Communication

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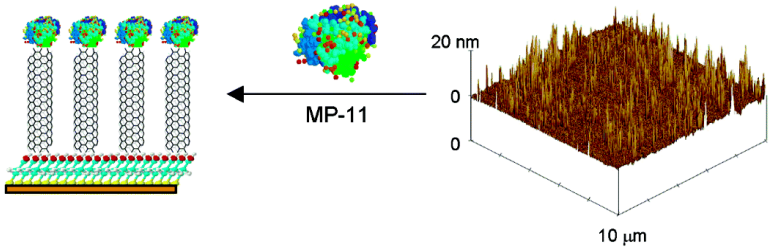

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Protein Electrochemistry Using Aligned Carbon Nanotube Arrays

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Modifying electrode surfaces on the molecular scale to allow efficient electron transfer between the electrode and a redox protein is important in the understanding of the redox properties of proteins and also in the development of enzyme biosensors without mediators. The modification of electrodes with carbon nanotubes has been applied for this purpose because of the remarkable electrocatalytic properties of carbon nanotubes. From the perspective of electron-transfer properties, the open ends of carbon nanotubes have been likened to edge planes of highly oriented pyrolytic graphite (HOPG), while the walls are suggested to have properties similar to those of the basal planes of HOPG. Certainly, in studies of the electrochemical properties of biologically relevant molecules such as hydrogen peroxide,[1,2] NADH,[2] and dopamine,[3] carbon nanotube-modified electrodes have shown superior performance as compared to other carbon electrodes. With the twin benefits of their electrocatalytic properties and their small size, carbon nanotubes are an attractive material in bioelectrochemistry,[4] especially as the possibility exists to bring the nanotubes close to the redox centers of the proteins. Multiwalled carbon nanotubes (MWNT) have shown good communication with redox proteins where not only the redox active center is close to the surface of the protein, such as cytochrome *c*,[5−7] azurin,[5] and horseradish peroxidase,[8,9] but also it is embedded deep within the glycoprotein such as glucose oxidase.[10,11] In all of these studies, however, the electrode is modified with nanotubes by drop coating a random tangle of nanotubes onto the electrode surface resulting in an unknown spatial relationship between the redox proteins and the nanotubes. In this Communication, we present a strategy for investigating the electron-transfer properties of redox enzymes, in this case, microperoxidase MP-11, attached to the end of aligned single-walled carbon nanotubes (SWNT).

The fabrication of the SWNT electrodes with MP-11 attached is summarized in Figure 1. A polycrystalline gold electrode was cleaned electrochemically in 0.05 M sulfuric acid, as described previously,[12] and placed in an aqueous ethanol solution containing 1 mM cysteamine (Sigma, Sydney Australia) for 5 h to give a cysteamine self-assembled monolayer (SAM). The electrode was further modified with oxidatively shortened HiPco SWNTs (Carbon Nanotechnologies, Inc., USA). The as-supplied SWNTs, which resembled tangled hair in the TEM (see Figure 1a), were shortened as described previously[13−15] by sonicating SWNTs in a 3:1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 4 h (see Figure 1b). The result was a log-normal distribution of tube lengths (see Table 1). Raman spectra of the cut tubes still showed a band at 190 cm^{-1} which is attributed to the radial breathing mode of the SWNTs. The shortened tubes were filtered and washed with Milli Q water to bring the pH to greater than 5. Next, 0.2 mg of shortened SWNT was dispersed in 1 mL of dimethylformamide (DMF) with 0.5 mg of dicyclohexyl carbodiimide (DCC) to convert the carboxyl groups at the ends of the shortened SWNT into active carbodiimide esters.[14] The cysteamine-modified gold electrode was placed in the nanotube solution for 4 h, while the amines at the terminus of the SAM formed amide bonds with one end of the tubes. The result is shortened SWNT aligned normal to the electrode surface (Figure 1c). The AFM image shows that the aligned tubes assemble on the surface not as individual tubes but as bundles of around 5−20 tubes. Further evidence that these images represent aligned bundles of nanotubes is that with longer incubation times the surface density of features increases (Figure 1S, Supporting Information).

Microperoxidase MP-11 (Sigma, Sydney, Australia), a small (1.9 kDa) redox protein obtained by proteolytic digestion of horse heart cytochrome *c*,[16] was attached to the free ends of the tubes by incubation in a 0.5 mg/mL MP-11 solution in HEPES buffer (pH 7.5) at 4 °C overnight (Figure 1d). The redox active center, an iron protoporphyrin IX, in MP-11 is not shielded by a polypeptide, and hence electron transfer can easily be achieved. The electrochemistry

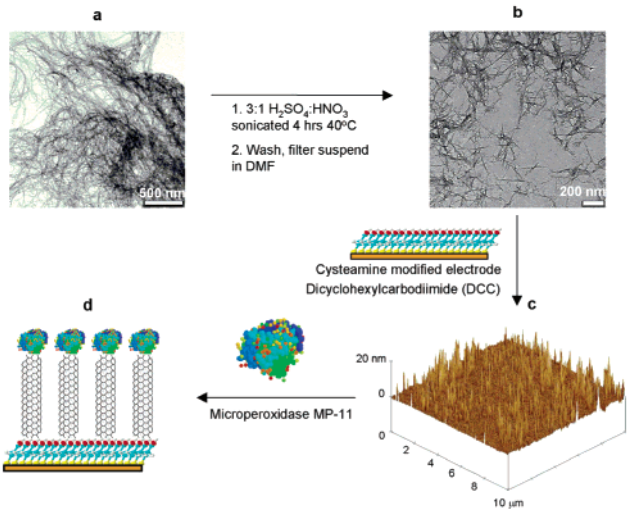

Figure 1. A schematic showing the steps involved in the fabrication of aligned shortened SWNT arrays for direct electron transfer with enzymes such as microperoxidase MP-11.

Table 1. Summary of SWNT Lengths and Electron-Transfer Rates with Different Electrode Modifications

modified electrode	mean SWNT length[a]	k_{ET} [b]
MPA		9.4 ± 0.59
cysteamine		6.1 ± 0.94
SWNTs (2 h cutting)	486 (755, 312)	3.1 ± 0.7
SWNTs (4 h cutting)	122 (170, 87)	2.8 ± 0.92
SWNTs (8 h cutting)	73 (93, 58)	3.9 ± 0.59

[a] (+1, −1 standard deviation)/nm. [b] ±95% confidence interval/s^{-1}.

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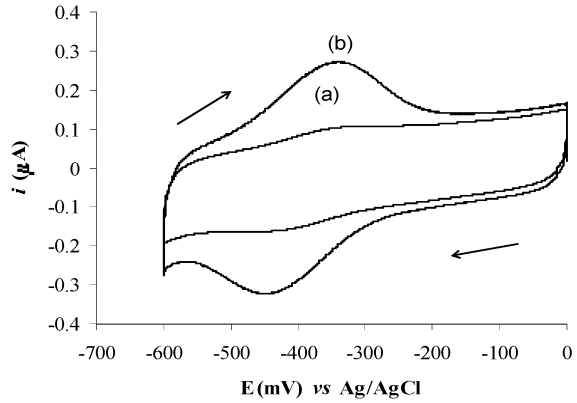

Figure 2. Cyclic voltammograms of (a) Au/cysteamine after being immersed in DMF and MP-11 solution and (b) Au/cysteamine/SWNTs/MP-11 in 0.05 M phosphate buffer solution pH 7.0 containing 0.05 M KCl under argon gas at a scan rate of 100 mV s^{-1} versus Ag/AgCl.

of the attached enzyme is shown in Figure 2. The characteristic cyclic voltammogram of MP-11 is observed with $E_{1/2}$ at −390 mV versus Ag/AgCl and a linear change in peak current with scan rate as expected for a surface bound species. The full width half-maximum of 140 mV, being greater than the theoretical value for a single bound species, implies a distribution of MP-11 molecules in different environments. The area under the reduction peak of the CV gave an MP-11 surface coverage of 35 pmol cm^{-2}. This coverage correlated well with the coverage of SWNTs of 32 pmol cm^{-2} estimated from the AFM images. The similarity in surface coverage suggested the electrochemistry was due to MP-11 attached to the ends of the tubes. The small size of MP-11 precludes the location of the enzyme on the surface being determined by AFM. Additional evidence for the redox activity being due to MP-11 molecules at the end of the tubes came from control. First, to show the observed electrochemistry was not due to MP-11 simply adsorbed on the cysteamine surface in the gaps between the nanotubes, MP-11 was adsorbed onto a cysteamine-modified electrode, and only a small current from MP-11 was observed (Figure 2a). Second, preparing a nanotube bed electrode by drop coating a nanotube dispersed in DMF on a gold electrode, followed by modification with MP-11, in the manner used for the aligned nanotubes, showed no electrochemistry. The absence of a Faradic process was unexpected as the electrochemistry of small redox molecules has been demonstrated through the walls[4,17] and previous studies have shown proteins adsorb onto the hydrophobic walls of carbon nanotubes.[5,17−19] The lack of observed electrochemistry is most likely a consequence of poor electron transfer across the walls of the SWNT to MP-11.

The efficiency of the nanotubes acting as molecular wires was determined by calculating the rate constant of heterogeneous electron transfer between MP-11 and the electrode using the method of Laviron[20] (see Table 1). The rate constant for electron transfer for the nanotube-modified electrodes was similar to that for MP-11 attached directly to a cysteamine-modified gold electrode (the same platform upon which the tubes are assembled) and to a 3-mercaptopropionic acid (MPA)-modified gold electrode (the same coupling of MP-11 as to the ends of the tubes). The similarity of rate constants demonstrates the efficiency of the shortened nanotubes as molecular wires; that is, the SWNTs do not add significant electrical resistance. The values were consistent with the range of values of most previous reports (between 1 and 20 s^{-1}; see, for example refs 16, 21). A further demonstration of the ability of the nanotubes to communicate with MP-11 comes from measurements of nanotube-modified electrodes where the nanotubes were cut to different lengths. Table 1 shows that, despite different distributions of tube lengths, the rate constants for heterogeneous electron transfer to the enzyme are essentially the same.

In summary, we have shown that shortened SWNTs can be aligned normal to an electrode by self-assembly and act as molecular wires to allow electrical communication between the underlying electrode and redox proteins covalently attached to the ends of the SWNTs. The high rate of electron transfer through the nanotubes to redox proteins is clearly demonstrated by the similarity in the rate constant for electron transfer to MP-11 regardless of whether SWNTs are present or not. This research represents a stepping stone toward achieving efficient electron transfer to enzyme redox centers, an important component of bioelectronic devices.

Supporting Information Available: Figure 1S, AFM images of aligned carbon nanotubes after 4 and 8 h of incubation in shortened SWNTs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wang, J. X.; Musameh, M.; Lin, Y. *J. Am. Chem. Soc.* **2003**, *125*, 2408−2409.
(2) Wang, J.; Musameh, M. *Anal. Chem.* **2003**, *75*, 2075−2079.
(3) Britto, P. J.; Santhanam, K. S. V.; Ajayan, P. M. *Bioelectrochem. Bioenerg.* **1996**, *41*, 121−125.
(4) Campbell, J. K.; Sun, L.; Crooks, R. M. *J. Am. Chem. Soc.* **1999**, *121*, 3779−3780.
(5) Davis, J. J.; Coles, R. J.; Hill, H. A. O. *J. Electroanal. Chem.* **1997**, *440*, 279−282.
(6) Wang, J. X.; Li, M. X.; Shi, Z. J.; Li, N. Q.; Gu, Z. N. *Anal. Chem.* **2002**, *74*, 1993−1997.
(7) Wang, G.; Xu, J. J.; Chen, H. Y. *Electrochem. Commun.* **2002**, *4*, 506−509.
(8) Zhao, Y. D.; Zhang, W. D.; Chen, H.; Luo, Q. M.; Li, S. F. Y. *Sens. Actuators, B* **2002**, *87*, 168−172.
(9) Yamamoto, K.; Shi, G.; Zhou, T. S.; Xu, F.; Xu, J. M.; Kato, T.; Jin, J. Y.; Jin, L. *Analyst* **2003**, *128*, 249−254.
(10) Guiseppi-Elie, A.; Lei, C. H.; Baughman, R. H. *Nanotechnology* **2002**, *13*, 559−564.
(11) Zhao, Y. D.; Zhang, W. D.; Chen, H.; Luo, Q. M. *Anal. Sci.* **2002**, *18*, 939−941.
(12) Gooding, J. J.; Erokhin, P.; Hibbert, D. B. *Biosens. Bioelectron.* **2000**, *15*, 229−239.
(13) Liu, J.; Rinzler, A. G.; Dai, H. J.; Hafner, J. H.; Bradley, R. K.; Boul, P. J.; Lu, A.; Iverson, T.; Shelimov, K.; Huffman, C. B.; Rodriguez-Macias, F.; Shon, Y. S.; Lee, T. R.; Colbert, D. T.; Smalley, R. E. *Science* **1998**, *280*, 1253−1256.
(14) Liu, Z.; Shen, Z.; Zhu, T.; Hou, S.; Ying, L.; Shi, Z.; Gu, Z. *Langmuir* **2000**, *16*, 3569−3573.
(15) Nan, X. L.; Gu, Z. N.; Liu, Z. F. *J. Colloid Interface Sci.* **2002**, *245*, 311−318.
(16) Lotzbeyer, T.; Schuhmann, W.; Katz, E.; Falter, J.; Schmidt, H.-L. *J. Electroanal. Chem.* **1994**, *377*, 291−294.
(17) Azamian, B. R.; Davis, J. J.; Coleman, K. S.; Bagshaw, C. B.; Green, M. L. H. *J. Am. Chem. Soc.* **2002**, *124*, 12664−12665.
(18) Balavoine, F.; Schultz, P.; Richard, C.; Mallouh, V.; Ebbesen, T. W.; Mioskowski, C. *Angew. Chem., Int. Ed.* **1999**, *38*, 1912−1915.
(19) Shim, M.; Kam, N. W. S.; Chen, R. J.; Li, Y. M.; Dai, H. J. *Nano Lett.* **2002**, *2*, 285−288.
(20) Laviron, E. *J. Electroanal. Chem.* **1979**, *101*, 19−28.
(21) Gooding, J. J.; Erokhin, P.; Losic, D.; Yang, W. R.; Policarpio, V.; Liu, J. Q.; Ho, F. M.; Situmorang, M.; Hibbert, D. B.; Shapter, J. G. *Anal. Sci.* **2001**, *17*, 3−9.

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