. A Pacific Nanotechnology (Santa Clara, CA) Nano-R SPM Atomic Force Microscope was used to measure height profiles of SWNT in dried samples to determine individualization. Samples were prepared by diluting 500 μL of sample in 3 mL of water. Samples were pipetted onto mica substrates and dried under vacuum prior to testing. LC/MS measurements were made with a Waters (Milford, MA) Q-Tof Premier.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00656.

Figure S1, AFM micrographs of dried samples; Figure S2, Fragment molecular weight distributions (PDF).

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Notes

The authors declare no competing financial interest.

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