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Research Letter

A novel ICP-MS strategy identifies arsenite as an inhibitor of selenocysteine-tRNA^{Sec} charging

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ABSTRACT — Arsenite (As(III)) is a widespread environmental contaminant that increases susceptibility to oxidative stress. We recently reported that As(III) suppresses the induction of glutathione peroxidases (GPx) by various selenium sources in cultured cells; however, its underlying mechanism remains unclear. GPx contains a selenocysteine (Sec) residue essential for catalytic activity, and Sec biosynthesis requires multiple steps of selenium metabolism. Selenite is directly incorporated into the Sec biosynthetic pathway via selenophosphate synthetase 2 (SEPHS2) and utilized for Sec-tRNA^{Sec} formation. Because Sec-tRNA^{Sec} decodes UGA codons, impaired synthesis of Sec-tRNA^{Sec} leads to nonsense-mediated decay or truncated translation of selenoprotein mRNAs. Here, we developed an inductively coupled plasma (ICP)-MS based method to evaluate Sec-tRNA^{Sec} and found that As(III) inhibits Sec charging of tRNA. As(III) markedly suppressed GPx protein induction with minimal effects on mRNA abundance. As(III) did not affect total tRNA^{Sec} levels; however, As(III) significantly decreased RNA-bound selenium released by deacylation, indicating reduced Sec-tRNA^{Sec} formation. These results suggest that As(III) impairs selenoprotein translation by inhibiting Sec charging of tRNA.

Key words: Sec-tRNA^{Sec}, Arsenite, Glutathione peroxidase, ICP-MS

INTRODUCTION

Arsenic is a naturally occurring environmental contaminant and a persistent public health concern worldwide (Singh *et al*., 2015). Among inorganic arsenicals, arsenite (As(III)) is considered highly toxic due to its high cellular uptake and reactivity toward thiol-containing biomolecules (Kitchin and Wallace, 2008). Chronic exposure to low-to-moderate concentrations of arsenic has been associated with increased susceptibility to oxidative stresses, impaired immune functions, and the development of multiple pathologies, including carcinogenesis (Kumagai and Sumi, 2007). Although a broad range of cellular targets have been proposed, the molecular mechanisms responsible for arsenic-induced redox imbalance and stress vulnerability remain not fully understood.

We recently reported that As(III) suppresses the induction of glutathione peroxidases (GPx) in cultured cells (Takashima *et al*., 2026). GPx is responsible for cellular defense against oxidative damage by reducing hydrogen peroxides and lipid peroxides using glutathione as substrate. Notably, GPx contains a single selenocysteine (Sec) residue at its catalytic site, and its protein expression strictly depends on the intracellular availability and biosynthesis of Sec (Ye *et al*., 2025; Takashima *et al*., 2026; Ito *et al*., 2024). Interestingly, the inhibitory effect of As(III) on GPx induction was observed regardless of the selenium source supplied to the cells, including inorganic selenium (selenite), organic selenium (selenocystine and selenoprotein P (SeP)). These findings suggested

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that As(III) may target intracellular selenium metabolism or the translational machinery for selenoproteins, especially the downstream of the catabolism after inorganic selenium (Takashima *et al*., 2026).

Selenocysteine, the 21st amino acid, is uniquely synthesized on its cognate tRNA ($tRNA^{Sec}$) and co-translationally incorporated at UGA codons via a dedicated recoding mechanism (Palioura *et al*., 2009; Low *et al*., 2000; Bulteau and Chavatte, 2015). After uptake, organic selenium (e.g., SeP or SeCys) is catabolized to selenide, whereas selenite is directly reduced to selenide via non-enzymatic reactions such as GSH-dependent reduction (Ito *et al*., 2024). Selenide is then activated by SEPHS2 and enters the Sec biosynthetic pathway. Because selenoproteins require Sec-tRNA for translation, impairment of this process can lead to nonsense-mediated mRNA decay, truncated polypeptides, or translational impairment. We previously observed that As(III) did not markedly decrease the expression of selenium metabolism–related enzymes, including SEPHS2 and PRDX6, indicating that transcriptional regulation of these pathways is unlikely to explain the observed suppression of GPx induction (Takashima *et al*., 2026). Therefore, the mechanism by which As(III) interferes with selenoprotein synthesis remains not understood.

Arsenic exposure has also been linked to ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation (Mishima and Conrad, 2022). Ferroptosis sensitivity is tightly modulated by intracellular redox balance, and GPx4, a selenoprotein, is recognized as a master suppressor of ferroptosis. Thus, arsenic-induced defects in selenium-dependent antioxidant systems may have broader implications for cellular stress responses and toxicity.

Despite the central role of Sec-tRNA in selenoprotein biosynthesis, analytical approaches to directly quantify Sec charging on tRNA remain limited. Most available techniques rely on radiolabeling (Gonzalez-Flores *et al*., 2013; Lee *et al*., 1990). Given that Sec is the only amino acid containing selenium (selenomethionine (SelMet), which is unintentionally incorporated into methionine, is also known) (Ogra *et al*., 2008), elemental mass spectrometry provides a unique analytical advantage for selective and highly sensitive detection. Inductively coupled plasma mass spectrometry (ICP-MS), in particular, enables attomole-level quantification of selenium species and has been widely used to analyze selenium distributions in biological matrices. However, ICP-MS-based methods have not been applied to charged tRNA species, leaving a major analytical gap in the field.

In this study, we developed a novel ICP-MS-based strategy to evaluate $Sec\text{-}tRNA^{Sec}$ and applied it to investigate the effects of As(III) on selenoprotein biosynthesis. Using this approach, we demonstrate that As(III) inhibits Sec charging on tRNA, leading to reduced GPx protein induction with limited effects at the mRNA level. Our findings provide new mechanistic insight into how arsenic disrupts selenium-dependent translation and suggest that interference with $Sec\text{-}tRNA^{Sec}$ biosynthesis represents a previously unrecognized mode of arsenic toxicity.

MATERIALS AND METHODS

Materials

Human fibrosarcoma (HT1080) cells were obtained from ATCC (CCL-12). Antibodies used in this study was as follows: Anti GAPDH Monoclonal antibody Peroxidase conjugated, 015-25473 (Wako Pure Chemical, Osaka, Japan); Anti-Glutathione Peroxidase 1 antibody, ab22604 (abcam, Cambridge, UK); and Anti-Glutathione Peroxidase 4 antibody, ab125066 (abcam). All other reagents used were of the highest grade available.

Primers used in this study for RT-qPCR were as follows: Human GPX1 forward: 5’- CAGTCGGTGTATGCCTTCTCG -3’; Human GPX1 reverse: 5’- GAGGGACGCCACATTCTCG -3’; Human GPX4 forward:5’ - GAGGCAAGACCGAAGTAAACTAC -3’; Human GPX4 reverse: 5’- CCGAACTGGTTACACGGGAA -3’; Human GAPDH forward: 5’- GCACCGTCAAGGCTGAGAAC -3’; and Human GAPDH reverse: 5’- TGGTGAAGACGCCAGTGGA -3’.

Cell culture conditions

HT1080 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; 08458-16, Nacalai Tesque, Kyoto, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA; Lot BCCC5944; containing 8.3 ppb Se) (Takashima *et al*., 2024), and 1% (v/v) penicillin–streptomycin solution. Cells were maintained in a humidified incubator at 37°C with 5% CO_2. For cell seeding, cells were cultured in medium containing 10 nM selenite.

Purification of total RNA and deacylation from tRNA

After the stimulus described in the figure legend, cells were washed with PBS and collected using IsogenII (Wako). RNA was extracted according to the manufacturer’s instructions. Briefly, RNase-free water was added to the IsogenII solution, which was then was separated into protein-containing pellets and RNA-containing supernatant by centrifugation. Supernatant

was taken and mixed with isopropanol. After centrifugation, RNA-containing pellets were washed with 70% ethanol and dissolved in nuclease-free water. For deacylation of tRNAs, total RNA was incubated in 20 mM Tris-HCl (pH 9.0) at 37°C for 40 min as previously described (Akiyama *et al*., 2022a). After deacylation, the reaction solution was mixed with 0.1 vol of 3M sodium acetate (pH 5.2) (Nacalai Tesque) and 1 vol of isopropanol (Nacalai Tesque), then centrifuged at 12,000 g for 10 min at 4°C. The supernatant was centrifuged using MV-100 (TOMY, Tokyo, Japan) for the removal of isopropanol, then dissolved in nuclease-free water and subjected to ICP-MS analysis as the sample derived from amino acids charged to tRNAs. After washing with 70% ethanol, the pellet was dissolved in nuclease-free water, then also subjected to ICP-MS analysis.

Determination of selenium and phosphorus concentration by ICP-MS

Prior to elemental analysis, 250 µL of 70% nitric acid was added to each sample, and the mixtures were digested using a microwave digestion system (ETHOS EASY; Milestone General, Kanagawa, Japan). Digestion was performed in sealed quartz vessels at 160°C for 30 min with a maximum pressure of 80 bar. After cooling to room temperature, the digested solutions were diluted with ultrapure water to a final volume of 1.0 mL, resulting in a final nitric acid concentration of 17.5%.

Elemental analysis was carried out using an Agilent 8900 triple quadrupole inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Santa Clara, CA, USA), as we previously reported (Ichikawa *et al*., 2025). Selenium was detected as the oxide ion ($^{78}Se^{16}O^{+}$, m/z 94) in oxygen reaction mode to avoid polyatomic interferences derived from argon species. Phosphorus was quantified by monitoring the oxide ion ($^{31}P^{16}O^{+}$, m/z 47). Helium collision gas and oxygen reaction gas were used in combination, and measurements were conducted in mass-shift mode to minimize potential spectral interferences. A mixed internal standard solution containing Be, Y, In, Te, and Bi (prepared in 17.5% nitric acid) was continuously introduced online during the analysis. Element concentrations were determined using external calibration with internal standard correction. Calibration standards were prepared from certified selenium and phosphorus standard solutions (Kanto Chemical Co., Inc., Tokyo, Japan). For quality control, calibration blanks were analyzed together with the samples, and signal stability during the analytical sequence was monitored using the internal standard signals.

Western blotting

After the stimulus described, cells were washed with PBS and collected with lysis buffer (0.05 M Tris-HCl (pH6.8) and 2% SDS). After that, cell lysates were incubated at 95°C for 5 min. Protein concentration was measured by DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA). It was mixed with 4x sample buffer (125 mM Tris-HCl (pH6.8), 10% Glycerol, 4% SDS, 0.025% Bromophenol blue), then incubated at 95°C for 5 min. Samples were loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. Proteins were transferred to a PVDF membrane (Immobilon, Merck Millipore, Darmstadt, Germany). The membrane was incubated with indicated primary antibodies at room temperature for 1 hr, then washed with TTBS (50 mM Tris-HCl (pH7.5), 150 mM NaCl, 0.05% Tween 20). After the incubation in secondary antibodies at room temperature for 1 hour, the membrane was washed with TTBS and treated with ImmunoStar LD (Wako), then detected using a LuminoGraph I (ATTO, Saitama, Japan).

Quantitative PCR

Total RNA was extracted as indicated in the section above. cDNA synthesis was performed using PrimeScriptTM RT Reagent Kit (Takara Bio, Shiga, Japan). Master mix (1.75 µL; 4 vol of 5xPrimerScript Buffer, 1 vol of PrimerScript RT Enzyme Mix I, 1 vol of 50 µM Oligo dT primer, 1 vol of 100 µM Random 6 mers) was mixed with 3.25 µL of RNA solution. It was incubated at 37°C for 30 min, then heated at 85°C for 5 sec for inactivation. For the qPCR, reaction mixture was prepared using KAPA SYBR Fast qPCR Kit (Kapa Biosystems, Wilmington, MA, USA). Forward/reverse primer (10 µM ; 0.5 µL), KAPASYBR (6.25 µL) and nuclease-free water (3.25 µL) was mixed as qPCR master mix. qPCR master mix (10.5 µL) was mixed with cDNA solution (2 µL) and subjected to qPCR reaction using a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). PCR condition is as follows: [1] 95°C for 30 sec; [2] 95°C for 5 sec; [3] 60°C for 30 sec; [4] 95°C for 15 sec; [5] 60°C for 30 sec; [6] 95°C for 15 sec- step from 2 to 3 was repeated 40 times.

Northern blotting

Total RNA was extracted as indicated in the previous section. Northern blotting was performed as we previously described (Takenaka *et al*., 2025; Akiyama *et al*., 2022b). The oligonucleotide probe for tRNA^{Sec} was synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA), then labeled with digoxigenin-11-dUTP using DIG

Oligonucleotide Tailing Kit (Roche Diagnostics, Mannheim, Germany) (Takenaka *et al.*, 2025). The sequence of the tRNA^{Sec} probe was 5'-GCCTGCACCCCAGACCACTGAGGATCATCCGGGC-3', which is complementary to position 1-34 of Sec-tRNASec (Chan and Lowe, 2016).

Statistical analysis

Band intensities were measured using ImageJ version 1.53i (National Institutes of Health, Bethesda, MD, USA). Statistical analyses were carried out with GraphPad Prism version 10.2.2 (GraphPad Software, San Diego, CA, USA). Differences among multiple groups were evaluated by one-way ANOVA followed by Tukey's post hoc test, while comparisons between two groups were analyzed using Student's t-test. Results are presented as the mean ± standard deviation (SD), and values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

To evaluate Sec-tRNA^{Sec} and its Sec-charging status in cells, we hypothesized deacylation from total RNA sample will allow selective analysis of aminoacyl-tRNA-binding amino acids. Because Sec is the only amino acid which contains selenium in the molecule, high sensitive elemental analysis using ICP-MS seems effective for detecting this (Fig. 1). Total RNA was extracted from cells and subjected to mild alkaline deacylation, allowing the charged amino acid to be released from tRNA molecules into the aqueous supernatant, while deacylated tRNAs and other RNAs remained in the pellet fraction. Phosphorus (P) is not contained in amino acid, thus it will only be found in precipitated RNA fraction if deacylation works. Selenium from Sec-tRNA^{Sec} was thereby expected to be detected in the selenium in the supernatant due to its elemental signature, which corresponds to Sec-tRNA^{Sec}.

The overall analytical concept is shown (Fig. 2A). In this experiment, we are analyzing the tRNA charging of Se-containing amino acids synthesized *de novo* (Sec) by selenite (50 nM for 24 hr). In the absence of selenite, the selenium content in RNA is approximately 1/20, approaching the detection limit and making quantification difficult, which indicates the contamination of SelMet is limited. At the cultured cell level, the addition of some selenium source is necessary in addition to FBS-derived selenium (data not shown). Before the deacylation, Se (0.1 ng Se/g RNA) and P (18 ng P/g RNA) were detected in total RNA sample. In this purification method, protein contamination which is evaluated by Abs260/280

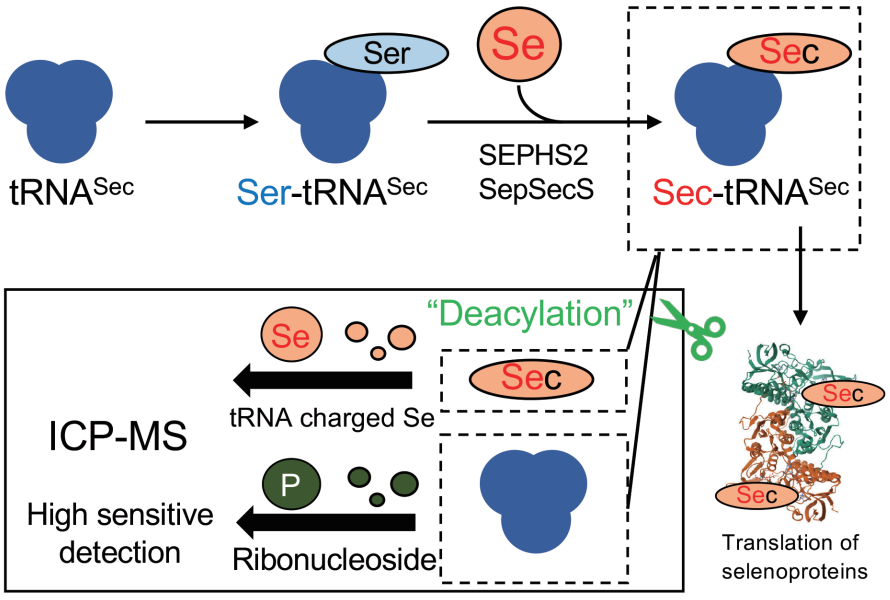


Fig. 1. Overview of the analytical strategy for Sec on tRNA^{Sec} using ICP-MS. Total RNA extracts were separated into an amino acid fraction derived from aminoacyl-tRNA and a ribonucleoside fraction. Measurement of selenium in the amino acid fraction indicates the amount of Sec on tRNA^{Sec}, while phosphorus in the ribonucleoside fraction can be used as an internal control.

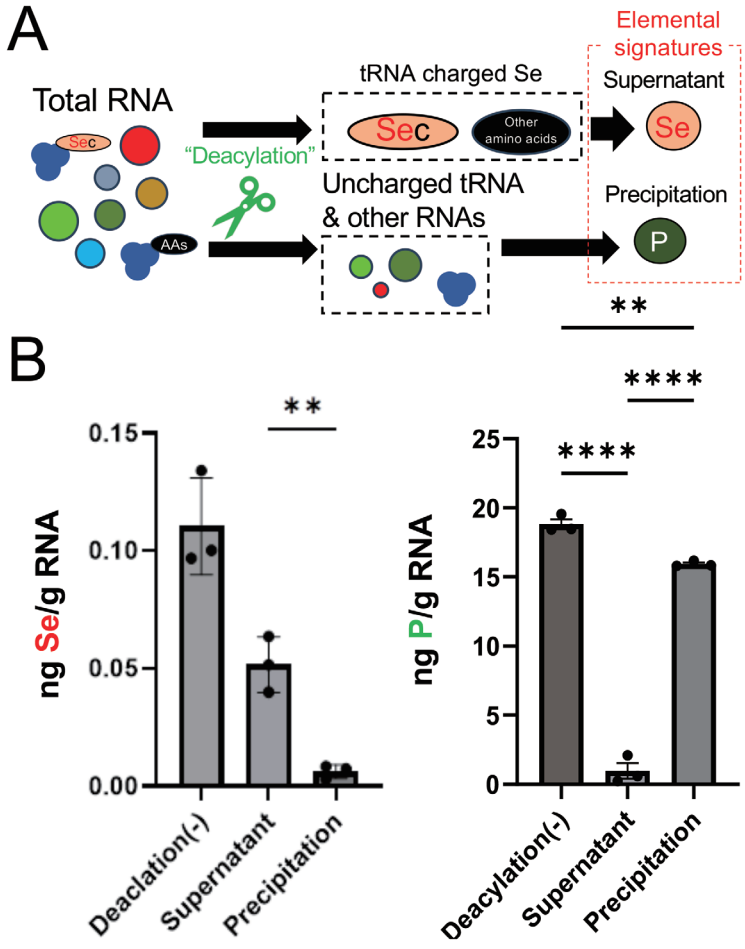


Fig. 2. Determination of Sec on tRNA^{Sec} using ICP-MS. (A) A brief scheme of the experimental procedure. Selenium in the amino acid fraction and phosphorus in the ribonucleoside fraction were used as elemental signatures for Sec on tRNA and for total RNA as an internal control, respectively. (B) HT1080 cells were treated with selenite (50 nM) for 24 hr and then fractionated into an amino acid fraction (supernatant) and a ribonucleoside fraction (precipitate). Input indicates the sample before fractionation. Data represent the mean ± S.D. (n = 3). Statistical analysis was performed using Tukey's test, and significance was indicated as **P < 0.01.

was over 1.9 and was at a negligible level; however, additional validation, such as electrophoretic analysis, may be required for rigorous assessment of RNA purity and this is the one of the limitations. After deacylation, samples were separated into supernatant and pellet fractions and analyzed by ICP-MS for selenium and phosphorus. As shown in Fig. 2B, Se was readily detected in the supernatant with very low detectable signal in the pellet, whereas P, originating from the nucleic acid backbone, was confined to the pellet fraction and almost undetectable in the supernatant. These data confirmed that the deacylation and separation steps effectively distinguished the charged Sec-tRNA derived selenium from bulk RNA sample, validating the analytical feasibility of this method for assessing Sec charging.

We next examined the effect of As(III) on selenoprotein expression. Western blot analysis demonstrated that induction of GPx1 and GPx4 by selenite was markedly suppressed by co-treatment with As(III) (Fig. 3A), as we previously reported (Takashima *et al*., 2026). In contrast, qPCR analysis revealed only minor reductions in GPx transcript levels under the same conditions (Fig. 3B), indicating that the inhibitory effect occurs predominantly at the post-transcriptional level. Consistent with these findings, Northern blotting of tRNA^{Sec} showed no appreciable changes in its abundance in response to either sel-

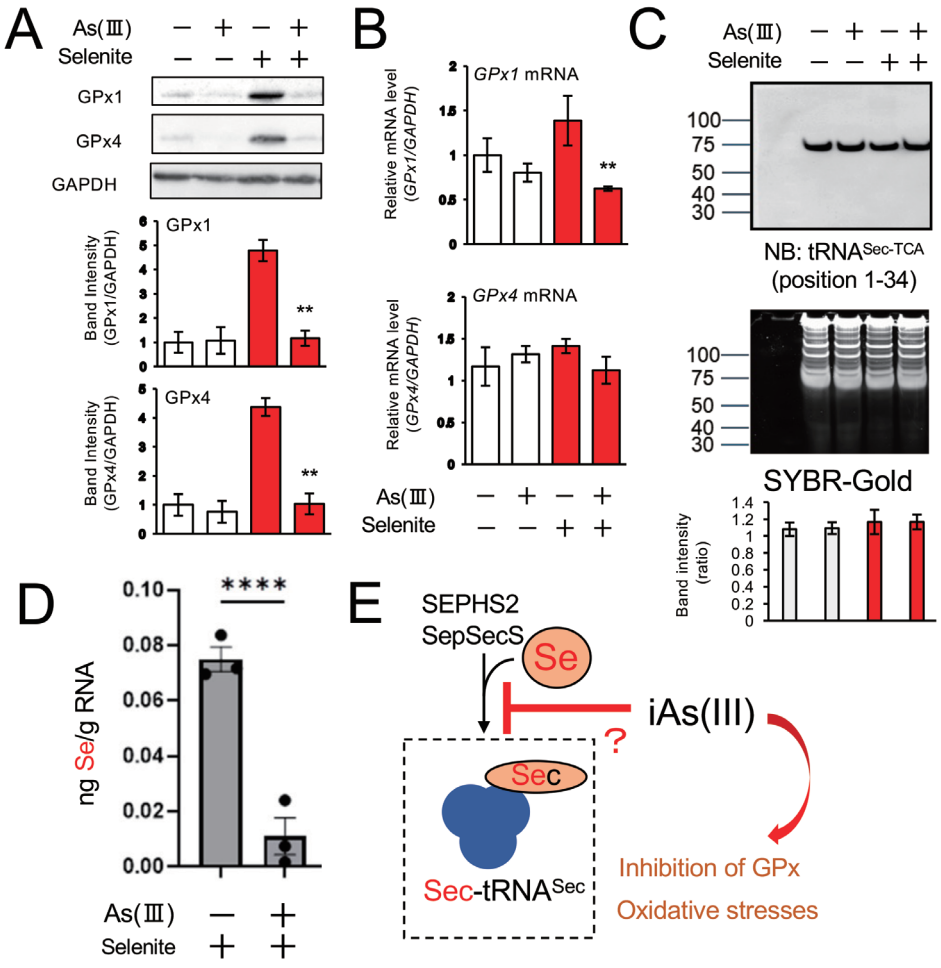


Fig. 3. Effect of the As(III) on the Sec charging to tRNA^{Sec}. (A) HT1080 cells were treated with selenite (50 nM) and As(III) (2.5 μM) for 24 hr. Representative Western blotting for GPx1 and GPx4 is shown. GAPDH was used as an internal control. Quantified band intensities normalized to GAPDH are shown in the bar graph below. Data represent the mean ± S.D. (n = 3). Statistical analysis was performed using Tukey's test, and significance is indicated as **P < 0.01. (B) RT–qPCR analysis of GPx1 and GPx4 under the same conditions as in (A). Data represent the mean ± S.D. (n = 3). Statistical analysis was performed using Tukey's test, and significance is indicated as **P < 0.01. (C) Northern blot analysis of tRNA^{Sec} under the same conditions as in (A). SYBR Gold staining was used as a loading control. Data represent the mean ± S.D. (n = 3). Statistical analysis was performed using Tukey's test; no significant difference was observed. (D) Selenium levels in the deacylated supernatant RNA fraction under the same conditions as in (A). Data represent the mean ± S.D. (n = 3). Statistical analysis was performed using Student's t-test, and significance is indicated as **P < 0.01. (E) Proposed mechanism underlying the inhibition of selenium metabolism by As(III).

enite or As(III) (Fig. 3C). Taken together, these results suggested that As(III) interferes with selenoprotein biosynthesis downstream of mRNA expression and tRNA availability, implicating a defect in the translational incorporation of Sec. We then quantified Sec-tRNA charging using the deacylation-ICP-MS assay as shown in Fig. 2. The selenium signal in the deacylated supernatant, corresponding to charged Sec, was markedly reduced in As(III)-treated cells (Fig. 3D). Because neither total tRNA levels nor GPx mRNA levels were little affected, these findings indicate that As(III) inhibits Sec charging on tRNA, accounting for the observed suppression of selenoprotein induction.

These results suggested Sec-tRNA charging as a previously unrecognized target of As(III) in selenium metabolism. Impairment of Sec charging has been linked to

decrease of GPx4, which is essential for suppressing ferroptosis, and its reduced translation may contribute to the pro-ferroptotic effect of arsenic reported in previous studies.

From a methodological point of view, the ICP-MS based assay offers high sensitivity to selenium as well as limitations. Because Sec is the only amino acid containing selenium, elemental mass spectrometry provides unique specificity for detecting Sec compared with other amino acids. In contrast, most other elements used (e.g., sulfur, carbon, nitrogen) are distributed across many amino acids, and this method is effective just only for Sec-tRNA^{Sec}. However, the method does not distinguish Sec from selenomethionine (SeMet). Although SeMet is misscharged to tRNA^{Met}, this would contribute to the background on the biological context, e.g., if SeMet was used as selenium sources, meaning of this assay will be change (Ogra *et al*., 2008, Hussein *et al*., 2022). At least in our condition, the contribution of SeMet is expected to be limited because we used selenite as selenium source, and this limitation should be considered when applying the method to metabolic models involving substantial SeMet uptake or protein turnover. When evaluating Sec tRNA in this system, conditions with controlled selenium sources are required.

The discrepancy in Se mass balance observed after fractionation (Fig. 2B) may reflect partial loss of Se during sample processing. One possible explanation is the formation of volatile Se species, such as hydrogen selenide (H_2Se), which could be lost during incubation or handling steps. In addition, Se-containing molecules, particularly low-molecular-weight or hydrophobic species, may adsorb nonspecifically to plasticware, leading to underestimation in recovered fractions. Furthermore, incomplete recovery during precipitation or transfer steps may also contribute to the observed gap. These technical factors should be considered when interpreting quantitative Se measurements in fractionated samples.

The chemical reactivity of Sec is high, and it easily undergoes oxidation. However, the chemical state of Sec on tRNA^{Sec} cannot be estimated by this assay and it is unclear to what extent the Sec are intact. There is potential interest in whether these modified Sec are used for translation. In the future, we would also like to evaluate the chemical form of Sec in tRNA using experimental methods, such as LC/MS.

The present findings raise important mechanistic questions regarding how As(III) impairs Sec charging. Sec-tRNA^{Sec} synthesis involves activation of selenite by SEPHS2, formation of selenophosphate, ligation to tRNA^{Sec}, and conversion to Sec-tRNA^{Sec}by SepSecS (Palioura *et al*., 2009). Arsenite is known to interact with thiol-containing enzymes and cofactors, suggesting that interference may occur at one or more enzymatic steps, particularly those requiring reduced cysteine residues or redox-sensitive cofactors. Further studies will be required to determine the specific molecular target(s) of As(III).

Technically, this approach enables a direct readout of Sec-tRNA charging in cells without reliance on radiolabeling or indirect inference from protein levels. This analytical strategy may be applicable to expanding investigations of selenium metabolism in toxicology, redox biology, ferroptosis, and nutritional selenium supplementation. For toxicological research in particular, the ability to quantify Sec charging opens possibilities for identifying environmental agents that selectively impair selenoprotein synthesis and redox homeostasis.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

The data in this study are included in the article/supplementary materials. Contact the corresponding author(s) directly to request the underlying data.

Author contributions

Conceptualization: Takashi Toyama
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Supervision: Takashi Toyama
Visualization: Takashi Toyama, and Reiko Makino
Writing – original draft: Takashi Toyama and Yasutoshi Akiyama
Writing – review and editing: Yoshihisa Tomioka, Takashi Toyama, Daigo Sumi and Yoshiro Saito

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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