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The contribution of selenocysteine to the peroxidase activity of selenoprotein S

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Abstract

Selenoprotein S (SelS, VIMP) is an intrinsically disordered enzyme that utilizes selenocysteine to catalyze the reduction of disulfide bonds and peroxides. Here it is demonstrated that selenocysteine is the residue oxidized by the peroxide substrate. It is possible to trap the reaction intermediate selenenic acid (SeOH) when the resolving cysteine is mutated. The selenocysteine allows SelS to rapidly reform its selenenylsulfide bond following its reduction, and to resist inactivation by H_2O_2. We propose that SelS's peroxidase mechanism is similar to that of atypical 2-Cys peroxiredoxin and that selenocysteine allows SelS to sustain activity under oxidative stress.

Selenoproteins are powerful oxidoreductases that utilize the reactive amino acid selenocysteine (Sec, U). Sec has lower pKa and stronger nucleophilicity than cysteine, which is frequently cited as a possible reason for the utilization of Sec in nature.[1, 2] Recently, the ability of selenoproteins to evade oxidative damage by free oxygen radicals was proposed to be superior to, and hence a key advantage over, that of other oxidoreductases.[3, 4] Here we examine this hypothesis and other unique features arising from the presence of Sec in the peroxidase's reaction mechanism of SelS.

The focus of this study, SelS, is an intrinsically disordered membrane enzyme[5] that is associated with the endoplasmic reticulum associated protein degradation pathway (ERAD) machinery and also resides in perinuclear speckles.[6–9] It is involved in inflammation and management of oxidative stress.[10, 11] Since many intrinsically disordered proteins are signaling proteins it is likely that SelS is involved in signal transduction though its physiological protein partner(s) are yet unknown. We have recently characterized SelS as a powerful reductase and moderate peroxidase *in vitro*.[12] A moderate peroxidase activity was reported for several other peroxidases whose physiological role is thought to be regulatory, as opposed to scavenging of oxidative oxygen species.[13]

SelS's peroxidase activity is related to that of atypical (monomeric) 2-Cys peroxiredoxin (Prxs).[13–15] In atypical Prxs, the peroxidatic Cys residue is first oxidized by H_2O_2 into sulfenic acid (S-OH), which is rapidly attacked by a nearby resolving Cys, leading to the formation of a disulfide bond.[16] We established that for SelS, the Sec188 is the peroxidatic residue, while Cys174 is the resolving residue, by examining the peroxidase activity of a series of mutants (Figure 1A). In all of the experiments described here, we employ a form of

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ASSOCIATED CONTENT

Supporting Information.

Supporting information included materials, methods, and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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SelS in which the transmembrane helix was deleted (Δ1–51, abbreviated as cSelS). Sec is inserted into the wild type cSelS employing *E. coli* genetic insertion machinery.[12] (For a scheme of all the mutants and a description of their properties see Figure S1 and Table S1). cSelS is a dimer in which Sec188 and Cys174 form an intramolecular selenenylsulfide bond. That Sec188 is the peroxidase residue is proved by the inactivity of the U188S and U188Δ mutants (in U188Δ translation is terminated after residue 187). Hence, Cys174 must be the resolving residue. The observation that cSelS C174S activity is nearly identical to that of the wild type suggests that cSelS C174S intermolecular diselenide bond (Figure S4) is surface accessible. It can be reduced by both hTrxR and hTrx *in vitro* (Figure 1A).

Due to the proposed similarity of SelS's reaction mechanism to that of atypical 2-Cys Prxs, we hypothesized that selenenic acid (Se-OH) is transiently formed during the reaction. Indeed, it is possible to trap that intermediate with dimedone (5,5-dimethyl-1,3-cyclohexanedione) if the resolving Cys is mutated into Ser to prevent the rapid formation of the selenylsulfide bond. Reduced cSelS C174S was incubated with H_2O_2 and dimedone for 20 min and examined by electrospray ionization mass spectrometry. A mass gain of 139 Da indicates labeling with dimedone (Figure 1B). Dimedone reacts exclusively with sulfenic acid or selenenic acid in proteins and since cSelS C174S has only one Sec and no Cys the adduct must reside on Sec188. This intermediate cannot be trapped in the wild type, most likely because the intermolecular reaction with dimedone is considerably slower than the intramolecular reaction with the resolving Cys174. The selenenic acid is thought to participate in several enzymatic reactions, but to the best of our knowledge, its presence has not been experimentally verified. Neither of the other mutants listed in Figure 1 reacted with dimedone in the presence of H_2O_2. Based on the information presented in Figure 1, we propose that the reaction mechanism of SelS involves reduction of the selenenylsulfide bond by thioredoxin (or a related protein partner), followed by an attack of H_2O_2 on Sec188, which leads to a transient selenenic acid species. This species is rapidly resolved by Cys174 (Scheme 1).

Next, we inspected the specific features that arise from the presence of Sec by examining the rate in which the wild type and U188C, U188S, U188Δ and C174S mutants reoxidize once reduced (that is, reform the selenenylsulfide, disulfide, and diselenide bonds). It is well known that molecular oxygen and trace metals present in the buffer can catalyze the formation of selenylsulfide and disulfide bonds in reduced proteins.[17] Reoxidation is believed to occur via the selenenic acid / sulfenic acid intermediate.[18] Hence, the reoxidation rate is indicative of how sensitive the protein is to the presence of trace metals, as well as the rate in which the intermediate is resolved. To measure the rates, we have followed the time course of the reoxidation of reduced proteins (in the absence of EDTA) by mass spectrometry or SDS-PAGE (Figure 2 and Figures S2–S6). For mass spectrometry detection, samples were drawn at different time intervals, and exposed Cys and Sec were alkylated with *N*-Ethylmaleimide (NEM). The ratio of oxidized protein and reduced protein (the only form that was alkylated) was measured. SDS-PAGE was used to measure the ratio of monomer to dimer in mutants where an intermolecular bond is formed. As Figure 2 demonstrates, the reoxidation of the wild type is essentially complete after 60 min, while that of U188C is considerably longer (the wild type is ~1500 times faster than U188C, Figure S7). It demonstrates that the Sec-containing enzyme reacts more rapid with metals and oxygen and is more sensitive to oxidation (Figure S8). Thus, *in vivo* the wild type SelS would be sensitive to the balance between the level of reactive oxygen species and the availability of Trx. This sensitivity could be advantageous when cSelS putatively acts in signal transduction.

Thiol-dependent peroxidases are inactivated by H_2O_2, via the irreversible modification of Cys residues to sulfinic and sulfonic forms. While some peroxidases are rather robust,

tolerating the presence of as much as 30 mM H_2O_2, others are hypersensitive, becoming fully inactivated at considerably lower exposures.[14, 19] Even the Sec-dependent GPx1 is inactivated by H_2O_2, though this was proposed to be a result of selenium elimination.[20] To gain further insight into the potentially advantageous role of Sec in SelS, we tested the ability of cSelS to maintain activity after exposure to high concentrations of H_2O_2. We have chosen to assay the reductase – not the peroxidase-activity of SelS since in our hands hTrxR was partially inactivated at high concentrations of H_2O_2 (data not shown). cSelS reductase activity was assayed using insulin turbidity tests[21], following 20 min incubations with various concentrations of H_2O_2, up to 50 mM and its subsequent removal by catalase. Figure 3A,B shows that for the wild type the reaction rate in the linear region (A_{650}> 0.2) did not significantly change irrespective of the H_2O_2 concentrations used during the incubations. In contrast, C174S, which also has a reductase activity (Figure S9), is more prone for inactivation (Figure 3C,D). Nevertheless, it still maintains about 80% of its activity after incubation at 50 mM H_2O_2. The increased resistance of the wild type to inactivation might provide an advantage for SelS, allowing it to sustain functionality under oxidative stress.

In conclusion, we demonstrated that SelS's peroxidase activity shares much in common with the atypical 2-Cys Prxs' reaction mechanism. It was shown that the reaction mechanism involves a selenenic acid. The catalytic cycle relies on an intramolecular selenenylsulfide. However, when the resolving Cys is damaged (here, mutated), SelS is capable of functioning by forming an intermolecular diselenide bond. We also provided evidence that SelS reoxidizes much faster than the Cys-variant, and that it can evade damage by oxidants. By quantitatively comparing the behavior of the Sec and Cys forms of SelS, it was demonstrated that these advantages are unique to the wild type Sec-containing enzyme and hence, could explain some of the potential advantages of utilizing Sec in SelS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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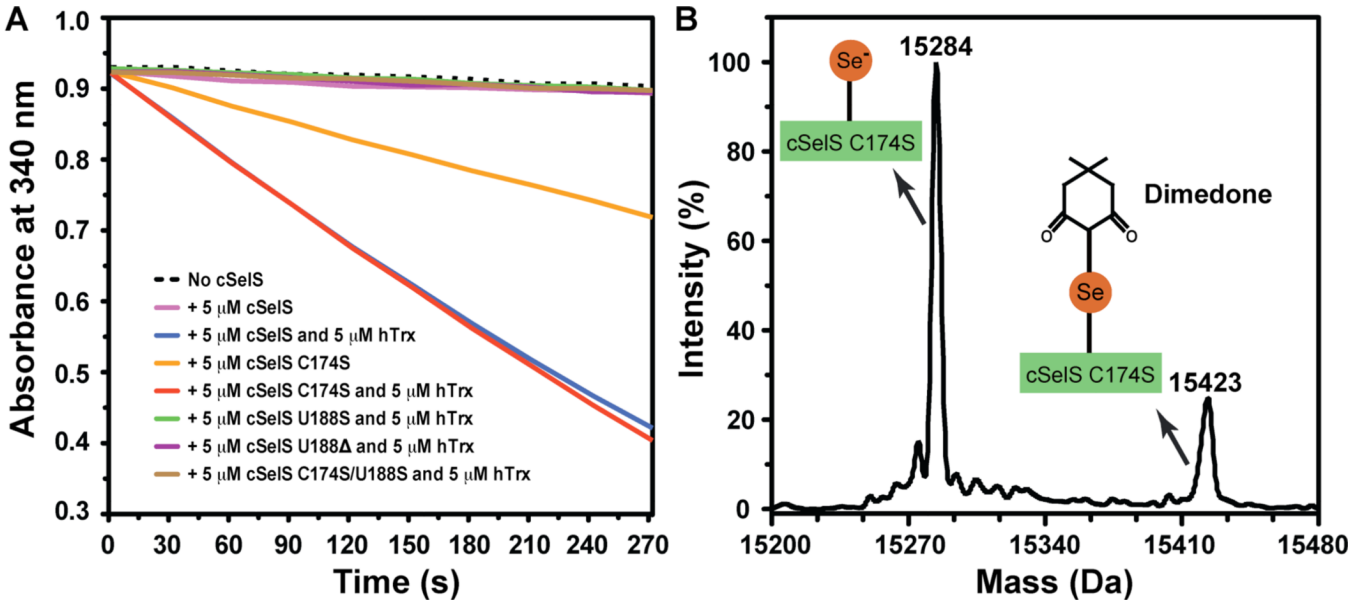


Figure 1.

(A) Identification of the peroxidatic and resolving residues in SelS using peroxidase assays. NADPH consumption is monitored in the presence of 8 nM hTrxR, 5 μM hTrx and 200 μM H_2O_2 and with and without cSelS, with and without thioredoxin, and with C174S, U188S, and U188Δ mutants. (B) Trapping of the reaction intermediate, selenenic acid, in cSelS C174S. Mass spectrum showing cSelS C174S (15284 Da) and its dimedone adduct (15423 Da).

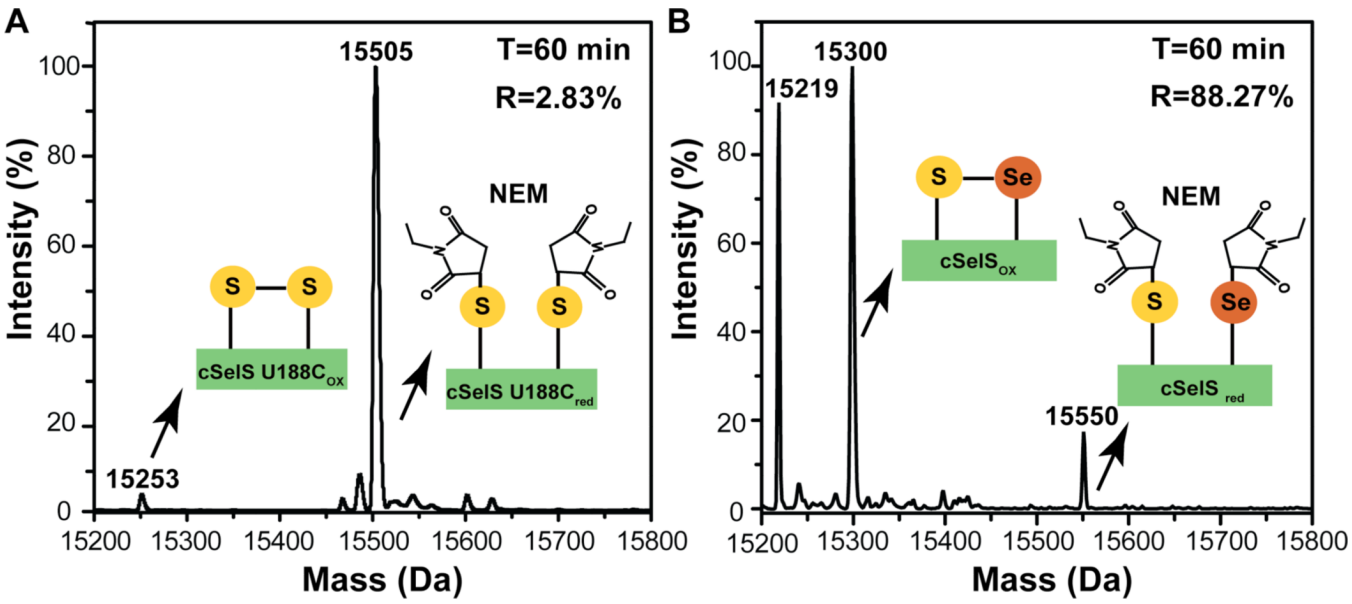


Figure 2.

Electrospray ionization mass spectrometry-based detection of the rate of formation of selenenylsulfide bond in cSelS and the disulfide bond in cSelS U188C. (A) After 60 min the majority of cSelS U188C was still reduced, allowing for full alkylation by NEM. Molecular weights: oxidized cSelS U188C 15253 Da and alkylated cSelS U188C 15505 Da. (B) After 60 min the majority of cSelS was oxidized. Molecular weights: alkylated cSelS U188Δ 15219 Da (a truncated, inactive form that arise during expression), oxidized cSelS 15300 Da, and alkylated cSelS 15550 Da. R denotes percent of oxidized protein.

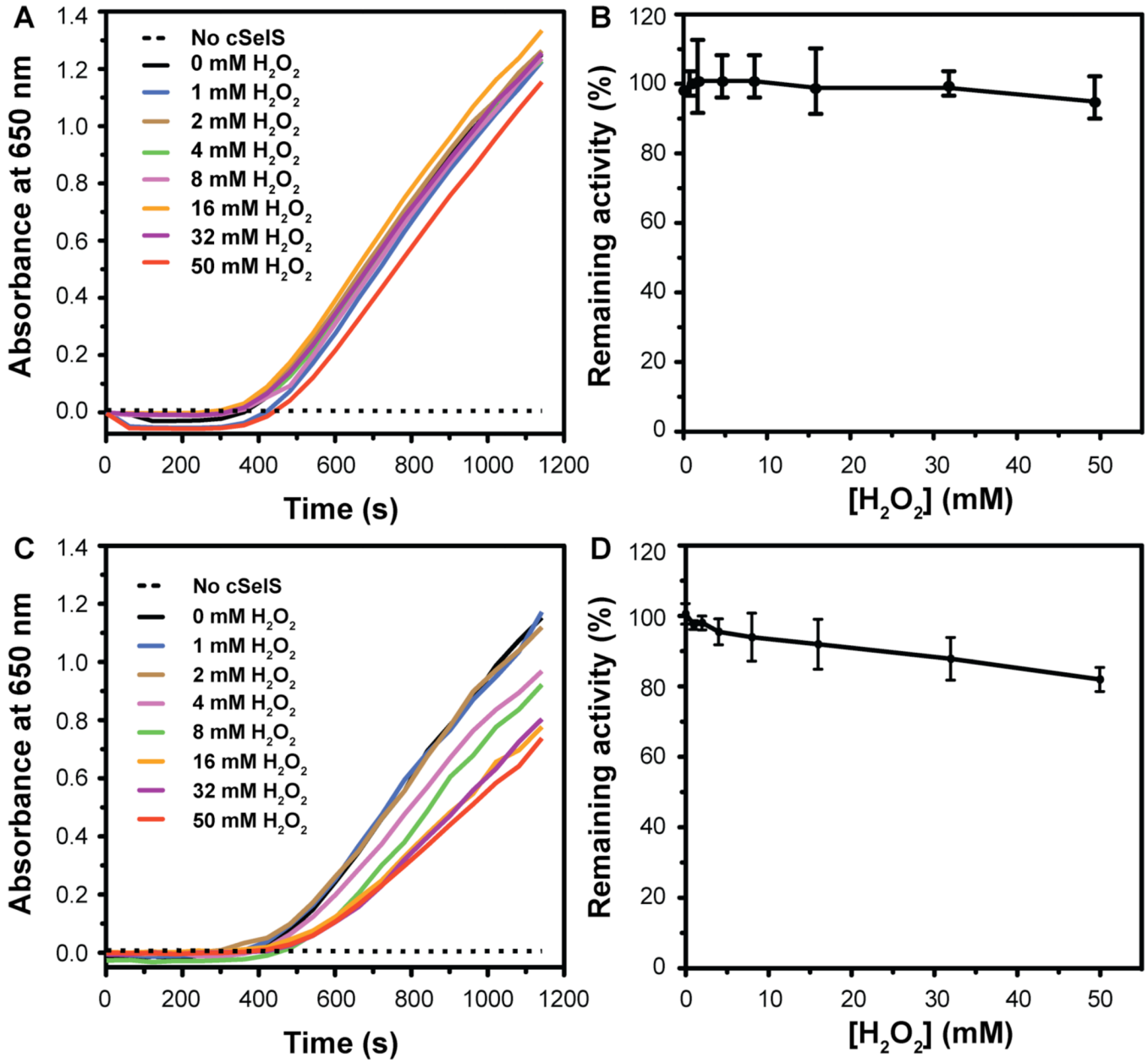
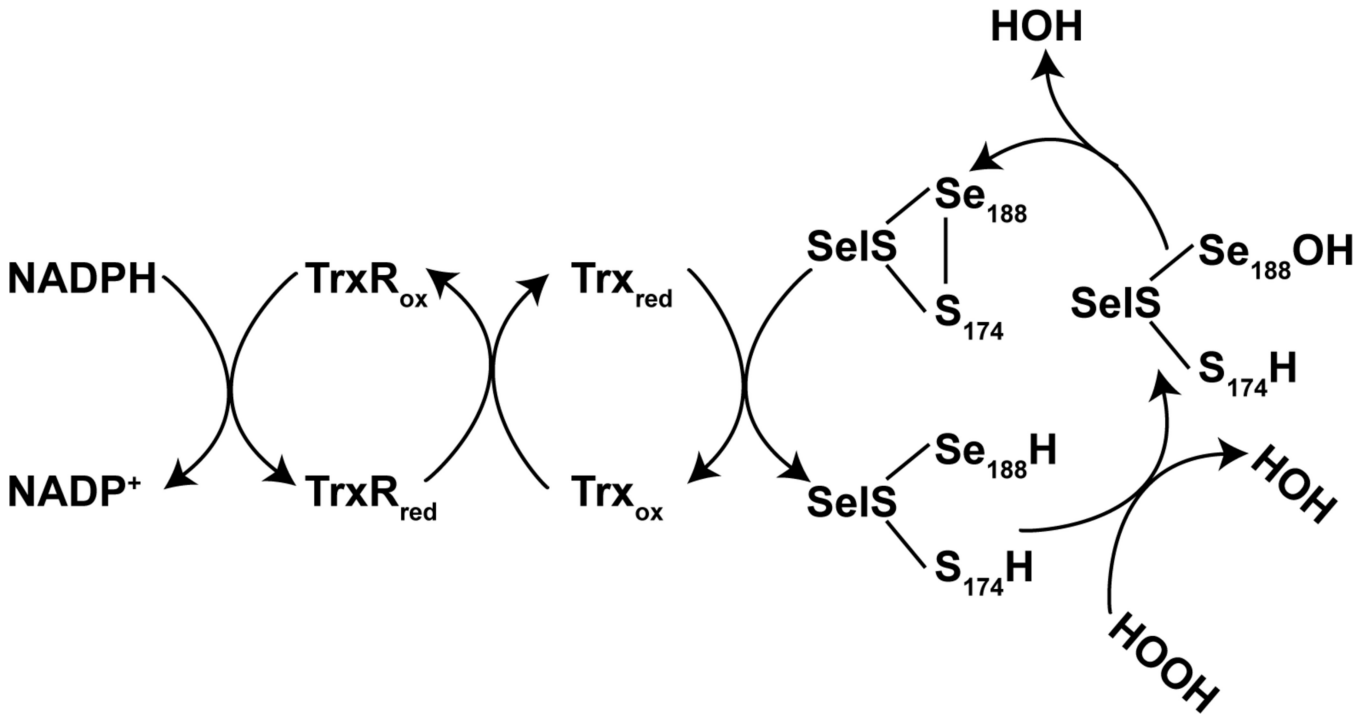


Figure 3.

cSelS resistance to inactivation by H_2O_2. The ability of cSelS to reduce insulin's intermolecular disulfide bond following incubations with H_2O_2 is monitored by recording the increasing turbidity caused by insulin's chain B aggregation. (A) cSelS reductase activity assay after incubations with H_2O_2. (B) Percentage of remaining activity of wild type cSelS after treatment with H_2O_2. (C) cSelS C174S reductase activity assay after incubations with H_2O_2. (D) Percentage of remaining activity of cSelS C174S after treatment with H_2O_2. The error bars represent the range of measurements (that is, highest and lowest values) among two repetitions, using two independent protein preparations.



Scheme 1.
Proposed reaction mechanism of SelS.