COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Single-walled carbon nanotubes acquire a specific lectin-affinity through supramolecular wrapping with lactose-appended schizophyllan†

Teruaki Hasegawa,[a] Tomohisa Fujisawa,[a] Munenori Numata,[a] Mariko Umeda,[a] Takahiro Matsumoto,[a] Taro Kimura,[c] Shiro Okumura,[c] Kazuo Sakurai[b] and Seiji Shinkai*[a]

[a] *Department of Chemistry, Graduate School of Engineering, Kyushu University, Hakozaki, 6-10-1, Fukuoka 812-8581, Japan. E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp; Fax: +81-92-642-3611; Tel: +81-92-642-3585*

[b] *Department of Chemical Processes and Environments, Faculty of Environmental Engineering, The University of Kitakyushu, Hibikino, 1-1, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0135, Japan*

[c] *Fukuoka Industrial Technology Center, Biotechnology and Food Research Institute, 1465-5 Aikawa, Kurume 839-0861, Japan*

Received (in Cambridge, UK) 20th May 2004, Accepted 23rd July 2004
First published as an Advance Article on the web 20th August 2004

Single-walled carbon nanotubes can be entrapped within a helical superstructure composed of schizophyllan bearing lactoside-appendages to show an excellent water-solubility as well as a specific lectin-affinity.

Much research effort has been launched on covalent functionalization of single-walled carbon nanotubes (SWNTs) to develop SWNT-based advanced materials, and various SWNT-derivatives carrying ferrocene, peptides, carbohydrates, *etc.* have been reported.[1,2] However, such covalent functionalization inevitably spoils the inherent structural and electrochemical properties of SWNTs. It is strongly desired, therefore, to find an easy, versatile, and non-destructive strategy to develop SWNT-based materials.

Schizophyllan (SPG), a natural β-1,3-glucan, exists as a triple-stranded helix (t-SPG) and random coiled single-strands (s-SPG) in water and DMSO, respectively.[3] Recently, we found that a stable SPG/SWNTs composite is formed when s-SPG in DMSO is diluted with SWNT-dispersed aqueous solution.[4] The most interesting feature of this composite includes a helical SPG-superstructure along the entrapped SWNTs that had never been observed in other composite systems.[5] In addition to the excellent water-solubility of the resulting composite, SPG has one clear structural advantage, *i.e.*, the presence of the pendent β-1,6-glucosides having $NaIO_4$-sensitive 1,2-diols that can be converted to aldehyde groups suitable for chemical modifications. When SPG derivatives carrying various functional-appendages are used for composite formation or "supramolecular wrapping", the resulting composites should acquire the specific functions inherent to the appendages (Fig. 1). It is undoubted, therefore, that this non-covalent strategy becomes a new and potential alternative to the conventional covalent-strategy. Herein, we describe one such example of SWNTs being able to acquire a specific lectin-affinity through a supramolecular wrapping with SPG carrying lactoside-appendages (SPG-Lac, Chart 1).[6]

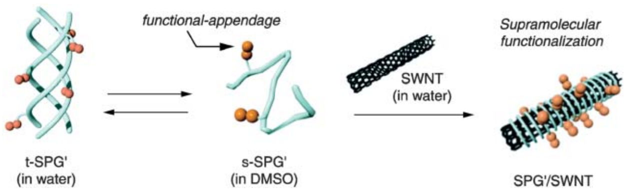

Fig. 1 Schematic illustration of the supramolecular functionalization of SWNT by using SPG-derivatives (SPG′).

† Electronic supplementary information (ESI) available: UV and NIR spectra of the composite, TGA profiles, AFM images of free SWNT and the composite incubated with ConA and WGA, a CLSM image of the composite incubated with FITC-RCA_{120} and QCM data for layer-by-layer adsorption. See http://www.rsc.org/suppdata/cc/b4/b407409b/

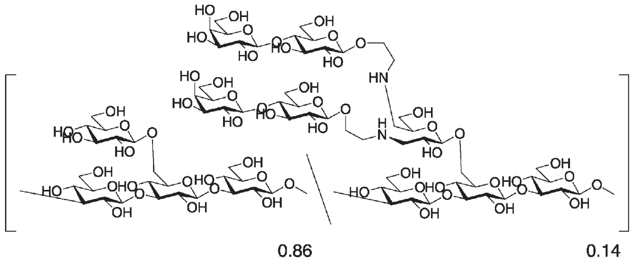

Chart 1 Structure of SPG carrying lactoside-appendages (SPG-Lac).

SPG-Lac/SWNTs composite was obtained by mixing SPG-Lac in DMSO with SWNTs dispersed in water.[7] The formation of the desired SPG-Lac/SWNTs composite was confirmed as follows: 1) the UV-vis spectrum of the resulting composite in water showed a broad absorption band at around 280 nm, which is characteristic of SWNTs; 2) SPG-Lac in the composite was quantified by color developing through a well-known phenol/sulfuric acid reaction system, indicating that almost half of the composite mass arises from SPG-Lac; 3) thermal gravimetric analysis (TGA) also proved that the composite is comprised of SPG-Lac and SWNTs. Furthermore, AFM observations (Fig. 2) show clear evidence for the composite formation: that is, SPG-Lac/SWNTs composite shows clear striped patterns proving the creation of the SPG-Lac helical superstructure along the entrapped SWNTs.[4]

Molecular recognition of SPG-Lac/SWNTs composite was assessed by surface plasmon resonance (SPR) using lectin-immobilized Au-surfaces.[8] In the case of free SWNT (Fig. 3-a), the increment of the resonance-unit is observed for all lectin-immobilized

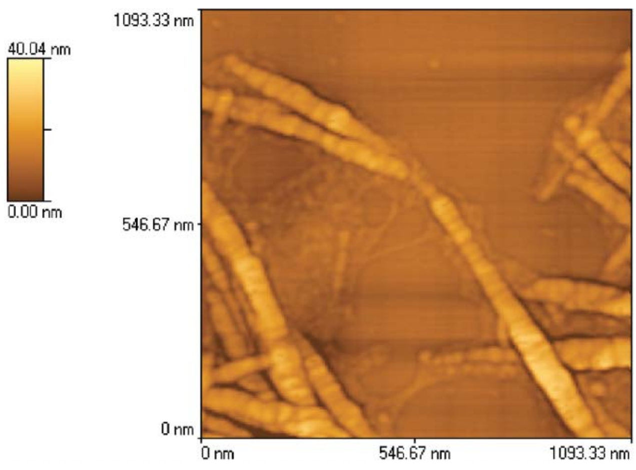

Fig. 2 AFM image of SPG-Lac/SWNTs composite: mica substrate.

DOI: 10.1039/b407409b

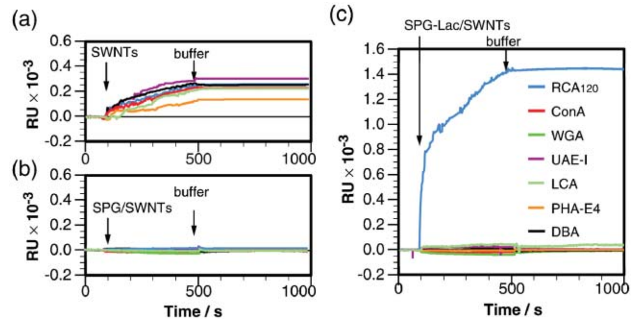

Fig. 3 SPR sensorgrams obtained for (a) free SWNT, (b) SPG/SWNTs composite, and (c) SPG-Lac/SWNTs composite: lectin-immobilized Au-surface, 1.0 mM Tris-HCl buffer (pH 7.2, [$CaCl_2$] and [$MnCl_2$] = 10 μM), 25 °C, flow rate = 10 μl min^{-1}, [SWNT] = 0.7 mg ml^{-1}.

surfaces, indicating that the change arises from the non-specific binding. Since detergent-containing buffer solution could release the SWNTs from the lectin-immobilized surfaces, this non-specific binding can be ascribed to hydrophobic interactions. In the case of SPG/SWNTs composite (Fig. 3-b), no or negligible binding can be observed for all lectins, clearly demonstrating that the hydrophobic nature of SWNTs is effectively shielded by the wrapping effect. The most fascinating data were obtained from SPG-Lac/SWNTs composite (Fig. 3-c), in which the specific binding is observed for the Au-surface immobilized with *ricinus communis* agglutinin (RCA_{120}, β-Lac-specific).[9,10] In contrast, the other lectin-immobilized surfaces showed negligible resonance changes, clearly demonstrating that the affinity of SPG-Lac/SWNTs composite is highly specific toward RCA_{120}.

The specific interactions between SPG-Lac/SWNTs composite and lectins were visualized by atomic force microscope (AFM). After incubation with RCA_{120} followed by purification with a centrifuge, SPG-Lac/SWNTs composite shows AFM images which feature small spheres clustering on the composite (Fig. 4). This unique morphology should arise from the dense clustering of RCA_{120} onto the surface of SPG-Lac/SWNTs composite to cover the inherent striped patterns of SPG. In contrast, no lectin-clustering phenomenon was observed for Concanavalin A (ConA, α-Man/Glc specific) and wheat germ agglutinin (WGA, β-GlcNAc specific).

The specific lectin-affinity of SPG-Lac/SWNTs composite was also confirmed by confocal laser scanning microscopic (CLSM) observations using fluorescein isothiocyanate (FITC)-labeled lectins. SPG-Lac/SWNTs composite incubated with FITC-RCA_{120} showed fluorescence and microscopic images which could be precisely overlapped with each other, indicating that FITC-RCA_{120} is clustered on SPG-Lac/SWNTs composite. As expected, no such clustering image was observed for FITC-ConA.

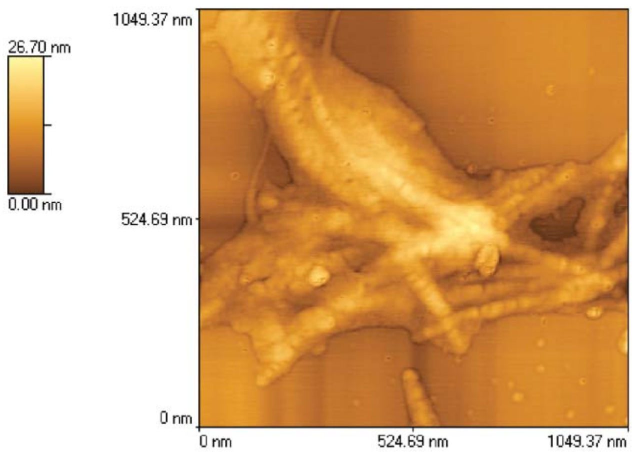

Fig. 4 AFM image of SPG-Lac/SWNT composite incubated with RCA_{120}: mica substrate.

The data described above clearly show that SWNTs acquire the specific lectin-affinity through supramolecular wrapping using SPG-Lac. This lectin-affinity of SPG-Lac/SWNTs composite is advantageous to fabricate various SWNT-based sensory systems or superstructures. For example, this system is readily applicable to construct a layer-by-layer structure composed of the SPG-Lac/SWNTs composite and RCA_{120} (See ESI). Quartz crystal microbalance (QCM) measurements using the RCA_{120}-immobilized Au-surfaces revealed a step-by-step decrease in the frequency, proving the construction of the expected layer-by-layer structure.

In conclusion, we applied supramolecular wrapping strategy to non-covalent introduction of functional groups onto SWNTs. In our strategy, SWNTs can acquire not only excellent water-solubility but also specific lectin-affinity through supramolecular wrapping with SPG-Lac without the perturbation of the π-conjugate system. Since various SPG-derivatives are easily accessible through reductive amination,[11] this non-covalent strategy can be applicable not only to many biochemical purposes but also to various photo- and electrochemical materials. Our research efforts are now focused on the development of such advanced materials based on SPG/SWNTs composite.

Notes and references

1 P. M. Ajayan, *Chem. Rev.*, 1999, **99**, 1787; Y.-P. Sun, K. Fu, Y. Lin and W. Huang, *Acc. Chem. Res.*, 2002, **35**, 1096; S. Niyogi, M. A. Hamon, H. Hu, B. Zhao, P. Bhowmik, R. Sen, M. E. Itkis and R. C. Haddon, *Acc. Chem. Res.*, 2002, **35**, 1105.

2 D. M. Guldi, M. Marcaccio, D. Paolucci, F. Paolucci, N. Tagmatarchis, D. Tasis, E. Vazquez and M. Prato, *Angew. Chem., Int. Ed.*, 2003, **42**, 4206; A. Bianco and M. Prato, *Adv. Mater.*, 2003, **15**, 1765; I. A. Banerjee, L. Yu and H. Matsui, *J. Am. Chem. Soc.*, 2003, **125**, 9542; K. Matsuura, K. Hayashi and N. Kimizuka, *Chem. Lett.*, 2003, **32**, 212.

3 T. Yanaki, T. Norisuye and H. Fujita, *Macromolecules*, 1980, **13**, 1462; T. Norisuye, T. Yanaki and H. Fujita, *J. Polym. Sci., Polym. Phys. Ed.*, 1980, **18**, 547; C. T. Chuah, A. Sarko, Y. Deslandes and R. H. Marchessault, *Macromolecules*, 1983, **16**, 1375; K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, 2000, **122**, 4520.

4 M. Numata, M. Asai, K. Kaneko, T. Hasegawa, N. Fujita, Y. Kitada, K. Sakurai and S. Shinkai, *Chem. Lett.*, 2004, 232.

5 A. Star, D. W. Steuerman, J. R. Heath and J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2002, **41**, 2508; O.-K. Kim, J. Je, J. W. Baldwin, S. Kooi, P. E. Pehrsson and L. J. Buckley, *J. Am. Chem. Soc.*, 2003, **125**, 4426; C. Lii, L. Stobinski, P. Tomasik and C. Liao, *Carbohydr. Polym.*, 2003, **51**, 93; M. J. O'Connell, P. Boul, L. M. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. D. Ausman and R. E. Smalley, *Chem. Phys. Lett.*, 2001, **342**, 265; X. Zhang, T. Liu, T. V. Sreekumar, S. Kumar, V. C. Moore, R. H. Hauge and R. E. Smalley, *Nano Lett.*, 2003, **3**, 1285; P. Petrov, F. Stassin, C. Pagnoulle and R. Jérôme, *Chem. Commun.*, 2003, 2904; N. Nakashima, S. Okuzono, H. Murakami, T. Nakai and K. Yoshikawa, *Chem. Lett.*, 2003, **32**, 456.

6 T. Hasegawa, M. Umeda, T. Matsumoto, M. Numata, M. Mizu, K. Koumoto, K. Sakurai and S. Shinkai, *Chem. Commun.*, 2004, 382.

7 Shortened SWNTs were used in this paper; M. Sano, A. Kamino, J. Okamura and S. Shinkai, *Science*, 2001, **293**, 1299.

8 Biacore biosensor and commercially available SPR chip immobilized with avidine–carboxymethyl dextran conjugate were used in the SPR assay. Before the binding assay, avidine-immobilized SPR-tips were allowed to interact with biotin-labeled lectins to yield lectin-immobilized surfaces.

9 Although SPG-Lac itself also induced the resonance change, the maximum (saturated) resonance change (*ca.* 400) was much smaller than that for SPG-Lac/SWNTs composite. It is clear that free or dissociated SPG-Lac is not a main contributor to the observed resonance change.

10 Dissociation of SPG-Lac/SWNTs composite from the RCA_{120}-immobilized surface was not observed even if buffer solution containing monomeric lactose (20 mM) was used in the dissociation phase. This stability is attributable to an enhanced RCA_{120}-affinity of SPG-Lac/SWNTs composite owing to a well-known "clustering effect" of carbohydrates.

11 T. Matsumoto, M. Numata, T. Anada, M. Mizu, K. Koumoto, K. Sakurai, T. Nagasaki and S. Shinkai, *Biochem. Biophys. Acta*, 2004, **1670**, 91.