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Synthesis of selenium nanowires morphologically directed by *Shinorhizobial* oligosaccharides

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ABSTRACT

Shinorhizobial cyclosophoraose (cyclic β-(1→2)-glucan) or succinoglycan monomer (SGM 2), which has one acetyl, pyruvyl, and succinyl group, functions as a morphology-directing agent for the synthesis of pure trigonal selenium nanowires by using ascorbic acid (vitamin C) as the reducing agent. The synthesis was achieved in water at room temperature. Under these experimental conditions, the diameters of the as-prepared Se nanowires were varied in the range of 34–120 nm by cyclosophoraose and of 33–66 nm by SGM 2, in which the nanowires were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Raman spectroscopy. Through this study, we propose that *Shinorhizobial* cyclic and linear oligosaccharides have morphologically directing functions for the synthesis of single-crystalline selenium nanowires by green chemical methods.

1. Introduction

One-dimensional (1D) nanostructural materials such as nanotubes, nanowires, and nanorods have recently received much attention due to their interesting properties and various potentials in scientific and technological applications.[1–6] Some biomolecules such as agarose, gellan, DNA, chitosan, starch, alginate, and cytochrome C have been also used due to the importance of eco-friendly synthetic process of the inorganic nanomaterials.[7–12]

Selenium (Se) is extensively known as an important elemental semiconductor as well as an essential element in biology and chemistry. Se also shows some physical properties, such as a relatively low melting point (~217 °C), a high photoconductivity ($\sim 8 \times 10^4$ S cm^{-1})[13], superconductivity of metallic selenium (below 6.7 K), and the anisotropy of the thermoconductivity.[22] For other commercial purposes, Se is also applied to rectifiers, solar cells, photographic exposure meters, and to xenography.[14]

A number of methods have been applied to synthesize Se nanowires during the past few years.[15–23] Among them, facile and environmentally benign processes were also achieved to synthesize the Se nanowires by using a protein[12] or a commercially available cyclic-(1→4)-α-D-glucan (β-cyclodextrin (β-CD)).[16] However, no study has yet been reported on the synthesis of Se nanowire directed by *Shinorhizobial* cellular oligosaccharides.

Cell-associated carbohydrates in nodule-forming bacteria are known to play important roles during bacteria–plant interactions.[24] As a nodule-forming bacterium, *Shinorhizobium meliloti* 1021 produces both cyclosophoraose (cyclic-(1→2)-β-D-glucan) and succinoglycan monomers (SGMs) in extra or intracellular environment. Cyclosophoraose, which was first discovered in 1942, is a family of unbranched cyclooligosaccharides that consist of a mixture of ring molecules with various sizes (Fig. 1a). SGMs originating from *S. meliloti* 1021 are linear octasaccharides with acetyl, pyruvyl, and/or succinyl groups as substituents. Among the SGMs, SGM 2 has one each of acetyl, succinyl, and pyruvyl groups (Fig. 1b).

The cyclic and linear oligosaccharides produced by some proteobacteria, including the bacteria, have also been studied in various biotechnological applications.[25–36] Based on the functions of these natural carbohydrates, we have extended their application to the synthesis of 1D nanostructures. In this study, we report that the *Shinorhizobial* oligosaccharides have morphology-directing ability to organize commercial SeO_2 compounds into single-crystalline Se nanowires under reduction by vitamin C in water at room temperature.

2. Results and discussion

2.1. Isolation, purification, and structural analyses of cyclosophoraose and SGM 2

Isolation, purification, and structural analyses of cyclosophoraose were carried out as described previously.[30–36] Ring sizes of

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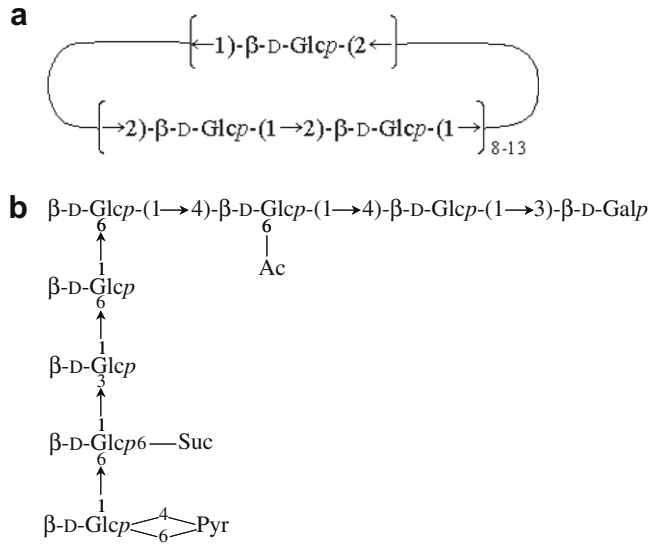

Figure 1. The proposed structures of (a) cyclosophoraose and (b) SGM 2[26,28,37] (Glc: glucose; Gal: galactose; Ac: acetyl; Suc: succinyl; Pyr: pyruvyl group).

neutral cyclosophoraose ranging from DP 17 to 27 were confirmed with matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS).[36] Based on the MALDI-MS, the number-average molecular weight of cyclosophoraose was determined as 3568.6.[35]

A detailed structural characterization of the purified SGMs was also carried out by nuclear magnetic resonance (NMR) spectroscopy, and the results were identical with the previous report.[26,37] From the structural information of the SGMs, we confirmed that the acetyl group is located at the C-6 position of the third glucose residue from the reducing terminus, the succinyl group is located at the C-6 position of the 6th and 7th glucose residue, and the pyruvyl group is linked to the 8th glucose residue through a 4,6-ketal linkage.

2.2. Characterization of as-prepared Se nanowires

In the present work, the general reaction for the formation of Se nanowire can be simplified as shown in Scheme 1. A brick-red suspension immediately appeared in all reaction mixtures in the absence or presence of the microbial carbohydrates, indicating the formation of amorphous Se in the form of spherical colloids. Then the colloidal particles of the amorphous Se were converted into Se nanostructures by the assistance of the *Shinorhizobial* oligosaccharides.

The morphology and the structure of the as-prepared Se nanowires were first investigated by scanning electron microscopy (SEM). Figure 2a and b show clear SEM images displaying the general morphology of the Se nanowires prepared successfully in the presence of *Shinorhizobial* cyclosophoraose and SGM 2, respectively. On the contrary, the mixtures of amorphous Se and short Se rods[16] were just observed when no *Shinorhizobial* carbohydrates were added (Fig. 2c).

The composition and the microstructure of the as-synthesized Se nanowires were also identified by X-ray diffraction (XRD). In the typical XRD patterns shown in Figure 3a, all the diffraction peaks could be indexed as pure trigonal phase with lattice constants of $a = 4.360$ Å and $c = 4.962$ Å for the product directed by

$$SeO_2 + H_2O \longrightarrow H_2SeO_3 + 2C_6H_8O_6 \longrightarrow Se\downarrow + 2C_6H_6O_6 + 3H_2O$$

Scheme 1. A general reaction scheme for the formation of the Se nanowires.

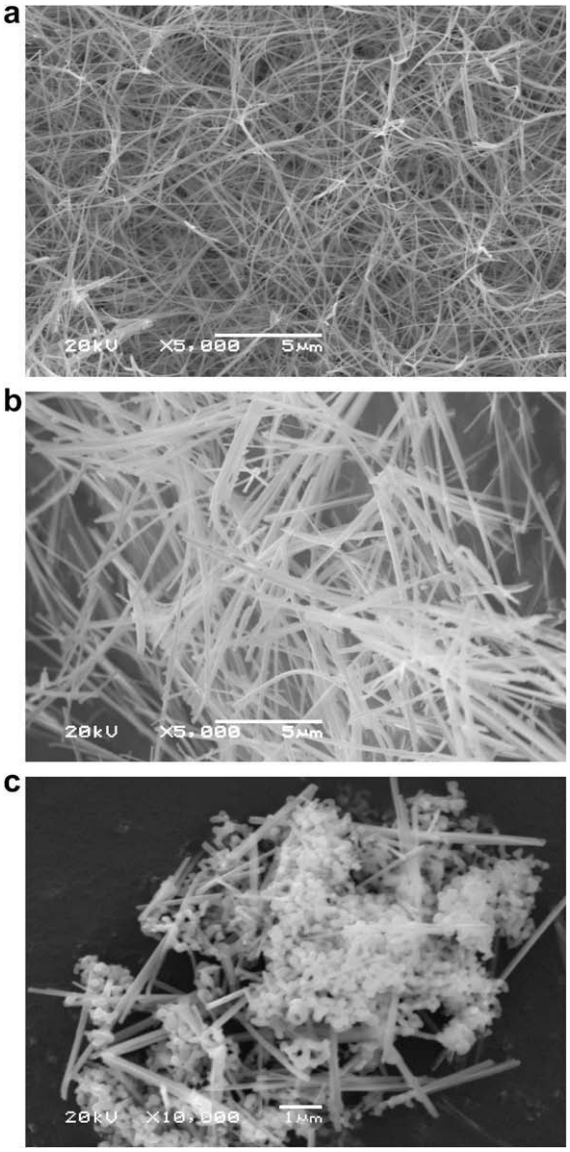

Figure 2. SEM images of the Se nanowires morphologically directed by (a) cyclosophoraose, (b) SGM 2, and (c) no *Shinorhizobial* oligosaccharides under the reduction by vitamin C in water at room temperature.

cyclosophoraose (upper trace) and $a = 4.368$ Å and $c = 4.958$ Å for the product by SGM 2 (lower trace) (JCPDS 06-0362). These results indicate that H_2SeO_3 reduced by vitamin C is successfully changed into Se nanowires under the assistance of the *Shinorhizobial* oligosaccharides in aqueous solution at room temperature.

To further investigate the crystal quality of the Se nanowires, we measured the Raman scattering spectra as shown in Figure 3b. Only one resonance peak around 238 cm^{-1}, which was observed for the products directed by cyclosophoraose (upper trace in Figure 3b) and SGM 2 (medium trace in Figure 3b), is a characteristic signature of trigonal Se, indicating the vibration of Se helical chains.[38] Through the Raman spectra, we confirmed that the as-prepared Se nanowires have a high crystal quality. In these spectra, the Se nanowires directed by the *Shinorhizobial* oligosaccharides did not give any signals of the 256 cm^{-1} peak for monoclinic Se and of the 264 cm^{-1} peak for amorphous Se.[22] However, both peaks at 256 cm^{-1} and 264 cm^{-1} as shown in the lower trace of Figure 3b were observed in the spectra obtained in the absence of the *Shinorhizobial* oligosaccharides.

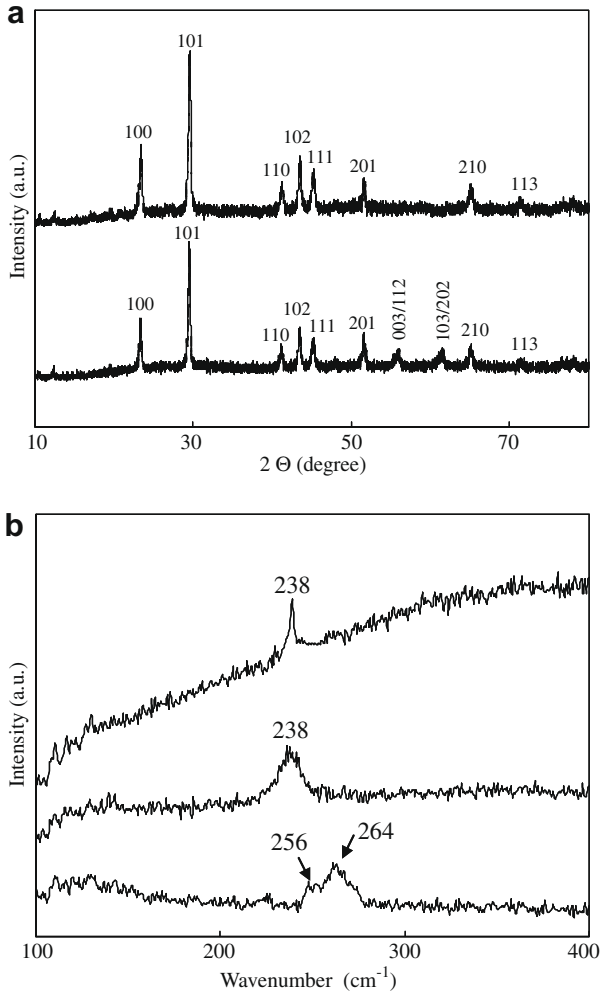

Figure 3. (a) XRD patterns of the Se nanowires obtained by the assistance of cyclosophoraose (upper trace) and SGM 2 (lower trace) under the reduction by vitamin C. (b) Raman scattering spectra of the Se nanowires synthesized by cyclosophoraose (upper trace), SGM 2 (medium trace), and no carbohydrate-added sample as control (lower trace).

The microstructures of the as-prepared nanowires were further analyzed with transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM). In the two TEM images, both bright and dark strips on the Se nanowires were observed, indicating the single-crystalline property of the products.[19] The lengths of the Se nanowires directed by cyclosophoraose (Fig. 4a) and SGM 2 (Fig. 4b) were up to several micrometers. The diameters of the Se nanowires were in the range of 34–120 nm by cyclosophoraose and of 33–66 nm by SGM 2 (Table 1). In the case of the wire directed by cyclosophoraose, the variety in the diameter may be attributed to the various ring sizes (DP 17–27)[36] of cyclosophoraose used in this study. Under the same experimental conditions, the diameters of the Se nanowires directed by α-, β-,[16] and γ-CD were less variable than those directed by cyclosophoraose (Table 1). The electron diffraction patterns were obtained by focusing the electron beam on an individual Se nanowire. Electron diffraction patterns, depending on different nanowires or different positions of a given individual nanowire, were essentially same as shown in insets of Figure 4c and d, indicating that the Se nanowires prepared by the assistance of the *Shinorhizobial* oligosaccharides were single crystalline. The spots in the insets correspond to the (1 0 0), (1 0 1), (0 0 1), (0 0 3) diffractions from trigonal Se. The occurrence of the (0 0 1) diffraction may be due to the double diffraction of the incident electron in the nanowire.[16,20,41] The electron diffraction patterns can be well indexed, indicating the formation of hexagonal Se, in agreement with the XRD results.

Figure 4c and d show the HRTEM images of the Se nanowire that give more detailed microstructural information such as the preferential growth direction and properties of the single-crystalline form of the Se nanowire. As shown in the images, the Se nanowires are structurally single-crystalline with the periodic fringe spacing of 0.60 Å for the product directed by cyclosophoraose and 0.45 Å for that by SGM 2 along the longitudinal axis ([0 0 1] zone axes) of the nanowires. These results indicate that the Se nanowires have the preferential growth direction along the [0 0 1] zone axes (*c*-axes of the lattices), and are consistent with the results reported previously.[15,16] The continuous fringes indicate that the Se nanowires have a low defect density, which indicates the high quality of the nanostructures. Combined with all the data of the Se nanowires obtained in this study, the lattice structure of trigonal Se can include an array of hexagonally packed spiral chains of Se atoms as shown in Figure 4e.

In addition to the *Shinorhizobial* oligosaccharides, in this study, we also investigated whether various cyclic and linear carbohydrates have a morphology-directing function or not, under the same experimental conditions (Table 1). Interestingly, the cyclic glucans produced by *Xanthomonas*[44] and *Bradyrhizobium*[45] species did not have morphology-directing function for the synthesis of the Se nanowires, and *Shinorhizobial* succinoglycan octasaccharides, SGMs 1 and 3 also did not. These facts suggest that individual linkage patterns of the carbohydrates, their characteristic 3D structures, and the differences in hydrogen-bonding networks in aqueous solution play important roles in determining the presence or absence of morphology-directing functions for the synthesis of Se nanostructures.

Although the growth mechanism of the Se nanowires is not yet clear, we consider that the crystalline Se nanowires are likely to grow by a phase-changing mechanism[16] during the reaction. Amorphous Se colloids can form at the initiation of the reaction and gradually dissolve in EtOH solution during aging,[16] resulting in the formation of trigonal Se nanowires. Thus, EtOH also appears to be an important medium for the growth of the Se nanowires. This is the reason why no crystalline nanowires of trigonal Se are synthesized when water was used instead of EtOH during aging.[16] Therefore, the carbohydrates having morphology-directing functions might primarily provide special environments to coalesce elemental Se, probably inducing the growth of crystalline Se nanowires via a metastable state[16,23] due to the dissolution in EtOH solution. The exact mechanistic studies for the synthesis of the Se nanowires by functional carbohydrates are in progress.

3. Conclusions

In this study, we show that *Shinorhizobial* cyclosophoraose or SGM 2 functions as a morphology-directing agent for the synthesis of Se nanowires in a single-crystalline form by using vitamin C as the reducing agent. The synthesis was achieved in water at room temperature. Single-crystalline Se nanowires were synthesized without exotic seed crystals and other toxic chemicals. The XRD patterns and the Raman scattering spectra measured from these nanowires indicate that the Se nanowires exist in the trigonal phase. During the reaction, the *Shinorhizobial* oligosaccharides were found to play crucial roles in the process of the growth of Se nanowires. As demonstrated by these successful cases, the morphology-directing functions toward inorganic nanomaterials by the *Shinorhizobial* oligosaccharides

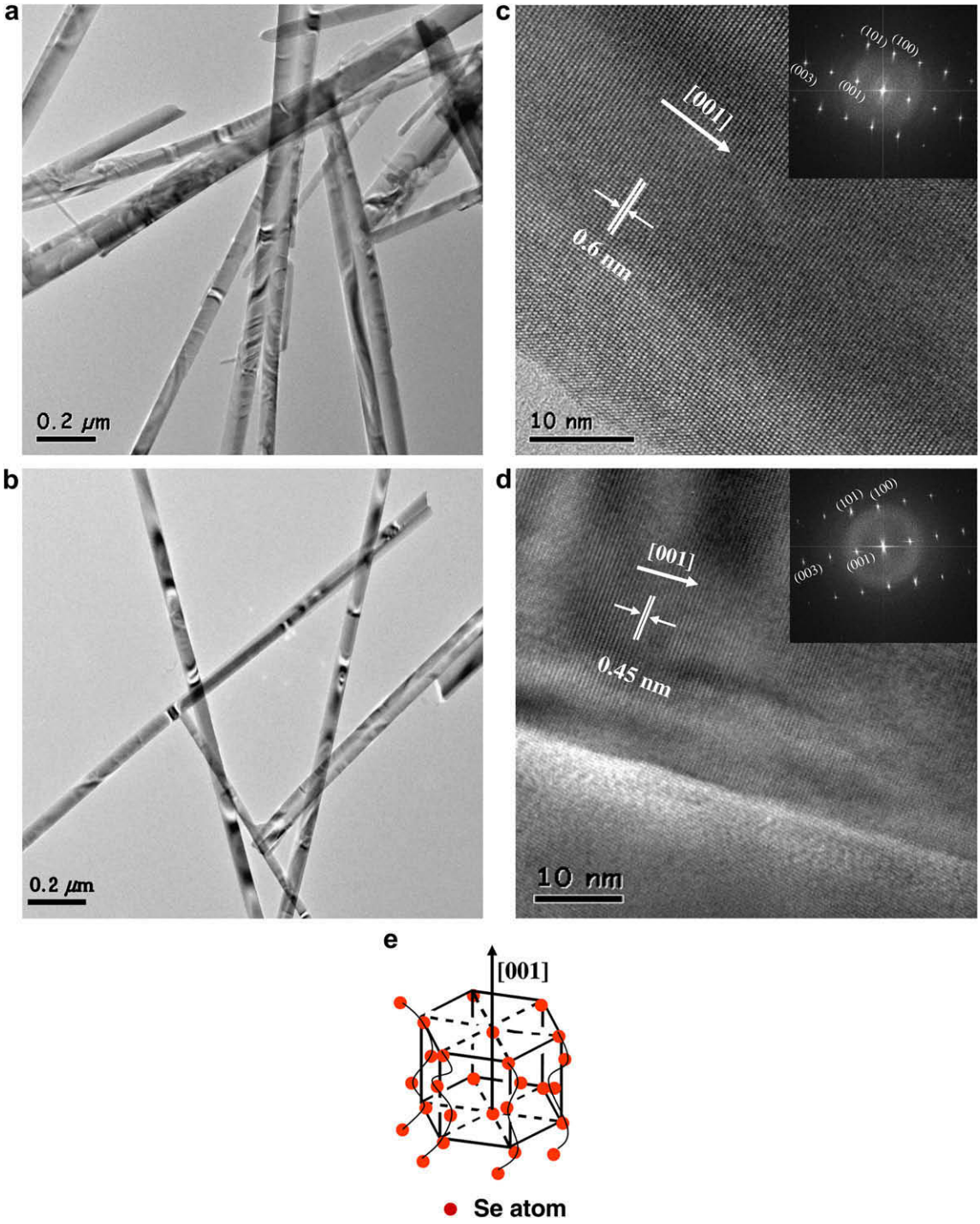

Figure 4. TEM images of the Se nanowires synthesized by the assistance of (a) cyclosophoraose and (b) SGM 2 and HRTEM images of the Se nanowires directed by (c) cyclosophoraose and (d) SGM 2 under the reduction by vitamin C. Insets of (c) and (d) represent the corresponding electron diffraction patterns of single Se nanowires synthesized by cyclosophoraose and SGM 2, respectively. (e) A schematic illustration showing the lattice structure of trigonal Se.

might also be extended to synthesize other elemental nanostructures. Also, the Se nanowires in the single-crystalline form synthesized under the experimental conditions used in this study might have some applications in nanoelectronics, nanooptoelectronics, nanosensors, and in nanobiotechnology.[42,43] Starting from this study, other naturally occurring unique carbohydrates having morphology-directing abilities will be searched for the synthesis of nanostructures in good yields using green chemical methods.

4. Experimental

4.1. General methods

Selenium dioxide (SeO_2, 99.9+%) was purchased from Sigma–Aldrich, Inc. Ultrapure water filtered from a water purification system (Direct-Q 3, Millipore) and HPLC-grade EtOH was used. The concentration of selenium dioxide (SeO_2, 30 mM), cyclosophoraose, and SGM 2 was 30 mM for each reagent. A straightforward

Table 1
Morphology of Se nanowires depending on various carbohydrates tested under the reduction of vitamin C.

Carbohydrates	XRD	Diameter[a] (nm)
Cyclosophoraose	Trigonal	34–120
α-Cyclosophorohexadecaose[b]	—	—
Cyclic β-(1→3),(1→6)-glucan[c]	—	—
α-CD	Trigonal	26–60
β-CD[d]	Trigonal	80–50
γ-CD	Trigonal	80–150
SGM 1[e]	—	—
SGM 2	Trigonal	33–66
SGM 3[f]	—	—
Succinoglycan polymers	—	—

[a] The diameters were determined by TEM results.
[b] α-Cyclosophorohexadecaose is cyclic oligosaccharides isolated from *Xanthomonas*.[27]
[c] Cyclic β-(1→3),(1→6)-glucan is cyclic oligosaccharides isolated from Bradyrhizobium species.[28]
[d] The results reported by Li and Yam.[16]
[e] SGM 1 is a *Rhizobial* oligosaccharides having acetyl and pyruvyl group as substituents.[26,28,37]
[f] SGM 3 is a *Rhizobial* oligosaccharides having acetyl, pyruvyl and two succinyl groups as substituents.[26,28,37]

green method was used to prepare Se nanowires as reported previously.[7,16] First, SeO_2 and the oligosaccharides were dissolved in ultrapure water (10 mL) and stirred for about 20 min to give clear solutions. The mixtures were separately added to the solutions of vitamin C (10 mL, 30 mM), which served as the reducing agent. After 12 h the mixtures were centrifuged and sequentially washed with pure water (3×) and EtOH (3×). The samples were suspended in EtOH and left to age for 6 h without stirring. After centrifugation, the samples were dried at room temperature.

4.2. Preparation of the *Shinorhizobial* oligosaccharides

S. meliloti 2011 was cultured in a 5-L jar fermenter containing GMS medium[39,40] at 30 °C for 72 h. Isolation and purification of the cyclosophoraose were achieved as in previous reports.[31–36] For the preparation of SGMs, *S. meliloti* 1021 was also cultured in 500 mL of GMS medium at 30 °C for 5 days.[26,28,37]

4.3. Characterization

The XRD pattern was recorded with a Japan Rigaku Rotaflex diffractometer equipped with a rotating anode and using Cu Kα radiation ($\lambda = 1.54056$ Å) over the 2θ range of 10–88°. Raman scattering spectrometer (T64000, HORIABA Jobin Yvon) with an Ar laser (514 nm) was also used. The SEM images were taken on a JEOL JSM 6380 scanning electron microscope. The TEM and SAED analyses were performed by a FEI TECNAI G2 transmission electron microscope with an accelerating voltage of 300 kV.

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