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Bioelectrochemical Single-Walled Carbon Nanotubes

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Single-walled carbon nanotubes constitute a new form of molecular diameter metallic or semiconducting wire. Their high conductance, tensile strength, and chemical stability have attracted much attention and excited postulation. Their potential application as molecular-scale components of future electronic devices has, in particular, drawn much speculation. They can be prepared by a variety of methods, and recently, significant progress has been made in both their chemical functionalization[1] and their manipulation. Although graphitic side-wall functionalization will unavoidably lead to a disruption of the nanotube delocalized π system, this offers a convenient and controllable means of tethering molecular species.[2]

We,[3−5] and others[6] have been interested in the biocompatibility and bioelectrochemical applications of carbon nanotubes and have been able to immobilize a variety of species on the external and internal surfaces of multiwalled nanotubes. In this communication we report the results of experiments in which we have analyzed the interactions between single-walled carbon nanotubes (SWNTs) and a variety of metalloproteins and enzymes. Carboxylate functionalities can be introduced into nanotube side walls by a variety of oxidizing procedures.[2] These moieties can subsequently be activated to condensation with amine functionalities, such as those exposed on the surfaces of water-soluble proteins, by direct reaction with carbodiimide reagents. In this study we have utilized oxidized, purified, and annealed SWNTs and have observed robust bioimmobilization with all.

Stable dispersions of nanotubes[7] were stirred with dilute protein solutions for a number of hours.[8] The samples were then filtered, washed with water, and dispersed on atomically flat substrates for characterization. Reactions were also carried out directly on the substrate surface[9] in an attempt to bypass the difficulties associated with effective resuspension of biomodified nanotubes. Artifacts arising from the presence of contaminating catalyst (or other) particles were eliminated by imaging the same surface-confined nanotube before and after protein exposure.

To elucidate the covalent, electrostatic, and nonspecific contributions to protein−SWNT interaction, reactions were carried out both in the presence and absence of the coupling reagent EDC and followed by prolonged incubations of the product material in high (1 M KCl) salt or surfactant levels (1−10 mass % Tween 20 or 1% Triton X-100).

Figure 1 shows a typical example of a 1.2-nm diameter pristine nanotube onto which cytochrome *c* molecules have been successfully immobilized noncovalently (in the absence of any coupling reagent). This *c*-type heme protein has a high net positive charge at physiological pH (pI 10.8). To both monitor this interaction with greater clarity and examine the possible role of protein charge, a study was carried out with ferritin, a larger structure with a pI of 4.6 (negatively charged at physiological pH). Ferritin is a spherical, 24-subunit iron storage protein with a key role in iron metabolism[10]

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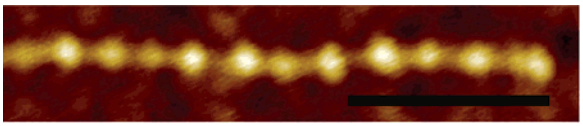

Figure 1. Cytochrome *c*-functionalized purified SWNT. Scale bar = 90 nm.

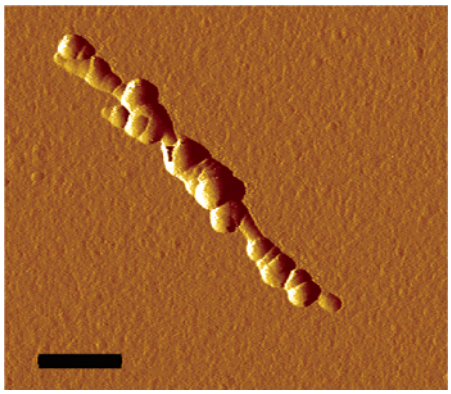

Figure 2. Amplitude AFM image of a ferritin-modified oxidized SWNT. The nanotube is heavily coated despite the expected presence of repulsive electrostatic forces. Scale bar = 150 nm.

and can be similarly immobilized on dispersed SWNTs. Having an outside diameter of 12.5 nm, ferritin labeling of nanotubes is more striking (Figure 2).

Interestingly, binding was observed at comparable levels both in the presence and absence of a coupling reagent, consistent with adsorption being predominantly noncovalent. It was not possible to significantly alleviate this physical immobilization by either washing the modified tubes or by carrying out the immobilization in the presence of surfactant. Vacuum-annealed nanotubes (in which surface-oxygen moieties are largely removed) possess similar bioimmobilization properties.[11] Control experiments carried out at highly orientated pyrolytic graphite (HOPG) surfaces produced strikingly similar results, observations consistent with the very comparable surface chemistries. These observations were made with a number of metalloproteins and enzymes (see below).

The electronic properties of SWNTs make them suitable candidates for the promotion of heterogeneous electron transfer, and promising results have been reported with both single- and multiwalled tubes.[4,12,13] Edge-plane graphite and glassy carbon (GC) electrodes were modified by methods similar to those published.[14] Background cyclic voltammograms in 100 mM potassium phosphate buffer (pH 7.5) revealed capacitance levels more than one 100-fold greater than observed at the bare underlying carbon surface under the same conditions. Broad oxidative and reductive peaks between −100 and −200 mV versus Calomel (SCE) were also observed, consistent with recent reports[12] and associated with the presence of oxygen-containing moieties on the tube surface (they

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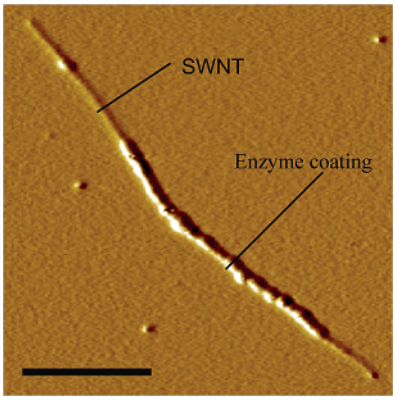

Figure 3. Amplitude AFM image of a glucose oxidase-modified SWNT. Scale bar = 200 nm.

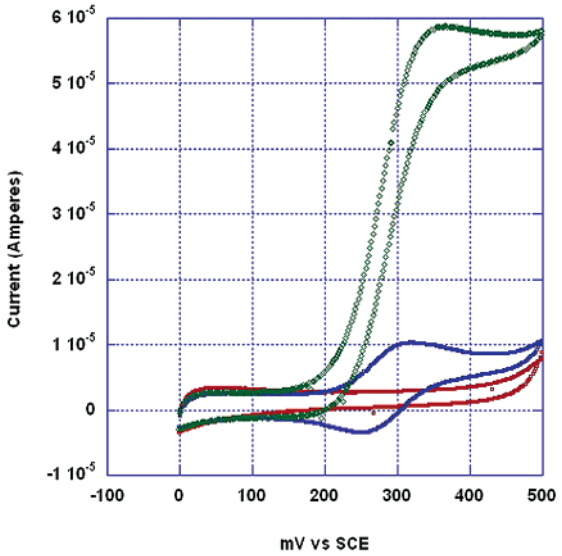

Figure 4. Voltammetric response of a GOX-SWNT-modified GC electrode in the absence (red) and presence (blue) of 0.5 mM FMCA. The catalytic response (green) is observed on the addition of 50 mM glucose.

are not observed on vacuum-annealed nanotubes).[11] The diffusive faradaic responses of $Ru(NH_3)_6^{3+}$ and ferrocene monocarboxylic acid (FMCA) and the adsorbed voltammetry of sulfonated anthraquinones observed at the SWNT-modified electrodes were more than 1 order of magnitude greater than at the underlying bare-carbon electrodes,[14] consistent with a significant increase in electroactive surface area.

High-capacity enzyme immobilization is central to much biosensor technology. Glucose oxidase is a 160 000 Da molecular mass dimeric flavoenzyme which catalyses the conversion of β-D-glucose to δ-D-gluconolactone and is used extensively in the clinical determination of blood glucose.[15] Physical immobilization of this enzyme on oxidized and dispersed SWNTs can be characterized by AFM (Figure 3). Figure 4 shows the voltammetric behavior of a glassy carbon electrode modified with GOX-SWNTs (glucose oxidase immobilized on SWNTs). The anodic response becomes catalytic on the addition of equilibriated β-D-glucose. The magnitude of this catalytic response is more than 10-fold greater than that observed on the same GC electrode surface in the absence of SWNTs (for the same substrate concentration) and is aided not only by the high levels of enzyme loading achievable on nanotubes but also by their transducing ability.

In conclusion, we have shown that it is possible to adsorb a variety of metalloproteins and enzymes on oxidized, purified (but not separately oxidized), and vacuum-annealed single-walled carbon nanotubes in aqueous solution. Although oxidative chemical activation of the nanotubes aids dispersion and thereby influences the subsequent degree of biofunctionalization, careful control experiments and AFM observations suggest that immobilization is strong, largely physical, and does not require covalent activation (equally robust immobilization occurs at vacuum-annealed SWNTs). The conductance and surface area characteristics of SWNTs offer significant advantages within the realm of heterogeneous electron transfer. Further to this, enzymes can be immobilized on the tubes with detectable retention of activity.

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Supporting Information Available: AFM micrographs of ferritin-modified vacuum-annealed nanotubes, and oxidized SWNTs after Tween 20 washes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) HiPco SWNTs (purchased from Carbon Nanotechnologies Inc., U.S.A.) were oxidized by refluxing in 3 M nitric acid at 120 °C for 13.5 h. Purity, as verified by TEM, Raman, and AFM, was high. These nanotubes could form, on sonication, stable suspensions in HPLC-grade deionized water (up to 0.1 mg/mL).
(8) A typical solution-phase protein-immobilization procedure: to a dispersion (0.03 mg/mL) of oxidized SWNTs in pure water was added a dilute solution of protein (50−100 μg/mL). The suspension was left to stand (2−20 h); then tubes were then washed thoroughly on a 0.4 μm polycarbonate membrane with HPLC-grade water, dispersed in water, and spin-coated onto oxidized silicon wafer for analysis.
(9) SWNTs were spin-coated onto oxidized silicon wafer, and the surface was then exposed to 1−100 μg/mL solutions of protein in potassium phosphate buffer for 1−3 h and then washed with HPLC grade water. Nanotubes were imaged before and after exposure to protein, using manually etched surface relocation markers.
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