Probing Protein-Carbohydrate Interactions using Carbon Nanotube Nanodevices for Rapid Bacterial Detection

H. Vedala, Y. Chen and A. Star
Department of Chemistry, University of Pittsburgh,
219 Parkman Avenue, Pittsburgh, PA, USA
astar@pitt.edu

ABSTRACT

We demonstrate a novel nanoelectronics device based on single-walled carbon nanotube field-effect transistor (NTFET) to probe the interactions between carbohydrates and their recognition proteins called lectins. These interactions are involved in a wide range of biological processes, such as cell-cell recognition as well as viral and bacterial infections. NTFETs were functionalized noncovalently with porphyrin-based glycoconjugates and change in electrical conductance was measured upon specific binding of bacterial lectins that present different carbohydrate preference, namely PA-IL, PA-IIL from *Pseudomonas aeruginosa* and a plant lectin Concanavalin A. However, no significant change in the device characteristics was observed when the devices were exposed to other lectins with different specificity. Detection of ConA binding to mannose-glycoconjugate functionalized NTFETs was highly sensitive and selective. Results were validated using various control experiments and microscopy techniques.

Keywords: carbon nanotubes, field-effect transistors, protein interactions, biosensors, bacterial detection

1 INTRODUCTION

Carbohydrates are major components of the cell membrane and are involved in diverse biological processes such as cell growth and development, cell-cell communication, pathogen binding, inflammation, tumor cell metastasis, immune responses, and mediating cell adhesion through carbohydrate-carbohydrate or carbohydrate-receptor interactions[1]. Lectins are sugar-binding proteins that play an important role in biological recognition involving glycoconjugates and possess high specificity for their cognate sugar moieties. In general, they have weaker interactions than antigen-antibody complexes and their dissociation constants are in the range of K_d = 10^{-6} - 10^{-7} M for glycoproteins[2-5]. Understanding and mimicking specific interactions between carbohydrates and lectins which are used in bacterial or viral adhesion[6-8] is a challenging task that could lead to improvements in pathogen detection and inhibition of bacterial or viral infections. The existing methods for probing lectin–carbohydrate interactions using biosensors are tedious, requiring extensive instrumental setup and technical expertise[9]. Accordingly, there are critical needs for developing effective new glycotechnologies and biosensors that are sensitive, rapid, simple, reliable and cost-effective.

In this regard, single-walled carbon nanotubes (SWNTs) because of their excellent electronic properties, high surface to volume ratio, and extreme sensitivity to surface adsorption events can be an ideal candidate for investigating carbohydrate interactions. Some recent examples have demonstrated the possible applications of carbon nanotubes (CNT) for the detection of pathogens or cancer biomarkers[10]. The sensitivity of CNT-containing devices is very impressive, and they in principle should be able to detect ultralow bacterial concentrations in few minutes. The specific interaction of CNTs with bacteria has been already studied by covalent[11] or noncovalent[12,13] functionalization with carbohydrate derivatives. Several reports have shown the interactions of carbohydrate-coated CNTs with lectins using glycoconjugates, glycolipids[14], glycopolymers[15]. The covalent functionalization with galactose dendrimers led to selective interactions with *Bacillus* spores[16]. However, the use of CNTs for electronic detection of carbohydrate-protein interactions has not been yet utilized. As it has been demonstrated by our group and others, NTFET devices can electronically transduce interactions with proteins and other biomolecules[17-20].

In this work, we investigate the specific binding of glycoconjugates and lectins using SWNTs configured into electrolyte gated field-effect transistors (FETs). SWNT networks act as conducting channels which transduce the binding between glycoconjugates and lectins into electrical signal. Figure 1a shows a schematic illustration of glycoconjugate-functionalized NTFET detection platform. Using these devices we study the interaction between porphyrin-based glycoconjugate carrying α-D-mannose epitope and its selective lectins Concanavalin A (ConA). ConA (25 kDa) lectin is tetrameric lectin, specific for mannose and glucose and is purified from jack-bean, *Canavalia ensiformis* [21,22]. To validate these results several control experiments and characterization methods such as UV-vis-NIR absorption spectroscopy were implemented.

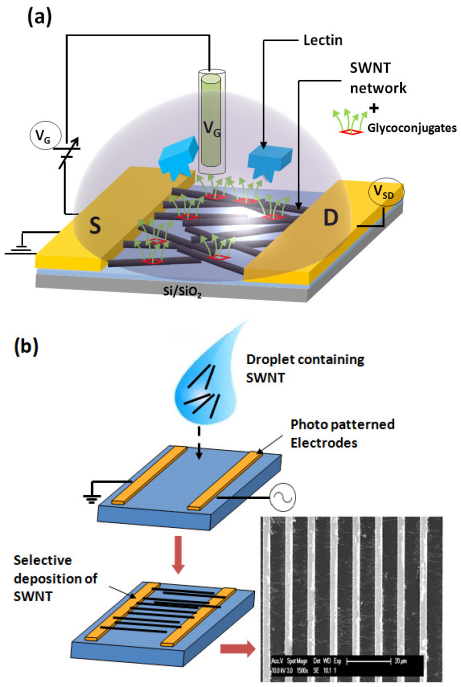

Figure 1: (a) Schematic illustration of glycoconjugate-functionalized single-walled carbon nanotubes (SWNTs)-FET for selective detection of lectins. (b) Schematic of dielectrophesis technique to fabrication SWNT FET device, SEM image of interdigitated electrodes.

2 EXPERIMENTAL

NTFET devices were fabricated by patterning interdigitated microelectrodes (source-drain spacing of 5 µm) on top of 200 nm oxide layer on Si substrates using photolithography and e-beam evaporation of 30 nm Ti and 100 nm of Au. SWNTs were obtained from Carbon Solutions Inc. and were used as conducting channels in these FETs. Alternating current dielectrophoresis (DEP) technique was used for selective deposition of SWNT networks from *N*,*N*-dimethylformamide (DMF) suspension onto each interdigitated microelectrodes pattern[23]. The dielectrophoresis parameters namely a.c frequency (10MHz), bias voltage (8 V_{pp}) and bias duration (60 s) were used to yield SWNT network devices with similar electrical conductance (0.1 – 1 mS)(Figure 1b). Each Si chip comprising of multiple FET devices was then placed onto a standard ceramic dual in-line package (CERDIP) and wirebonded. Two Keithley 2400 sourcemeters were used for FET measurements. The electrical performance of each device was investigated in electrolyte gated FET device configuration. The conductance of NTFET device was tuned using the electrolyte as a highly effective gate[24]. A small fluid chamber (1 mL) was placed over the NTFET device to control the liquid environment using phosphate buffer solution (PBS) (20 mM) at pH 7. A liquid gate potential (−0.75 V to +0.75 V) with respect to the grounded drain electrode was applied using an Ag/AgCl (3 M KCl) reference electrode submerged in the electrolyte. The drain current of the device was measured at a constant source-drain voltage of 50 mV. Transfer characteristics (i.e., conductance (G) versus gate voltage (V_g)) were measured to investigate the interactions between glycoconjugates and lectins. Figure 1b shows the scanning electron microscope (SEM) images (Phillips XL30 FEG) of a single device after DEP of SWNTs.

To detect lectins, NTFET devices were noncovalently functionalized with porphyrin-based glycoconjugates. The experimental details for their synthesis are described elsewhere[25]. Surface functionalization of NTFETs with each glycoconjugate was performed by incubating the Si chips in 2 µM solution of the glycoconjugates (in deionized water) for two hours followed by rinsing with deionized water. This was followed by incubating the chips for 40 minutes in different concentrations of lectin solutions prepared in PBS with 5 µM $CaCl_2$ and latter washed three times with PBS solution.

3 RESULTS AND DISCUSSION

We measured a total of 20 NTFET devices. The liquid gate FET measurements of bare NTFETs exhibited either a p-type or ambipolar device characteristics (Figure 2). Because of the presence of metallic SWNTs in the network the ON/OFF ratio of the device was below 10^2. After functionalization with the porphyrin-based glycoconjugates, a decrease in ON conductance with a slight negative shift in gate voltage was observed (Figure 2a). This indicates that the noncovalent interactions between metalloporphyrin core and SWNT dominate the signal. Furthermore, the decrease in device conductance can be attributed to the screening of charge in the SWNT network as a result of the presence of electron donating Zn-porphyrin molecules[26]. In a control experiment, zinc tetraphenyl porphyrin (ZnTPP) showed a similar response on transfer characteristics of NTFETs confirming the dominant role of Zn-porphyrin and its π-π interaction with SWNT sidewalls (Figure 3a).

Additionally, UV-vis-NIR absorption spectroscopy was employed to investigate the interaction between SWNTs and metalloporphyrin. Besides S_1, S_2 and M_1 bands corresponding to SWNTs an additional peak at 425 nm was observed for SWNT-α-D-mannose conjugates confirming the formation of the desired complex. We also observed an increase in the S_1 band intensity by 6.5% and 13.6% when SWNTs were functionalized with α-D-mannose and its specific binding lectin, ConA, respectively. This increase in S_1 intensity and decrease in the NTFET conductance (Figure 2b) can be attributed to the refilling of the partially depleted SWNT valence band with electronic density donated by the binding molecules)[26].

After glycoconjugate functionalization, devices were treated with specific binding lectins and their controls. Figure 2a shows the response of a α-D-mannose

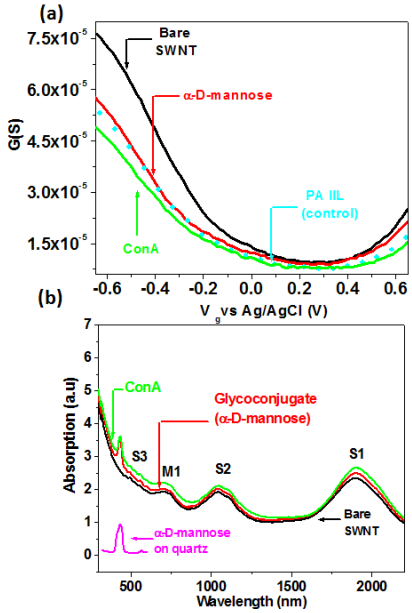

Figure 2: Electronic detection of carbohydrate-lectin interactions: G vs Vg data for NTFET devices functionalized (a) with α-D-mannose glycoconjugate and measured after incubation with 2 µM ConA lectin (PA IIL as control). (b) UV-vis-NIR absorption spectra of pristine SWNTs after functionalization with r-D-mannose and after ConA (mannose specific lectin)[25].

glycoconjugate functionalized device to various lectins. Upon incubation with non-specific lectin (PA-IIL, 2 µM) the transfer characteristics remained unaffected. However, when treated with the mannose specific lectin (ConA, 2 µM) a decrease in ON conductance was observed indicating the selective interaction between the glycoconjugate and the lectin. The decrease in ON conductance can be attributed to the net negative charge of the ConA (isoelectric point of 5) at the measured pH of 7 and charge transfer to SWNTs .

In a different control experiment, when a NTFET device was functionalized with only ZnTPP and treated with ConA, the transfer characteristics remained unchanged(Figure 3b). This observation indicates that the responses obtained in Figure 2 are indeed from specific interaction between glycoconjugates and lectin and not due to non-specific adsorption on Zn-porphyrins or SWNTs. No significant change in the transfer characteristics was observed when NTFET devices were incubated for longer duration (~18 hr)[25].

Additionally, the sensitivity of NTFET devices was investigated by plotting the G vs V_g for ConA and other lectins (namely β-D-galactose, α-L-fucose)[25]. A detection limit of 2 nM of specific lectin was obtained for β-D-galactose functionalized NTFETs towards its specific lectin PA-IL. The detection limit of NTFET devices was comparable to other techniques traditionally used for different lectin detection such as optical microarray (1.4 nM), electrochemical surface plasmon resonance (E-SPR) (41 nM)(references listed in [25]). To further understand the kinetics of the glycoconjugate-lectin interactions, and to determine dissociation constant (K_d), as a function of C_{lectin} were plotted. The dissociation constant of K_d ~ 6.8 µM was obtained for galactosylated NTFETs towards its specific lectin PA-IL.

In conclusion, we have demonstrated a novel detection platform using NTFET devices for highly selective detection of interactions between glycoconjugates and bacterial lectins, which exhibit specific multivalent binding to carbohydrates. The interaction between lectins and glycoconjugates was transduced as change in the device conductance. Noncovalent functionalization of the devices with glycoconjugates facilitated in preventing non-specific protein adsorption and highly selective lectin binding. Using standard microfabrication techniques, multiple devices having different glycoconjugates functionalized can be developed for simultaneous and rapid detection of specific lectins. These lectins can then be specifically attach to the carbohydrates on the bacterial surface thus leading to bacteria detection. Currently, we are in the process of integrating these devices with microfluidic systems for rapid identification of bacterium within a crude sample from a water system, soils or human specimens. We envision the use of this nanodetection platform for early detection of disease outbreaks and preservation of public health.

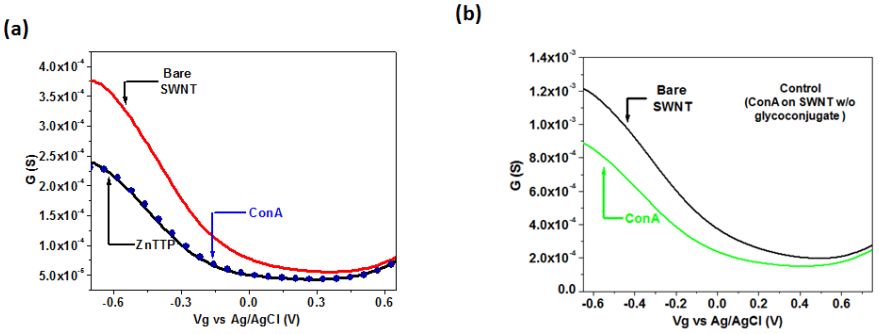

Figure 3: Control experiments: (a) G vs Vg curves measured for bare SWNT, and after 2 µM ConA lectin (in PBS (pH7) with 5 µM Ca2+) attachment[25]. (b) *G* vs *V*g curves for bare SWNT after ZnTTP functionalization (without glycoconjugate) and after ConA (mannose specific lectin).

REFERENCES

1. H. Lis, N. Sharon, Chem. Rev. 98, 637,1998.
2. R. J. Pieters, Org. Biomol. Chem., 7, 2013,2009.
3. A. Varki, Glycobiology 3, 97,1993.
4. A. K. Dwek, Chem. Rev. 96, 683, 1996.
5. C. R. Bertozzi and L. L. Kiessling, Science,291, 2357, 2001.
6. J. J. Lundquist and E.J. Toone, Chem. Rev., 102, 555, 2002.
7. A. Imberty and A. Varrot, Curr. Opin. Struct. Biol. 18, 567, 2008.
8. N. Sharon, Biochim. Biophys. Acta, 1760, 527, 2006.
9. R. Jelinek and S. Kolusheva, Chem. Rev. 104, 5987, 2004.
10. A. Gustavo, R. Jordi, D. Ali and F. Rius, Angew. Chem. Int. Ed. 48, 7334,2009.
11. J. Bahr and J. J. Tour, Mater. Chem.,12, 1952, 2002.
12. P. Wu, X. Chen, N. Hu, U. C. Tam, O. Blixt, A. Zettl and C. R. Bertozzi, Angew. Chem. Int. Ed. 47, 5022, 2008.
13. T. Hasegawa, T. Fujisawa, M. Numata, M. Umeda, T. Matsumoto, T. Kimura, S. Okumura, K. Sakurai and S. Shinkai, Chem. Commun. 2150, 2004.
14. X. Chen, U.C. Tam, J. L. Czlapinski, G. S. Lee, D. Rabuka, A. Zettl and C. R. Bertozzi, J. Am. Chem. Soc. 128, 6292, 2006.
15. P. G. Luo, H. Wang, L. Gu, F. Lu, Y. Lin, K. A. Christensen, S. T. Yang and Y.-P. Sun, Nano 3, 3909, 2009.
16. H. G. Sudibya, J. Ma, X. Dong, S. Ng, L. Li, X. Liu and P. Chen, Angew. Chem. Int. Ed. 48, 2723,2009.
17. K. Bradley, M. Briman, A. Star and G. Grüner Nano Lett. 4, 253,2004.
18. A. Star, J.-C. P.Gabriel,K. Bradley and G. Grüner, Nano Lett. 3, 459,2003.
19. M. Shim, N. Kam, R. Chen, Y. Li, and H. Dai Nano Lett. 2, 285, 2002.
20. I. Heller, A. Janssens, J. Männik, E. Minot, S. G. Lemay and C. Dekker Nano Lett.,8, 591, 2008.
21. A. Imberty, M. Wimmerová, E. P. Mitchell and N. Gilboa-Garber Microbes Infect. 6, 221,2004.
22. C. Chemani, A. Imberty, S. de Bentzman, P. Pierre, M. Wimmerová, B.P. Guery and K. Faure K. Infect. Immun. 77, 2065,2009.
23. Z. B. Zhang, X.J. Liu, E.E. Campbell and S.L. Zhang J. Appl. Phys. 98, 056103, 2005.
24. S. Rosenblatt, Y. Yaish, J. Park, J. Gore, V. Sazonova and P.L. McEuen Nano Lett. 2, 869. 2002.
25. H. Vedala, Y. Chen, S. Cecioni, A. Imberty, S. Vidal and A. Star, Nano Lett., Nano Lett. 11, 170, 2011.
26. S. Hecht, R.J. Ramirez, M. J. Briman, E. Artukovic, K. S. Chichak and J. S. Stoddart, and G. Grüner, Nano lett., 6, 2031, 2006.
27. S. Cecioni, S. Faure, U. Darbost,I. Bonnamour, H. Parrot-Lopez, O. Roy, C. Taillefumier, M. Wimmerová, J. Praly, A. Imberty, S. Vidal, Chem.Eur.J. (in press)
28. M. J. Waner, M.Gilchrist and M. Schindler,M.J. Dantus, Phys. Chem. B, 102, 1649, 1998.