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Selenium metabolism in *Escherichia coli*

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***Escherichia coli* will reduce selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) to elemental selenium Se^0. Selenium will also become incorporated into proteins as part of the amino acids selenocysteine or selenomethionine. The reaction of selenite with glutathione produces selenodiglutathione (GS-Se-GS). Selenodiglutathione and its subsequent reduction to glutathioselenol (GS-SeH) are likely the key intermediates in the possible metabolic fates of selenium. This review presents the possible pathways involving selenium in *E. coli*. Identification of intermediates and potential processes from uptake of the toxic oxyanions through to their detoxification will assist us in understanding the complexities of metalloid oxyanion metabolism in these bacteria.**

Keywords: *Escherichia coli*, metabolic rates of selenium, selenium

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; GSSeSG, selenodiglutathione

Selenium, found in group VIA of the Periodic Table, is a naturally occurring element. Required as a trace element within the biosphere, selenium is found in four inorganic oxidation states. Selenate, SeO_4^{2-} (+VI valence state), and selenite, SeO_3^{2-} (IV), are highly soluble in water and are known to be toxic to biological systems at relatively low concentrations. By contrast, elemental selenium, Se^0 (+0), is essentially non-toxic and highly insoluble in water. Selenide, Se^{2-}(–II), is both highly toxic and reactive but is readily oxidized to Se^0. Thus, selenium behaves as a double edged sword in biological systems, in which a fine line must be negotiated between concentrations that are physiologically essential and those that are toxic. Specific scavenger, uptake, storage and removal systems are available in bacteria for many elements. Other metals, including selenium, find ways into cells by utilizing systems dedicated to similar elements or chemical species.

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This mini review compiles the metabolic fate of selenium in *Escherichia coli*; from uptake as an oxyanion, through potential metabolic processes, both essential and toxic. *E. coli* strains can grow in the presence of $> 32\,000\ \mu g\ ml^{-1}$ selenate and at concentrations $< 1600\ \mu g\ ml^{-1}$ selenite. The significance of each of the various reactions discussed here would be reflected in the growth environment and depend on the relative concentration of selenium oxyanions and the concentrations of other physiologically relevant oxyanions (sulfate, sulfite, nitrate, nitrite, etc.). Figure 1 shows the metabolic pathway and also includes some reactions that are likely to occur but have yet to be demonstrated *in vivo*. The detoxification of selenite through reduction leads to deposition of elemental Se^0 within the cell. Selenium granules are frequently associated with the cell wall and/or membrane (Gerrard *et al*. 1974). Several enzymes in bacteria utilize selenium either in the form of selenocysteine or a bound oxyanion. These enzymes have been reviewed elsewhere (Heider & Böck 1993). The overall microbiological cycling and ecology of selenium in the environment has also been reviewed (Doran 1982).

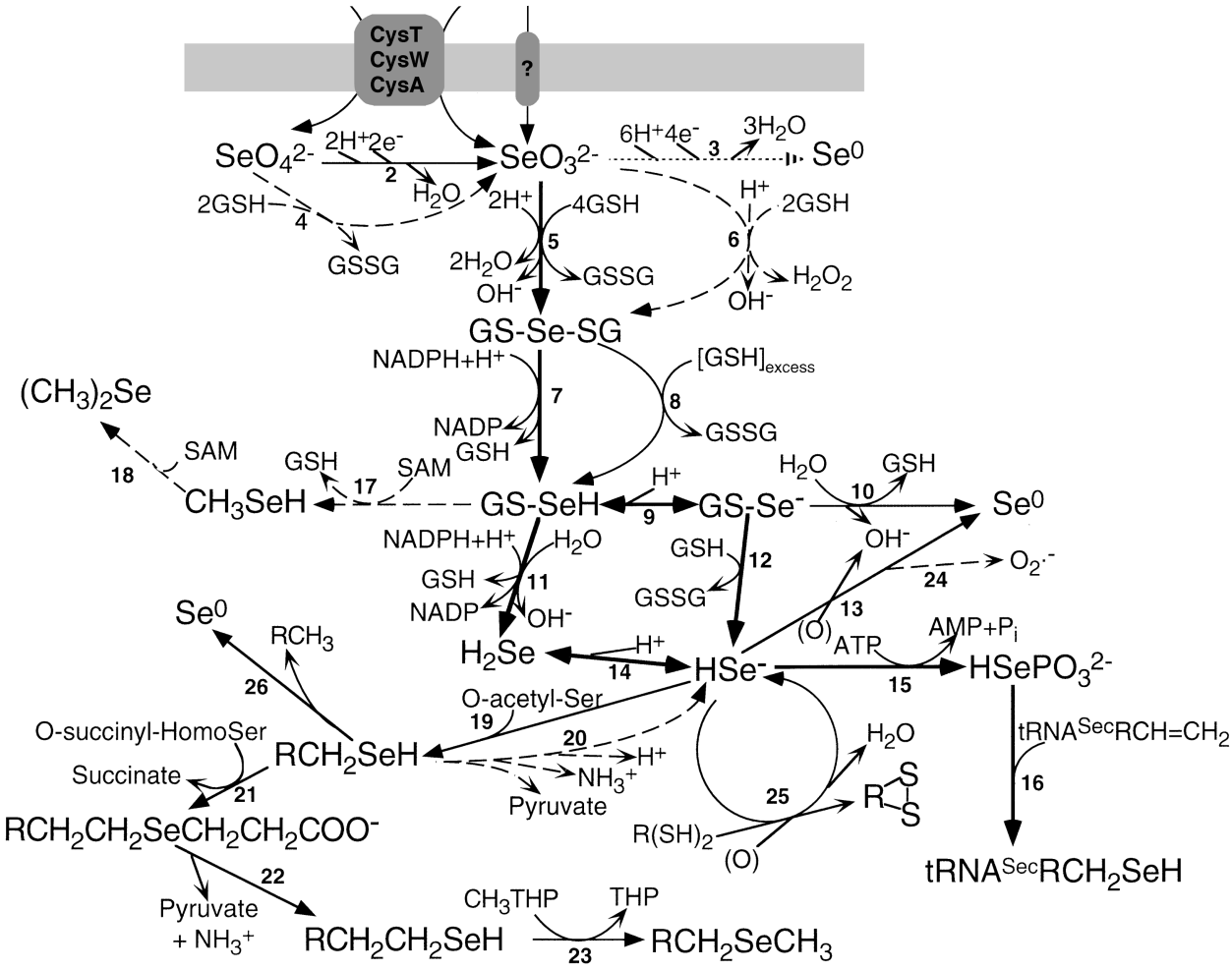

Figure 1. Metabolism of selenium. Numbers designate specific reaction steps and the description of each reaction is described in the body of the review. Dashed lines (– – – – –) refer to probable reactions which have yet to be demonstrated *in vivo*. Dotted lines (· · · · ·) refer to reactions which likely do not occur *in vivo*. Abbreviation used: Reactions **17** and **18**: SAM, *S*-adenosly-L-methionine. Reaction **23**; THP, tetrahydropteroyl $(Glu)_n$. In products from reactions **16**, **19–23**, **26**, R = $(NH_3^+)CHCOO^-$; in reaction **25** R = $(NH_3^+)CHCOO^-$ as well as the further constituents of the peptide or protein (see text). Reactions **27–30** are not shown in this figure and their contributions to this scheme are discussed in the text.

Selenate [**1**]* is thought to enter the cell through the sulphate permease system (*cysA*, *cysU*, *cysW*) (Heider & Böck 1993, Kredich 1996, LaRossa 1996). Mutations in these genes give rise to selenate resistance. Selenite can also enter the cell through this sulphate transporter; however, an alternative yet undefined carrier likely exists because repression of sulfate permease expression does not inhibit selenite uptake completely.

It is possible that some reduction of the oxyanion in the periplasm can occur. The periplasmic nitrate reductase (*napA*) from *Paracoccus denitrificans* and *Rhodobacter sphaeroides sp. denitrificans* has been shown to possess selenate to selenite reduction activity (Avazeri *et al.* 1995, Richardson *et al.* 1997). The *E. coli* NapA also possesses this activity, although the specific activity is low (Turner *et al.* unpublished results).

Once in the cell, selenate can be reduced to selenite [**2**] by the nitrate reductases A and Z (*narGHIJ* and *narZUWV*, respectively) (Avazeri *et al.* 1997). Although these enzymes are expressed at high levels anaerobically, nitrate reductase A still accumulates under aerobic conditions. Although nitrate reductase has been shown to have tellurite (TeO_3^{2-}) to tellurium (Te^0) reductase activity, the

*numbers refer to reactions in Figure 1.

equivalent reaction with selenite [**3**] is not catalyzed by this enzyme (Avazeri *et al*. 1997). Therefore, other reactions must occur in order further to reduce SeO_3^{2-} to Se^0 and SeH^-.

It is clear that once inside the cell, the selenium (both from selenate and selenite) will be incorporated into the amino acids methionine and cysteine as selenocysteine [**19**] and selenomethionine [**21, 22** and **23**]. For these reactions to occur the selenium oxyanions must be reduced to selenide. This reduction of the oxyanions to selenide was initially thought to follow the sulfate-reduction pathway (Scala & Williams 1962). However, selenate and selenite resistances were only found in *cysK* mutants which encode for *O*-acetylserine (thiol)-lyase [**19**] (Fimmel & Loughlin 1977, LaRossa 1996). Additionally, no selenite reductase activity has been reported or observed for the sulfite reductase coded for by *cysI* and *cysJ* in *E coli*. Another consideration is the relative concentrations of sulfur and selenium compounds in the environments (typically a 1000-fold difference), suggesting a need for specific enzymes and pathways to sufficiently supply the cell with its selenium metabolites. Only CysK of the *cys* genes is utilized as an entry point of selenium into the free amino acid selenocysteine [**19**].

Selenomethionine is thought to be synthesized from the free amino acid selenocysteine by the same pathway as methionine. This pathway utilizes cystathione γ-synthase (*metB*) [**21**], β-cystathionase (*metC*) [**22**] and methionine synthase (*metE* and *metH*) [**23**].

Selenocysteine is an essential active site residue in select redox proteins such as formate dehydrogenase. Selenide can be incorporated into the amino acid selenocysteine through a specific pathway (reviewed by Böck *et al*. 1991, Heider & Böck 1993, Böck & Sawers 1996). The gene *selD* encodes selenophosphate synthetase, which produces an 'activated form of selenium [**15**], selenophosphate. Selenium from this activated oxyanion is used for charging the serine-$tRNA^{Sec}$ with Se by selenocysteine synthetase (*selA*), generating selenocystyl-$tRNA^{Sec}$ [**16**].

CysK-mediated incorporation of selenium into selenocysteine generates the free amino acid, whereas the specific *sel* system produces the specific aminoacyl-$tRNA^{Sec}$. Since L-cysteine is quite toxic to most enteric bacteria, it is likely that free selenocysteine would also be highly toxic. Direct incorporation of selenocysteine into proteins, through misacylation of $tRNA^{Cys}$, thus replacing cysteine, would significantly alter activities and functions. This is a consequence of the considerably lower pK_a of the selenol, RSeH (5–6) compared with the thiol, RSH (8–9). To avoid the toxicity of cysteine and to enable its use as a carbon source, two enzymatic activities are available for cysteine degradation (McFall & Newman 1996), namely L-cysteine desulfhydralase (L-cysteine hydrogen sulfide-lyase (deaminating)) and tryptophanase (*tnaA*), both of which catalyze the following reaction:

$$\text{L-cysteine} + H_2O \rightarrow \text{pyruvate} + NH_3 + H_2S \quad [\mathbf{27}]$$

It is possible that these two enzymes could also catalyze the degradation of the free amino acid selenocysteine [**20**], although such activity has not yet been reported. Selenocysteine and selenomethionine lyase activities (reaction products are alanine and H_2Se or CH_3SeH) have been observed in other species of bacteria (Chocat *et al*. 1983). Recently, a selenocysteine lyase has been cloned from *E. coli* (Mihara *et al*. 1997) [**26**] and affords the degradation of selenocysteine and another pathway for the production of elemental selenium. Selenomethionine, when incorporated into proteins by replacing methionine through misacylation of $tRNA^{Met}$, does not appear to be toxic to *E. coli* (Scala & Williams 1962). Catabolism of selenomethionine by glutamine transaminase from bovine liver has been reported (Blarzino *et al*. 1994); again such an activity has yet to be identified in *E. coli*.

Selenite reacts with thiols following the Painter reaction (Painter, 1971):

$$4RSH + H_2SeO_3 \rightarrow RS\text{-}Se\text{-}SR + RSSR + 3H_2O \quad [\mathbf{28}]$$

Glutathione (L-γ-glutamyl-L-cysteinyl-L-glycine; GSH) is a prime candidate for RSH in this reaction within *E. coli* [**5**], for as much as 90% of the total reduced thiol concentration is glutathione (~27 μmoles g^{-1} cells) (Fahey *et al*. 1978). Glutathione is considered to be the main component in the pathway of selenium metabolism as the first in a series of reduction reactions which converts selenite to selenide. The chemical mechanism and kinetic description of the reaction of thiols with selenite have been described by Kice and coworkers (Kice *et al*. 1980, 1991). Several other Se intermediates are possible for this reaction ($GSSeO_2^-$ and GSOSeSR). The produce GS-Se-SG (referred to as bis(glutathionyl)selenide, glutathiol selenotrisulfide or selenodiglutathione) is the major product formed as shown by ^{77}Se NMR (Milne *et al*. 1994). It has been proposed that selenate may also react slowly with glutathione [**4**] (Shamberger 1985), although the nitrate reductase(s) catalyzed reaction(s) [**2**] is far more efficient and likely the key reaction which allows for the selenium from selenate to become incorporated.

The reaction of selenite with glutathione has been studied extensively *in vitro* (Ganther 1968, Sandholm & Sipponen 1973 Kramer & Ames 1988, Milne *et al*. 1994). The stablility of GS-Se-SG is dependent on the molar ratio of reduced glutathione to SeO_3^{2-}, as well as on pH. Further reduction of selenodiglutathione to GSSeH can occur if GSH is in a molar excess of greater than 4:1 [**8**]. Selenols have a low pK_a in the 5–6 range. Thus, at neutral pH, a glutathiolselenolate is generated which can decompose to produce elemental elenium, Se^0 [**9** and **10**] as the terminal product. Se^0 is observed as a characteristic orange–red colour of cultures grown in the presence of selenium oxyanions.

A consequence of the reaction of selenite with glutathione is the production of H_2O_2 and $O_2^{\bullet -}$ (Kramer & Ames 1988). This helps to explain the oxidizing ability and toxicity of selenite. Hydrogen peroxide can be formed in a reaction similar to reaction **5** if the concentration of GSH is lower [**6**]. Superoxide is produced *in vitro* if the thiol is in excess. This gives HSe^- as a product, and a reaction pathway through **8**, **9**, and **12** gives $O_2^{\bullet -}$ being produced in a reaction similar to **13**:

$$2HSe^- + 2.5O_2 \rightarrow 2Se^0 + H_2O + 2O_2^{\bullet -} \qquad [\mathbf{24}]$$

Alternatively, further reduction of selenodiglutathione to selenide can be enzymatically catalyzed. Selenodiglutathione was found to be a substrate of glutathione reductase of yeast (Ganther 1971). It has also been found to be a substrate for mammalian thioredoxin reductase (Björnstedt *et al*. 1992, Kumar *et al*. 1992); however, *E. coli* thioredoxin reductase does not directly utilize this compound as a substrate. Selenodiglutathione can oxidize *E. coli* thioredoxin [**29**], which can be reduced and recycled by thioredoxin reductase in an NADPH dependent manner [**30**] (Kumar *et al*. 1992, Björnstedt *et al*. 1995):

$$\text{thioredoxin}(RSH)_2 + GSSeSG \rightarrow$$
$$GSH + GSSe + \text{thioredoxin(S-S)} \qquad [\mathbf{29}]$$

$$\text{thioredoxin(S-S)} + NADPH \rightarrow$$
$$\text{thioredoxin}(RSH)_2 + NADP^+ \qquad [\mathbf{30}]$$

It has not been shown directly that *E. coli* glutathione reductase can utilize selenodiglutathione. However, it is clear that reactions **7** and **11** occur in an NADPH dependent manner, whether via thioredoxin and thioredoxin reductase or by glutathione reductase. Thus, the result of the reduction of selenite with reduced glutathione is selenodiglutathione, which is further reduced to glutathioselenol (GS-SeH). Further reduction of this compound leads to hydrogen selenide and to selenium, providing the necessary reactive intermediates for selenium incorporation into amino acids. An important consideration is the consequence of generating the reactive intermediate hydrogen selenide (HSe^-). This compound is highly reactive and in the presence of oxygen can cause further oxidation of spatially close thiol pairs in peptides and proteins via the redox-cycling of selenide [**25**] Shamberger 1985).

Due to the reactivity of selenite with thiols it is important to consider other reactions which may occur with cysteines and cysteine pairs within proteins in the periplasm before seeing glutathione in the cytoplasm. A group of proteins which has potential to react with selenite is the Dsb proteins which catalyze disulphide bond formation (Missiakas & Raina 1997). Additionally, thioredoxin, which has been found concentrated at adhesion junctions and is easily released into the periplasm (Bayer *et al*. 1987), could also react with selenite. Both thioredoxin and Dsb proteins contain cysteine pairs in a C-X-X-C motif which, upon reaction with selenite, could potentially produce the protein-S-Se-S-protein stable cyclic adduct similar to that of an arsenical reacted protein (Brown *et al*. 1987). The existence of such possibilities still needs to be explored.

Bacteria grown in the presence of selenite have a distinctive garlic-like odor. The origin of this odor is methylated forms of selenium. These compounds are produced in an *S*-adenosylmethionine dependent manner from thioselenol (GSSeH) through a methane selenol [**17**] to dimethyl selenide [**18**] (Doran 1982). However, the specific methylase(s) involved have not been identified in *E. coli*.

Understanding the complete metabolism and biochemical fate of selenium is of primary importance in the mechanism of the toxicity of this element and its effects on various enzymes and metabolic pathways involving selenium. Figure 1 represents what we know to date regarding the biochemical steps in the metabolism of selenium; however, some aspects are still speculative and have not been demonstrated *in vivo*, and it is clear that further work is required to fully understand all possible fates of selenium in this model bacterium.

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