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Review Article

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 et al., 2018) [36]. 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.

1. 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.

2. 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.

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

Abbreviations: CO, carbonmonoxide; $^{-}$CN, cyanide; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane regulator; C, one-letter abbreviation for cysteine; Cys, cysteine; $^{-}$SCys, cysteinate; Cys-SOH, cysteine sulfenic acid; Cys-SO_2^-, cysteine sulfinic acid; Cys-SO_3^-, cysteine sulfonic acid; DUOX, dual oxidase; *E. coli*, *Escherichia coli*; GSH, glutathione; GS•, glutathione radical; GSSG, glutathione disulfide; GPX, glutathione peroxidase; H, hydrogen; H_2ases, hydrogenases; H_2O_2, hydrogen peroxide; HOBr, hypobromous acid; HOCl, hypochlorous acid; HI, hypoiodous acid; HOSCN, hypothiocyanous acid; $^{-}$SCN, hypothiocyanite; Fe, one-letter abbreviation for iron; LPO, lactoperoxidase; mcm^5se^2U, 5-carboxymethylaminomethyl-2-selenouridine; mcm^5s^2U, 5-carboxymethylaminomethyl-2-thiouridine; mnm^5se^2U, 5-methylaminomethyl-2-selenouridine; mnm^5s^2U, 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, selenocysteinate; Sec, selenocysteine; Sec-SeOH, selenenocysteine selenenic acid; Sec-SeO_2^-, selenocysteine seleninic acid; Sec-SeO_3^-, selenocysteine selenonic acid; se^2c^5Ura, 2-selenouracil-5-carboxylic acid; s^2c^5Ura, 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

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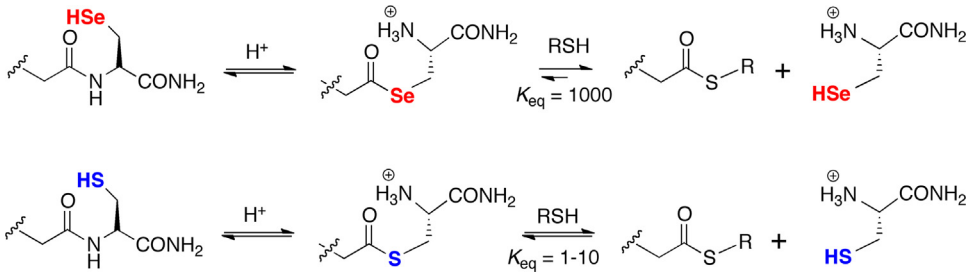

Fig. 1. Use of a C-terminal selenocysteine to generate thioesters used for acyl-transfer reactions in comparison to the same reaction catalyzed by cysteine. The equilibrium constant for transthioesterification is 100–1000 fold faster for selenoesters in comparison to thioesters. Selenoesters are therefore superior acyl-transfer reagents in comparison. Here Sec is in the carboxamide form.

example of this is the use of Sec in acyl-transfer reactions of the type shown in Fig. 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 Fig. 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].

3. 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

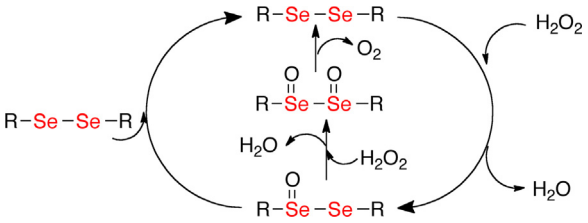

Fig. 3. Catalytic decomposition of H_2O_2 by selenocystine as proposed by Tappel (adapted from [25].

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 Fig. 3.

Unfortunately, the catalytic cycle proposed by Tappel in Fig. 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 Fig. 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 the 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

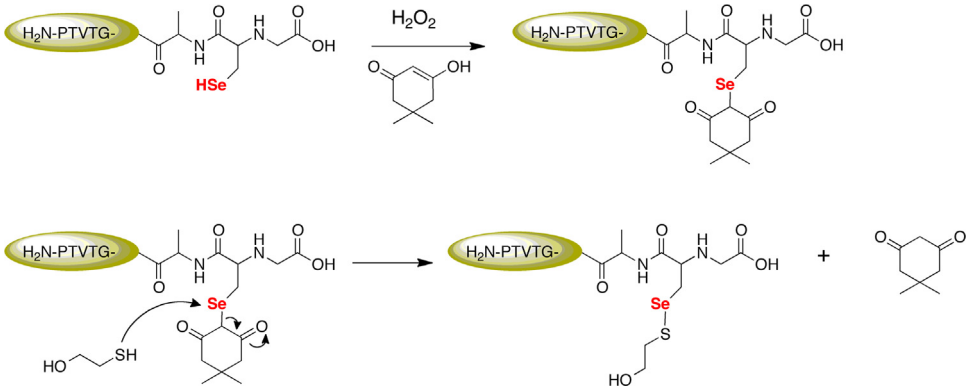

Fig. 2. (top panel) A Sec-containing peptide is treated with H_2O_2 in the presence of dimedone, resulting in an alkylated Sec residue. (bottom panel). The dimedone adduct can be removed by the addition of excess thiol [23].