Using cytochrome c₃ to make selenium nanowires

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We report on a new method to make nanostructures, in this case selenium nanowires, in aqueous solution at room temperature. We used the protein cytochrome c₃ to reduce selenate (SeO₄²⁻) to selenium (Se⁰). Cytochrome c₃ is known for its ability to catalyze reduction of metals including U⁷⁺→U⁶⁺, Cr⁶⁺→Cr³⁺, Mo⁶⁺→Mo⁵⁺, Cu⁴⁺→Cu²⁺, Pb⁴⁺→Pb²⁺, Hg²⁺→Hg⁰. Nanoparticles of Se⁰ precipitated from an aqueous solution at room temperature, followed by spontaneous self-assembling into nanowires. Cytochrome c₃ was extracted from the sulfate-reducing bacteria Desulfovibrio vulgaris (strain Hildenborough) and isolated by the procedure of DerVartanian and Legall. The tetraheme cytochrome c₃ was reduced by sodium dithionite (Na₂S₂O₄). The redox potentials of the heme cofactor range from -50mV to -350mV. The center of each heme of the cytochrome c₃ contains an iron atom that can be reduced and oxidized reversibly.

1.5Fe³⁺-cytochrome c₃ + 3SeO₄²⁻ + 4H₂O = 1.5Fe²⁺-cytochrome c₃ + 6SO₄²⁻ + 8H⁺ (1)

The reduced cytochrome c₃ catalyzed the reduction of selenate:

1.5Fe²⁺-cytochrome c₃ + SeO₄²⁻ + 8H⁺ = 1.5Fe₃³⁺-cytochrome c₃ + Se⁰ + 4H₂O (2)

Cytochrome c₃ reduction (equation 1) and oxidation (equation 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 c₃ was placed in a Hewlett-Packard 8452 diode array spectrometer and monitored by UV-visible light. Then selenate was added. The overall reaction was very fast as indicated by instantaneous oxidation of cytochrome c₃, figure 1.

![Figure 1. Oxidation of cytochrome c₃ (Cyt c₃-reduced) in a sodium dithionite solution after addition of an excess of sodium selenate.](image)

The overall reaction was very fast as indicated by instantaneous oxidation of cytochrome c₃, figure 1. Precipitation of Se⁰ was indicated after one week when the solution turned red. The monoclinic modification of selenium is red. A few drops of the selenium suspension were deposited onto a carbon-coated grid and rinsed with deionized water to remove soluble salts. 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. The precipitate was analyzed for chemical composition and morphology. Crystal structure information was obtained using selected area electron diffraction (SAED). Nanoparticles with spherical shape and an average diameter of 50 nm were found. SAED showed the precipitate to consist of a mixture of well-crystallized monoclinic and amorphous particles. Eventually, exposure of the crystallized...
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particles to the electron beam led to loss of crystallinity.

Selenium nanowires were discovered in a solution with a stoichiometric ratio of dithionite/selenate slightly less than one, i.e., reducing conditions were maintained after reduction of selenate. A week after nanoparticles started to form, the first nanowires appeared. The wires were about 60 nm in diameter and up to 1 μm long (Figure 2a). 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, closely matching those of monoclinic selenium. EDS analysis showed that the wires consisted of selenium with some sulfur and traces of tellurium as impurities (Figure 2b).

Figure 2a. TEM image of monoclinic (red) Se⁰ nanowires formed by reduction of SeO₄²⁻ by cytochrome c₇. Inset: SAED pattern of Se⁰ nanowires.

Figure 2b. EDS spectrum of Se⁰ nanowires with S and Te impurities.

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

Figure 4. TEM image showing several twisted Se⁰ nanowires.

Figure 3 shows a wire at higher magnification, revealing that it is built of nanosized particles (see arrows). Dark lines are grain boundaries. Sometimes several wires are twisted (Figure 4). The sample in figure 3 is composed of one strand of spherical particles. Thicker wires contain several nanoparticles in parallel (Figure 5). The increase in diameter may be due to attachment of nanoparticles to single strand wires. Further aging of the precipitate led to formation of more wires with an average
diameter below 100 nm and a length of a few micrometers. Se$^0$ particles and wires were stable in aqueous solution after ten weeks of storage. Electron beam irradiation caused the nanowires to become amorphous but they did not disassemble.

![Amorphous Se$^0$](image)

Figure 5. TEM image showing nanoparticles in a "thick" (100 nm wide) Se$^0$ wire.

The mechanism for one-dimensional assemblage of crystalline Se$^0$ nanoparticle is not known in detail. However, the properties of elemental selenium and its chemical homologue sulfur are well known and provide insight into the mechanism. After reduction of selenate, the solution is clear and colorless. Se$^0$ is expected to be in colloidal state in analogy to sulfur. Colloidal solutions of S$^0$ are clear, colorless, and very stable. The red precipitate of selenium visible after a week and the presence of sulfur in it suggest that the monoclinic Se$_m$ modification formed. Se$_m$ forms solid solutions with sulfur, Se$_g$ does not. Red glassy- and red crystalline selenium 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$_m$ 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.

A similar phenomenon of particle assemblage in one direction was observed by Zhou et al.$^9$ 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 two 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 $c_5$ 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 remains to be studied. Sodium dithionite can be eliminated by electrochemical reduction of cytochrome $c_5$, thereby purifying the system. A particular advantage of this new process is that nanocrystals are formed in one chemical reaction at room temperature.

References

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