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Selenium versus sulfur: reversibility of chemical reactions and resistance to permanent oxidation in proteins and nucleic acids[†]

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Abstract

This review highlights the contributions of Jean Chaudière to the field of selenium biochemistry. Chaudière was the first to recognize that one of the main reasons that selenium in the form of selenocysteine is used in proteins is due to the fact that it strongly resists permanent oxidation. The foundations for this important concept was laid down by Al Tappel in the 1960's and even before by others. The concept of oxygen tolerance first recognized in the study of glutathione peroxidase was further advanced and refined by those studying [NiFeSe]-hydrogenases, selenosubtilisin, and thioredoxin reductase. After 200 years of selenium research, work by Marcus Conrad and coworkers studying glutathione peroxidase-4 has provided definitive evidence for Chaudière's original hypothesis [Ingold, I., Berndt, C., Schmitt, S., Doll, S., Poschmann, G., Buday, K., Roveri, A., Peng, X., Porto Freitas, F., Seibt, T., Mehr, L., Aichler, M., Walch, A., Lamp, D., Jastroch, M., Miyamoto, S., Wurst, W., Ursini, F., Arnér, E. S. J., Fradejas-Villar, N., Schweizer, U., Zischka, H., Angeli J. P. F, and Conrad, M. (2018) Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis, *Cell* 172, 1–14.]. While the reaction of selenium with oxygen is readily reversible, there are many other examples of this phenomenon of reversibility. Many reactions of selenium can be described as “easy in – easy out”. This is due to the strong nucleophilic character of selenium to attack electrophiles, but then this reaction can be reversed due to the strong electrophilic character of selenium and the weakness of the selenium-carbon bond. Several examples of this are described.

Graphical Abstract

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Introduction

This review is derived from a lecture that the corresponding author gave at the 11th *International Symposium on Selenium in Biology and Medicine* in Stockholm, Sweden in 2017, to celebrate the 200th anniversary of the discovery of selenium by Berzelius. This short review will focus on a key chemical property of selenium that very likely explains its use in nature; the reactions of selenium are readily reversible, especially with oxygen. We will also highlight the work of Jean Chaudière, one of the first to recognize the selenium-oxygen connection.

Selenocysteine: reversibility of reactions

One of the most important forms of selenium in biology is that of selenocysteine (Sec)¹, the 21st amino acid in the genetic code [1, 2]. Insertion of Sec into a protein requires multiple accessory factors and the use of a special stem-loop sequence in the mRNA to decode a UGA stop codon into a sense codon for Sec [3–13]. Because insertion of Sec into a protein is bioenergetically costly, Sec must be able to do chemistry that its relative cysteine (Cys) cannot perform. This point is taken up below.

¹ABBREVIATIONS AND TEXTUAL FOOTNOTES

CO, carbonmonoxide; ⁻CN, cyanide; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane regulator; C, one-letter abbreviation for cysteine; Cys, cysteine; ⁻SCys, cysteinylate; Cys-SOH, cysteine sulfenic acid; Cys-SO₂⁻, cysteine sulfinic acid; Cys-SO₃⁻, cysteine sulfonic acid; DUOX, dual oxidase; *E. coli*, *Escherichia coli*; GSH, glutathione; GS•, glutathione radical; GSSG, glutathione disulfide; GPX, glutathione peroxidase; H, hydrogen; H₂ases, hydrogenases; H₂O₂, hydrogen peroxide; HOBr, hypobromous acid; HOCl, hypochlorous acid; HI, hypoiodous acid; HOSCN, hypothiocyanous acid; ⁻SCN, hypothiocyanite; Fe, one-letter abbreviation for iron; LPO, lactoperoxidase; mcm⁵se²U, 5-carboxymethylaminomethyl-2-selenouridine; mcm⁵s²U, 5-carboxymethylaminomethyl-2-thiouridine; mmm⁵se²U, 5-methylaminomethyl-2-selenouridine; mmm⁵s²U, 5-methylaminomethyl-2-thiouridine; *M_r*, molecular ratio; MPO, myeloperoxidase; NADPH, β-nicotinamide adenine dinucleotide phosphate-reduced; N, nitrogen; NOX, NADPH oxidase; Ni, nickel; PMN, polymorphonuclear neutrophil; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; Se, selenium; ⁻SeCys, selenocysteinylate; Sec, selenocysteine; Sec-SeOH, selenocysteine selenenic acid; Sec-SeO₂⁻, selenocysteine seleninic acid; Sec-SeO₃⁻, selenocysteine selenonic acid; se²c⁵Ura, 2-selenouracil-5-carboxylic acid; s²c⁵Ura, 2-thiouracil-5-carboxylic acid; Se-tRNA, 2-selenouridine-containing tRNA; S, sulfur; SI_r, silent, catalytically ready; SU, silent, catalytically unready; S-tRNA, 2-thiouridine-containing tRNA; Trx, thioredoxin; TXNRD, thioredoxin reductase; U, one-letter abbreviation for selenocysteine;

In the broadest sense, the reason for the use of Sec in biology is due to the fact that it can participate in reactions that can be reversed. An example of this is the use of Sec in acyl-transfer reactions of the type shown in Figure 1. Macmillan has shown that Sec placed at the C-terminus of a peptide attacks the carbonyl amide of the backbone much more readily than Cys to form a selenoester [14]. This selenoester can then undergo a transthioesterification reaction to form the more stable thioester, which can then be attacked by a variety of nucleophiles thereby transferring the acyl-group to an acceptor. The equilibrium constant for formation of a thioester from a selenoester is 1000, while the equilibrium constant for the analogous reaction involving sulfur is in the range of 1–10 [15, 16]. This type of reaction might be viewed as “easy in-easy out” because the strongly nucleophilic selenolate accelerates the rate of attack on the backbone carbonyl to form the selenoester, and then the selenoester is an excellent acyl-transfer reagent due to the lack of π -bonding in the Se–CO bond [15, 17, 18]. Selenoesters, like thioesters are “ketone-like” due to this lack of π -bonding [19]. Selenoprotein K most likely takes advantage of this property to facilitate transfer of palmitoyl groups [20, 21].

Another example of reversibility is the alkylation of selenenic acids by dimedone. Selenenic acids can be alkylated by dimedone, but the label is not stable as is shown in Figure 2. Oxidation of the selenol results in a transient selenenic acid (Sec-SeOH) that is rapidly alkylated by dimedone. However, because α -keto selenides (as is the case with dimedone) have been shown to be reactive towards nucleophiles [22], we tested whether the Sec-dimedone label could be removed by thiol. We were able to remove the label with a 10-fold excess of thiol [23]. This is unlike the situation in Cys-dimedone adducts, which are stable towards thiols [24].

Reversible reaction of selenocysteine with oxygen

Al Tappel was one of the first to recognize that the reversible reaction of Sec with H_2O_2 (or oxygen in general) might be key to its use in biology [25]. Key to this early insight was Tappel's recognition of the biochemical connection of selenium with vitamin E as selenium supplementation could prevent or attenuate some diseases in animals caused by vitamin E deficiency [26–29]. Because the concentration of selenium in a cell is much lower than that of sulfur, Tappel reasoned that selenium must be more biologically reactive than sulfur. Tappel pointed to the fact that alkyl selenides had been reported to be much better scavengers of oxidants in paraffin oil in comparison to alkyl sulfides [30].

Tappel was able to show that the amino acid selenocystine (the oxidized form of selenocysteine) rapidly consumed H_2O_2 , while cystine (the oxidized form of cysteine) did not [25]. In his experimental system, he noted that selenocystine was not completely consumed by H_2O_2 . Based on this observation, he proposed a catalytic mechanism by which selenocystine could decompose H_2O_2 as shown in Figure 3.

Unfortunately, the catalytic cycle proposed by Tappel in Figure 3 could not be verified by Roy and coworkers [31]. However, their data suggested that selenomethionine could act in a catalytic fashion, similar to what is shown for selenocystine in Figure 3 [30]. Nevertheless,

the work of Tappel provided an early model for how Sec-containing glutathione peroxidases (GPX) might work, discovered nearly a decade later [32, 33].

Jean Chaudière is the next actor in the story of the catalytic reaction of oxygen and Sec. In seminal work in this area, Chaudière and coworkers showed that while the cysteine-mutant GPX had much lower activity than the Sec-containing GPX, the cysteine-mutant was inactivated by its substrate (alkyl hydroperoxides or H_2O_2), but the Sec-containing GPX was not [34]. The very important conclusion that Chaudière reached was that "...the most significant results of this study are...a weak activity of [Cys]GPx and a marked tendency to inactivation in the presence of hydroperoxides, which underlines the selective advantage of SeGPx which may have evolved to catalyze the fast reduction of hydroperoxides without undergoing significant self-inactivation." [34]. Thus, Chaudière was the first to realize that nature had selected Sec to be used in enzymes because it resisted inactivation by oxidation. This prescient insight has been experimentally verified in the following decades.

Chaudière predicted that the reason for the inactivation of the Cys-GPX was due to oxidation of the Cys residue to Cys-sulfinic acid, followed by β -elimination to form dehydroalanine as shown in Figure 4.

While Chaudière was correct about the rapid inactivation of Cys-GPX, the mechanism of inactivation may involve formation of Cys-sulfonic acid ($Cys-SO_3^-$). Evidence for this comes from work by Bellelli and coworkers in their study of the Cys-mutant GPX from *S. mansoni* [35]. In this study, the Cys-mutant GPX was expressed as a recombinant protein in *E. coli*. However, the enzyme was inactive at all stages of purification, even if reducing agent was included from the moment the cells were lysed at the beginning of the purification procedure. Subsequent crystallization and analysis of the X-ray crystal structure of the Cys-mutant GPX revealed that the peroxidatic Cys residue was in the sulfonic acid form [35]. This could suggest that over oxidation of the Cys residue to the sulfonic acid form is faster than β -elimination.

Perhaps the ultimate verification of Chaudière's hypothesis came from the group of Marcus Conrad earlier this year, as his group showed that mouse embryonic fibroblast cells that only expressed the Cys-mutant GPX underwent peroxide induced ferroptosis, while wild type cells expressing the Sec-GPX did not [36]. Mass spectrometric analysis of the Cys-mutant GPX confirmed that the active site Cys residue was overoxidized to both the Cys-sulfinic ($Cys-SO_2^-$), and Cys-sulfonic acid ($Cys-SO_3^-$) forms [36]. It has been shown that Sec-containing GPX avoids inactivation because the selenenic acid is protected from further oxidation by rapid attack by a backbone amide nitrogen to form a cyclic selenenylamide [37]. The selenenylamide can be reduced by glutathione (GSH) to restore the active selenol form of Sec-GPX. This is also an example of a reversible reaction that selenium can participate in.

The *in vivo* evidence provided by Conrad through the study of GPX4 is further supported by *in vitro* evidence through the study of other selenoenzymes such as thioredoxin reductase (TXNRD) and selenosubtilisin [38, 39]. This *in vitro* evidence is reviewed in [17].

Extension of Chaudière's hypothesis to innate immunity

As discussed above there is very good evidence that the selenoenzyme GPX4 protects against peroxide induced ferroptosis. Ferroptosis is a type of programmed cell death that is dependent on both reactive oxygen species (ROS) and iron [40]. GPX4 helps to protect against ferroptosis by protecting against lipid peroxidation [36]. GPX4 may also help to protect against ferroptosis induced Huntington's Disease [40], Sedaghatian-type spondylometaphyseal dysplasia, and seizures due to its critical role in antioxidant defense [36]. Apparently GPX4 is essential to a particular cell type, parvalbumin positive interneurons, which are rich in polyunsaturated fatty acids (PUFAs). These PUFAs are very sensitive to lipid peroxidation and they require protection against lipid induced peroxidation by GPX4 [36].

The model described by Conrad and coworkers for a selenoenzyme to protect a specific cell type against ROS has similarities to work done by Day and coworkers who found evidence that the selenoenzyme TXNRD was essential to protect lung epithelial cells against hypothiocyanous acid (HOSCN) toxicity [41–43]. In the model presented by Day, thiocyanate (SCN^-) from lung epithelial cells is exported by the cystic fibrosis transmembrane conductance regulator (CFTR) protein into the epithelial lung fluid, where it acts as an antioxidant by reacting with H_2O_2 produced by various oxidases, resulting in the production of HOSCN, a potent bactericide.

HOSCN is chemically distinct from hypohalous acids HOI, HOBr, and HOCl (abbreviated as HOX) and H_2O_2 . It is much more acidic, and has a lower redox potential compared with those of HOX. It also displays differential reactivity over a large pH range, being much more reactive at acidic pH. For example, HOSCN reacts with thiols with rate constants in the range of 10^3 – $10^5 \text{ M}^{-1} \text{ s}^{-1}$ at pH: 7.4, but this increases to $\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at pH 4.85. In comparison, the rate constants for HOX are 10^7 – $10^9 \text{ M}^{-1} \text{ s}^{-1}$ over the same pH range. These biophysical properties result in HOSCN being very selective for low pK_a protein thiols [44–48]. The ability of HOSCN to react with low pK_a thiols may mean that key metabolic enzymes such as glucose-6-phosphate dehydrogenase, and thioredoxin-fold (Trx-fold) proteins of the bacteria cell are specifically targeted. Trx-fold proteins are of special interest because they have reactive, low pK_a thiols that could be targeted by HOSCN and are extremely important to cell viability. Some examples of these key enzymes are: DsbA (protein folding), ribonucleotide reductase (dNTP production), and thioredoxin (Trx – redox homeostasis) [49, 50]. Perhaps most importantly, HOSCN is a unique bactericide because it can selectively oxidize protein thiols of lung bacterial pathogens without causing inflammation to the host, as would be the case for oxidants such as HOCl produced by myeloperoxidase (MPO). While HOSCN is a very good bactericide, the host cell needs protection against this oxidant. Day and coworkers have presented evidence that TXNRD, containing a low pK_a selenol, is also a target of HOSCN, but the Sec residue is not oxidized because TXNRD converts HOSCN into SCN^- and water [41]. The possible role of Sec-containing TXNRD in HOSCN detoxification and innate immunity is explained in Figure 5.

If HOSCN is critical for defense against bacterial pathogens that colonize the lung as suggested by Day [41–43], then it may be important in the pathology of cystic fibrosis (CF).

CF disease is caused by defects in the CFTR protein. A defect in the CFTR protein would result in low levels of SCN^- in the epithelial lung fluid, resulting in a loss of the antioxidant function of SCN^- and increased inflammation to the host by HOCl. CF disease is characterized by excessive inflammation, oxidative stress and bacterial colonization of the lung, leading to lung insufficiency and premature death [51]. Loss of extracellular SCN^- would also mean decreased levels of HOSCN that would result in increased bacterial proliferation in the lung.

Just as the Sec residue in GPX4 imparts resistance to irreversible inactivation by H_2O_2 mediated inactivation, so too must the Sec residue impart resistance to HOSCN mediated oxidation [38]. The reason why Sec is key to avoiding permanent oxidation by HOSCN is that oxidation of Sec by HOSCN results in a TXNRD-Sec-Se-SCN intermediate that can either be attacked by a resolving Cys residue, releasing SCN^- , or be hydrolyzed by water, resulting in a TXNRD-Sec-Se-OH intermediate. This is an identical intermediate to that found in GPX4 during the detoxification of lipid hydroperoxides. The TXNRD-Sec-Se-OH intermediate is highly electrophilic and is immediately attacked by a resolving Cys residue to produce water and an oxidized TXNRD. If TXNRD contained Cys instead, then a TXNRD-Cys-S-OH intermediate would result. The S-oxide is much less electrophilic in comparison to the Se-oxide and this means that a TXNRD-Cys-S-OH intermediate is much more likely to be overoxidized and inactivated instead of being reduced as discussed above and reviewed in [17]. We note that HOSCN-mediated defense against bacterial colonization of the lung supplements the work of lung neutrophils, which are largely responsible for defense against bacterial lung infection.

Selenium as a one-electron oxidant: contributions by Chaudière

While Sec acts catalytically towards 2-electron oxidations as discussed above, Chaudière discovered that selenols (like Sec) can act catalytically with respect to 1-electron oxidations as well [52]. Chaudière showed that selenocystamine, a diselenide, could be reduced by GSH to produce a free selenolate. The free selenolate could then react with molecular oxygen to produce superoxide and a selanyl radical as shown in Figure 6. The selanyl radical could either dimerize to the diselenide, restarting the cycle, or be reduced to a selenolate by GSH, yielding glutathione radical ($\text{GS}^\bullet$). The selenolate could then react with oxygen to start the cycle over again. It is this redox cycle that most likely explains the toxicity of selenium compounds due to consumption of GSH, resulting in oxidative stress [53].

Evidence for Chaudière's hypothesis in [NiFeSe]-hydrogenases

While the study of Sec-containing enzymes/proteins in animals has been the subject of intense study over almost five decades, an often overlooked area in the field is the study of [NiFeSe]-hydrogenases that occur in anaerobic bacteria [54]. Shortly after Chaudière's prediction about the use of Sec in GPX, Maroney and coworkers developed the identical hypothesis through the study of nickel complexes that were designed to serve as models for the active site of [NiFeSe]-hydrogenases [55]. As shown in Figure 7, exposure of the sulfur-containing nickel complex to oxygen resulted in oxidation of the thiolato ligand to sulfates, while there was no reaction in same oxidation of the selenato complex [55]. A

review of the work done on [NiFeSe]-hydrogenases with respect to Chaudière's original hypothesis is given below.

[NiFeSe]-hydrogenases

Hydrogenases (H₂ases) are oxido-reductase enzymes found in archaea, bacteria, and a few eukarya that catalyze the two-electron redox chemistry of molecular hydrogen (Eq. 1) [56–58].

The function, structure, and biochemical properties of H₂ases have been extensively reviewed [59, 60]. Briefly, H₂ases may be utilized in biological systems to either supply reducing equivalents for the reduction of substrates (H₂-uptake H₂ases), or utilize protons as terminal electron acceptors via the formation of H₂ (H₂-evolving H₂ases). When separated by membranes, they may also be deployed to store energy in the form of proton gradients [61]. The two-electron oxidation of H₂ involves heterolytic cleavage of the H-H bond into a hydride (typically bound to a metal) and a proton, and is assisted by the presence of a basic group in the active site. Formation of H₂ involves the two-electron reduction of a proton to a hydride at a metal center and subsequent protonation. The H₂ases are often classified by their metal content into NiFe-, FeFe- and Fe-H₂ase groups, the latter of which is a small group that requires methenyltetrahydromethanopterin as a hydride carrier [62, 63]. The NiFe- and FeFe-H₂ases feature a bimetallic active site where the metals are bridged by two thiolates, and feature CN[−] and CO ligands in the Fe sites [59]. The prototypical periplasmic NiFe-H₂ase (Group 1) [56] consists of a small subunit containing FeS clusters for electron transport (typically a Fe₄S₄(SCys)₄ cluster proximal to the NiFe site, a medial Fe₃S₄(SCys)₃ cluster, and a distal Fe₄S₄(SCys)₃(His) cluster) and a large protein subunit containing the NiFe active site [59]. The active site features a heterodinuclear metal cluster where the Fe is ligated by two CN[−] ligands and a CO ligand, bridged to Ni via two cysteinate (−SCys) ligands, and where Ni is coordinated by two additional terminal −SCys ligands (Figure 8a) [59, 64]. The Cys ligands are derived from two conserved CXXC motifs, one pair from near the N-terminus of the large subunit and the other pair from near the C-terminus, with each CXXC motif providing one bridging and one terminal thiolate S-donor ligand. There is a third non-protein bridging group that varies with the oxidation state and the source/treatment of the enzyme, and has been reported as hydroperoxide [65, 66], hydroxide [65–67], or oxide [68], or sulfide [69, 70]. Recent spectroscopic data point to hydroxide as the native bridge in both resting oxidized states, Ni-A and Ni-B [71]. In any event, this bridge is the site of hydride formation during catalysis [64, 72, 73]. The geometry of the Ni site can be viewed as a distorted five-coordinate pyramidal structure with the bridging ligands and one terminal −SCys (first −SCys from the N-terminal CXXC motif) forming the base and the other terminal −SCys ligand (the first Cys in the C-terminal CXXC) in the apical position. This apical −SCys ligand provides the base for catalysis (Figure 8a) [72].

The typical O₂-sensitive enzyme (*e.g.*, periplasmic H₂ases from *Desulfovibrio* species) are reversibly deactivated by exposure to air [59]. During reductive activation and catalysis, the enzyme exhibits several intermediates (Figure 9) [59]. The most oxidized states are Ni-A and Ni-B, which exhibit distinct Ni(III) EPR signals and CN⁻ and CO stretching frequencies that are sensitive to the redox state of the enzyme (Figure 9). Additionally, the Ni-A form exhibits a long lag phase in the kinetics of reductive activation of the enzyme while the Ni-B form is rapidly activated. One-electron reduction of Ni-A and Ni-B generate EPR silent intermediates (SU – catalytically unready and SI_r – catalytically ready, respectively). The SI_r state is converted to SI_a, the EPR silent state representing the oxidized form of the enzyme that is active in catalysis (Figure 9), via loss of the bridging OH⁻ group. One-electron reduction of SI_a leads to Ni-C, which exhibits a Ni(III) EPR signal (Figure 9) and contains a bridging hydride ligand [73]. The fully reduced Ni-R state can be obtained via one-electron reduction of Ni-C, and features both the bridging hydride and protonation of the apical ⁻SCys ligand prior to proton transfer (Figure 8a) [72].

Among the NiFe-H₂ases is a subgroup that features substitution of one of the nickel SCys ligands by selenocysteinate, (⁻SeCys) (Figure 8b) [54]. [NiFeSe]-H₂ases have been found exclusively in sulfate-reducing and methanogenic microorganisms and belong to two classifications, Group 1 (periplasmic or associated with the cytoplasmic membrane, designated Hys) and Group 3a (F₄₂₀ reducing enzymes, designated Fru) and 3c (methyl viologen reducing enzymes, designated Vhu) [54]. Crystal structures of NiFeSe H₂ases (*e.g.*, Figure 8b) are available from two organisms, *Desulfomicrobium baculatum* [67, 74] and *Desulfovibrio vulgaris* Hildenborough [75–77], both of which are sulfate-reducers. These two NiFeSe H₂ases belong to Group 1 and feature the heterodimeric quaternary structure described above. The discussion below focuses on these enzymes, for which structures are available.

The group 3 enzymes, for which there are no structures, have a more complicated quaternary structure, but a subunit with high homology (*e.g.*, 27% identity) with the large subunit in the NiFe heterodimeric enzymes that contains a similar heterodinuclear NiFe center can be identified [54]. The crystal structures of the NiFeSe enzymes and sequence alignments of NiFe-H₂ases and NiFeSe H₂ases show that ⁻SeCys is substituted for the ⁻SCys ligand in the apical position (site of protonation, Figure 8b). Model studies of some group 3 enzymes suggest that ⁻SeCys may also occupy a bridging position [54]. Other than this substitution, the quaternary structure and protein folds of the NiFe and NiFeSe enzymes are similar and maintain analogous proton- and electron-transfer pathways [67, 75, 78].

The active site of the [NiFeSe]-H₂ases is structurally very similar to the NiFe active site structure (Figure 8), with the possible exceptions of the OH⁻ bridge, which is absent in the structure of the reduced *D. baculatum* enzyme [66], but present in more oxidized forms [68], and the enzyme from *Desulfovibrio vulgaris* (Hildenborough) [75] where it is absent even in oxidized forms, and heterogeneity in the positions of the Ni center and the ⁻SeCys ligand [74, 75, 77]. Heterogeneity in the position of Ni and Se is observed even in recombinant NiFeSe enzyme that was isolated anaerobically and where no oxidative modifications are present [77].

The substitution of the apical SCys^- by SeCys^- seems strategic, as it is the Cys residue that serves as a base in catalysis that is substituted, and has profound effects on the properties of the NiFe active site. These effects include increased catalytic activity, decreased O_2 sensitivity, the absence of the EPR active oxidized states Ni-A and Ni-B found in NiFe H_2 ases, and less product inhibition by H_2 in hydrogen evolution [54].

The changes in catalytic properties of the NiFeSe enzyme can be rationalized to a large extent in terms of the chemistry of selenocysteine [17]. The lower $\text{p}K_a$ of Sec (5.24 for Sec vs. 8.25 for Cys [79, 80]) favors H_2 production at physiological pH [67]. The lower basicity of Sec relative to Cys makes the former less able to polarize H_2 for heterolytic splitting while making Sec a better source of protons for protonating H^- . At the same time, the increased nucleophilicity and polarizability of the SeCys^- ligand with respect to SCys^- increases the electron density on the cluster through increased covalency in the Ni-Se bond, making the formation of H^- more favorable. The increased electron density in the cluster is apparent in lower the ν_{CO} frequencies for Fe-CO in species in the catalytic cycle where direct comparisons between NiFeSe and NiFe H_2 ases are possible (Figure 9), and might account for the lack of a solvent-derived OH^- bridge given the attendant decreased Lewis acidity of the metal cluster.

The changes in redox chemistry and stability toward reaction with O_2 are quite striking. In contrast to the NiFe H_2 ases, the NiFeSe enzymes do not normally exhibit Ni-A or Ni-B and are isolated in air in EPR silent states that nonetheless require reductive activation for catalysis, and do not exhibit the long lag that is typical of Ni-A (*vide supra*, Figure 9) [81, 82]. Hydrogen evolution by [NiFeSe]- H_2 ase cannot be sustained in the presence of O_2 without applying low potentials. The structures of NiFeSe enzymes also reveal a variety of oxidative modifications depending on the conditions [74, 76, 77]. These modifications include oxidation of SeCys^- ligand to persulfides, selenenate or seleninate, oxidation of the remaining terminal SCys^- to a disulfide (with a bridging SCys^- in the same CXXC motif), sulfenate, sulfinic acid, and oxidation of the proximal FeS cluster. A crystal structure of the *D. vulgaris* NiFeSe enzyme isolated in air reveals that the remaining terminal SCys^- residue is oxidized to a sulfinate ligand, but the SeCys^- ligand is not oxidized [75]. These features indicate a much lower degree of O_2 stability than true O_2 -tolerant enzymes, which can oxidize H_2 in the presence of O_2 [59]. The O_2 -tolerant NiFe enzymes form only the readily reduced Ni-B form upon aerobic oxidation, show pronounced H_2 inhibition for H_2 evolution, and are associated with a unique proximal $\text{Fe}_4\text{S}_3(\text{SCys})_6$ cluster that is capable of delivering two reducing equivalents [59]. The [NiFeSe]- H_2 ases also feature an altered FeS cluster arrangement wherein the medial cluster is also a Fe_4S_4 cluster, but do not contain the unique proximal cluster found in O_2 -tolerant NiFe H_2 ases).

The association of Sec with increased O_2 -tolerance is supported by structural characterization of a protein variant where the SeCys^- ligand is replaced by SCys^- [77]. In this variant, even brief exposure to O_2 led to oxidation of the apical SCys^- to sulfinic acid and the basal terminal SCys^- ligand to sulfenate, conditions under which the wild type [NiFeSe]- H_2 ase showed no oxidative damage [77]. Similarly, the decreased catalytic activity of NiFe H_2 ases with respect to the NiFeSe enzyme could be reproduced in the Sec $\rightarrow$ Cys variant

[77]. Interestingly, a sample of the Sec $\rightarrow$ Cys variant isolated aerobically showed the presence of an OH^- bridge between Ni and Fe [77].

The connection between Sec and stability with respect to reaction with O_2 seems clear from its chemical properties [17] and from model studies designed to address aspects of the oxidative damage seen in H_2 ases [55]. Although readily oxidized (formation of a diselenide is $\sim 4\times$ faster than the analogous disulfide in model Ni complexes) [83], because of weaker π -bonding, Se oxoanions are less stable, and thus have a lower thermodynamic driving force for formation, and are more easily reduced than the sulfur analogs [17]. Thiolate ligands, particularly in Ni complexes [55, 84–89], react slowly and generally irreversibly with O_2 to form complexes of sulfur oxoanions: R-SO^- (sulfenate), R-SO_2^- (sulfinate), and R-SO_3^- (sulfonate). One common mechanistic pathway involves nucleophilic attack of RS^- on O_2 leading to the formation of a transient dioxothiirane intermediate [88]. Cleavage of the O-O bond leads to formation of the sulfinate anion, R-SO_2^- , with the incorporation of both O atoms from the O_2 molecule. This chemistry is particularly common in low-spin Ni(II) complexes, where all the π -symmetry orbitals on Ni(II) are filled and no π -bonding with the S 3p orbitals is available, rendering the thiolate ligands more nucleophilic relative to other transition metal complexes. This chemistry is also distinct from oxidations by peroxide, which require one O_2^{2-} unit for each S-O bond formed [90].

With respect to the H_2 ase active site structure, the terminal thiolates should be more nucleophilic than bridging thiolates [84, 87], where the negative charge is shared between two metals. This has been confirmed in a model complex where only oxidation of the terminal thiolates with O_2 is observed (Figure 7a) [87]. Thus, substitution of a terminal SCys^- by SeCys^- would have the effect of protecting the more reactive site from oxidation to form oxygenates. Indeed, analogous ligands with Se donors are much less reactive toward the formation of oxygenates than the corresponding thiolates, and do not give rise to the same oxidation products (Figure 7b) [55]. This is precisely the chemistry that is seen in the native NiFeSe enzyme (no oxidation of Se) and in the Sec $\rightarrow$ Cys variant (sulfinate formation at the apical position) [77]. Thus, substitution of a terminal SCys^- ligand in the NiFeSe H_2 ases makes sense from the standpoint of protection from oxidation, particularly the catalytic apical Cys residue.

The association of the NiFeSe enzymes with sulfate-reducing bacteria suggests another intriguing possibility. It is possible that NiFeSe H_2 ases evolved to use the H_2S produced from sulfate reduction to repair oxidative damage (Figure 10). Formation of a selenenate ligand, R-Se=O^- , could be reduced by HS^- in a manner similar to the reduction of Sec-SeOH in the glutathione peroxidase consensus mechanism (Figure 10a) [17]. The observation of several forms of NiFeSe H_2 ases with sulfide in the active site, including bound to SeCys, support this possibility [54, 75], as does the identification of a specific H_2S binding site found $\sim 7 \text{ \AA}$ from the active site in both enzyme crystal structures that could serve as a reservoir for this molecule [67, 75]. Thus, it is possible that resistance to oxidation in these enzymes requires the presence of H_2S , and that mechanistic studies conducted in the absence of H_2S have not addressed the native intracellular form of the enzyme. Further, the presence of H_2S and incorporation of a SeCys^- ligand may also provide a mechanism for repair of an oxidized terminal SCys^- ligand, in a manner similar to that proposed for

methionine reduction by methionine sulfoxide reductase (Figure 10b) [17]. Such reactions require flexibility in the structure for $^{-}\text{SeCys}$, a feature that is also borne out by the existing crystal structures of the NiFeSe H₂ases (*vide supra*).

Why is selenium in tRNA?

Another biological macromolecule in which selenium is found is in tRNA where it occurs in the form of 5-methylaminomethyl-2-selenouridine (mnm⁵se²U) and 5-carboxymethylaminomethyl-2-selenouridine (mcm⁵se²U) [91, 92]. Just as in proteins, the selenium atom replaces sulfur, but in this case the replacement is sulfur of the more common modified nucleosides 5-methylaminomethyl-2-thiouridine (mnm⁵s²U) and 5-carboxymethylaminomethyl-2-thiouridine (mcm⁵s²U) [93, 94].

Given that it is now firmly established that one reason that selenium replaces sulfur in proteins/enzymes is due to its ability to resist permanent oxidation and impart resistance to oxidative inactivation as discussed above, Payne and coworkers wondered whether this rationale also applies to selenouridine [95].

To investigate this hypothesis, they synthesized water-soluble analogs of selenouridine and thiouridine. These analogs are 2-selenouracil-5-carboxylic acid (se²c⁵Ura) and 2-thiouracil-5-carboxylic acid (s²c⁵Ura) [95]. In one experiment shown in Figure 11, they showed that se²c⁵Ura had glutathione peroxidase activity, similar to ebselen, a selenium containing selenamide. This experiment showed that selenouridine is capable of redox cycling and forming a selenenic acid, which can then be reduced by GSH to form a selenosulfide intermediate, which can then be reduced by a second mole of GSH to return to the original form. The sulfur-containing analog did not have any glutathione peroxidase activity under the conditions tested.

Payne and coworkers also showed that s²c⁵Ura readily lost sulfur when exposed to H₂O₂, but se²c⁵Ura formed a diselenide. When these analogs were exposed to H₂O₂ in the presence of GSH or ascorbate, s²c⁵Ura was still desulfurized, but se²c⁵Ura did not lose selenium [95]. The results with these model compounds are identical to the data described above for Sec-containing enzymes, which is the presence of selenium imparts resistance to irreversible oxidative modification.

Interestingly, both selenouridine and thiouridine are found in the wobble position (position 34) of the anticodon stem loop of three tRNA species: tRNA^{Gln}_{UUG}, tRNA^{Lys}_{UUU}, and tRNA^{Glu}_{UUC}. Both modifications have been shown to destabilize the U/G wobble pair and increase the affinity of the U/A base pair [96], leading to greater translational fidelity during protein synthesis. However, the relative affinity of selenouridine for adenosine is higher in comparison that of thiouridine [96].

One hypothesis that arises from the higher strength of the 2-selenouridine:adenosine base pair relative to a 2-thiouridine:adenosine base pair is that while 2-thiouridine and 2-selenouridine have the same biochemical function since they both translate AAA, CAA, and GAA codons, the use of 2-selenouridine may be a preferred way for the cell to respond to oxidative stress. This is because the *in vitro* data shows that the oxidation of 2-selenouridine

is reversible and this may make 2-selenouridine-containing tRNA (Se-tRNA) resistant to oxidative inactivation enabling the Se-tRNA to translate essential proteins during conditions (oxidative stress) in which the S-tRNA cannot. Perhaps the proteins that are translated under stress conditions by Se-tRNA are involved in the stress response. This rationale provides a connection of the chemistry of selenium, which is uniquely suited to deal with ROS, to its use in tRNA. This hypothesis is currently being explored by our laboratory. This model is shown in Figure 12.

There is precedence for this idea as a research team led by Hidalgo has found that thiouridine in tRNA^{Lys} is needed to translate stress proteins [97]. They found that stress proteins in yeast have biased codon usage for AAA (Lys) codons compared to AAG. In a related manner, Sorenson has found that modifications to the wobble position of tRNA^{Glu} are involved in enhancing the rate of translation [98], and Dedon and Begley review how codon biased translation is influenced by modification of the wobble position [99, 100].

Currently, there are few references in PubMed about selenouridine, a reflection of how little research has been done in this area. The authors hope the discussion above spurs growth in this field.

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Highlights

- Reactions of selenium are readily reversible in comparison to sulfur
- Contribution of Jean Chaudière to selenium biochemistry is highlighted
- Contribution of Al Tappel to selenium biochemistry is highlighted
- Selenium protects certain cell types
- Potential role of thioredoxin reductase in cystic fibrosis

Figure 1.

Use of a C-terminal selenocysteine to generate thioesters used for acyl-transfer reactions in comparison to the same reaction catalyzed by cysteine. The equilibrium constant for transthioesterification is 100–1000 fold faster for selenoesters in comparison to thioesters. Selenoesters are therefore superior acyl-transfer reagents in comparison. Here Sec is in the carboxamide form.

Figure 2.
(top panel) A Sec-containing peptide is treated with H₂O₂ in the presence of dimedone, resulting in an alkylated Sec residue. (bottom panel). The dimedone adduct can be removed by the addition of excess thiol [23].

Figure 3.
Catalytic decomposition of H_2O_2 by selenocystine as proposed by Tappel (adapted from [25]).

Figure 4.

Possible mechanism for the inactivation of Cys-GPX as made by Chaudière. Oxidation of the active site Cys residue to Cys-sulfinic acid could be followed by rapid β -elimination and formation of dehydroalanine.

Figure 5.

The role of Sec-TXNRD in HOSCN detoxification, relevance to innate immunity, and possible role in CF disease. There are four key ideas expressed in the figure. Each idea corresponds to a red number. (1) HOSCN is produced by lactoperoxidase (LPO) by the reaction of SCN^- and H_2O_2 . HOSCN is an ideal bactericide because it selectively oxidizes low pK_a thiols at lower concentrations compared to HOCl and H_2O_2 and specifically targets thiols on key bacterial enzymes such as the low M_r Cys-containing TXNRD (2) Sec-TXNRD in the lung epithelial cell metabolizes HOSCN into SCN^- and water. The Sec residue of TXNRD imparts resistance to HOSCN-mediated inactivation. (3) Defects in CFTR result in low levels of SCN^- in the airway fluid. (4) SCN^- minimizes lung inflammation because it is an antioxidant that can scavenge hypohalous acid (HOX) or acts as a competitive substrate for haloperoxidases MPO and LPO. Hypohalous acids such as HOCl are non-selective oxidants and cause damage and inflammation to host tissues (represented by $\blacklozenge$). SCN^- effectively lowers the concentration of HOCl produced under inflammatory conditions that occurs during bacterial infection. Note that in the figure a red T-shaped symbol indicates inhibition and DUOX = dual oxidase, NOX = NADPH oxidase, and PMN = polymorphonuclear neutrophil.

Figure 6.

Catalytic reaction of a diselenide to produce superoxide by way of a one-electron mechanism. The superoxide undergoes rapid dismutation to form hydrogen peroxide, which then be reduced by selenolate, forming water. The net result of these reactions is to consume glutathione (GSH), causing oxidative stress *in vivo*. Note that $GSSG$ is the abbreviation for oxidized glutathione. This figure was adapted from [52].

Figure 7.

Oxygen reactivity of model Ni(II) thiolate complexes of relevance to hydrogenase oxidations. a) A dinuclear complex exhibiting S-oxidation at the terminal thiolate ligands. b) Analogous thiolate and selenolate complexes showing oxidation of a thiolate to a sulfinate ligand and the lack of reactivity of the selenolate analog.

Figure 8. Structures of the active sites of a) reduced *Desulfovibrio vulgaris* Myazaki F NiFe H₂ase (PDB 4U9H) showing the position of a bridging hydride and protonated apical Cys₅₄₆. b) Structure of the aerobically isolated oxidized state of the NiFeSe H₂ase from *Desulfovibrio vulgaris* (Hildenborough) (PDB 3ZE7) showing oxidation of C75 to a sulfinic ligand. Color Scheme: Ni, green; Fe, magenta; Se, orange; S, yellow; O, red; N, blue; C, gray; H, white. The protein-derived Cys ligands are truncated at the C β atom for clarity.

Figure 9.

Redox and catalytic intermediates for NiFe H₂ases (left) and NiFeSe H₂ases (right). The structures shown are representations of consensus structures drawn from crystal structures and spectroscopic data discussed in the text. IR and EPR data for NiFe H₂ases are from studies of *Desulfovibrio vulgaris* Myazaki F [59]. IR and EPR data for NiFeSe H₂ase are from studies of *Desulfovibrio vulgaris* (Hildenborough) [78].

Figure 10.

Hypothetical repair mechanisms for oxidation of the terminal chalcogenate ligands in the active site of NiFeSe H₂ase. a) repair of -SeCys oxidation with H_2S derived from sulfate reduction in sulfate-reducing bacteria. b) repair oxidized -SCys by the -SeCys ligand and H_2S .

Figure 11.

Glutathione peroxidase activity of ebselen (black), se^2c^5Ura (red), and s^2c^5Ura (blue) as measured by consumption of NADPH in a glutathione reductase coupled assay as described in [95]. The sulfur-containing analog (s^2c^5Ura) has no activity in this assay, the selenium-containing analog (se^2c^5Ura) has activity intermediate between that of ebselen and s^2c^5Ura .

Figure 12.

Model for the involvement of 2-selenouridine-containing tRNA (Se-tRNA) in translation during oxidative stress in comparison to 2-thiouridine-containing tRNA (S-tRNA). A) A mRNA containing a lysine codon is being translated under conditions of oxidative stress by S-tRNA. Under these conditions the S-tRNA can be dethiolated to uridine, resulting in a loss of function and a mistranslation error, resulting in a protein with altered or impaired function. B) In the case of translation of the same codon by Se-tRNA under conditions of oxidative stress, but in the presence of an antioxidant like GSH, the Se-tRNA resists permanent oxidation, maintaining function. This results in the translation of a functional protein.