NANO LETTERS

2002
Vol. 2, No. 4
285−288

Functionalization of Carbon Nanotubes for Biocompatibility and Biomolecular Recognition

Moonsub Shim, Nadine Wong Shi Kam, Robert J. Chen, Yiming Li, and Hongjie Dai*

Department of Chemistry, Stanford University, Stanford, California 94305

Received December 5, 2001; Revised Manuscript Received January 12, 2002

ABSTRACT

The interface between biological molecules and novel nanomaterials is important to developing new types of miniature devices for biological applications. Here, the streptavidin/biotin system is used to investigate the adsorption behavior of proteins on the sides of single-walled carbon nanotubes (SWNTs). Functionalization of SWNTs by coadsorption of a surfactant and poly(ethylene glycol) is found to be effective in resisting nonspecific adsorption of streptavidin. Specific binding of streptavidin onto SWNTs is achieved by co-functionalization of nanotubes with biotin and protein-resistant polymers.

Recent years have witnessed a significant interest in biological applications of novel solid-state nanomaterials.[1−6] The unique physical properties of molecular- or nanoscale solids (dots or wires) when utilized in conjunction with the remarkable biomolecular recognition capabilities could lead to miniature biological electronics and optical devices including probes and sensors. Not only could these devices exhibit advantages over existing technology in size but also in performance. Several issues are important regarding nanomaterial/biosystems. One of them is biocompatibility, especially for in-vivo applications of implantable bioelectronic devices. Another is specificity that requires biofunctionalization of nanomaterials for recognition of only one type of target biomolecule in solution and rejection of others. Central to tackling these issues is surface functionalization of nanomaterials and elucidating the interfaces and interactions between nanomaterials and biosystems.

Single-walled carbon nanotubes (SWNTs) are novel molecular scale wires exhibiting useful properties for various potential applications including miniature biological devices. For instance, nanotubes can be used as electrodes for detecting biomolecules in solutions, similar to commonly used conventional carbon based electrode materials. Also, the electrical properties of SWNTs are sensitive to surface charge transfer and changes in the surrounding electrostatic environment, undergoing drastic changes by simple adsorptions of certain molecules or polymers.[7−10] SWNTs are therefore promising for chemical sensors for detecting molecules in the gas phase and biosensors for probing biological processes in solutions. Nevertheless, significant effort is required in order to understand interactions between nanotubes and biomolecules and how to impart specificity and selectivity to nanotube-based bioelectronic devices.

Motivated by the biological application prospects of solid-state nanomaterials, this work investigates (1) nonspecific binding (NSB) of proteins to SWNTs, (2) functionalization of nanotubes for resisting nonspecific interactions, and (3) enabling specific binding of proteins to functionalized nanotubes. We find that streptavidin nonspecifically binds to as-grown SWNTs and show that prevention of NSB of streptavidin on SWNTs is achieved by coating nanotubes with a surfactant and poly(ethylene glycol), PEG. Selective binding of streptavidin is introduced by co-functionalization of SWNTs with PEG and biotin. The results have implications to the nanotube biocompatibility[11] issue and specificity of potential bioelectronic devices based on nanotubes.

In an earlier communication, we have shown that protein binding to SWNTs is reliably enabled via a noncovalent sidewall functionalization scheme.[5] In this manner, a variety of proteins have been successfully immobilized on SWNTs functionalized by 1-pyrenebutanoic acid succinimidyl ester. The pyrene moiety adsorbs onto the sidewalls of SWNTs via $\pi-\pi$ interaction, and the succinimidyl ester group reacts with amine groups on lysine residues of proteins to form covalent amide linkages.[5] Multiwalled carbon nanotubes have been functionalized with 4-hydroxynonenal (4-HNE) to induce adsorption of 4-HNE antibody.[11] However, certain proteins are found to bind to SWNTs even without sidewall functionalization. A published example is the binding of a fullerene-specific monoclonal antibody to SWNTs.[6] Such

* Corresponding author. Phone: (650) 723-4518. Fax: (650) 725-0259. E-mail: hdai1@stanford.edu.

10.1021/nl015692j CCC: $22.00
Published on Web 01/25/2002

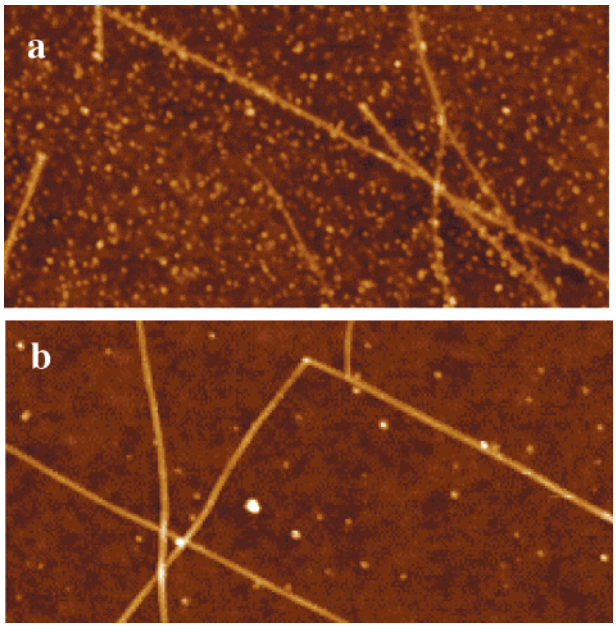


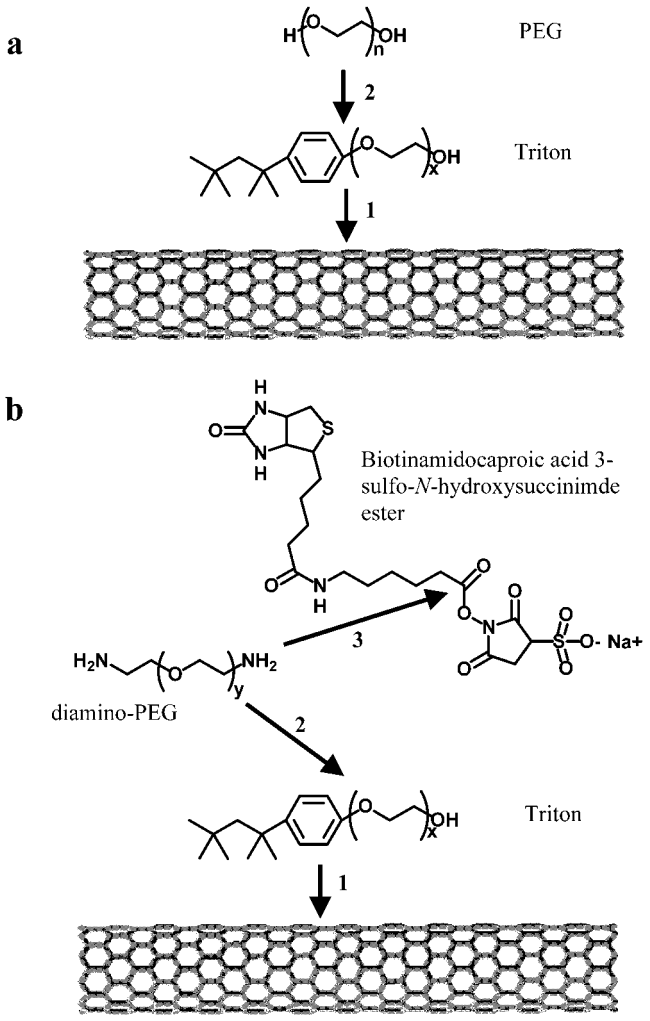
Figure 1. (a) AFM image of streptavidin (dots along the SWNTs) adsorbed nonspecifically on as-grown SWNTs (line-like features). The diameters (topographic heights) of SWNTs shown in all of the Figures are 1 to 3 nm. (b) AFM image showing nonspecific streptavidin binding is prevented by coating SWNTs with a surfactant, Triton X-100, and a well-known protein resistant polymer PEG (Scheme 1a). Both images are 0.5 × 1 μm.

binding points to attractive interactions between certain proteins and as-grown SWNTs. It is interesting and important to address how to prevent NSB of proteins on SWNTs and at the same time introduce selective and specific binding.

We use streptavidin as our model protein in this work. Streptavidin has been shown previously to adsorb spontaneously and close pack onto multiwalled carbon nanotubes.[12] Nonspecific binding of streptavidin on as-grown SWNTs is observed here as shown in Figure 1a, where the atomic force microscopy (AFM) images show adsorbed proteins on SWNTs after exposure to a solution of streptavidin. Protein adsorption is carried out by immersing bare SWNTs grown on SiO_2 substrate from discrete nanoparticles[13] in a 10 mM phosphate buffer solution (pH 7) of 0.7 μg/mL streptavidin for 1 h followed by thorough H_2O rinsing. The high density of nonspecifically adsorbed streptavidin (spots along the lengths of nanotubes in Figure 1a) points to high affinity of the proteins toward nonfunctionalized nanotubes via hydrophobic interactions.[12] The result also suggests that streptavidin is an excellent system for investigating nanotube sidewall modification for resisting NSB of proteins.

A variety of polymer coatings and self-assembled monolayers have been used to prevent nonspecific binding of proteins on surfaces for biosensor and biomedical device applications.[14−16] Among them, PEG is one of the most effective and widely used. We find that PEG (average MW = 10 000, Aldrich chemicals), similar to other polymers[10,17,18] can be irreversibly adsorbed on SWNTs. The resulting PEG coated nanotubes exhibit certain degree of protein resistance. However, appreciable adsorption of streptavidin is still observed (data not shown), indicating that the coverage of

Scheme 1. (a) Functionalization of SWNTs for Preventing Non-specific Binding of Protein[a] and (b) Strategy for Introducing Selective Binding of Streptavidin with Prevention of Non-specific Binding[b]



[a] A surfactant Triton-X 100 is first adsorbed onto the sidewalls of SWNTs followed by adsorption of PEG. [b] Triton-X 405 is adsorbed on SWNTs followed by amine-terminated PEG covalently linked to biotin.

PEG on SWNT sidewalls is not complete or uniform. To circumvent this problem, we find that a surfactant, Triton X-100 or Triton X-405 (Aldrich chemicals) can be adsorbed onto SWNTs as a wetting layer to significantly enhance PEG adsorption on nanotubes (Scheme 1a). Figure 1b shows that essentially no streptavidin adsorbs on SWNTs that are treated with both the surfactant and PEG. Thus, coadsorption of Triton and PEG on SWNTs is found to be highly effective in preventing nonspecific adsorption of streptavidin on nanotubes.

Triton is a surfactant molecule containing an aliphatic chain and a short hydrophilic PEG unit. Binding of surfactant molecules to a nanotube is driven by hydrophobic interactions and has been widely utilized for making stable suspensions of SWNTs in aqueous solutions. Adsorption of the Triton surfactant alone on nanotubes results in partial resistance to nonspecific adsorption of streptavidin on SWNTs without affecting NSB on the substrate (see Sup-

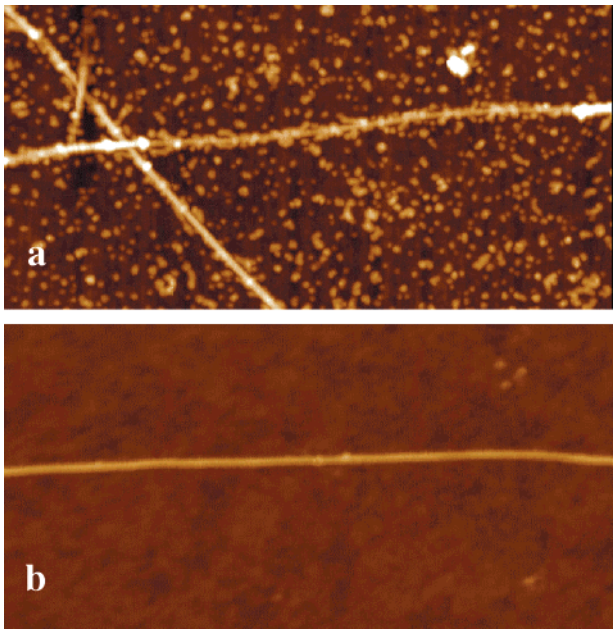


Figure 2. (a) AFM image showing specific adsorption of streptavidin by co-functionalization of SWNTs with a protein repelling layer and biotin (Scheme 1b). SWNTs are first coated with surfactant Triton X-405 then by diamino-PEG followed by covalent linkage of succinimidyl ester terminated biotin to the amine moieties of the PEG. (b) Control experiment where SWNTs are co-functionalized with PEG and biotin as in (a) but exposed to streptavidin that has been plugged with biotin prior to adsorption onto SWNTs. Images are 0.5 × 1 μm.

porting Information). Functionalization by both Triton and PEG, on the other hand, is specific to SWNTs and responsible for high resistance of nanotubes to NSB of streptavidin. Nevertheless, we also find that during our process, PEG does adsorb onto the SiO_2 substrate to render the background surface resistant to streptavidin to a certain degree (see Supporting Information).

Next, we explore functionalization of SWNTs with ligands for specific binding of proteins together with polymers to eliminate nonspecific protein−nanotube interactions. Streptavidin exhibits extremely high affinity for biotin (dissociation constant $\sim 10^{-15}$), and the streptavidin/biotin system has been widely studied and utilized in biology and bioengineering. We adsorb Triton and PEG on SWNTs, and the biotin moiety is then added to the PEG chains (Scheme 1b) by using amine-terminated PEG (diamino-PEG, average MW = 3400, Shearwater Polymers) and covalently linking it with an amine-reactive biotin reagent, biotinamidocaproic acid 3-sulfo-*N*-hydroxysuccinimide ester (Sigma).[19] For the nanotubes coated with Triton/PEG-biotin, selective binding of streptavidin is observed as revealed by the high density of adsorbed proteins along the lengths of the nanotubes after exposure to a streptavidin solution (Figure 2a). No appreciable adsorption is seen when SWNTs functionalized in the same manner are exposed to streptavidin that has been plugged with 4 equiv of free biotin (Figure 2b). This result demonstrates that it is possible to functionalize nanotubes for specific protein recognition while eliminating or minimizing nonspecific protein binding.

It is known that the interactions between proteins and surfaces are complex, involving many types of noncovalent forces that are electrostatic, hydrogen bonding, hydrophobic, or entropic in nature related to surfaces as well as surrounding water molecules.[14−16] Nevertheless, this is a topic crucial to many areas of biological and medical science and technology. While there have been numerous studies of protein adsorption on macroscopic surfaces, interactions between nanomaterials such as SWNTs and proteins are just beginning to be explored. As an aside, through this work, we have noticed an interesting phenomenon related to the size of nanomaterials. We have found that relatively small proteins such as streptavidin (∼60 kDa) can adsorb more readily to the sidewalls of SWNTs. Fibrinogen, on the other hand, is a large protein (∼340 kDa) and is well known to strongly adsorb onto hydrophobic surfaces.[16] However, we have not observed appreciable adsorption of fibrinogen on the highly hydrophobic as-grown SWNTs. This is attributed to the much larger size of fibrinogen relative to the diameter of SWNTs (∼2 nm). The noncovalent nature of the interactions and small interaction area with the nanotube can account for the low affinity of large proteins to SWNTs.

We have also investigated functionalization of nanotubes by other types of polymers and surfactants for resisting NSB of proteins on SWNTs and found that the effectiveness of protein resistance depends on the coverage and uniformity of the polymer layer adsorbed on nanotubes. A systematic investigation on the adsorption behavior of various types proteins on SWNTs functionalized by various polymers and surfactants is ongoing and will be presented elsewhere.

In summary, this letter presents our first investigation of interactions between single-walled carbon nanotubes and proteins and ideas of nanotube modification for protein resistance and specific binding. Functionalization of nanotubes by surfactant and polymer species significantly reduces nonspecific protein adsorption, while co-immobilization of ligands or antibodies can impart specificity in protein binding on nanotube surfaces. Further research along these lines will pave the way to novel biocompatible nanomaterials and provide a guide to developing miniature biodevices whose operation will be based on the unique physical properties of nanomaterials and high recognition capabilities of biomolecules.

Acknowledgment. This work is supported by the Lucille Packard Foundation, the Alfred Sloan Foundation and a Terman Fellowship.

Supporting Information Available: AFM images of streptavidin adsorption on carbon nanotubes functionalized with only PEG (a) and with only Triton-X100 (b). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.
(2) Chan, W.; Nie, S. *Science* **1998**, *281*, 2016−2018.
(3) Taton, T.; Mirkin, C.; Letsinger, R. *Science* **2000**, *289*, 1757−1760.

(4) Cui, Y.; Wei, Q.; Park, H.; Lieber, C. *Science* **2001**, *293*, 1289−1292.
(5) Chen, R.; Zhang, Y.; Wang, D.; Dai, H. *J. Am. Chem. Soc.* **2001**, *123*.
(6) Erlanger, B. F.; Chen, B.; Zhu, M.; Brus, L. E. *Nano Lett.* **2001**, *1*, 465.
(7) Kong, J.; Franklin, N.; Zhou, C.; Chapline, M.; Peng, S.; Cho, K.; Dai, H. *Science* **2000**, *287*, 622−625.
(8) Collins, P. G.; Bradley, K.; Ishigami, M.; Zettl, A. *Science* **2000**, *287*, 1801−1804.
(9) Kong, J.; Dai, H. *J. Phys. Chem. B* **2001**, *105*, 2890−2893.
(10) Shim, M.; Javey, A.; Kam, N. W. S.; Dai, H. *J. Am. Chem. Soc.* **2001**, *123*, 11512−11513.
(11) Mattson, M. P.; Haddon, R. C.; Rao, A. M. *J. Mol. Neurosci.* **2000**, *14*, 175.
(12) Balvlavoine, F.; Schultz, P.; Richard, C.; Mallouh, V.; Ebbeson, T. W.; Mioskowski, C. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1912−1915.
(13) Li, Y.; Kim, W.; Zhang, Y.; Rolandi, M.; Wang, D.; Dai, H. *J. Phys. Chem.* **2001**, *105*, 11424−11431.
(14) Harris, J. M.; Zalipsky, S. *Poly(ethylene glycol): Chemistry and Biological Applications*; American Chemical Society: Washington, DC, 1997.
(15) *Biocompatible polymers, metals and composites*; Szycher, M., Ed., Technomic: Lancaster, PA, 1983.
(16) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605−5620, and references therein.
(17) Star, A.; Stoddart, J.; Steuerman, D.; Diehl, M.; Boukai, A.; Wong, E.; Yang, X.; Chung, S.; Heath, J. R. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1721−1725.
(18) O'Connell, M. J.; Boul, P.; Ericson, L. M.; Huffman, C.; Wang, Y.; Haroz, E.; Kuper, C.; Tour, J.; Ausman, K.; Smalley, R. E. *Chem. Phys. Lett.* **2001**, *342*, 265−271.
(19) SWNT samples on SiO_2 substrates are immersed in an aqueous solution of Triton (10 wt %) then in 5 mM solution of diamino-PEG in H_2O for 1h each followed by >3 h immersion in 5 mM solution of biotinamidocaproic acid 3-sulfo-*N*-hydroxysuccinimde ester in pH 7.5 phosphate buffer (0.1 M). Streptavidin or plugged streptavidin is carried out by immersing the samples in ∼0.7 μg/mL solution of protein in pH 7.0 phosphate buffer. A thorough rinsing with running H_2O is carried out after each step. Plugged streptavidin solution is made in pH 7.0 phosphate buffer solution by mixing 6:1 ratio of biotin and streptavidin to give ∼0.7 μg/mL of streptavidin.

NL015692J