

Short Conceptual Overview

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Selenomethionine metabolism and its toxicity in yeast

Abstract: The importance of selenium for organisms can be explained by its existence as selenocysteine in the catalytic centers of glutathione peroxidase and thioredoxin reductase. Another selenoamino acid, selenomethionine, is the major form of selenium in foods, and organisms that require selenium as a nutrient directly metabolize selenomethionine to a reactive form of selenium or store it in general proteins. Selenium is recognized as an essential nutrient for human and animal health; however, its excessive uptake harms mammals and the cytotoxic mechanism of selenium remains unclear. Recent progress in the development of selenium-enriched yeast and selenomethionine-resistant mutant to produce selenomethionine-containing proteins for X-ray crystallography has provided new insights into the molecular mechanism of selenomethionine toxicity. In this review, we describe the metabolism of seleno-compounds in yeast and discuss the cytotoxicity caused by selenomethionine against yeast from a metabolic viewpoint.

Keywords: selenium; selenium-enriched yeast; selenium metabolism; selenium toxicity; selenomethionine.

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List of abbreviations

AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; Hcy, homocysteine; Se, selenium; Sec, selenocysteine; SeHcy, selenohomocysteine; Se-AdoHcy, *Se*-adenosylselenohomocysteine; Se-AdoMet, *Se*-adenosylselenomethionine.

Introduction

Selenium (Se) is an essential element for many organisms, but its excessive uptake is detrimental. Toxicity caused by Se has been prevalent for many years. In the 1930s, Se-amino acids were already assumed to be toxic compounds from plants grown in seleniferous soils (1). Chronic ingestion of these Se-polluted plants causes ‘blind staggers’ and ‘alkali disease’ in cattle and sheep (2). Early studies on determination of the form of Se present in seleniferous plants suggested that this element may be present in proteins as an integral part of amino acids, possibly by replacement of sulfur in cysteine and methionine (3).

Regarding the biological importance of Se, it is primary thought to be related to its presence in selenoproteins, including glutathione peroxidase and thioredoxin reductase, both of which incorporate a selenocysteine (Sec) in their catalytic centers. Epidemiologic studies have suggested that Se deficiency causes lung (4), bladder (5), and prostate cancers (6, 7). In addition, Se and selenoproteins have been reported to participate in immune function (8, 9) and sperm maturation (10). Humans and animals take up Se from plants, which absorbed it from the soil to produce organic Se compounds. Therefore, Se deficiency can occur in regions with relatively low Se levels in soil.

At present, Se-enriched yeast is widely used as a nutritional supplement. The advantages of using yeast for Se supplementation include the production of Se-enriched yeast at an industrial scale, which is more convenient than the use of other organisms such as Se-accumulating plants. In addition, *Saccharomyces cerevisiae* cultured with inorganic Se compounds, such as selenite (SeO₃²⁻) or selenate (SeO₄²⁻), produces organic Se compounds that are much less toxic and more bioavailable than the original salts (11). Furthermore, yeast accumulates these compounds at levels as high as 3000 µg Se/g of dry biomass (12). The predominant form of Se in such yeasts is protein-bound selenomethionine (SeMet) (13).

SeMet-containing proteins contribute to the solution for phase problems in X-ray crystallography using a

multi-wavelength anomalous dispersion method established in 1990 (14). Most recently, target proteins for structure analyses have been shifted to proteins from mammalian cells, the expression of which often fails in *Escherichia coli*. Several trials have reported the production of SeMet-containing proteins in mammalian (15–17), insect (18), and yeast cells (19–22); however, most studies primarily emphasize on the practical use of the specified host cells and have not fundamentally overcome SeMet toxicity. This review discusses SeMet metabolism in yeast and the molecular mechanism of SeMet toxicity, which has been discovered over the past few years.

De novo SeMet synthesis

Se can exist in four oxidation states: elemental form [Se (0)], selenide [Se²⁻ (II)], selenite [SeO₃²⁻ (IV)], and selenate [SeO₄²⁻ (VI)]; however, selenide is a major form in nature, typically in proteins that contain Se-amino acids such as SeMet and Sec. To synthesize SeMet, selenate and selenite must be taken up into a cell and reduced to selenide.

Studies on microorganisms demonstrated that selenate uptake is mediated by sulfate transporters (23, 24). Recently, selenite uptake was reported to be mediated by phosphate transporters in wheat (25) and by silicon transporters in rice (26). In the case of yeast, it is assumed that selenite is passively incorporated into the cells because several kinds of Se compounds were detected in selenite-treated cells by metabolomic analysis (27), even though the transporters for sulfite and selenite uptake have not been identified.

As shown in Figure 1, the first step in selenate reduction is the formation of adenylylselenate by ATP sulfurylase, which has been shown to utilize selenate as a substrate *in vitro* (28). Adenylylselenate reduction is thought to occur via a mechanism analogous to the sulfur assimilation pathway. The adenylylselenate is phosphorylated again by adenylylsulfate kinase to yield 3'-phosphoadenylyl selenate and is subsequently reduced to selenide by 3'-phosphoadenylyl sulfate reductase and homocysteine (Hcy) synthase. Once selenate is converted to adenylylselenate, it also can be reduced to selenide in a non-enzymatic reaction. This reaction pathway has been well documented in plants by Terry et al. (29), and it appears that the non-enzymatic reaction also occurs in yeast (30). The selenide is introduced into *O*-acetylhomoserine by Hcy synthase, which is encoded by *MET17* in yeast. The protein Met17 was first reported to be a bifunctional enzyme that can also catalyze sulfide incorporation

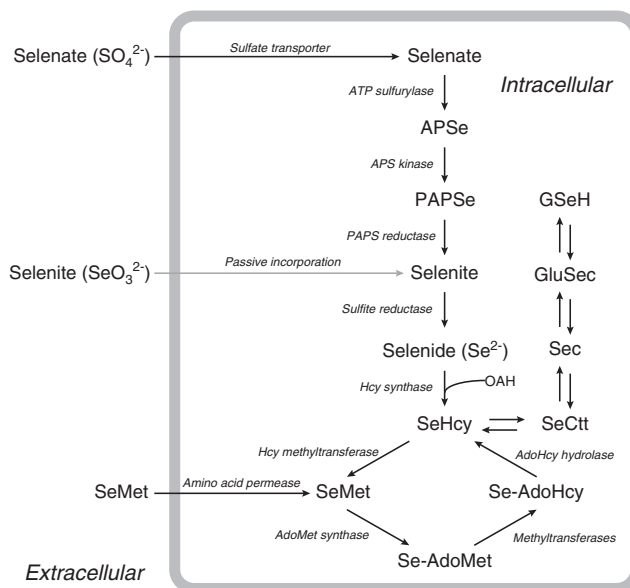


Figure 1 Selenium (Se) metabolism in *Saccharomyces cerevisiae*. Extracellular selenate and selenomethionine (SeMet) are actively incorporated into the cell by transporters for their S analogs, whereas selenite is passively incorporated. The enzymatic reductions of selenate and selenite are shown. Please refer to the review by Terry et al. (29) for the non-enzymatic reduction pathway. APS, adenylylsulfate; APSe, adenylylselenate; GluSec, γ -glutamylselenocysteine; GSeH, selenogluthathione; OAH, *O*-acetylhomoserine; PAPS, 3'-phosphoadenylyl sulfate; PAPSe, 3'-phosphoadenylyl selenate; SeCtt, selenocystathionine.

into *O*-acetylserine; however, yeast lacks serine-acetyltransferase activity to produce *O*-acetylserine (31, 32). Accordingly, selenohomocysteine (SeHcy) is a branching point in the Se-amino acid biosynthetic pathway (Figure 1). SeMet is directly synthesized from SeHcy by the cobalamin-independent Hcy methyltransferase, which is encoded by *MET6*. The cobalamin-independent Hcy methyltransferase from *E. coli* has been shown to methylate SeHcy at rates similar to those of Hcy methylation (33).

SeMet metabolism

Once incorporated into cells, SeMet can enter the sulfur metabolic pathway, where it is converted to Se analogs of S compounds, although it is primarily used for protein synthesis. It has been difficult to analyze intracellular Se compounds because the concentrations of these compounds are often three orders of magnitude lower than those of their S analogs (34). In addition, the lack of authentic standards for most Se compounds adds to the difficulty. Recent advances in high-sensitivity and

high-resolution mass spectrometry have allowed us to identify trace amounts of Se compounds, based on the characteristic isotopic patterns of Se. Using liquid chromatography orbitrap mass spectrometry, Rao et al. (27) demonstrated the distribution of Se compounds in selenate-, selenite-, and SeMet-treated yeast. Se compound production was much higher in SeMet-treated cells than in selenate- and selenite-treated cells. When the yeast cells were treated with SeMet, the intracellular SeMet levels increased very quickly (<15 min), whereas the levels of other Se compounds [SeHcy, selenogluthathione, γ -glutamylselenocysteine, and Se-adenosylselenohomocysteine (Se-AdoHcy)] peaked at 2 h after the addition of SeMet. The principal form of Se at that moment was Se-AdoHcy (70.1% of the total Se compounds), whereas the percentages of other Se compounds were <10%. Se-AdoHcy also accumulates in selenate- and selenite-treated yeast (more than 90% of the total Se compounds), although selenide can enter both the methyl cycle and the GSH synthetic pathway via SeHcy (Figure 1). Se-AdoHcy can be produced from Se-adenosylselenomethionine (Se-AdoMet) with the methylation of nucleic acids, proteins, lipids, and secondary metabolites. Evidence shows that methyltransferases can transfer methyl groups from Se-AdoMet to nucleic acids, including rRNA, tRNA, and DNA (35). Although Se-AdoHcy accumulates at high levels in SeMet-treated yeast, the percentage of SeHcy remains at 8.5% of the total Se compounds. Accordingly, Se-AdoHcy can be hydrolyzed by the S-adenosylhomocysteine (AdoHcy) hydrolase, which is encoded by *SAH1* in yeast, but the hydrolase activity of the Sah1 protein against Se-AdoHcy may be much less than that against AdoHcy. In addition to the Se compounds identified by Rao et al. (27), 5'-methylselenoadenosine was observed in the SeMet-treated yeast by capillary electrophoresis time-of-flight mass spectrometry (36), indicating that Se from SeMet can enter the methionine salvage pathway. Taken together, these results suggest that SeMet behaves like methionine in the metabolic pathway, but the distributions of Se compounds are quite different from those of their S analogs. This may be caused by the reduced enzymatic activities at each metabolic step, particularly selenocystathionine synthesis by cystathionine β -synthase and SeHcy synthesis by AdoHcy hydrolase.

SeMet-containing proteins

Unlike selenoproteins, SeMet-containing proteins are not required for cell growth. SeMet is incorporated into

general protein because it can be transferred onto tRNA^{Met} as efficiently as methionine (37); however, the overall rate of protein synthesis is reduced because selenomethionyl-tRNA^{Met} was less effective as a substrate than methionyl-tRNA^{Met} in translation process (38).

Methionine substitution with SeMet in proteins does not significantly alter the kinetic properties of enzymes, but influences their physical properties. For instance, a β -galactosidase protein in which 50% of the methionine residues were replaced by SeMet showed K_m and V_{max} values similar to those for the wild-type enzyme; however, the SeMet-containing β -galactosidase was less stable in response to both heat and urea denaturation, compared with the wild-type enzyme (39). Similarly, both the SeMet and the methionine derivatives of thymidylate synthase from *E. coli* exhibited essentially the same kinetic and binding properties; however, the SeMet-containing enzyme was twofold less stable than the wild-type enzyme (40). One reported exception to these findings was phosphomannose isomerase from *Candida albicans*, which contains four methionine residues in the vicinity of the active site; the SeMet-containing enzyme had a fourfold higher K_m than the methionine-containing enzyme (41).

SeMet-resistant yeast mutants

Despite their lesser biological importance, proteins that contain SeMet instead of methionine are valuable tools in structural biology, particularly for phase determinations of crystal according to the single- or multi-wavelength anomalous dispersion method. The use of SeMet-containing proteins for X-ray crystallography was originally reported in 1990 (14), and, currently, this technique is widely used for phase determinations of protein crystals. SeMet-containing proteins are routinely prepared in *E. coli* cells that are cultured with SeMet. However, preparation of the mammalian proteins containing SeMet is still hard work because it is more difficult to express the proteins in eukaryotic systems than in *E. coli* cells. In addition, eukaryotic proteins, which require post-translational modification, often fail to be expressed in *E. coli* cells. To improve these issues, studies have reported the production of SeMet-containing proteins in eukaryotic cells (15–22). Most recently, SeMet-resistant yeast mutants have been reported for the production of SeMet-containing proteins (42–44).

Malkowski et al. (42) demonstrated that the deletion of both *SAM1* and *SAM2*, which encode the isomers of S-adenosylmethionine (AdoMet) synthase, conferred

increased SeMet resistance and allowed the production of SeMet-containing proteins at 95% occupancy.

In a different approach, Bockhorn et al. (43) screened a collection of single-gene deletion mutants of *S. cerevisiae* and demonstrated that a mutant lacking cystathionine γ -lyase activity (*cys3* Δ) exhibited the highest resistance to SeMet and had the ability to incorporate SeMet (88.3 \pm 6.7%) that was equal to or slightly higher than that of the *sam1* Δ *sam2* Δ cells (74.4 \pm 15.3%).

A *Pichia pastoris* mutant, which exhibits SeMet resistance, has also been reported to produce SeMet-containing proteins (44). Unlike the *S. cerevisiae* *sam1* Δ *sam2* Δ double mutant and *cys3* Δ single mutant, the SeMet-resistant *P. pastoris* mutant did not exhibit a cysteine and AdoMet auxotrophic phenotype and expressed the SeMet-containing human lysozyme at 65% occupancy, which was sufficient for single-anomalous dispersion phasing. An analysis of a *S. cerevisiae* mutant that presented the same phenotype as that of the *P. pastoris* mutant identified a mutant allele of the *MUP1* gene, which encodes the high-affinity methionine permease (*mup1-100*) (45). Although methionine uptake was reduced in the *mup1* mutant, the mutant could incorporate SeMet into an overexpressed epidermal growth factor peptide at 73% occupancy. SeMet uptake in the *mup1* mutant was most probably mediated by amino acid permeases with broad substrate specificities. These results suggest the importance of moderate SeMet uptake by amino acid permeases other than Mup1 for the alleviation of SeMet toxicity.

Toxicity

Isolation and analysis of SeMet-resistant yeast mutants have provided a promising procedure to express recom-

binant proteins with high levels of SeMet incorporation and important clues for understanding the molecular mechanisms of SeMet toxicity. All of the abovementioned SeMet-resistant yeast mutants, including *sam1* Δ *sam2* Δ (42), *cys3* Δ (43), and *mup1-100* (45), have mutations in genes that are involved in sulfur amino acid metabolism. Most interestingly, Malkowski et al. (42) also found that the *met6* Δ *sam1* Δ *sam2* Δ triple mutant, in which neither AdoMet nor methionine was synthesized, exhibited better growth than the *met6* Δ single mutant. This result indicates that SeMet toxicity does not come from the existence of SeMet in proteins, but strongly suggests that Se-AdoMet itself or one of its metabolic products inhibits yeast cell proliferation. Furthermore, capillary electrophoresis time-of-flight mass spectrometry of metabolites in SeMet-treated yeast demonstrated that the intracellular thiol compound levels decreased significantly within 10 min, whereas the diselenide and selenosulfide compound levels increased (36). The SeMet-induced growth defect was recovered by extracellular addition of cysteine and genetic modification of yeast cells, in which serine-acetyltransferase and *O*-acetylserine sulfhydrylase from *E. coli* were expressed to increase the intracellular cysteine levels (Figure 2) exhibiting SeMet resistance. In addition, SeMet resistance was further increased by *CYS3* gene deletion in the wild-type strain that expressed serine-acetyltransferase and *O*-acetylserine sulfhydrylase, although *CYS3* deletion in the strain did not change the intracellular levels of thiol compounds. Accordingly, Sec is likely to be a primary toxic compound in yeast.

In selenoprotein synthesis, Sec is synthesized from serine on tRNA^{Sec} and is incorporated into nascent polypeptide chains in response to the UGA codon (46, 47). The existence of Sec at the catalytic centers of selenoproteins, instead of cysteine, is now assumed to be due to the

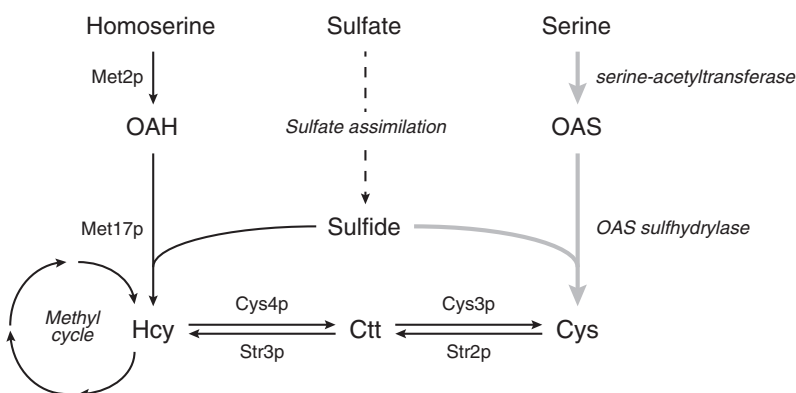


Figure 2 The sulfur metabolic pathway in *Saccharomyces cerevisiae*.

Metabolic routes are indicated by black arrows. The *de novo* synthetic pathway of cysteine that is introduced to increase the intracellular cysteine levels is indicated by gray arrows. Ctt, cystathionine; OAS, *O*-acetylserine.

advantage that it confers on enzymatic reactions at physiological pH levels, at which the selenol group shows high nucleophilicity. In addition to the above speculation, it appears that the Sec biosynthetic machinery has evolved to trap the toxic amino acid Sec in proteins, so that it does not exist as a free selenol compound.

In summary, SeMet metabolism and the molecular mechanism behind SeMet toxicity were elucidated using high-resolution mass spectrometry and genetic analyses. However, other toxic mechanisms may exist in humans and animals because the findings of this study are only based on studies with yeasts, which do not require Se for growth. Further metabolomic analyses of these organisms

will elucidate the general mechanism of SeMet toxicity. Moreover, a metabolomic approach would provide new insights into the importance of Se for human health.

Acknowledgments: This article was dedicated to Dr. Yoshifumi Jigami who greatly contributed to molecular biology of yeast.

Conflict of interest statement: There is no conflict of financial and other interests.

Received October 10, 2013; accepted October 28, 2013

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