Selective Interactions of Sugar-Functionalized Single-Walled Carbon Nanotubes with *Bacillus* Spores

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ABSTRACT It was reported previously that monosaccharide-functionalized single-walled carbon nanotubes (SWNTs) could interact with *Bacillus anthracis* (Sterne) spores with the mediation of a divalent cation such as Ca^{2+} to result in significant spore aggregation and reduction in colony forming units. In this work a more systematic investigation was performed on interactions of the SWNTs functionalized with individual mannose and galactose moieties and their various dendritic configurations with *B. anthracis* and *B. subtilis* spores in the presence and absence of a divalent cation. Significant differences and selectivity between the *Bacillus* spores and between different sugars and their configurations were observed. The relevant results are presented, and their mechanistic implications are discussed.

KEYWORDS: single-walled carbon nanotubes · *Bacillus* spore · anthrax · aggregation · carbohydrate

Potential applications of nanomaterials in biology and biodefense have attracted much recent attention.[1−5] In many cases, the nanomaterials are designed and used as scaffolds for carrying or displaying bioactive functional groups,[2−9] which facilitate more effective and/or selective interactions of these nanoscale bioconjugates with targeted biological species. For example, polymeric nanoparticles tethered with mannose moieties were found to bind to *Escherichia coli* cells to result in massive cell agglutination, while there were no similar binding and agglutination with the use of free mannose molecules.[6]

Single-walled carbon nanotubes (SWNTs) are interesting nanomaterials of small diameters and extremely large aspect ratios. Because of the unique structure, SWNTs represent an ideal pseudolinear platform for displaying multiple copies of bioactive molecules, such as DNA,[10,11] proteins and peptides,[12−14] and carbohydrates,[9,15,16] enabling more effective interactions with organisms such as cells and other biological species that are not available to the free bioactive molecules. For example, through supramolecular wrapping with lactose-appended schizophyllan, SWNTs could acquire specific lectin-affinity.[16]

Bacillus spores (especially *Bacillus anthracis*) and their surface structures relevant to binding with bioactive species have been an ongoing research emphasis of the counter-bioterrorism community.[17] A number of investigations have been reported on interactions of the spores with peptides,[18−21] lectins,[22] and carbohydrates.[23] The genetically closely related *Bacillus subtilis* has often been used as a simulant for *B. anthracis*.[24] Although the current knowledge is still limited on the spore surface characteristics of these two species of *Bacillus* genus, it has been suggested that the spore surface is mainly composed of glycoproteins and carbohydrates.[25−27] For example, one surface glycoprotein found to be a structural component of *B. anthracis* exosporium contained rhamnose, 3-*O*-methyl rhamnose, and galactosamine.[25,26] Quinovose and other unique sugars were isolated from the *B. subtilis* spore.[27]

As reported previously,[5] monosaccharide (mannose or galactose)-functionalized SWNTs could interact with *B. anthracis* (Sterne) spores with the mediation of a divalent cation such as Ca^{2+} to result in significant spore aggregation and substantial reduction in colony forming units (CFU).[5] The aggregation of spores could be useful to the ongoing effort on countering anthrax-based bioterrorism because it is known that aggregated spores would pose significantly less or diminished threat in terms of the most lethal inhalation anthrax infection.[28,29]

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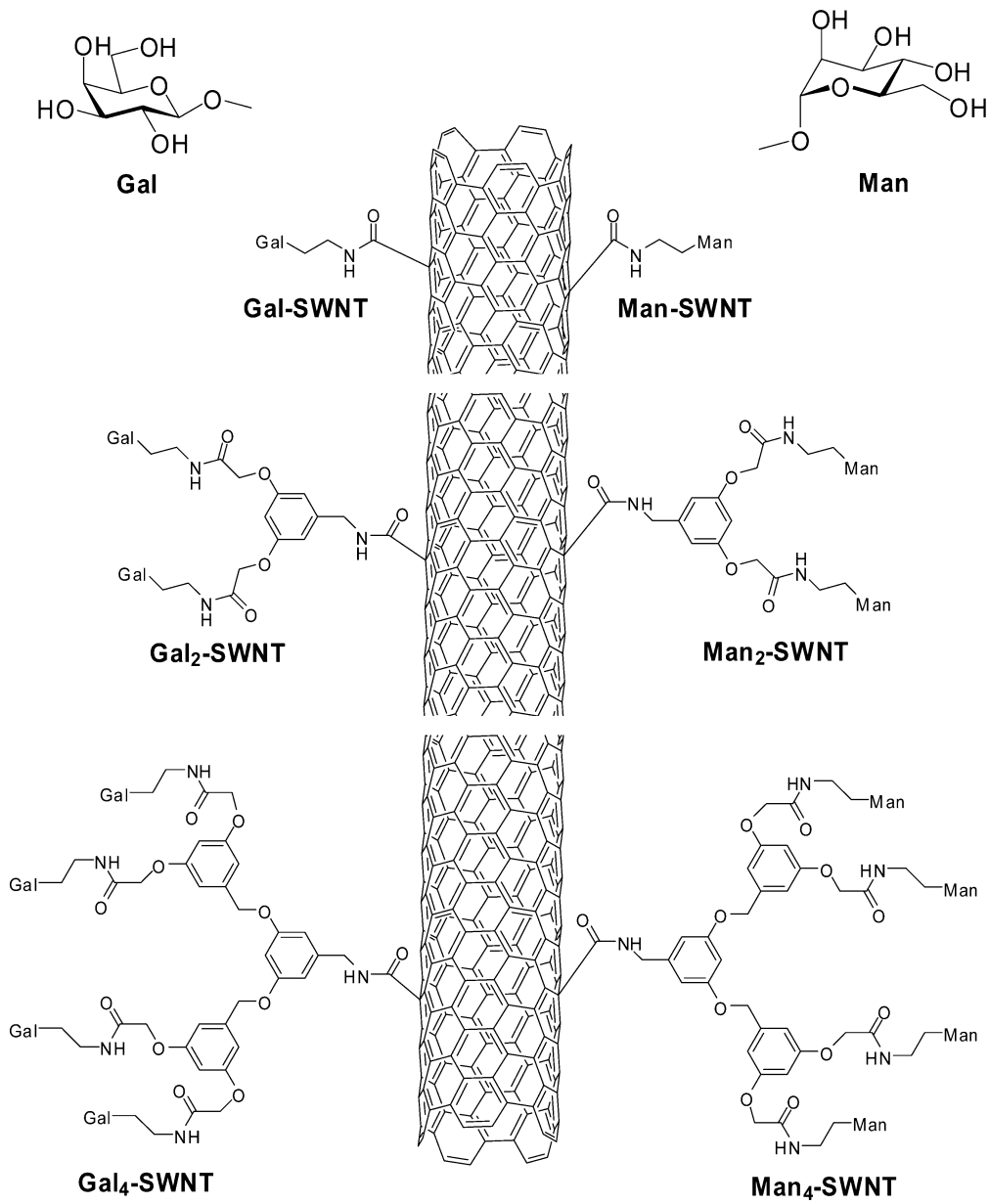

Scheme 1. Sugar-functionalized SWNTs.

Here we report a more systematic study of the mannose- and galactose-functionalized SWNTs in various configurations[30] and their interactions with the *Bacillus* spores in the presence and absence of a divalent cation. Significant differences between *B. anthracis* and *B. subtilis* and between different sugars and their configurations were observed. The relevant results are presented, and their mechanistic implications are discussed.

RESULTS AND DISCUSSION

We previously reported the Ca^{2+}-mediated aggregation of *B. anthracis* spores by monosaccharide-functionalized SWNTs.[5] In a more systematic study reported here, SWNTs functionalized with monosaccharide molecules (Man-SWNT or Gal-SWNT) or with the sugar dendrons (Man_2-SWNT, Gal_2-SWNT, Man_4-SWNT, and Gal_4-SWNT) (Scheme 1) were dispersed in aqueous solution for interactions with suspended *B. anthracis* or *B. subtilis* spores with or without the presence of a divalent cation. Major differences or significant variations in the interactions were observed for the different functionalized SWNTs and between the two *Bacillus* spores.

Cation Mediated Binding. In the presence of Ca^{2+} both Man-SWNT and Gal-SWNT could bind to *B. subtilis* spores for their aggregation into large clumps of 20−100 μm in size (Figure 1), similar to the aggregation of *B. anthracis* spores.[5] Since the size of a single spore is on the order of one micrometer, these clumps should each contain hundreds to thousands of aggregated spores. The SEM imaging at a higher resolution allowed a closer look at the spore aggregates, in which the spores were tangled by the nanotubes in a spider weblike morphology (Figure 2).

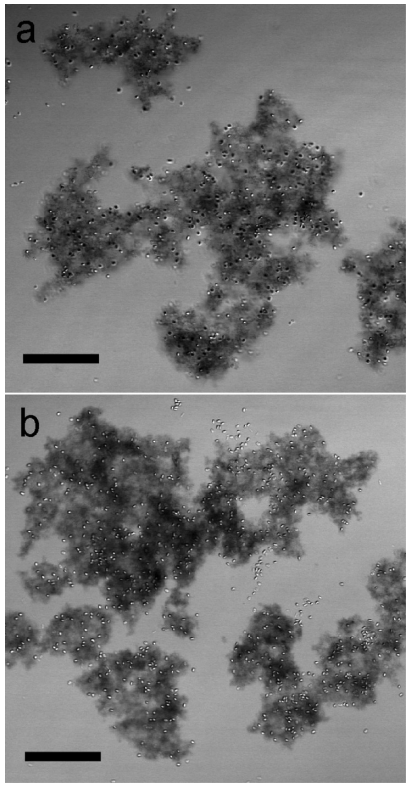

Figure 1. Optical microscopy images (40 μm for both scale bars) for *B. subtilis* spores aggregated by (a) Man-SWNT and (b) Gal-SWNT in the presence of Ca^{2+}.

The aggregation of *B. subtilis* spores was accompanied by reduction in CFUs at different Ca^{2+} concentrations under otherwise the same experimental conditions. For example, at a constant Man-SWNT concentration (0.2 mg/mL mannose equivalent), the CFU reduction was 71% for 25 mM Ca^{2+}, 78% for 50 mM Ca^{2+}, 80% for 100 mM Ca^{2+}, and reaching a more significant 87% reduction for a higher Ca^{2+} concentration of 250 mM (Figure 3). The role of Ca^{2+} might be the mediation of carbohydrate-carbohydrate interactions by forming coordinate bonds among the sugars, as suggested in the literature.[31−34] As a well-established example on the aggregation of live sponge cells in seawater, the presence of physiological Ca^{2+} was found to be necessary to mediate carbohydrate-carbohydrate interactions.[35]

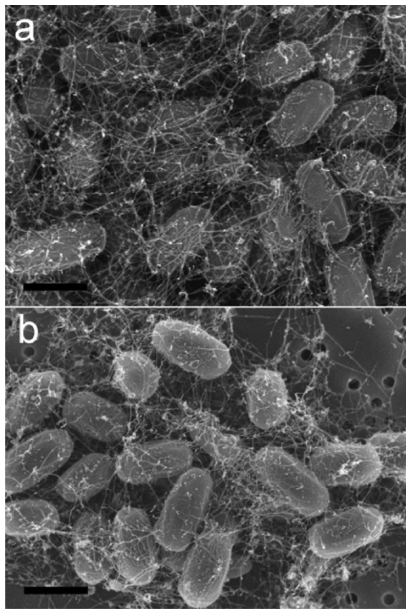

Figure 2. SEM images (1 μm for both scale bars) for *B. subtilis* spores aggregated by (a) Man-SWNT and (b) Gal-SWNT in the presence of Ca^{2+}.

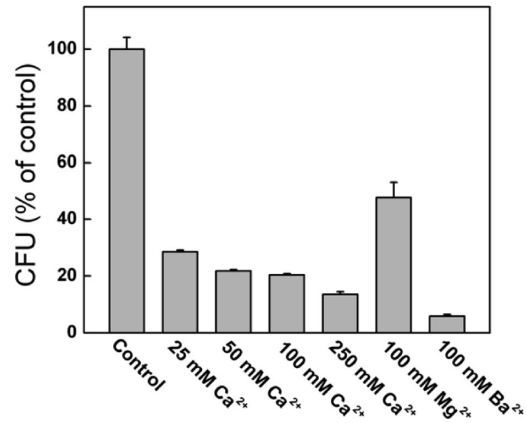

Figure 3. The CFU reduction of *B. subtilis* spores by Man-SWNT at different Ca^{2+} concentrations or in the presence of other divalent cations.

The same coordinating effect on carbohydrates could be achieved with other divalent cations.[34] In a comparison of other cations Mg^{2+} and Ba^{2+} with Ca^{2+} at the same concentration of 100 mM, the effectiveness in the CFU reduction of *B. subtilis* spores by Man-SWNT was in the order of $Mg^{2+} < Ca^{2+} < Ba^{2+}$ (Figure 3). This might be due to the same order of variation in the diameter of the divalent cations, which as suggested in the literature[34] could affect the cation-mediated carbohydrate−carbohydrate interactions, with a cation of a larger diameter being more effective. However, cations such as Na^{+} could not mediate the interactions, consistent with what is known in the literature,[36] nor divalent cations alone without sugar-functionalized nanotubes, as expected.

Although we did not intentionally change conditions during our experiments, we frequently observed aggregates of Man-SWNT with spores (either *B. anthracis* or *B. subtilis*) in the presence of Ca^{2+} that were rodlike and 20−50 μm in length by 5−10 μm in diameter by optical microscopy (Figure 4). We speculate that the formation of this specific rodlike shape was related to the nanotubes being linear in nature. Further investigations are necessary to determine the factors that drive the formation of these aggregates and to understand their mechanistic operations.

The sugar dendron-functionalized SWNTs, despite their higher relative sugar contents and the sugars being in pairs or quartets, were found to be generally ineffective or less effective in binding and aggregating

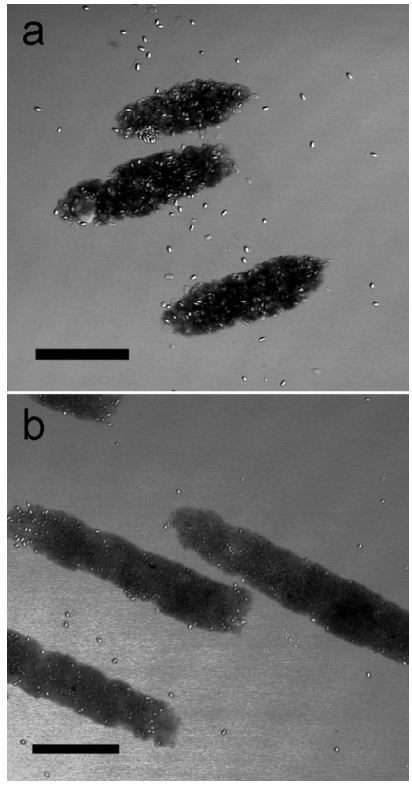

Figure 4. Optical microscopy images (30 μm for both scale bars) on the frequently observed rodlike aggregates of (a) *B. subtilis* and (b) *B. anthracis* spores by Man-SWNT in the presence of Ca^{2+}.

the *Bacillus* spores in the presence of Ca^{2+}. For *B. anthracis* (Sterne) spores, specifically, no significant aggregation was observed with Man_2-SWNT or Gal_2-SWNT and even less so with their tetrasugar counterparts. The results from the corresponding CFU reduction assays were equally negative (essentially no reduction). On the other hand, the same dendron-functionalized SWNTs were more active toward *B. subtilis* spores, exhibiting some binding and aggregation effects in the presence of Ca^{2+},[30] though clearly less effective than their monosaccharide counterparts Man-SWNT and Gal-SWNT. For example, in CFU reduction assays the use of Man_2-SWNT resulted in about 50% CFU reduction for the *B. subtilis* spores in the presence of Ca^{2+} (100 mM), compared to the 80% and 67% CFU reductions with the use of Man-SWNT and Gal-SWNT, respectively, under the same experimental conditions (Figure 5). Similar experiments with the use of Man_4-SWNT resulted in no meaningful aggregation of the *B. subtilis* spores, with the corresponding CFU reduction assays showing CFU changes within experimental uncertainty.

The observed aggregation and CFU reduction of the *Bacillus* spores (*B. anthracis* and *B. subtilis*) were attributed to divalent cation-mediated multivalent carbohydrate−carbohydrate interactions,[33,34,37,38] or more specifically, the carbohydrates tethered to SWNTs with those expressed on the spore surface. The interactions were not disrupted by the presence of the corresponding free sugars as competition. The role of Ca^{2+} mediation was confirmed by the experiment in which the aggregation of *Bacillus* spores was reversed with the addition of EDTA, a strong chelating agent for Ca^{2+} and the other divalent cations. Experimentally, while the *Bacillus* spores aggregated substantially using Man-SWNT and Gal-SWNT with Ca^{2+} under the specific experimental conditions described above, the subsequent addition of an aqueous EDTA (sodium salt) solution (20 mM, 100 μL) with gentle shaking resulted in an immediate and complete redispersion of both the spores and nanotubes (no aggregates at all). The observation was consistent with what was known in the literature on similar carbohydrate−carbohydrate interactions.[31−34,37,38]

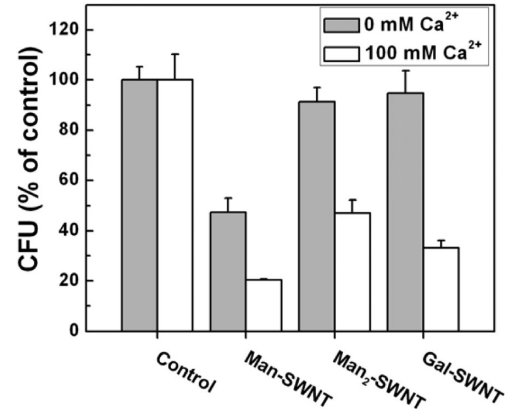

Figure 5. The CFU reduction of *B. subtilis* spores by Man-SWNT, Man_2-SWNT, and Gal-SWNT in the absence and presence (100 mM) of Ca^{2+}.

Binding without Cation. In the absence of any divalent cations, the two *Bacillus* spores exhibited pronounced differences in the binding and aggregation experiments. As reported previously on the basis of preliminary experimental data,[5] more systematic investigations in this study confirmed no binding or aggregation of Man-SWNT and Gal-SWNT with *B. anthracis* (Sterne) spores without the mediation of a divalent cation. Interestingly, however, this was not the case for *B. subtilis* spores.

Man-SWNT could bind to and aggregate *B. subtilis* spores without the cation mediation. Experimentally, upon the gentle mixing of an aqueous solution of Man-SWNT and an aqueous suspension of *B. subtilis* spores by slow rotation for 16 h, an aliquot of the mixture was used to prepare specimens for optical microscopy analyses. As shown in Figure 6, there were aggregates of *B. subtilis* spores due to the binding of Man-SWNT in optical microscopy images. A closer examination in SEM imaging revealed that the aggregated spores were tangled by the nanotubes in a weblike morphology (Figure 7). The results from the corresponding CFU reduction assays without any cations suggested that the aggregation of *B. subtilis* spores by Man-SWNT resulted in about a 53% reduction (Figure 5).

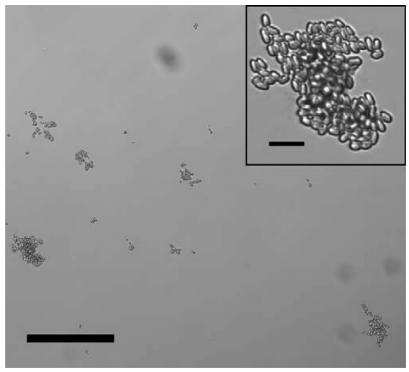

Figure 6. Optical microscopy images (scale bar = 40 μm) for *B. subtilis* spores aggregated by Man-SWNT in the absence of any divalent cation. Inset: a closer look (scale bar = 4 μm).

The observed interactions of Man-SWNT with *B. subtilis* spores must be due to specific binding, not simple adsorption or other nonspecific driving forces, because the same interactions were not found with the use of Gal-SWNT under similar experimental conditions, namely that in the absence of any divalent cations Gal-SWNT exhibited no meaningful effects on *B. subtilis* spores in an aqueous suspension. Experimentally, when Gal-SWNT instead of Man-SWNT was used in the same experimental procedure described above, the mixture remained homogeneous after the gentle rotation for 16 h. The corresponding specimen was analyzed by imaging with optical microscopy and electron microscopy, which suggested no detectable aggregates of *B. subtilis* spores. This was confirmed by the results from the CFU reduction assay showing no meaningful reduction (Figure 5).

The sugar dendron-functionalized SWNTs also did not bind to *B. anthracis* (Sterne) spores in the absence of any divalent cations, the same as their monosaccharide counterparts. Similarly, Gal_2-SWNT and Gal_4-SWNT were the same as Gal-SWNT, exhibiting no binding and aggregation effects on *B. subtilis* spores without any cation mediation. On the other hand, Man_2-SWNT could bind to *B. subtilis* spores without any divalent cations, but was less effective than Man-SWNT in the binding and aggregation of the spores. According to results from CFU reduction assays, there was only less than 10% CFU reduction with the use of Man_2-SWNT, in comparison to the 53% reduction with the use of Man-SWNT (Figure 5). Man_4-SWNT was found to be even less effective in the aggregation of *B. subtilis* spores, with results from the CFU reduction assay showing only some variations within the experimental uncertainty margins.

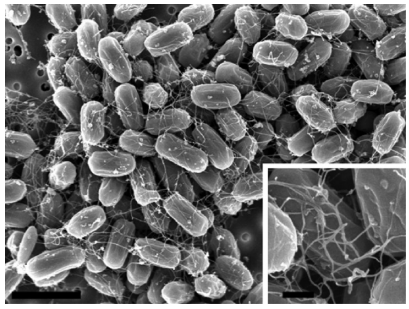

Figure 7. SEM images (scale bar = 2 μm) for *B. subtilis* spores aggregated by Man-SWNT in the absence of any divalent cation. Inset: at a higher resolution (scale bar = 300 nm).

More Discussion. The results presented above suggest that both *B. anthracis* (Sterne) and *B. subtilis* spores could be aggregated effectively by the monosaccharide-functionalized SWNTs (Man-SWNT and Gal-SWNT) in the presence of a divalent cation, without any pronounced selectivity. However, the high selectivity became evident in the absence of any divalent cations, with the binding and aggregation observed only for Man-SWNT with *B. subtilis* spores. It should be noted that the binding would not necessarily lead to aggregation (as correctly pointed out by a reviewer), though for the specific purpose in this work only aggregation as a result of the binding was monitored. Two ways for the binding leading to aggregation might be considered. One could be that some sugar-functionalized nanotubes bound to multiple spores; and the other could be due to similar carbohydrate interactions between the sugar-functionalized nanotubes. The former likely dominated the aggregation of *B. subtilis* by Man-SWNT without cation, as the starting Man-SWNT solution was homogeneous. For the cation-mediated aggregation there were probably contributions from both because similar aggregation was observed in Man-SWNT and Gal-SWNT solutions upon the cation addition.[39] In any case, simply functionalized SWNTs (such as those with oligomeric polyethylene glycol[40,41]) containing no carbohydrate moieties had no response to the presence of cations for any aggregation of the spores.

As discussed above, the mediation of Ca^{2+} or another divalent cation was likely for carbohydrate−carbohydrate interactions between those displayed through tethers on SWNTs and those expressed on the *Bacillus* spore surface. The reversibility with the use of EDTA to take the divalent cation out of the mediation was supportive to such a mechanistic explanation. In the literature the Ca^{2+}-mediated carbohydrate−carbohydrate interactions have been explained in terms of a "zipper" model.[33,42] The carbohydrate moieties in this model are equivalent to stumps of the zipper, which must be in complementary conformations for strong enough binding. The role of divalent cations is like that of the "zipper puller" to provide the driving force for the interactions and to stabilize the complementary carbohydrate conformations that are required for stronger interations.[33,42] Such a model may explain the divalent cation-mediated interactions of the sugar-functionalized SWNTs with the *Bacillus* spores, for which the model emphasizes the complementary conformations of the carbohydrate moieties

but largely ignores the chemical identities of the carbohydrates (Man *vs* Gal).

For the selective binding of Man-SWNT with *B. subtilis* spores, it is tempting or even logical to suggest their being attributed to carbohydrate−protein interactions of the nanotube-tethered mannoses with the specific protein receptors on the *B. subtilis* spore surface. The observed high specificity, not available for Gal-SWNT on the carbohydrate side or *B. anthracis* on the spore side, seems consistent with carbohydrate−protein binding, which is known to be more specific.[43,44] While no literature reports were found with our best effort for any existing experimental evidence on the presence of carbohydrate-binding proteins on the spore surface, there were previous studies suggesting that many pathogens have surface proteins binding to specific carbohydrates. Fox example, the *E. coli* strain ORN178 contains mannose-specific adhesin FimH of type 1 pili that could bind selectively to mannose-functionalized nanoparticles.[6,45] In addition, it might be argued that the larger CFU reduction of *B. subtilis* with Ca^{2+} by Man-SWNT than Gal-SWNT was due to additional contributions from the mannose-protein binding. However, more systematic investigations are needed for more quantitative conclusions.

Individually both carbohydrate−carbohydrate and carbohydrate−protein interactions are known for their low binding affinity,[37] so that the multivalent display of the carbohydrates is necessary. The sugar dendrons were prepared for the purpose of displaying mannose and galactose in pairs or quartets, thus intuitively for significantly improved multivalency. It was surprising that the sugar dendron-functionalized SWNTs, especially Man_4-SWNT and Gal_4-SWNT, were generally ineffective in the binding and aggregation of the *Bacillus* spores either with or without the divalent cation mediation. Two possibilities may be considered. One is that the paired sugar moieties may have stronger interactions between themselves; and the other is that tethering the sugar moieties in pairs or quartets may have caused some kind of steric hindrance unfavorable to the targeted interactions (namely a lack of or poorer complementary conformations for the carbohydrate moieties in terms of the zipper model[33,42]). On a related note, it was reported previously[5] and confirmed again in this study that the binding and aggregation of the *Bacillus* spores are unique to the mannose and galactose displayed through tethers on the nanotube surface, not available to the mannose- or galactose-functionalized polymeric nanobeads (on the order of 100−200 nm in diameter). The obvious selectivity and related issues require further investigations for their mechanistic understandings.

EXPERIMENTAL SECTION

Sugar-Functionalized SWNTs. The synthesis and characterization for the functionalized SWNTs (Scheme 1) with mannose (Man-SWNT) or galactose (Gal-SWNT) moieties or their dendrons (Man_2-SWNT, Gal_2-SWNT, Man_4-SWNT, or Gal_4-SWNT) have been reported previously.[30]

Bacillus Spores. *B. anthracis* spores (Sterne 34F2, a nonvirulent strain, but still requiring careful handling in a biosafety level-2 laboratory) were supplied by Colorado Serum Company (Denver, Colorado). *B. subtilis* ATCC33234 spores were obtained from American Type Culture Collection (Manassas, VA). Spores were prepared by using a modified procedure from the literature.[21,46] Briefly, *Bacillus* cells were grown at 37 °C on solid Difco sporulation medium (DSM) until sporulation was essentially complete. Vegetative cells and spores were collected from medium plates, washed extensively with cold (4 °C) sterile distilled water, and pelleted by centrifugation. The pellet was resuspended in a solution of Tris-Mg buffer (pH 8.0) that contained lysozyme at a concentration of 1 mg/mL. Subsequently, the suspension was shaken slowly for 1 h at 37 °C. *N*-Laurylsarcosine was then added to a concentration of 2%, and the solution was incubated for 30 min. The spores were then pelleted and washed four times with sterile cold distilled water. Finally the spores were resuspended in cold sterile distilled water and stored at 4 °C until further use. The purification of spores was checked through spore staining and phase contrast microscopy.

Binding Assays. By following experimental procedures described previously,[5] spore aggregation assays for Man-SWNT, Gal-SWNT, Man_2-SWNT, Gal_2-SWNT, Man_4-SWNT, and Gal_4-SWNT were carried out in the presence or absence of a divalent cation. In a typical experiment without cation, an aqueous suspension of either *B. anthracis* or *B. subtilis* spores (10^5 CFU/μL, 20 μL) was added with a sugar-SWNT solution (sugar equivalent concentration ~0.2 mg/mL, 20 μL) or distilled water (20 μL) as control. For similar experiments with Ca^{2+}, the spore and sugar-SWNT mixture was added (after 30 min) with an aqueous $CaCl_2$ solution (100 mM, 10 μL). All resulting mixtures (samples or control) were gently mixed with slow rotation for 16 h, followed by both a visual examination and with a microscope.

For optical microscopy analyses, a small aliquot (10 μL) of the sample from above binding assay was dropped onto a glass slide and covered with a coverslip. The specimen was imaged in the differential interference contrast (DIC) mode on a Leica DMIRE2 microscope equipped with a Leica TCS SP2 laser scanning device and Leica HC PL APO 10×/0.30 and 20×/0.70 objectives.

For scanning electron microscopy (SEM) analyses, the sample mixture from above binding assay was centrifuged at 6800*g* for 5 min. The pellet was washed with distilled water in several centrifuging-suspending cycles, and the final pellet was suspended for the fixing and postfixing treatment according to established procedures.[47] The final specimen upon critical point drying was mounted on an aluminum stub with double-sided carbon tape and coated with platinum. SEM images were obtained on a Hitachi S4700 field-emission SEM system.

CFU Reduction Assays. A suspension of *Bacillus* spores (2.6×10^8 in 40 μL) was mixed with a sugar-SWNT sample (40 μL) or with distilled water as control. After 30 min, an aqueous $CaCl_2$ solution (100 mM, 20 μL) was added with gentle mixing, followed by rotation at room temperature for 16 h. The resulting mixtures (samples and control) were centrifuged at a low speed (25*g*) for 1 min. A portion of each supernatant (40 μL) was used for the assay. Upon serial dilution, the diluted samples (0.1 mL each in triplicate) were spread evenly on solid trypticase soy agar (TSA) plates. The plates were allowed for incubation at 37 °C overnight. The colonies on the plates were counted, and the percentage CFU reduction was calculated in reference to the control.

Similarly for the binding assay without a divalent cation, a suspension of *B. subtilis* spores (2×10^8 in 40 μL) was mixed with a solution of the selected sugar-SWNT sample (40 μL) or with distilled water as control. After being rotated at room tem-

perature for 16 h, the mixtures (samples and control) were treated in the same procedures as described above for the assays with Ca^{2+}.

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REFERENCES AND NOTES

1. Niemeyer, C. M. Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128–4158.
2. Katz, E.; Willner, I. Integrated Nanoparticle−Biomolecule Hybrid Systems: Synthesis, Properties, and Applications. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042–6108.
3. Luo, P. G.; Tzeng, T.-R.; Qu, L.; Caldwell, E.; Latour, R. A.; Stutzenberger, F.; Sun, Y.-P. Quantitative Analysis of Bacterial Aggregation Mediated by Bioactive Nanoparticles. *J. Biomed. Nanotechnol.* **2005**, *1*, 291–296.
4. Akasaka, T.; Watari, F. Carbohydrate Coating of Carbon Nanotubes for Biological Recognition. *Fullerenes, Nanotubes, Carbon Nanostruct.* **2008**, *16*, 114–125.
5. Wang, H.; Gu, L.; Lin, Y.; Lu, F.; Meziani, M. J.; Luo, P. J.; Wang, W.; Cao, L.; Sun, Y.-P. Unique Aggregation of Anthrax (*Bacillus anthracis*) Spores by Sugar-Coated Single-Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2006**, *128*, 13364–13365.
6. Qu, L.; Luo, P. G.; Taylor, S.; Lin, Y.; Huang, W.; Anyadike, N.; Tzeng, T.-R. J.; Stutzenberger, F.; Latour, R. A.; Sun, Y.-P. Visualizing Adhesion-Induced Agglutination of *Escherichia coli* with Mannosylated Nanoparticles. *J. Nanosci. Nanotechnol.* **2005**, *5*, 319–322.
7. Qu, L.; Gu, L.; Li, H.; Taylor, S.; Elkin, T.; Luo, P. G.; Tzeng, T.-R. J.; Jiang, X.; Latour, R. A.; Stutzenberger, F.; Williams, A.; Sun, Y.-P. Galactosylated Polymeric Nanoparticles: Synthesis and Adhesion Interactions with *Escherichia coli*. *J. Biomed. Nanotechnol.* **2005**, *1*, 61–67.
8. Elkin, T.; Jiang, X.; Taylor, S.; Lin, Y.; Gu, L.; Yang, H.; Brown, J.; Collins, S.; Sun, Y.-P. Immuno-Carbon Nanotubes and Recognition of Pathogens. *ChemBioChem* **2005**, *6*, 640–643.
9. Gu, L.; Elkin, T.; Jiang, X.; Li, H.; Lin, Y.; Qu, L.; Tzeng, T.-R. J.; Joseph, R.; Sun, Y.-P. Single-Walled Carbon Nanotubes Displaying Multivalent Ligands for Capturing Pathogens. *Chem. Commun.* **2005**, 874–876.
10. Lu, Q.; Moore, J. M.; Huang, G.; Mount, A. S.; Rao, A. M.; Larcom, L. L.; Ke, P. C. RNA Polymer Translocation with Single-Walled Carbon Nanotubes. *Nano Lett.* **2004**, *4*, 2473–2477.
11. Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. J. Carbon Nanotubes as Multifunctional Biological Transporters and Near-Infrared Agents for Selective Cancer Cell Destruction. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *112*, 11600–11605.
12. Huang, W.; Taylor, S.; Fu, K.; Lin, Y.; Zhang, D. H.; Hanks, T. W.; Rao, A. M.; Sun, Y.-P. Attaching Proteins to Carbon Nanotubes *via* Diimide-Activated Amidation. *Nano Lett.* **2002**, *2*, 311–314.
13. Kam, N. W. S.; Dai, H. J. Carbon Nanotubes as Intracellular Protein Transporters: Generality and Biological Functionality. *J. Am. Chem. Soc.* **2005**, *127*, 6021–6026.
14. Pantarotto, D.; Partidos, C. D.; Hoebeke, J.; Brown, F.; Kramer, E.; Briand, J. P.; Muller, S.; Prato, M.; Bianco, A. Immunization with Peptide-Functionalized Carbon Nanotubes Enhances Virus-Specific Neutralizing Antibody Responses. *Chem. Biol.* **2003**, *10*, 961–966.
15. Matsuura, K.; Hayashi, K.; Kimizuka, N. Lectin-Mediated Supramolecular Junctions of Galactose-Derivatized Single-Walled Carbon Nanotubes. *Chem. Lett.* **2003**, *32*, 212–213.
16. Hasegawa, T.; Fujisawa, T.; Numata, M.; Umeda, M.; Matsumoto, T.; Kimura, T.; Okumura, S.; Sakurai, K.; Shinkai, S. Single-Walled Carbon Nanotubes Acquire a Specific Lectin-Affinity Through Supramolecular Wrapping with Lactose-Appended Schizophyllan. *Chem. Commun.* **2004**, 2150–2151.
17. Matsumoto, G. Anthrax Powder: State of the Art. *Science* **2003**, *302*, 1492–1497.
18. William, D. D.; Benedek, O.; Turnbough, C. L. Species-Specific Peptide Ligands for the Detection of *Bacillus anthracis* Spores. *Appl. Environ. Microbiol.* **2003**, *69*, 6288–6293.
19. Turnbough, C. L. Discovery of Phage Display Peptide Ligands for Species-Specific Detection of *Bacillus* Spores. *J. Microbiol. Methods* **2003**, *53*, 263–271.
20. Brigati, J.; Wiliams, D. D.; Sorokulova, I. B.; Nanduri, V.; Chen, I.-H.; Turnbough, C. L.; Petrenko, V. A. Diagnostic Probes for *Bacillus anthracis* Spores Selected from a Landscape Phage Library. *Clin. Chem.* **2004**, *50*, 1899–1906.
21. Knurr, J.; Benedek, O.; Heslop, J.; Vinson, R. B.; Boydston, J. A.; McAndrew, J.; Kearney, J. F.; Turnbough, C. L. Peptide Ligands That Bind Selectively to Spores of *Bacillus subtilis* and Closely Related Species. *Appl. Environ. Microbiol.* **2003**, *69*, 6841–6847.
22. Cole, H. B.; Ezzell, J. W.; Keller, K. F.; Doyle, R. J. Differentiation of *Bacillus anthracis* and Other Bacillus Species by Lectins. *J. Clin. Microbiol.* **1984**, *19*, 48–53.
23. Tarasenko, O.; Islam, S.; Paquiot, D.; Levon, K. Glyco-conjugates for the Recognition of *Bacillus* Spores. *Carbohydr. Res.* **2004**, *339*, 2859–2870.
24. Valentine, N.; Wunschel, S.; Wunschel, D.; Petersen, C.; Wahl, K. Effect of Culture Conditions on Microorganism Identification by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry. *Appl. Environ. Microbiol.* **2005**, *71*, 58–64.
25. Fox, A.; Stewart, G. C.; Waller, L. N.; Fox, K. F.; Harley, W. M.; Price, R. L. Carbohydrates and Glycoproteins of *Bacillus anthracis* and Related Bacilli: Targets for Biodetection. *J. Microbiol. Methods* **2003**, *54*, 143–152.
26. Fox, A.; Black, G. E.; Fox, K.; Rostovtseva, S. Determination of Carbohydrate Profiles of *Bacillus anthracis* and *Bacillus cereus* Including Identification of *O*-Methyl Methylpentoses by Using Gas Chromatography-Mass Spectrometry. *J. Clin. Microbiol.* **1993**, *31*, 887–894.
27. Driks, A. *Bacillus subtilis* Spore Coat. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 1–20.
28. Druett, H. A.; Henderson, D. W.; Packman, L.; Peacock, S. Studies on Respiratory Infection I. The Influence of Particle Size on Respiratory Infection with Anthrax Spores. *J. Hygiene* **1953**, *72*, 24–31.
29. Canter, D. A. Addressing Residual Risk Issues at Anthrax Cleanups: How Clean Is Safe? *J. Toxicol. Environ. Heal. Part A* **2005**, *68*, 1017–1032.
30. Gu, L.; Luo, P. G.; Wang, H.; Meziani, M. J.; Lin, Y.; Veca, L. M.; Cao, L.; Lu, F.; Wang, X.; Quinn, R. A.; *et al.* Single-Walled Carbon Nanotube as Unique Scaffold for Multivalent Display of Sugars. *Biomacromolecules* **2008**, *9*, 2408–2418.
31. Bovin, N. H. Carbohydrate−Carbohydrate Interactions. In *Glycoscience: Status and Perspectives*; Gabium, H. J., Gabium S., Eds.; Chapman & Hall: Weinheim, Germany, 1997; p 277.
32. Angyal, S. J. Complexes of Metal Cations with Carbohydrates in Solution. *Adv. Carbohydr. Chem. Biochm.* **1989**, *47*, 1–43.
33. Rojo, J.; Morales, J. C.; Penadés, S. Carbohydrate−Carbohydrate Interactions in Biological and Model Systems. *Top. Curr. Chem.* **2002**, *218*, 45–92.
34. Reynolds, A. J.; Haines, A. H.; Russell, D. A. Gold Glyconanoparticles for Mimics and Measurement of Metal Ion-Mediated Carbohydrate−Carbohydrate Interactions. *Langmuir* **2006**, *22*, 1156–1163.
35. Bucior, I.; Scheuring, S.; Engel, A.; Burger, M. M. Carbohydrate−Carbohydrate Interaction Provides Adhesion Force and Specificity for Cellular Recognition. *J. Cell Biol.* **2004**, *165*, 529–537.
36. Koshy, K. M.; Boggs, J. M. Investigation of the Calcium-Mediated Association between the Carbohydrate Head Groups of Galactosylceramide and Galactosylceramide I3

Sulfate by Electrospray Ionization Mass Spectrometry. *J. Biol. Chem.* **1996**, *271*, 3496–3499.
37. Bucior, I.; Burger, M. M. Carbohydrate−Carbohydrate Interactions in Cell Recognition. *Curr. Opin. Struct. Biol.* **2004**, *14*, 631–637.
38. de la Fuente, J. M.; Eaton, P.; Barrienos, A. G.; Menendez, M.; Penades, S. Thermodynamic Evidence for Ca^{2+}-Mediated Self-Aggregation of Lewis X Gold Glyconanoparticles. A Model for Cell Adhesion *via* Carbohydrate−Carbohydrate Interaction. *J. Am. Chem. Soc.* **2005**, *127*, 6192–6197.
39. Gu, L. Functionalized Carbon Nanotubes for Biological Applications. Ph.D. Dissertation. Clemson University, Clemson, SC, 2008.
40. Huang, W.; Fernando, S.; Allard, L.; Sun, Y.-P. Solubilization of Single-Walled Carbon Nanotubes with Diamine-Terminated Oligomeric Poly(ethylene glycol) in Different Functionalization Reactions. *Nano. Lett.* **2003**, *3*, 565–568.
41. Fernando, K. A. S.; Lin, Y.; Sun, Y.-P. High Aqueous Solubility of Functionalized Single-Walled Carbon Nanotubes. *Langmuir* **2004**, *20*, 4777–4778.
42. Spillmann, D. Carbohydrates in Cellular Recognition: From Leucin-Zipper to Sugar-Zipper? *Glycoconjugate J.* **1994**, *11*, 169–171.
43. Lis, H.; Sharon, N. Lectins: Carbohydrate-Specific Proteins That Mediate Cellular Recognition. *Chem. Rev.* **1998**, *98*, 637–674.
44. Lasky, L. A. Selectins: Interpreters of Cell-Specific Carbohydrate Information during Inflammation. *Science.* **1992**, *258*, 964–969.
45. Lin, C.; Yeh, Y.; Yang, C.; Chen, C.; Chen, G.; Chen, C.; Wu, Y. Selective Binding of Mannose-Encapsulated Gold Nanoparticles to Type 1 Pili in *Escherichia coli*. *J. Am. Chem. Soc.* **2002**, *124*, 3508–3509.
46. Zaman, M. S.; Goyal, A.; Dubey, G. P.; Gupta, P. K.; Chandra, H.; Das, T. K.; Ganguli, M.; Singh, Y. Imaging and Analysis of *Bacillus anthracis* Spore Germination. *Microsc. Res. Tech.* **2005**, *66*, 307–311.
47. Dykstra, M. J. *Biological Electron Microscopy, Theory, Techniques, and Troubleshooting*; Kluwer Academic/Plenum Publishers: New York, 1992; pp 237−242.