



Biological accumulation of tellurium nanorod structures via reduction of tellurite by *Shewanella oneidensis* MR-1

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HIGHLIGHTS

- ▶ *Shewanella oneidensis* MR-1 reduced tellurite to elemental tellurium.
- ▶ Crystal Te(0) nanorods accumulated intracellularly.
- ▶ Te(IV) caused changes in cell membrane rigidity by shifting the compositions of lipid components.
- ▶ *Shewanella* may provide an alternative method for Te(0) nanorod preparation.

ARTICLE INFO

Article history:

Received 30 April 2012

Received in revised form 27 August 2012

Accepted 28 August 2012

Available online 4 September 2012

Keywords:

Shewanella

Tellurium crystals

Tellurite reduction

ABSTRACT

The dissimilatory metal-reducing bacterium, *Shewanella oneidensis* MR-1, reduced tellurite (Te(IV), TeO₃²⁻) to elemental tellurium under anaerobic conditions resulting in the intracellular accumulation of needle shaped crystalline Te(0) nanorods. Fatty acid analyses showed that toxic Te(IV) increased the unsaturated fatty acid composition of the lipid components of the cell membrane, implying a deconstruction of the integrity of the cellular membrane structure. The current results suggest that dissimilatory metal reducing bacteria such as *S. oneidensis* MR-1 may play an important role in recycling toxic tellurium elements, and may be applied as a novel selective biological filter via the accumulation of industry-applicable rare materials, Te(0) nanorods, in the cell.

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1. Introduction

Recently, tellurium and tellurium-containing compounds have been used extensively in various fields such as petroleum refining, electronics, optics, glass production, and sensor production (Sen et al., 2009; Tang et al., 2006; Turner et al., 2012; Wang et al., 2011). However, expanded use of tellurium has increased the likelihood of environmental contamination (Chasteen et al., 2009). In the environment, tellurium exists as elemental tellurium (Te(0)), in inorganic forms such as telluride (Te(II), Te²⁻), tellurite (Te(IV), TeO₃²⁻), and tellurate (Te(VI), TeO₄²⁻), and in organic forms such as dimethyl telluride (CH₃TeCH₃). The elemental state, Te(0), is insoluble in water and has low bioavailability and toxicity, however its soluble oxyanions tellurite and tellurate, particularly Te(IV), are highly toxic to both eukaryotic and prokaryotic cells at concentrations as low as 1 µg/ml (Chasteen et al., 2009; Zannoni

et al., 2007). Another issue is that studies pertaining to the functional role of tellurium in biological systems has lagged behind that of selenium (Se) which is located in the same column of the periodic table – this lack of research has occurred because of the lower crustal abundance, oxyanion solubility, and biospheric mobility of Te (Chasteen et al., 2009). This point is even more significant when compared to the amount of research that has been conducted on other metals and metalloids in the context of the environment, such as chromium, mercury, cadmium, and copper, which become toxic at concentrations about 100-fold higher than Te(IV) (Chasteen et al., 2009). It has been recently discussed that the reduction of Te(IV) to insoluble and less toxic Te(0) is considered to be a potentially effective strategy for relieving the high toxicity of Te(IV) in the environment (Wang et al., 2011; Zannoni et al., 2007).

Since biological interactions with inorganic tellurium compounds were first reported (Chasteen et al., 2009), a number of different species of Te(IV)-resistant bacteria were isolated from tellurium-contaminated environments (Baesman et al., 2009; Borsetti et al., 2003; Ollivier et al., 2008) that possessed genetic determinants (Te^R) for Te(IV) resistance on the bacterial chromosomes or on plasmids (Chasteen et al., 2009; Taylor, 1999; Trutko

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et al., 2000; Zannoni et al., 2007). The mechanisms of bacterial resistance include: direct extrusion of Te(IV), decreasing Te(IV) uptake, enhancing Te(IV) efflux, converting Te(IV) to less toxic forms which are volatile or alkylated, and reduction of Te(IV) to insoluble elemental Te(0) (Chasteen et al., 2009). Although the genetic and physiological basis of Te(IV) resistance have been investigated in a diverse range of microorganisms, a unified mechanism of response has yet to be reported (Chasteen et al., 2009; Zannoni et al., 2007).

In this paper, the dissimilatory metal-reducing bacterium, *Shewanella oneidensis* MR-1, is shown to reduce Te(IV) with intracellular accumulation of Te(0) nanocrystals in the bacterial cells. In addition, exposure of the cells to toxic Te(IV) leads to changes of the cellular membrane lipid components with an increased amount of unsaturated fatty acids, implying a decrease in the integrity of the cellular membrane structure. As such, this research could present the biological potential for the remediation and recovery of novel biogenic Te(0) nanostructures in the environment and/or in industrial wastewater contaminated with tellurium species via an environmentally-friendly process.

2. Methods

2.1. Chemicals, bacterial strains, and culture conditions

All chemicals and reagents were purchased from Sigma–Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). The facultative anaerobic bacterium *S. oneidensis* MR-1 and their mutants were kindly provided from Dr. Jim Fredrickson of Pacific Northwest National Laboratory, Washington, USA. All bacteria were grown aerobically on Luria–Bertani (LB) broth at 30 °C with shaking at 200 rpm for 12 h. Cells were centrifuged (9000g for 10 min), washed with sterile HEPES buffer (10 mM, pH 7.0), and resuspended in HEPES buffer to achieve an optical density (OD) of 2.0 at a wavelength of 600 nm. Cells were subsequently inoculated into serum bottles at 1% of the total volume (100 ml) of sterilized HEPES-buffered basal medium (Lee et al., 2007b), which contained 10 mM of sodium lactate (0.22 µm filter sterilized) and 100 µM of sodium tellurite as the electron donor and acceptor, respectively. All incubations were performed in triplicate and carried out in the absence of light without agitation at 30 °C.

2.2. Analytical methods

The bacterial culture medium was periodically sampled during incubation to determine the concentration of lactate, acetate, and tellurite in solution. For each sample, 1 ml of culture medium was collected using sterile syringes at the selected time and then immediately passed through a 0.22 µm membrane filter (MFS-25, Advantec MFS, Inc., Dublin, CA). The aqueous phase was diluted with high-purity 2% (vol/vol) HNO₃ and analyzed for tellurite concentration by inductively coupled plasma-mass spectrometry (ICP-MS, 7500ce, Agilent Technology, Palo Alto, CA). Lactate and acetate were quantified by high-performance liquid chromatography (Shimadzu, Tokyo, Japan), which was equipped with a SPD-10A UV detector (Shimadzu, Tokyo, Japan) and a Shodex RSpak KC-811 (8.0 mm ID × 300 mm) column (Shodex, Tokyo, Japan). The mobile phase was 5 mM sulfuric acid with a flow rate of 0.5 ml/min, and the UV detection was performed at 210 nm. All measurements were conducted in triplicate.

2.3. Electron microscopic analysis

The bacterial cells were periodically sampled during the incubation to determine the formation and accumulation of tellurium

nanostructures. Samples were collected at a selected time and centrifuged at 9000g for 5 min. The pellets were washed three times and resuspended with deionized water, and dropped onto a silica wafer for SEM imaging, which was operated at 10 kV (SEM, XL30-FEG, Philips, Eindhoven, The Netherlands). For TEM imaging, washed cells were fixed with 3% (vol/vol) glutaraldehyde for 2 h and 1% osmium tetroxide (OsO₄) for 3 h at 4 °C and placed onto carbon-coated 200-mesh copper grids. The images of whole mounts were obtained at 200 kV using a JEOL JEM-2100 high resolution TEM (JEOL, Tokyo, Japan). The cross-sectioned specimens were taken from cells fixed with 2% (wt/vol) uranyl acetate, and embedded in LR White resin.

2.4. Fatty acid analysis

S. oneidensis MR-1 was grown with 100 µM of Te(IV) or 10 mM of fumarate as the electron acceptors at 30 °C for 24 h. Cells were harvested by centrifugation at 9000g at 4 °C for 10 min and washed with sterile HEPES buffer. The total fatty acids were saponized, methylated, and extracted from 40 mg (dry weight) of cells by a standard protocol (Klein et al., 1999; Miller, 1982). Samples were analyzed using an Agilent 6890 Gas Chromatography (Agilent Technologies, Santa Clara, CA). Samples were separated on an HP-1 cross-linked methyl siloxane column (30 m × 0.320 mm × 0.25 µm). Compounds were identified by comparison of fatty acid spectral database provided by Sherlock MIS software (Microbial ID, Inc., DE). Quantitative data were determined by comparison of individual peak areas and comparison with a known concentration of internal standard (Sigma–Aldrich, St. Louis, MO). This analysis was performed at the Korean Collection Center of Microorganisms, Korea.

3. Results and discussion

3.1. Bacterial reduction of Te(IV)

The reduction of Te(IV) by *S. oneidensis* MR-1 were measured under anaerobic conditions (Fig. 1). Bacterial growth measurement by absorbance in the presence of Te(IV) was influenced by precipitation of Te(0) nanostructures in the medium. Instead, the metabolic activity was monitored by quantifying the amount of the electron donor lactate in the presence or absence of Te(IV) as an electron acceptor in the culture medium. At the end of the incubation, there was decrease of lactate in the culture medium with incubation. In contrast, there was no significant oxidation of lactate observed in the control experiments lacking Te(IV). In parallel experiments, more than 90% of Te(IV) was reduced in 120 h of incubation (Fig. 1). With the reduction of Te(IV) by *S. oneidensis* MR-1, the color of the incubation medium changed to black, as was previously reported (Baesman et al., 2007; Klonowska et al., 2005; Ollivier et al., 2008; Yurkov et al., 1996). In contrast, color change and Te(IV) reduction were not observed in the control experiments where inoculation of heat-killed bacterial cells was conducted or where no inoculation of bacterial cells was used. The current experiments suggested that *S. oneidensis* MR-1 was actively involved in the reduction of Te(IV) as the final electron acceptor while consuming the electron donor lactate.

Several different cytochrome deficient mutants of *S. oneidensis* MR-1 (Coursolle and Gralnick, 2010; Fredrickson et al., 2008; Shi et al., 2007) were subsequently tested in order to gain further understanding of the biochemical mechanism of Te(IV) reduction. Te(IV) reduction by mutants deficient in the genes: *cymA*, *mtrA*, *mtrB*, *mtrC*, and *omcA* was 22%, 23%, 25%, 28%, and 38%, respectively, of the initial concentration of Te(IV) over a 120 h incubation period (Table 1). These results indicated that the cellular components, CymA, MtrA, MtrB, MtrC, and OmcA, which are

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