RESEARCH ARTICLE

Selenium metabolism and toxicity in the yeast *Saccharomyces cerevisiae*

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Abstract

Selenium (Se) is an essential trace element of considerable interest in humans from both a nutritional and a toxicological perspective because of the narrow margin between intakes that result in efficacy and toxicity. It is used as selenocysteine in a few selenoproteins with important physiological functions. Moreover, at supranutritional doses, Se-containing compounds have attracted interest as potential anticancer agents with high efficacy and selectivity against cancer cells. Thus, Se is becoming a widely used dietary supplement. However, accumulating evidence indicate that adverse health effects are associated with excess dietary supplementation. Therefore, characterizing the toxicity of Se metabolic intermediates are important steps to better understand both the beneficial and toxic mechanisms of Se. This review focuses on the metabolism of Se and the biological mechanisms explaining the toxicity of important Se-metabolites in the yeast *Saccharomyces cerevisiae*. Conclusions drawn from these studies support the use of yeast as a valuable model system to elucidate the mode of action and the biological effects of supranutritional Se in higher eukaryotes.

Keywords: selenite, selenide, selenomethionine, selenocysteine, methylselenol

1-Introduction

Selenium (Se) is an essential trace element for many living species, including humans. It is required to synthesize a few selenoproteins, in which Se is specifically incorporated as the amino acid selenocysteine (SeCys) [1]. So far, 25 selenoproteins have been identified in humans [2]. Many of them possess redox properties and function as antioxidants in which SeCys is the catalytic residue [3]. Se deficiency has been associated with cardiomyopathy, increased risk of mortality, poor immune function and cognitive decline [4]. Moreover, the last decades have witnessed a growing interest in Se biology because of its reported beneficial effects in prevention against cancer and other diseases at supranutritional intake levels [5, 6]. Se is becoming a widely used dietary supplement for humans and livestock[7]. Potential benefits are, however, not without risk because of the relatively narrow window between intakes that result in efficacy or toxicity [8, 9], as high levels of blood selenium have been associated with an increased risk of developing certain types of cancer, hypertension, chronic diseases such as diabetes, and neurodegenerative diseases [10].

Selenium-enriched yeast (Se-yeast) produced by growing *Saccharomyces cerevisiae* in selenite- or selenate-enriched media, is a recognized source of organic Se used to supplement the dietary intake of this important trace mineral. One of the advantages of using yeast for Se supplementation is its high capacity of accumulation of Se resulting in the production of organic Se-enriched products at an industrial scale. Metabolization of selenocompounds *in vivo* gives rise to multiple different metabolites, as observed in Se-yeast [11, 12]. The biological activity of different Se species, as well as their toxicity in yeast and in humans, depends upon their transformation into different active products [13]. Understanding the metabolic pathways and toxicity of Se in yeast is important, not only for the optimization of the production process of Se-yeast, but also to produce yeast enriched in specific Se metabolites that may be beneficial for human health. Moreover, yeast can be used as a model system to understand the mode of action and the biological effects of supranutritional Se in higher eukaryotes [14, 15].

2-Inorganic selenium transport in yeast

Inorganic Se is commonly found in four oxidation states: +6 (e. g. selenate, SeO_4^{2-}), +4 (selenite, SeO_3^{2-}), 0 (Se^0, elemental Se) and -2 (selenide, H_2Se/HSe^-) [16]. Inorganic Se compounds do not have specific transporters for uptake in *S. cerevisiae*. Elemental selenium, which is insoluble, is not expected to be transported across membranes. Volatile H_2Se is believed to cross membranes by diffusion [17].

Selenic acid (H_2SeO_4) is a strong diacid (both pKa being <2), thus at physiological pH, selenate is exclusively found as selenate (SeO_4^{2-}) anion. SeO_4^{2-} is taken up by sulfate transporters [18]. Two membrane transporters (encoded by *SUL1* and *SUL2*) take up external sulfate with high-affinity (5-10 µM). Single mutants exhibited only a small decrease in sulfate uptake. Double mutants have no detectable sulfate transport and are highly resistant to selenate. Both *SUL1* and *SUL2* are under the control of the transcriptional activator Met4p. *MET4* is negatively regulated by sulfur amino acids, creating a negative feedback loop that

decreases sulfur assimilation when sulfur metabolites are sufficiently abundant [19]. Thus, *SUL1* and *SUL2* are repressed in growth medium containing > 0.1 mM methionine [20]. The activity of both sulfate permeases is inhibited by selenate and chromate. Sulfate permeases have nearly the same affinity for sulfate, selenate [20], and chromate [21].

Selenious acid is a weak diacid with pKa_1 and pKa_2 of 2.57 and 6.60, respectively. This means that H_2SeO_3, hydrogenoselenite ($HSeO_3^-$) and selenite (SeO_3^{2-}) occur in solution, in proportions depending on the pH. An initial study on the kinetics of selenite uptake suggested the existence of two transport systems: a high-affinity system and a low-affinity system operating at different selenite concentration [22]. These systems were later characterized as the high- and low-affinity phosphate transporters [23]. In *S. cerevisiae*, the inorganic phosphate (Pi) acquisition system is composed of five transporters [24]. The high-affinity transport system (K_M~10-25 µM), composed of Pho84p and Pho89p, is transcriptionally up-regulated by the phosphate signal transduction pathway (PHO) in response to Pi starvation [25]. The Pho84p transporter operates preferentially at neutral and acidic pH, while Pho89p is functional at alkaline pH. The transporters Pho87p, Pho90p, and Pho91p are constitutively transcribed and take up phosphate with low affinity (K_M~1-2 mM) [26]. Their activity is post-transcriptionally down-regulated at low phosphate conditions by Spl2p, a member of the *PHO* regulon. Thus, this regulatory mechanism results in cells that use either the high-affinity (when Pi concentration in the growth medium is < 0.5 mM) or the low-affinity transport systems, depending on phosphate availability [27]. Comparison of the Vmax/ K_M of the transporters for Pi or for selenite showed that while Pho84p is slightly more efficient than the low-affinity carriers for selenite transport, it has a much higher affinity for Pi than for selenium. Thus, the high-affinity transporter is very selective for Pi, whereas the low-affinity system is much less discriminating [23]. As a consequence of the regulation of the phosphate transport system, selenite toxicity depends on the phosphate concentration in the medium. Selenite toxicity is high in very low Pi conditions and decreases with increasing Pi concentration up to 0.4 mM Pi. When phosphate concentration in the culture medium is further increased, the transport of phosphate (and of selenite) is progressively taken over by the low-affinity carriers and selenite uptake and toxicity increase. Another membrane transporter, the monocarboxylate transporter Jen1p, takes up selenite efficiently when yeast cells are cultured in a non-glucose medium [28]. Jen1p catalyzes transport of cellular metabolites such as acetate, pyruvate and lactate. Its expression is up-regulated when yeast cells are grown using non-fermentative carbon sources, conditions which lead to increased selenite toxicity [29].

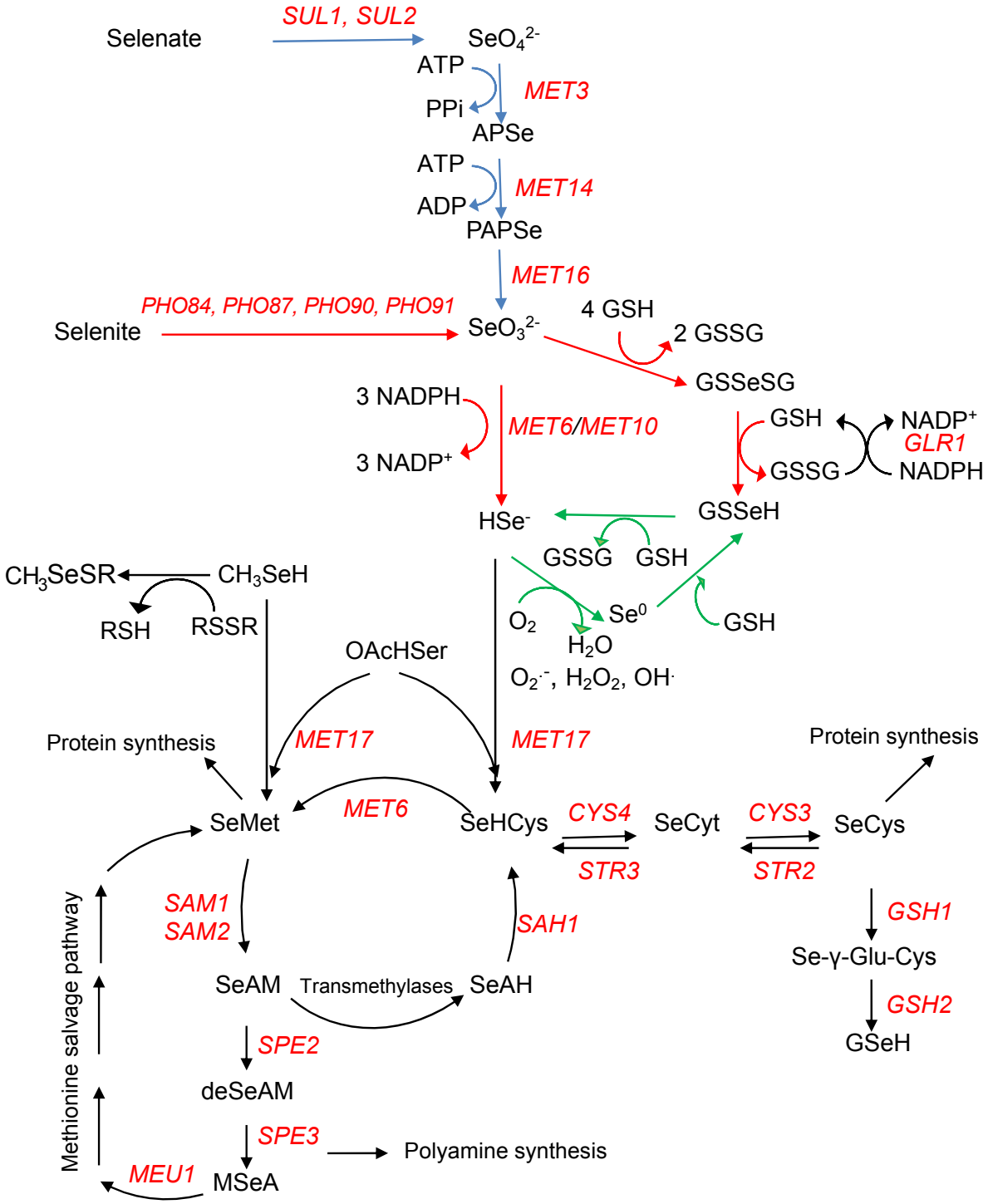

Figure 1. Transport and metabolism of Se in *S. cerevisiae* (adapted from [15]). Names of genes involved in Se transport or enzymatic reactions are indicated in red. Selenate uptake and reduction pathway is indicated with blue arrows. Selenite transport and reduction to selenide is indicated with red arrows. Redox cycling of selenide with oxygen and GSH is indicated with green arrows. Organic Se primary metabolic pathways are represented with black arrows. Abbreviations not used in the main text are as follows: APSe, adenylyl-selenate; PAPSe, phosphoadenylyl-selenate; SeAM, *Se*-adenosylmethionine, deSeAM, decarboxylated SeAM; MSeA, methylselenoadenosine; SeCyt, selenocystathionine.

3- Inorganic selenium toxicity in *S. cerevisiae*

Once inside cells, selenate is reduced to selenite by the sulfate reduction pathway (Fig. 1, blue arrows). Selenite can be reduced to hydrogen selenide either enzymatically by sulfite reductase or by glutathione (GSH) according to the scheme presented in Fig. 1 [17, 30, 31]. Selenite reacts spontaneously with GSH to produce selenodiglutathione (GSSeSG) which is further reduced by GSH into glutathione selenenylsulfide (GSSeH). The latter either spontaneously dismutates into Se^0 and GSH or is further reduced to yield H_2Se/HSe^- (Fig. 1, red arrows). In several bacteria and animal cells, hydrogen selenide provides Se for the synthesis of selenoproteins after activation to selenophosphate. This pathway does not exist in yeast or plants, which do not possess selenoproteins. H_2Se/HSe^- is readily oxidized by oxygen. This reaction produces Se^0, which can be reduced by GSH with regeneration of H_2Se/HSe^- that will initiate a new cycle of oxidation/reduction. These redox cycles produce reactive oxygen species (ROS) [32], and consume intracellular antioxidants such as thioredoxin and GSH and, consequently, the reducing cofactor NADPH (Fig. 1, green arrows) [33].

A large body of evidence, accumulated in the course of the last decades, indicates that the toxicity of selenite/selenide is mainly caused by DNA damage [34, 35]. In yeast, Letavayova et al. showed that selenite induces double strand breaks (DSB) and chromosome fragmentation [36]. To explain these results, the authors proposed that selenite induces single-strand damage that is converted to DSB upon DNA replication. Such lesions can be repaired by homologous recombination (HR). Accordingly, a *rad52* (a key gene in the HR pathway) mutant was found to be hypersensitive to sodium selenite [37, 38]. *RAD9*, a DNA-damage checkpoint gene, required for transient cell-cycle arrest and activation of DNA repair mechanisms in response to DSBs [39] was also found to be extremely sensitive to selenite. Genome-wide studies of yeast gene deletion mutants confirmed the importance of DNA repair systems, especially the homologous recombination pathway, in the resistance to selenite or selenide exposure [40, 41].

In vitro studies [41, 42] showed that selenide directly induced DNA single-strand breaks. In contrast, selenite did not break DNA unless it was mixed with GSH, indicating that reduction of selenite into HSe^- accounts for selenite-induced DNA damage *in vivo*. DNA fragmentation was strictly oxygen-dependent and was inhibited by mannitol, a hydroxyl radical scavenger, but not by superoxide dismutase or catalase [41]. This suggests that hydroxyl or hydroxyl-like radicals produced upon oxidation of selenide by oxygen are the cause of DNA damage and inorganic Se toxicity. A recent study by Dereven'kov *et al*. suggests that GSSeH produced by redox cycling of HSe^- with GSH reacts with hydrogen peroxide to generate hydroxyl radicals [43].

In addition to DNA damage, selenite exposure promotes redox imbalance and oxidative stress [44]. Genome-wide analyses of the *S. cerevisiae* transcriptome revealed that selenite treatment up-regulated genes involved in the oxidative stress response under the control of the Yap1p transcription factor [45, 46]. Yeast redox state is controlled by the thioredoxin pathway and the glutathione system composed of GSH, GSH reductase

and glutaredoxins [47]. Several genes belonging to the glutathione redox pathway, such as *GSH1*, *GLR1*, *GRX1, GRX2, GRX3*, *GRX5* and *YAP1* were shown to play a role in tolerance to selenite exposure [29, 39, 48, 49]. This is likely linked to the oxidation of GSH by the reductive metabolism of selenite/selenide resulting in a severe decrease of the reduced/oxidized ratio of all low-molecular weight thiols (glutathione, cysteine, homocysteine, γ-glutamyl-cysteine and cysteinyl-glycine) [50]. In contrast, deletion of *YCF1*, encoding a vacuolar transporter, which detoxifies heavy metals by sequestration in the vacuole as GSH-conjugates, confers increased resistance to selenite exposure [39]. To explain this paradoxical result, it was shown that transport to the vacuole of selenodiglutathione (GSSeSG) by Ycf1p results in cytosolic GSH depletion [51].

4- Toxicity of seleno-amino acids

Because of the chemical similarity between Se and sulfur (S), most enzymes involved in sulfur metabolism do not discriminate between the two chalcogen elements (see Fig. 1 for Se metabolic pathways in yeast). Thus, the yeast *MET17* gene product, encoding O-acetylhomoserine sulfhydrylase, catalyzes the incorporation of inorganic Se into O-acetylhomoserine (OAcHSer) to form selenohomocysteine (SeHCys), which is then used by methionine synthase (Met6p) to synthetize SeMet. SeCys is produced from SeHCys or SeMet by the transulfuration pathway. SeMet can be activated and transferred onto tRNA by methionyl-tRNA synthetase or used as substrate for *S*-adenosyl-methionine synthetase (encoded by *SAM1/2*) with similar efficiency to methionine [52]. Similarly, cysteinyl-tRNA synthetase can aminoacylate its tRNA using SeCys instead of cysteine. Thus, both SeMet and SeCys can be incorporated in polypeptide chains [53, 54]. An analysis of the Se content of Se-enriched yeast showed that around 60% of the Se was incorporated in proteins as SeMet and 10–15% as SeCys [54]. Numerous Se-containing low-molecular weight compounds, including non-proteinous SeMet account for the remaining Se.

Several studies using yeast mutants show that SeMet itself is not toxic [55, 56]. Although misincorporation of SeMet in proteins might in principle generate toxicity, it has been shown that the substitution of more than 90% of protein methioninyl residues by SeMet does not elicit significant toxicity in yeast, excluding a toxic effect of SeMet inserted into proteins in place of methionine [57-59]. For example, a *sam1sam2* mutant, in which the conversion of methionine to *S*-adenosylmethionine is blocked, exhibited reduced SeMet toxicity compared with wild-type yeast and increased production of protein during growth in SeMet, although replacement of methionine by SeMet was nearly complete [60]. These results suggest that a metabolite of SeMet is responsible for the toxicity of this compound [61].

Bockhorn et al. [59] screened a collection of single-gene deletion mutants of *S. cerevisiae* for resistance to SeMet and demonstrated that a mutant lacking cystathionine γ-lyase activity (Δ*cys3)* showed the highest resistance to SeMet, suggesting that SeCys and/or a downstream metabolite of SeCys is responsible for SeMet toxicity. Another genome-wide screen revealed that tolerance against SeMet mainly involves

mechanisms related to the folding or removal of damaged proteins, suggesting that SeMet induces a proteotoxic stress [53]. SeMet was also shown to induce an accumulation of protein aggregates by a mechanism that requires de novo protein synthesis. Protein aggregation was suppressed in a Δ*cys3* mutant unable to synthetize selenocysteine, suggesting that aggregation results from the metabolization of SeMet to SeCys followed by translational incorporation in the place of cysteine [53]. In support of this hypothesis, introduction in *S. cerevisiae* cysteinyl-tRNA synthetase of a mutation reducing SeCys recognition increased resistance to the toxic effects of SeMet [62]. Thus, misincorporation of SeCys in nascent polypeptides, resulting in protein misfolding and aggregation is likely to be the main pathway for seleno-amino acids toxicity. Random replacement of cysteine by SeCys may induce misfolding by formation of non-native intermolecular or intramolecular selenylsulfide or diselenide bridges resulting in the formation of insoluble protein adducts [53].

In addition to protein aggregation, selenols produced from SeMet metabolism (SeHCys, SeCys and selenoglutathione (GSeH)) can induce an oxidative stress by reacting with low molecular thiols or selenols in the presence of oxygen to form mixed selenylsulfides or diselenides and generate superoxide radicals. Thus, mass spectrometry-based metabolomic studies in SeMet-treated cells showed that most of the selenols detected were in the oxidized forms and that low-molecular weight reduced thiols were significantly decreased, with concomitant increase in selenylsulfide compounds [63].

5- Toxicity of methylselenol

Among organic Se compounds, methylselenol (CH_3SeH, MeSeH) is of particular importance because it has been postulated that the anticancer properties associated with high Se intakes were mediated primarily by this compound [64]. Because of its volatility and reactivity, MeSeH is difficult to manipulate. Therefore, its toxicity is generally studied by using precursor molecules such as methylseleninic acid ($CH_3SeCOOH$, MSA) or dimethyldiselenide ($CH_3SeSeCH_3$, DMDSe), which are reduced to MeSeH by intracellular thiols. Recently, to identify metabolic pathways affected by MeSeH, we studied the toxicity mechanisms of these precursors in *S. cerevisiae*. Our results show that MeSeH can serve as substrate for OAH-sulfhydrylase, which rapidly converts it into SeMet. Thus, in wild-type yeast cells, MeSeH enters the Se amino acid pool and its toxicity is mediated by the resulting SeMet [65]. MeSeH precursors were also toxic, although at higher concentration, in a Δ*met17* mutant devoid of OAH- sulfhydrylase activity. In this strain, a different mechanism of toxicity was uncovered. MeSeH is a strong reductant that can act as a catalyst able to disrupt disulfide bonds in proteins and to activate or inactivate redox-regulated proteins [66]. We show that in Δ*met17* cells, exposure to DMDSe caused a reductive endoplasmic reticulum (ER) stress, resulting in incorrect disulfide bond formation in newly synthesized proteins, which leads to accumulation and aggregation of unfolded proteins [67]. These results suggest that MeSeH generated from DMDSe in the reducing environment of the cytosol diffuses across the ER membrane resulting in a reductive stress in this compartment.

6- Conclusion

Studies using the yeast *S. cerevisiae* as a model organism demonstrate that the toxicity of Se compounds depends on the mode of action of their metabolites. The biological mechanisms explaining the toxicity of three important metabolites of Se have been characterized in this organism. Inorganic Se species that are metabolized into hydrogen selenide induce ROS-mediated DNA damage, GSH depletion and oxidative stress. Compounds that can be incorporated in the seleno-amino acid pool generate SeCys that induces cytosolic protein aggregation when it is mistakenly inserted into nascent polypeptides in the place of cysteine. Finally, MeSeH induces an ER stress by shifting the ER redox balance towards more reduced conditions, which results in protein misfolding in this compartment. In animal or human cells, selenite was also shown to induce ROS-dependent DNA strand breaks and/or base oxidation. Likewise, cytotoxicity of methylselenol is due to its ability to induce an ER stress, both in yeast or animal cells [68]. These results, therefore, support the notion that studies in yeast can contribute to elucidate the mechanisms of Se toxicity in higher eukaryotes, including humans.

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