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Single-Walled Carbon Nanotube-Based Chemiresistive Affinity Biosensors for Small Molecules: Ultrasensitive Glucose Detection

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In recent years, there has been increasing interest in the use of one-dimensional (1D) nanostructures such as nanowires, nanobelts and nanotubes as transducer elements in affinity (bio)sensors. Use of nanomaterials provides high sensitivity with a low limit of detection and (in conjunction with a molecular-recognition element) high selectivity for label-free, rapid, low-cost, multiplexed, point-of-care/field detection of various analytes. Single-walled carbon nanotubes (SWNTs) are one such class of nanomaterials that have been used extensively as sensing elements because of their excellent electrical properties, their ultrahigh ratio of surface area to volume, and the extreme sensitivity of their surface atoms to any surface adsorption/reaction events. SWNTs modified with biorecognition molecules, such as antibodies, aptamers, or DNA, have been successfully used to detect various targets, including proteins,[1] viruses,[2] bacteria,[3] yeast,[4] DNA/RNA,[5] and even mammalian cancer cells.[6] The majority of these SWNT-based biosensors are affinity sensors wherein the binding of the analyte (generally a large, charged antigen) to the bioreceptor immobilized on the surface of the SWNTs leads to a change in conductance of the SWNT channels.

Weakly charged or uncharged small molecules constitute a large group of analytes of interest in the fields of environmental monitoring and health care. The detection of these analytes using SWNT-based chemiresistive/field-effect transistor (FET) sensors using the traditional modes of affinity-based sensing might be ineffective, as their binding to the recognition molecule might not generate a measurable change in conductance/resistance. Nanobiosensors that can detect and quantify such small molecules with high sensitivity and selectivity are therefore urgently needed. In an effort to achieve these objectives, we have for the first time employed the displacement immunoassay/sensor format[7] on a SWNT-based chemiresistive platform and demonstrated its effectiveness. In the displacement mode of operation, the SWNTs are initially functionalized with an analogue of the target analyte that has a lower affinity for binding to the biological recognition molecule than the actual analyte does; this is followed by binding to the biological recognition element, such as an antibody. Upon addition of the sample analyte to the device, the analyte competes with the analogue for the bioreceptor and displaces it from the SWNT channels, leading to a change in the sensor conductance.

Figure 1 shows a schematic of the displacement-based chemiresistive affinity (bio)sensor. The principle behind the displacement mode-based sensors is similar to the competitive mode in immunoassays. The well-characterized glucose−concavalin A−dextran system[8] was evaluated as a model system to demonstrate the displacement-based chemiresistive mode of sensing. Concanvalin A (ConA), a plant lectin that binds noncovalently to some

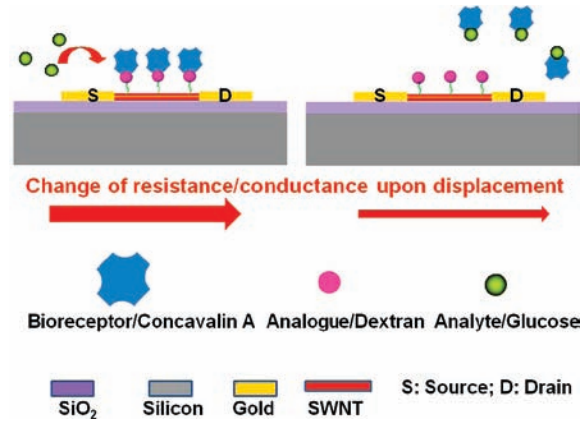

Figure 1. Schematic of the displacement-based chemiresistive biosensor.

carbohydrates, is a metalloprotein with four carbohydrate binding pockets that can exist in a dimeric form at low pH and a tetrameric form at neutral pH.[9] Dextran, a polysaccharide, and glucose, a monosaccharide, are among the carbohydrates that reversibly bind to ConA, with glucose displaying a higher binding affinity. Thus, upon introduction of glucose to a ConA−dextran complex, glucose displaces dextran from ConA (Figure 1). The binding of ConA to carbohydrates results in changes to its conformation that alter its isoelectric point to far from neutral pH, leading to an accumulation of positive charge.[10] On the other hand, both glucose and dextran are electrically neutral over a wide pH range in their free forms as well as when bound to ConA. Thus, when this system is used in the chemiresistive configuration, binding and removal of ConA from the SWNTs results in a conductance change because of its positive charge.

The process started with alternating-current (AC) dielectrophoretic alignment of SWNTs across a pair of 3 μm spaced microfabricated gold electrodes. In brief, this procedure involved addition of a 0.1 μL drop of SWNTs suspended in dimethyl formamide and application of an AC voltage at a frequency of 4 MHz (0.3 V peak-to-peak amplitude) across the electrodes. The aligned SWNTs were then annealed in place by heating at 300 °C for 1 h in an inert environment maintained by a continuous flow of nitrogen gas containing 5% hydrogen. This was followed by modification with dextran by overnight incubation at room temperature with 1 wt % phenoxydextran (DexP) in water, incubation with 0.1% Tween20 to block any naked/bare sites on the SWNTs to prevent any nonspecific adsorption, and a final incubation with 14 μM ConA solution prepared in 10 mM phosphate buffer supplemented with 0.5 mM $CaCl_2$ and 0.1 mM $MnCl_2$ (PB) for 2 h at room temperature ($CaCl_2$ and $MnCl_2$ were added because the metalloprotein ConA requires

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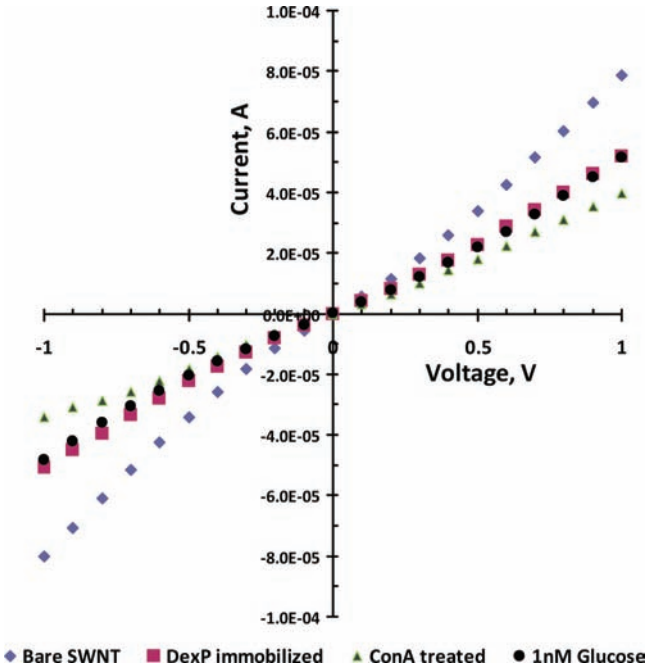

Figure 2. $I-V$ characteristics of the biosensor at various stages of fabrication and upon addition of glucose.

Mn^{2+} and Ca^{2+} for binding).[11] Because dextran cannot bind to the SWNTs by itself, the hydrophobic dextran derivative DexP was synthesized (see the Supporting Information) to noncovalently modify the SWNTs.[12] The fabrication and sensing processes were monitored by recording the current−voltage ($I-V$) characteristics of the device between +1 and −1 V after each step using a semiconductor parameter analyzer. As shown in Figure 2, the current in the SWNT device at a given voltage decreased upon incubation with both dextran and ConA. The former decrease is a consequence of the modification of the SWNTs with the DexP molecules by means of $\pi-\pi$ stacking interactions between SWNTs and phenoxy groups, and the latter is attributed to the accumulation of positive charge on the ConA molecules and/or a scattering-potential effect resulting from its binding to dextran.[13]

To examine the displacement principle of detection and the functionality of the biosensor for glucose, the biosensor was incubated with 1 nM glucose in PB for 1 min and washed three times with PB and once with deionized water, after which the $I-V$ data were recorded. As shown in Figure 2, the conductance of the device reverted to the original value for the dextran-modified SWNTs, confirming the displacement sensing modality for the weakly charged, small-sized glucose molecules. The specificity of the biosensor was also evaluated by measuring the response of DexP- and Tween20-blocked SWNTs without conjugated ConA upon addition of glucose, Mg^{2+}, and Ca^{2+}. As shown in Figure 3, the sensor's conductance remained unaffected upon incubation with 1 nM glucose, 0.1 mM $MnCl_2$, and 0.5 mM $CaCl_2$ in 10 mM phosphate buffer, confirming the sensor response to be the result of the highly specific competition between dextran and glucose for ConA binding sites. Figure 4A shows the normalized response of the biosensor $[(R - R_0)/R_0$, where R and R_0 are the resistances after exposure to glucose and buffer, respectively, as determined from the reciprocals of the slopes of the $I-V$ curves from +0.1 to −0.1 V] as a function of analyte concentration in the buffer. The response was linear over the range from 1 pM to 1 nM and had a sensitivity of 0.039 per pM glucose (the slope of the calibration plot). This

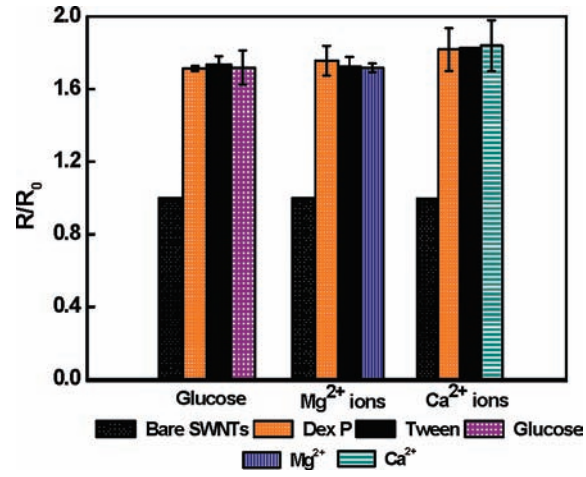

Figure 3. Response of SWNTs modified with DexP and Tween20 but not ConA to 1 nM glucose, 0.1 mM $MnCl_2$, and 0.5 mM $CaCl_2$ prepared in 10 mM phosphate buffer.

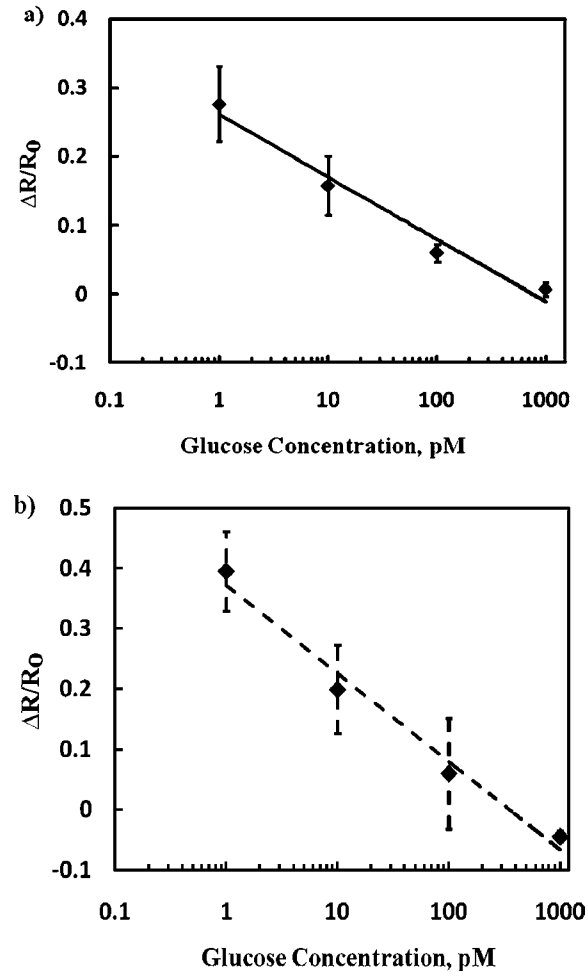

Figure 4. Biosensor calibration for glucose in (a) 10 mM phosphate buffer and (b) human plasma. Data points are averages of four independent sensors (for each investigation) prepared at different times, and error bars represent ±1 standard deviation. The regression coefficient for the buffer sample was 0.97, and that for the spiked plasma sample was 0.98.

lower detection limit is superior to the limits of 50 nM and 3.7 mM reported for ConA−cyclodextrin/dextran solution-based assays using fluorescence resonance energy transfer between CdTe quantum dots and gold nanoparticles[14] and IR absorbance of carbon

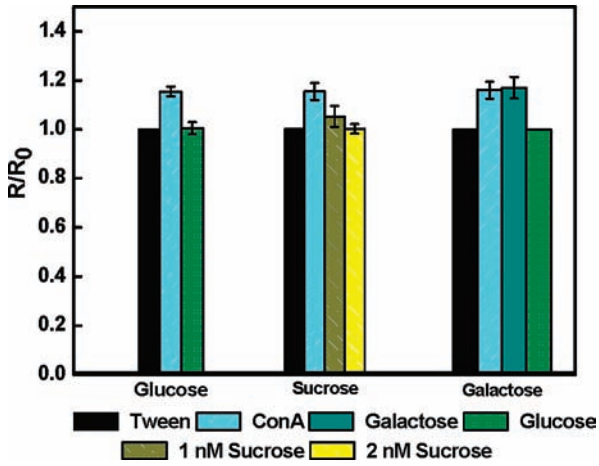

Figure 5. Biosensor selectivity for various sugars (1 nM in 10 mM phosphate buffer). Data points are averages of three independent sensors prepared at different times, and error bars represent ±1 standard deviation.

nanotube deaggregation,[12] respectively, and is attributed to the high sensitivity of the chemiresistive/FET mode of transduction.

The biosensor selectivity was evaluated against the sugars sucrose and galactose. As illustrated in Figure 5, a 2-fold higher concentration (2 nM) of sucrose, a disaccharide of glucose and fructose, was required to return the device resistance to the original value. On the other hand, there was no decrease in the device resistance upon incubation with 1 nM galactose, a monosaccharide. However, when the same sensor was treated with 1 nM glucose, ConA was completely displaced from the sensor surface, confirming the sensor functionality. These selectivity results are in agreement with the literature results[8] that ConA has a lower affinity for sucrose than for glucose and no affinity for galactose. Although the mechanism of operation of the sensor is well proven, these sugar specificity tests further augment this fact. Furthermore, a nearly identical sensitivity in an analysis of glucose spiked in human plasma (Figure 4B) demonstrated no interference from plasma components (i.e., no matrix effect) and thus the potential application of the sensor for blood glucose measurements.

In conclusion, we have built a SWNT-based sensor displaying two novel aspects: (1) the adaptation of the displacement mode of biosensing to a chemiresistive sensor for detection of small molecules that would otherwise be difficult to detect by the chemiresistive/FET transduction principle and (2) an enzyme-free chemiresistive glucose sensor with sensitivity in the picomolar range and exquisite selectivity. The ability to detect such low glucose concentrations using the reported sensor would find potential applications in monitoring glucose in unconventional body fluids such as interstitial fluid extracted by iontophoresis, tears, saliva and urine and at intracellular concentrations at the single-cell level in metabolomic studies.[15] While the displacement detection principle has been demonstrated for glucose, it can also be applied to other weakly charged or uncharged molecules. Furthermore, the biosensor sensitivity can be amplified by augmenting the charge of the displaced moiety.

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Supporting Information Available: Phenoxydextran synthesis, SWNT suspension preparation, and instrumentation details. This material is available free of charge via the Internet at http://pubs.acs.org.

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