Metalloid Reductase of *Pseudomonas moravenis* Stanleyae Conveys Nanoparticle Mediated Metalloid Tolerance

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Supporting Information

ABSTRACT: A glutathione reductase (GSHR)-like enzyme in *Pseudomonas moraviensis* stanleyae was previously implicated as underlying the bacterium's remarkable SeO_3^{2-} tolerance. Herein, this enzyme is sequenced, recombinantly expressed, and fully characterized. The enzyme is highly adapted for selenodiglutathione substrates ($K_m = 336 \mu\text{M}$) compared to oxidized glutathione ($K_m = 8.22 \text{ mM}$). The recombinant expression of this enzyme in the laboratory strains of *Escherichia coli* conveys a 10-fold increase in IC_{90} for SeO_3^{2-} . Moreover, selenium nanoparticles are observed when the enzyme is overexpressed in the cells exposed to SeO_3^{2-} , but not in the corresponding no-enzyme controls. The analyses of the structural homology models of the enzyme reveal changes in the parts of the enzyme associated with product release, which may underlie the Se substrate specialization. Combined, the observations of adaptation to Se reduction over oxidized glutathione reduction as well as the portability of this nanoparticle-mediated SeO_3^{2-} tolerance into other cell lines suggest that the *P. moraviensis* GSHR may be better described as a GSHR-like metalloid reductase.

INTRODUCTION

The enzymatic conversion of soluble inorganic ions into insoluble forms is accomplished by enzyme centers such as those found in ferritin, magnetosomes, and silicateins. This enzymatic alteration in the solubility state facilitates the synthesis of biogenic inorganic materials.^{1–3} These naturally occurring catalysts, in concert with accessory proteins, can exhibit control over subsequent material composition, oxidation state, morphology, and structure. These natural precedents suggest that the intentional engineering of biological diversity could underlie the engineered diversity in biogenically synthesized inorganic materials. Such inorganic materials—synthesized by laboratory-evolved or engineered biomolecules (peptides, proteins, and nucleic acids) attract attention in catalysis, self-assembly, and in biocontrast (labeling) applications.^{4–8}

Many self-contained biological systems, for synthesizing an inorganic nanostructure, will generally require an oxidoreductase activity, enabling the conversion of inorganic ions from soluble to insoluble oxidation states. Ferritins and DNA-binding proteins accomplish this with the ferroxidase enzymatic centers.^{9,10} Mercuric reductases accomplish this with substrate reduction through an active dithiol,¹¹ which exhibits striking similarities to other enzymes such as lipoamide dehydrogenase.¹²

We recently reported on the ability of glutathione reductase (GSHR) to enzymatically reduce selenite (SeO_3^{2-}) to zerovalent red selenium in a nicotinamide adenine dinucleotide

phosphate (NADPH)-dependent reaction.¹³ Similar, although diminished, activity was observed for the same enzyme in reducing tellurite (TeO_3^{2-}) to elemental Te. Our prior work identified the selenite reductase activity in *Pseudomonas moravenis* stanleyae. This microbe attracted our attention because it is found as an endophyte in the selenium-tolerant plant, *Stanleya pinnata*. When cultured independently in liquid media, it tolerates SeO_3^{2-} supplementation in liquid culture up to 10 mM. This is 10-fold more than the SeO_3^{2-} tolerance of most other microbes. We attributed the observed selenite reduction activity of *P. moravenis* to a GSHR-like enzyme on the basis of proteomic mass spectrometry of an in-gel in situ selenium reductase activity.

GSHRs generally belong to the family of pyridine nucleoside-dependent oxidoreductases. This enzyme family also, notably, includes another well-characterized metal-reducing enzyme—mercuric reductase.¹⁴ Within this enzyme family, the active sites are highly conserved. The typical active site peptide sequences are CXXXXC for type I and CXXC for type II enzymes.¹⁵ These classes of enzymes have demonstrated their ability to reduce a variety of metal ions to zerovalent forms, including Se, Hg, Te, Fe, Cr, and U.^{16–19} GSHR is reported to reduce Au(III) to the zerovalent form as well.²⁰ Thus, the class of pyridine nucleoside-dependent oxidoreductases may repre-

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Table 1. Sequence and Structural Homology^a

	PM MTLR	PF GSHR	EC GSHR	SC GSHR	HS GSHR
PM MTLR		93.10%/1.56	74.14%/1.23	60.78%/1.27	64.71%/1.31
PF GSHR	98.00%/1.71		77.59%/1.05	75.47%/1.12	68.00%/1.07
EC GSHR	67.42%/1.44	68.31%/1.42		81.48%/0.87	79.63%/0.85
SC GSHR	70.92%/1.56	73.48%/1.33	74.61%/1.17		84.91%/0.56
HS GSHR	70.34%/1.44	70.63%/1.24	78.97%/1.07	75.77%/0.92	

^aSequence similarity (left value) and RMSD calculations (right values). PF—*P. fluorescens*, EC—*E. coli*, SC—*S. cerevisiae*, and HS—*H. sapiens*.

Figure 1. Substrate activity assays: (a) GRLMR, (c) GSHR, with the corresponding Lineweaver–Burke plots: (b) GRLMR, (d) GSHR.

sent an evolutionarily adaptable platform of inorganic ion reductases, with modifications to the enzyme, altering the metal-ion selectivity. Such a catalytic center, with alterable precursor selectivity, is of interest in biogenic inorganic nanoparticle (NP) synthesis.

In a prior study, we characterized the commercially sourced *Saccharomyces cerevisiae* GSHR for selenite reductase activity, showing the ability of the enzyme to oxidize NADPH while reducing SeO_3^{2-} to $\text{Se}(0)$ NPs.¹³ In the present study, we characterize a homologous metalloid reductase from the seleno-specialist *P. moraviensis*. We find that the substrate selectivity of the metalloid reductase (K_m) shows a substantially larger preference for GS-Se-SG relative to all other reported GSHR enzymes. These enzymatic properties can be partially rationalized in terms of sequence and the corresponding homology-modeled structure of the enzyme. We also observe that expressing this enzyme in the laboratory strains of *E. coli* (BL21, SS320) results in an increased tolerance to SeO_3^{2-} , as well as the presence of Se NPs in these cells. Overall, our data suggest that the enzyme may be best described as a GSHR-like metalloid reductase (GRLMR).

RESULTS AND DISCUSSION

The altered substrate specificity of GRLMR enzymes, favoring selenodiglutathione (GS-Se-SG) over oxidized glutathione (GSSG) as a substrate, could underlie the remarkable SeO_3^{2-} tolerance of *P. moraviensis stanleyae*. We therefore

characterized the *P. moraviensis stanleyae* GRLMR enzyme identified previously. The DNA sequence of the enzyme was acquired through a full-genome sequencing (ACGT Inc., Wheeling, IL). The sequencing was conducted using de novo paired-end sequencing.²¹ This revealed a genome in which 70.3% of the nucleobases have, at the most, a 1:1000 probability of misassignment. Figure S1 shows the “Quality Score” (Q score) for each sequenced base with $Q = -\log_{10}(e)$. The Q scores are derived from a phred-like error probability assessment of each individual nucleotide.²²

A basic local alignment search tool search of the genomic sequence, using the *Pseudomonas* R-28S GSHR as a reference, identified one GSHR-like sequence, with 93% sequence homology. The sequence alignment of this GRLMR DNA using Serial Cloner shows a high similarity (98.00%) to *Pseudomonas fluorescens* GSHR and modest similarity (67–71%) to *Escherichia coli*, *Stanleya cerevisiae*, and *Homo sapiens* GSHR DNA. The sequence similarities are summarized in Table 1, and full alignments are shown in the Supporting Information, Figure S2. The DNA sequence, combined with the homology modeling of the structure, suggests that all of the structural domains of type I pyridine nucleotide-dependent oxidoreductases, including catalysis, dimerization, nucleotide binding, and substrate/product-binding domains,^{15,23,24} are present in this enzyme. Such a homology would suggest that the GRLMR enzyme would conduct substrate reduction for GSSG, and related molecules, in a fashion similar to previously

reported GSHR reduction pathways via an active-site dithiol.^{25,26} This GRLMR shows 19% DNA sequence homology to mercuric reductase from *Pseudomonas aeruginosa*.

Homology modeling using intensive parameters on the Phyre 2 server²⁷ suggests that the structures of the *Pseudomonas*-derived GSHR and GRLMR enzymes are homologous to the other GSHR enzymes, despite modest DNA sequence divergence. Table 1 shows the root-mean-square deviation (RMSD) of the atomic position values for a set of GSHR homology models and/or crystal structures. Overall, the RMSD values for these structures are similar, suggesting an overall structural homology between GRLMR, and GSHR from *P. fluorescens*, *E. coli*, *H. sapiens*, and *S. cerevisiae*.

Under the hypothesis that the GRLMR enzyme has altered selectivity relative to other characterized GSHRs, we characterized the enzyme kinetics of both GRLMR and *S. cerevisiae* GSHR. We expressed GRLMR recombinantly in the *E. coli* BL21 cells. Following a 6X-histidine tag purification, we determined the Michaelis–Menton constants (K_m) for both GRLMR and the commercially sourced *S. cerevisiae* GSHR (Sigma, G3664). The K_m value of each enzyme was determined for each of the three substrates: SeO_3^{2-} , GSSG, and GS-Se-SG. It has been previously reported that the GSHR enzymes have the ability to reduce these three substrates,¹³ with the proposed mechanisms for the GSH-based substrates.^{25,26} While SeO_3^{2-} (Alfa Aesar, 12585) and GSSG (Sigma, G4376) are commercially available, we synthesized GS-Se-SG according to the previous published methods.²⁶

The enzymatic rates for both enzymes with both the GSSG and GS-Se-SG substrates are plotted in Figure 1, panels (a,c). The data are plotted as NADPH cofactor consumption, observed experimentally as the depletion of a spectroscopic peak characteristic of NADPH (but not NADP^+) at 340 nm. The decay rate, as measured at 340 nm, was converted to a normalized reaction rate. Lineweaver–Burke plots (shown as insets) were generated for each enzyme to determine $V_{\max}$ and K_m for the corresponding substrates. We chose to utilize the Lineweaver–Burke analysis over the more current method of nonlinear regression because our data was not accurately modeled using this technique. Points after 0.2 mM for GS-Se-SG were not used for the determination of K_m because of particle scattering altering the observed rate. Without having the data points nearing the asymptote, the nonlinear regression analysis suffered from large errors and major variations in the calculated constants. When nonlinear regression was used for the analysis of all GSSG data points, the reciprocal plots showed an uneven distribution, indicating that the enzyme kinetics were not modeled correctly using this method. Upon further investigation, we discovered that the reported K_m values for SC GSHR were calculated using the Lineweaver–Burke analysis.²⁸ Using the Lineweaver–Burke analysis granted us consistency across our data analysis and allowed for comparison with the literature, giving us the most accurate and comparable values relative to the other common kinetic analyses. The determined K_m values for each enzyme/substrate combination are shown in Table 2.

For the GRLMR enzyme, we observe a remarkable specialization of the enzyme for GS-Se-SG over GSSG. Specifically, the K_m value of GRLMR is 25 times more favorable for GS-Se-SG as compared to GSSG. This difference in K_m for the two substrates strongly implies that the enzyme is specialized for Se reduction over GSSG reduction. For *S.*

Table 2. Enzyme Kinetics^a

enzyme/substrate	GRLMR K_m	SC GSHR K_m (μM)	GRLMR $V_{\max}$	SC GSHR $V_{\max}$
GSSG	8.22 mM	103	2.62	0.107
GS-Se-SG	336 μM	133	0.187	0.137

^a $V_{\max}$ reported in ($\mu\text{M}/\text{min}$)/(μg of enzyme).

cerevisiae GSHR, we determined similar K_m values for GSSG and GS-Se-SG substrates, with the enzyme showing a slightly greater affinity for GSSG. The values we find for both substrates are consistent with the previous findings from other research groups.^{13,28,28} The K_m value of GRLMR for SeO_3^{2-} has not been previously reported, to our knowledge. The physiological relevance of the K_m value for SeO_3^{2-} is questionable, as Se salts such as SeO_3^{2-} are converted to GS-Se-SG via GSH reduction *in vivo*.²⁹ We established here the K_m value for SeO_3^{2-} for both GRLMR and *S. cerevisiae* GSHR because we identified GRLMR on the basis of the SeO_3^{2-} reductase activity, but note that the value is sufficiently unfavorable compared to the GSH-based substrates that it is unlikely to be physiologically relevant.

The value of $V_{\max}$ for the GS-SG and GS-Se-SG substrates track in tandem up to the initial concentrations of 0.20 mM. Deviations begin near a concentration of 0.30 mM, where the rate for GS-Se-SG has reached its maximum velocity, whereas the rate for oxidized glutathione continues to increase until roughly 0.60 mM substrate concentration. The maximum velocity observed for GSSG is approximately 14 times greater than the $V_{\max}$ for GS-Se-SG, but this occurs at a larger substrate concentration for GSSG, accounting for the lower affinity. It is possible that the fall-off in rate that we observe at higher GS-Se-SG concentrations is artifactual, arising from the interference in the optical assay by the selenium NPs produced during the experiment.

Overall, the K_m values we find suggest a strong substrate preference for GS-Se-SG for the GRLMR enzyme. This is in contrast to the other characterized GSHR enzymes, where there is essentially no differentiation between the substrates. The cellular concentrations of GSSG would typically be much higher than GS-Se-SG at sublethal amounts of selenium *in vivo*; thus, the SC GSHR enzyme would turn over GSSG much more frequently and does not lead to selenium resistance as in the *P. Moraviensis* system. To our knowledge, this is the first finding of an enzyme specialized for reduction of GS-Se-SG over any other substrate. Such a specialized enzyme could be used for nanomaterial development such as biogenic quantum dots and, most notably, as a starting point for the development of a functionalized clonable NP.

We hypothesized that if GRLMR conveys SeO_3^{2-} tolerance to *P. moraviensis*, then the recombinant expression of this enzyme may convey a similar tolerance to the host organism for GRLMR expression. To evaluate this hypothesis, we transformed the lab expression strains of *E. coli*, BL21 and SS320, with the isopropyl β -D-1-thiogalactopyranoside (IPTG)-inducible expression vector described above. For the GRLMR enzyme-expressing *E. coli*, we determined selenite tolerance as the concentration of SeO_3^{2-} that kills 90% of the cells (IC_{90}). We also examined the cells microscopically for the presence or absence of SeNPs.

For IC_{90} determination, identical volumes of cells were plated on lysogeny broth (LB) agar and LB agar + SeO_3^{2-} , each in triplicate, and grown overnight at 37 °C. The following

Figure 2. IC₉₀ assays reported in percent inhibition vs selenite concentration: (a) BL21 *E. coli* cell lines with a GFP plasmid; (b) BL21 cells with the GRLMR plasmid; and (c) SS320 cells with the GRLMR plasmid.

day, the colony-forming units (cfus) were counted. IC₉₀ was calculated as the percentage of cfus present relative to an identical control supplemented with 1.0 μM SeO_3^{2-} . We determined 1.0 μM SeO_3^{2-} as necessary for maximizing the number of observed cfus. We hypothesize that such a supplementation is necessary because the overexpression of a SeO_3^{2-} -reducing enzyme in these cells reduces and makes unavailable the essential amount of Se needed as a micro-nutrient for optimum growth.³⁰

Figure 2 shows relative growth inhibition as a function of selenite concentration. The $\log_{10}[\text{SeO}_3^{2-}]$ gives a linear concentration dependence for SeO_3^{2-} growth inhibition. This allows the determination of the inhibitory concentration of selenite that eliminates 90% of cell growth (IC₉₀). We found an IC₉₀ of 21.3 ± 9.80 mM under the conditions of GRLMR overexpression, whereas an IC₉₀ of 1.89 ± 0.46 mM is observed in a corresponding control experiment. This result is at least somewhat cell-line-independent. When GRLMR is recombinantly overexpressed in *E. coli* SS320, we observe an IC₉₀ of 18.3 ± 19.50 mM. GRLMR scavenges the cells of the micronutrient levels of selenium which can stunt growth and cause cell death. This inherently causes large deviations in cfu formation from run to run. Even with the large error bars, a statistically significant increase in SeO_3^{2-} tolerance is induced by the presence of the GRLMR plasmid, and we believe that the SS320 data is justified because of the similarly observed tolerance in the BL21 cell line.

We note that the presence of the recombinantly expressed enzyme results in the liquid cultures taking on the red color characteristic of the red allotrope of zerovalent selenium, whereas cultures grown with SeO_3^{2-} without the recombinantly expressed enzyme do not take on this color. To illustrate this, Figure 3 shows the cell cultures expressing GRLMR (left panel) or the green fluorescent protein (GFP) (right panel) in the presence of SeO_3^{2-} supplementation at 5.0 mM, after 3 h of exposure. This “bulk color” change suggests that the cells expressing the recombinant enzyme may also be forming Se(0) NPs, just as we previously observed for the *P. moravenis* strain.

The examination of cells by scanning electron microscopy (SEM) revealed the presence of selenium NPs in cells expressing the recombinant enzyme and grown in Se-supplemented media. Figure 4 shows the scanning transmission electron micrographs of glutaraldehyde-fixed dry-mounted BL21 *E. coli* cells expressing GFP or GRLMR after growth in SeO_3^{2-} -supplemented media. Both GFP and GRLMR cells show dark inclusions, with more inclusions observed in the GRLMR-expressing cell line. The dark

Figure 3. Separation of selenium NP growth. BL21 cells with (right) and without (left) the GRLMR plasmid after a 3 h exposure to 5.0 mM selenite-supplemented LB.

Figure 4. SEM images (a,b) with EDS overlay (c,d) of fixed cells after 3 h exposure to 5 mM selenite. (a,c) Cells without the GRLMR plasmid. Scale bars are 5 μm . (b,d) Cells with the GRLMR plasmid. Scale bars are 0.5 μm . The corresponding EDS spectra of cells without GRLMR (gray) and additional peaks from cells with the GRLMR plasmid (red) on bottom. Cu peaks are present in our sample, as carbon-coated Cu mesh grids were used.

Table 3. Key Glutathione Pocket Residues

enzyme	PM MTLR	PF GSHR	EC GSHR	SC GSHR	HS GSHR
key product-binding site residues	α -Ser β L-Lys β R-Glu	α -Ser β L-Lys β R-Glu	α -Met β L-Lys β R-Lys	α -Cys β L-Lys β R-Asn	α -Met β L-Lys β R-Ser

Figure 5. Red— α residue, teal— β residues, and yellow—GSH. (a) Yeast GSHR substrate-binding pocket with bound GSH. (b) *E. coli* GSHR substrate-binding pocket. (c) GRLMR substrate-binding pocket.

inclusions seen in both cell lines near the cell walls are most likely the cellular nuclei because of their cellular placement and single appearance per cell. Electron-dispersive spectroscopy (EDS) mapping confirms that the dark inclusions are Se-rich for the GRLMR cells, whereas any inclusions observed in the GFP-expressing cells show no evidence of Se presence. The corresponding EDS spectra further confirm these conclusions by the presence of signature selenium peaks being present for the GRLMR cells and the absence of these peaks for the GFP cells. Complete EDS mapping for the control and GRLMR cells can be seen in Figure S6. The intracellular Se-rich NPs have an amorphous surface similar to our previously reported results,¹³ suggesting a partial cytosol-exposed surface. This appears to be unique to our enzyme-synthesized particles because inorganic/bioinorganic methods tend to make smooth-surfaced particles and require capping agents for stabilization.^{31,32} The transportability of enzymatic function to foreign cell lines further demonstrates the ability for this enzyme's potential application as a clonable NP contrast generator.

Previously, we observed that some fraction of NPs synthesized by *S. cerevisiae* GSHR were associated with the particle fraction.¹³ We examined GRLMR for a similar behavior. Selenium particles were synthesized in vitro and were separated from the solution using centrifugation. The analysis of the protein content in the solution and the particles were determined by Bradford assay (Bio-Rad). Overall, approximately 10% of the enzyme is associated with the selenium NPs. This is a smaller fraction than we observed for *S. cerevisiae* GSHR (~18%), suggesting that the *P. moravenis*-derived enzyme is more efficient at turning over or releasing the particles that they create. Overall, this contributes to the picture of GRLMR being specialized for conveying Se tolerance.

Differences in the product-/substrate-binding pocket of this family of enzymes may underlie any observed differences in substrate specificity and enzyme activity. The key residues in the product-/substrate-binding areas of GSHR are shown in Table 3, and the models of these are shown in Figure S3. The structural alignment between GRLMR and *P. fluorescens* reveals an RMSD of 1.71 Å for the full enzyme and 1.56 Å for the product-/substrate-binding pocket. These values show the largest deviation between any of the enzymes considered here, but to the best of our knowledge, no crystal structure of

Pseudomonas GSHR has been obtained which would affect the generated structure models.

The product-/substrate-binding pocket for GSHR contains a set of evolutionarily conserved residues, most notably including a cysteine that is implicated in glutathionylation regulatory mechanisms. There are three residues that dominate the binding interaction, one on an α -helix, and two on parallel β -strands (β L, β R). Comparing our *Pseudomonas* enzymes, we see that they contain the same key residues; α -Ser, β L-Lys, and β R-Glu (Figure 5).³³

This absence of a sulfur-containing residue (Figure 1) in the *P. moravenis* GRLMR suggests that the enzyme is not subject to the glutathionylation regulatory mechanism well-established for canonical GSHR enzymes such as *S. cerevisiae* GSHR. Glutathionylation of enzymes is a common post-translation modification for proteins in signaling pathways and survival gene modification.^{34–37} This reversible post-translation modification is the binding of glutathione to an unpaired cysteine residue.³⁸ Such a modification alters the enzyme activity, presumably as a regulation mechanism.^{39,40} In the case of *S. cerevisiae* GSHR, glutathionylation at C239 (Figure 5) inhibits the enzyme. Chemically blocking the glutathionylation pathway is shown to increase the GSHR activity by a factor of 2.1.⁴¹ Overall, the absence of the possibility of a glutathionylation regulation mechanism for the GRLMR enzyme suggests that it is distinct from the other GSHR enzymes.

The selenium metabolism literature highlights several examples of species within the *Pseudomonas* genus with remarkable tolerance to Se. In many cases, GSHR enzymes are implicated in the tolerance. GSHR was responsible for reducing selenite and tellurite to insoluble NPs using the O-2 strain of *Pseudomonas maltophilia*;⁴² GSHR and thioredoxin reductase are responsible for selenite and selenate reduction in *Pseudomonas seleniipraecipitans*.^{43–46} The highly conserved sequence across species within the *Pseudomonas* genus, including conservation in the product/substrate binding pocket, is suggestive that the ability to handle the normally toxic amounts of SeO_3^{2-} may be a general feature of the *Pseudomonas* genus. This Se tolerance may arise from the nature of GSHRs in this genus.

■ CONCLUSIONS

In summary, we have characterized GRLMR from the bacterium *Pseudomonas moraviensis stanleyae*. The kinetic studies showed an overall decrease in substrate affinity for GRLMR relative to the *S. cerevisiae* GSHR, but an overall increased affinity for GS-Se-SG over GSSG. The transportability of the gene was tested by transforming the lab strain *E. coli* with GRLMR. Selenite tolerance increased 10-fold compared to the cells without the gene, and elemental red selenium was formed when GRLMR was present. SEM/EDS further confirmed this by showing the selenium particles associated with the cells containing the gene. Product association experiments showed a decrease in product retention when compared to the *S. cerevisiae* GSHR, which ultimately allows for increased product release and contributes to the overall Se tolerance.

■ MATERIALS AND METHODS

Identification/Isolation. An LB agar plate with colonies of the original *P. moraviensis* cell line was submitted for full-genome sequencing. The DNA sequence from the most closely related enzyme identified by MALDI-MS was used to identify the sequence of our enzyme of interest. This sequence was cloned into a pD441-CH *E. coli* vector, and a standard heat-shock protocol was used to transform BL21 *E. coli*.

Standard protein purification was conducted by growing cells in 1 L of LB to an optical density at 600 nm (OD_{600}) of 0.6 and inducing protein expression with 1 mM IPTG for 2 h. The cells were collected and resuspended in 25 mL of lysis buffer and lysed by tip sonication. The soluble cell lysate was collected and nickel-agarose beads were used to isolate and wash our expressed protein.

GS-Se-SG Synthesis. The protocol from Ganther was followed for the synthesis of selenodiglutathione.²⁶ To 24 mL of 0.1 M HCl, 400 moles of HNaSeO_3 was added and cooled to 4 °C. Another solution of 0.1 M GSH was cooled to 4 °C and added quickly to the selenite solution. The mixture was allowed to react at 4 °C for 20 min. A 2.5 mL of 2 M NaOAc was added to obtain a final pH of 4.5. A C18 column was used to separate the products using pH 2.0 HCl. Thin-layer chromatography was used to check the contents of the lyophilized fractions. The isolated GS-Se-SG was identified and the amount quantified using UV-vis absorption at 263 nm. (S.4)

K_m. 1 mL reactions were conducted in 1× phosphate-buffered saline with 0.1 mM NADPH and 15 μg of either purified enzyme or *S. cerevisiae* GSHR purchased from Sigma. The substrates tested were GSSG, GS-Se-SG, and selenite. Their concentrations were varied between reactions, and the depletion of the NADPH peak at 340 nm was monitored every 2 s after the contents were mixed.

Transportability. IC₉₀'s were determined by standard plating experiments. In short, cultures of BL21 cells containing either a plasmid with GRLMR or a generic reporter gene were grown overnight in LB at 37 °C. The following morning, 100 μL of this starter culture was added to 2.5 mL of fresh LB and grown for roughly 2.5 h to reach an OD_{600} of 0.6. Various amounts of selenite were added to each culture, and exposure was continued for 24 h. After the exposure, the cells were diluted 10⁶-fold, and 20 μL of each dilution was plated in triplicate on 1× Kanamycin LB agar. The plates were put in an

oven at 37 °C, and colonies were grown overnight and counted the following day.

Scanning Transmission Electron Microscopy (STEM).

A volume of 3 mL of BL21 cells containing either a metalloid reductase gene or GFP reporter gene was grown separately in 10 mL culture tubes overnight containing LB medium (Teknova) supplemented with Kanamycin at 25 μg/mL. The following morning, the culture was added to a 125 mL Erlenmeyer flask containing LB medium supplemented with Kanamycin (25 μg/mL). The cells were grown for 2.5 h, and 100 mM Na_2SeO_3 (Alfa Aesar, 98+%) was added to reach a final concentration of 5 mM. The cells were collected by centrifuging for 20 min at 4000 rpm and 4 °C after 3 h of growth with selenite. The cells were washed with 20 mM Tris (pH 7.4) (Fischer) three times followed by resuspension in 1 mL of fixing solution (2% glutaraldehyde (25% Sigma-Aldrich) and 2.5% formaldehyde); the fixing solution was allowed to react for 12 h at 4 °C. The fixing solution was centrifuged and the pellet was washed five times in 20 mM Tris (pH 7.4). The cells were resuspended in 1 mL of 20 mM Tris (pH 7.4). The aliquots (4 μL) were mounted on 400 mesh Cu grids with 50 nm C coating and washed two times with H₂O. The dry-mounted cells on transmission electron microscopy grids were loaded onto a STEM holder. The STEM images were taken with a JEOL JSM-6500-F scanning electron microscope at an accelerating voltage of 15 kV.

EDS. EDS was performed on the *P. moraviensis stanleyae* cells with SEM, as described above. EDS was collected on a NORAN System 7 X-ray microanalysis detector with a time interval of 1 s.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00826.

Genome sequencing scores, sequence alignment, generated model templates, glutathione-binding sites, GS-Se-SG UV-vis, K_m assay UV-vis, and SEM/EDS (PDF)

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ABBREVIATIONS

DPS, DNA-binding protein; GSHR, glutathione reductase; NADPH, nicotinamide adenine dinucleotide phosphate; GRLMR, glutathione reductase-like-metalloid reductase; BLAST, basic local alignment search tool; RMSD, root-mean-square deviation; NP, nanoparticle; IPTG, isopropyl β -D-1-thiogalactopyranoside; cfus, colony-forming units; GFP, green fluorescent protein; SEM, scanning electron microscopy; EDS, electron-dispersive spectroscopy; LB, lysogeny broth; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; OD, optical density; TLC, thin-layer chromatography; PBS, phosphate-buffered saline

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