Using Cytochrome c_3 To Make Selenium Nanowires

A. Abdelouas,[†] W. L. Gong,[†] W. Lutze,*,[‡]
J. A. Shelnutt,[§] R. Franco,[||] and I. Moura[||]

*Center for Radioactive Waste Management (CeRaM),
The University of New Mexico,
1001 University Blvd., SE,
Albuquerque, New Mexico 87106;
Department of Chemical and Nuclear Engineering,
The University of New Mexico, Farris Engineering
Center, Albuquerque, New Mexico 87131;
Biomolecular Materials and Interfaces Department,
Sandia National Laboratories,
Albuquerque, New Mexico 87185-1349; and
Department of Chemistry, The University of New
Mexico, Albuquerque, New Mexico 87131; and Centro
de Química Fina e Biotecnologia, Departamento de
Química, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa,
2825 Monte de Caparica, Portugal*

The photoelectric and semiconductor properties of selenium are well-known. Selenium is used, e.g., in rectifiers, solar cells, photographic exposure meters, and xerography.[1] There is increasing interest in electronic, magnetic, mechanical, and chemical properties of nanoscale structures such as quantum dots and wires.[2,3] New applications for selenium may emerge. Techniques for nanowire fabrication include nanofabrication,[4] electrodeposition of metallic wires into pores and track-etched polymer membranes,[5,6] nanowire formation using self-organized nanoholes of anodically oxidized alumina,[7,8] and electron beam lithography.[9−11] A review on noval methods of nanoscale wire formation has been published recently.[12]

[†] Center for Radioactive Waste Management (CeRaM), The University of New Mexico.
[‡] Department of Chemical and Nuclear Engineering, The University of New Mexico.
[§] Biomolecular Materials and Interfaces Department, Sandia National Laboratories.
[||] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.
(1) Johnson, J. A.; Saboungi, M. L.; Thiyagarajan, P.; Csencsits, R.; Meisel, D. J. *Phys. Chem. B* **1999**, *103*, 59.
(2) Stewart, D. R.; Springzak, D.; Marcus, C. M.; Duruoz, C. I.; Harris, J. S., Jr. *Science* **1997**, *278*, 1784.
(3) Awschlom, D. D.; DiVincenzo, D. P. *Phys. Today* **1995**, *48*, 43.
(4) Prokes, S. M.; Wang, K. L. *Mater. Res. Soc. Bull.* **1999**, *24*, 13.
(5) Martin, C. R. *Chem. Mater.* **1996**, *8*, 1739.
(6) Ferre, R.; Ounadjela, K.; George, J. M.; Piraux, L.; Dubois, S. *Phys. Rev.* **1997**, *B56*, 7791.
(7) Zhang, Z. B.; Ying, J. Y.; Dresselhaus, M. S. *J. Mater. Res.* **1998**, *13*, 1745.
(8) Schwarzacher, W.; Kasyutich, O. I.; Evans, P. R.; Darbyshire, M. G.; Yi, G.; Fedosyuk, V. M.; Rousseaux, F.; Cambril, E.; Decanini, D. J. *Magn. Magn. Mater.* **1999**, *199*, 185.
(9) Dial, O.; Cheng, C. C.; Scherer, A. J. *Vacuum Sci. Technol.* **1998**, *16*, 3887.
(10) Sone, J.; Fujita, J.; Ochiai, Y.; Manako, S.; Matsui, S.; Nomura, E.; Baba, T.; Kawaura, H.; Sakamoto, T.; Chen, C. D.; Nakamura, Y.; Tsai, J. S. *Nanotechnology* **1999**, *10*, 135.
(11) Ochiai, Y.; Manako, S.; Fujita, J.; Nomura, E. *NEC Res. Dev.* **1999**, *40*, 388.
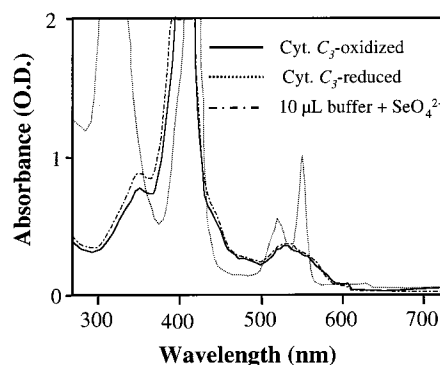(12) *MRS Bull.* **1999**, *24* (8, August).

Figure 1. Oxidation of cytochrome c_3 (Cyt c_3-reduced) in a sodium dithionite solution after addition of an excess of sodium selenate.

We report on a new method to make nanostructures in aqueous solution at room temperature, in this case selenium nanowires. We used the protein cytochrome c_3 to reduce selenate (SeO_4^{2-}) to selenium (Se^0). Cytochrome c_3 is known for its ability to catalyze reduction of metals including $U^{VI} \rightarrow U^{IV}$, $Cr^{VI} \rightarrow Cr^{III}$, $Mo^{VI} \rightarrow Mo^{IV}$, $Cu^{II} \rightarrow Cu^0$, $Pb^{II} \rightarrow Pb^0$, and $Hg^{II} \rightarrow Hg^0$.[13−15] Nanoparticles of Se^0 precipitated from an aqueous solution at room temperature, followed by spontaneous self-assembling into nanowires. Cytochrome c_3 was extracted from the sulfate-reducing bacteria *Desulfovibrio vulgaris* (strain Hildenborough) and isolated by the procedure of DerVartanian and Legall.[16]

The tetraheme cytochrome c_3 was reduced by sodium dithionite ($Na_2S_2O_4$). The redox potentials of the heme cofactor range from -50 to -350 mV. The center of each heme of the cytochrome c_3 contains an iron atom that can be reduced and oxidized reversibly. The following reactions indicate the catalytic function of cytochrome c_3:

$$1.5(Fe^{III}\text{-cytochrome } c_3)^{4+} + 3S_2O_4^{2-} + 6H_2O =$$
$$1.5Fe^{II}\text{-cytochrome } c_3 + 6SO_3^{2-} + 12H^+ \quad (1)$$
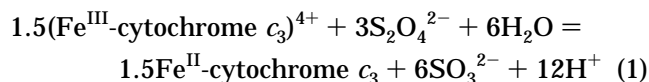
The reduced cytochrome c_3 catalyzed the reduction of selentate:

$$1.5Fe^{II}\text{-cytochrome } c_3 + SeO_4^{2-} + 8H^+ =$$
$$1.5(Fe^{III}\text{-cytochrome } c_3)^{4+} + Se^0 + 4H_2O \quad (2)$$

Cytochrome c_3 reduction (eq 1) and oxidation (eq 2) were conducted at 25 °C in a solution with a stoichiometric ratio of selenate/dithionite slightly greater than one. The solution with dithionite and cytochrome c_3 was placed in a Hewlett-Packard 8452 diode array spectrometer and monitored by UV−visible light. Selenate was then added. The overall reaction was fast as

(13) Lovley, D. R.; Widman, P. K.; Woodward, J. C.; Phillips, E. J. P. *Appl. Environ. Microbiol.* **1993**, *59*, 3572.
(14) Lovley, D. R.; Phillips, E. J. P. *Appl. Environ. Microbiol.* **1994**, *60*, 726.
(15) Abdelouas, A.; Gong, W. L.; Lutze, W.; Nuttall, E. H.; Sprague, F.; Strietelmeier, B. A.; Travis, B. J. *Sci. Total Environ.* **2000**, *250*, 21.
(16) Devartanian, D. V.; LeGall, J. *Biochim. Biophys. Acta* **1974**, *346*, 29.
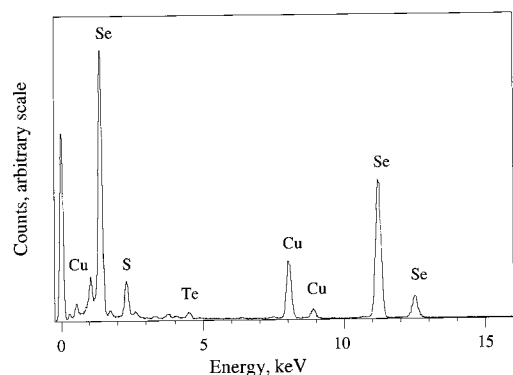
Communications

Chem. Mater., Vol. 12, No. 6, 2000 **1511**

a
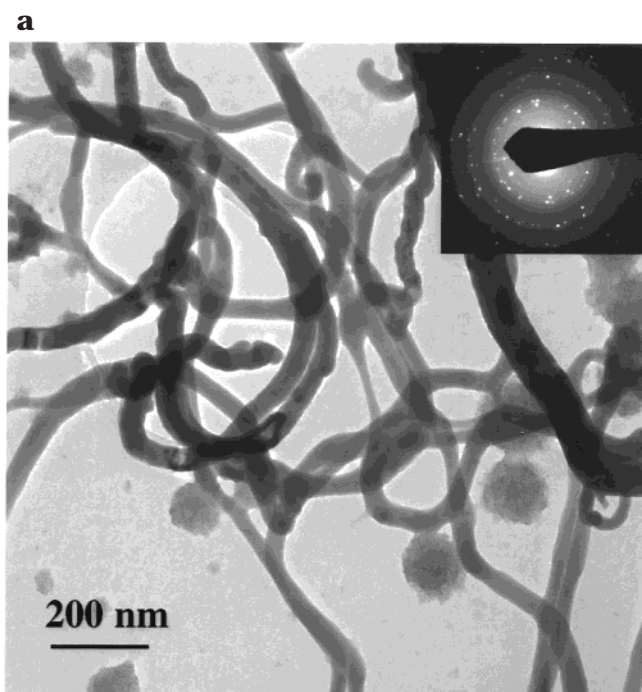
b

Figure 2. (a) TEM image of monoclinic (red) Se⁰ nanowires formed by reduction of SeO_4^{2-} by cytochrome c_3. Inset: SAED pattern of Se⁰ nanowires. (b) EDS spectrum of Se⁰ nanowires. S and Te are impurities from the chemicals.

Figure 3. TEM image of a Se⁰ nanowire composed of a chain of nanoparticles (wire: 40 nm wide, >1 μm long). Arrows point at grain boundaries.

Figure 4. TEM image showing nanoparticles in a "thick" (100 nm wide) Se⁰ wire.

indicated by instantaneous oxidation of cytochrome c_3. The spectra of reduced and oxidized cytochrome c_3 are shown in Figure 1. At the end of the experiment the solution was colorless but turned red after 1 week. Precipitation of Se⁰ was presumed because the monoclinic modification of selenium is red. A few drops of the red solution with suspended solid material were deposited on a carbon-coated grid. The grid was carefully rinsed with deionized water to remove soluble salts. We did not test whether organic material (cytochrome c_3) was still attached to the precipitate. The grid was placed into a JEOL JEM-2010 transmission electron microscope (TEM), equipped with an Oxford Link ISIS energy dispersive spectrometry (EDS) system. The microscope was operated at 200 keV. Selected-area electron diffraction was used to obtain structural information. We did not have enough material to use X-rays for diffraction analysis.

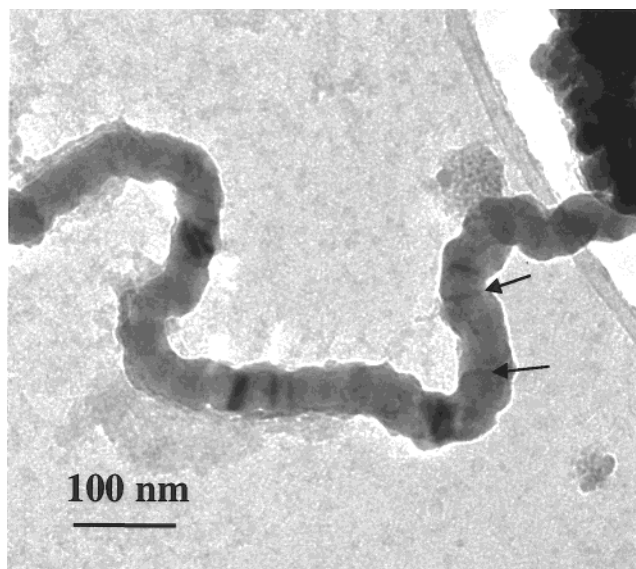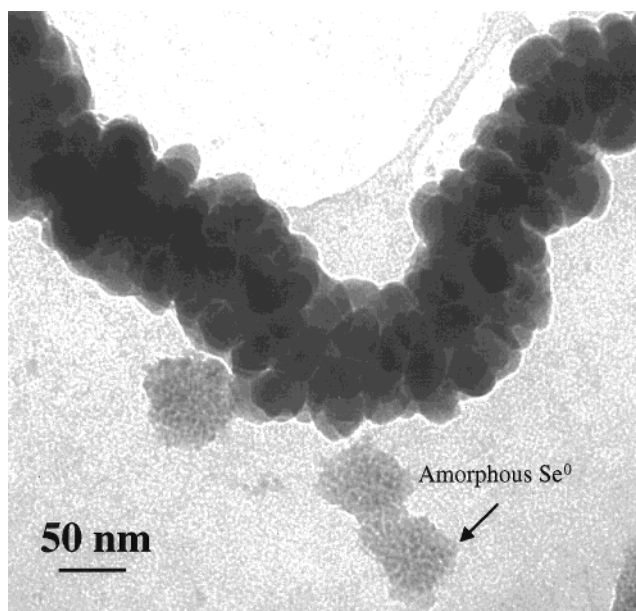The solid red material consisted of nanowires and of nanosize particles with a spherical shape. The wires were about 60 nm in diameter and typically 1 μm long (Figure 2a), the particles were about 50 nm in diameter. They appeared to be agglomerates of smaller particles (see cluster in Figure 4). With time, more red particles self-assembled and more nanwires formed. EDS analysis showed that the wires consisted of selenium with some sulfur and traces of tellurium (Figure 2b). Sulfur was an impurity in sodium dithionite, tellurium in sodium selenate.

The nanowires showed different morphologies. The sample in Figure 3 was composed of one strand of spherical particles. Thicker wires contained several nanoparticles in parallel (Figure 4). The increase in diameter may be due to attachment of nanoparticles to single strand wires. Se⁰ particles and wires were stable in aqueous solution even after 10 weeks of storage. Aging did not produce thicker but slightly longer wires.

The electron diffraction pattern in Figure 2a shows that the solid material was crystalline. During extended

exposure of the sample to the electron beam, the contrast of the diffraction pattern faded and diffuse rings appeared instead. Hence, the nanowires were susceptible to radiation damage and amorphization but they did not disassemble. The *d* spacings of this material were (in nm): 0.377, 0.325, 0.303, 0.283, 0.263, 0.220, 0.202, and 1.82. Although indexing of the electron diffraction pattern was not entirely unambiguous, the closest match with selenium crystal structures was that with the monoclinic modification, i.e., red selenium.

In a few experiments, SAED yielded diffuse diffraction patterns instantaneously, whereas amorphization of the crystalline material occurred only after some time. This suggested that amorphous Se^0 was present but we estimated that its fraction was small compared with that of the crystalline material (see round particles in Figures 2a and 4).

The mechanism for one-dimensional assemblage of crystalline Se^0 nanoparticle is not yet known. In particular, it is not yet clear whether cytochrome c_3 has a role in the formation of the nanowires. However, the properties of elemental selenium and its chemical homologue sulfur are well-known and may provide insight into the mechanism. After reduction of selenate, the solution was clear and colorless. Se^0 was expected to be in colloidal state in analogy to sulfur. Colloidal solutions of S^0 are clear, colorless, and very stable. The red selenium precipitate visible after a week and the presence of sulfur in it suggest that the monoclinic Se_∞ modification formed. Se_∞ forms solid solutions with sulfur, Se_β does not. Red glassy and red crystalline seleniums are metastable. Observation of amorphous precursors and amorphization under irradiation of crystalline nanoparticles of red Se is in agreement with Se phase behavior. Transformation of red Se into gray Se, the only thermodynamically stable modification of Se, requires higher temperature. Red monoclinic Se forms Se_8 rings stacked in one crystallographic direction. It may be speculated that stacking of nanocrystals in the same direction provides an energetically favorable link between them. This would support preferential assemblage of nanocrystals in one direction. Grain boundaries between nanocrystals are visible and pointed out by the arrows in Figure 3.

A similar phenomenon of particle assemblage in one direction was observed by Zhou et al.[17] who studied the formation of silver nanoparticles using a method known as solid−liquid-phase arc discharge. Aging of a solution containing silver nanoparticles led to the formation of silver nanowires after 2 weeks. The wires were about 80 nm in diameter and 14 μm long. Zhou et al. suggested that the formation of clusters of tadpole-like or column-like morphology, together with the cooling of the solution prevented these shapes from becoming spherical.

The catalytic function of cytochrome c_3 to accelerate certain reduction reactions may find further application in the field of nanomaterials synthesis. As an example, we found that nanoparticles of copper ($<10−70$ nm) and lead (40 nm) form instantaneously, using the procedure described above. Formation of nanowires with these materials remains to be studied. Sodium dithionite can be eliminated by electrochemical reduction of cytochrome c_3, thereby purifying the system. A particular advantage of this new process is that nanocrystals are formed in one chemical reaction at room temperature.

(17) Zhou, Y.; Yu, S. H.; Cui, X. P.; Wang, C. Y.; Chen, Z. Y. *Chem. Mater.* **1999**, *11*, 545.