

## Selenocysteine a key amino acid for some redox enzymes.

### Review

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### Dedicated to Professor Marcello Tiecco on the occasion of his retirement

#### 1. Introduction

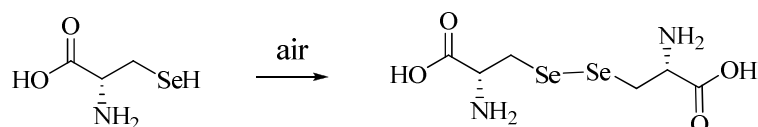
In biology selenium was long considered as a toxic element until 1957 when its role as micronutrient for bacteria, mammals and birds was reported. [1] More recently many books, reviews and reports appeared in the literature describing the identification of various selenoproteins involved in a wide number of biochemical processes as well as the nutrient importance of selenium. [2]

Selenium is predominantly present in biological systems in the form of naturally occurring amino acids selenocysteine (Sec) and selenomethionine that are incorporated in some proteins with a relevant role in redox equilibria. Berry *et al.* demonstrated that enzymatic activity of some selenoproteins is strongly reduced by replacement of catalytic active Sec residues with a cysteine (Cys), confirming the biological relevance of selenium. [3]

Various oxidation states of selenium within proteins have been observed such as selenol, selenenic acid, selenoxide, selenenyl sulphide and diselenide.[4]

From a chemical point of view the different pKa (5.2 for selenols and 8.0 for thiols) is responsible of full dissociation at physiological pH and consequently of an increased nucleophilicity of the selenolate in the enzyme's active site in respect to the undissociated thiol.

In contrast to the thiol group in Cys the selenol moiety in the free amino acid is very unstable and oxidizes spontaneously in air to produce the corresponding diselenide L-selenocysteine (Scheme 26). This is probably a direct consequence of the best stability of diselenide bond respect to the disulfide one. [5] (scheme 1)



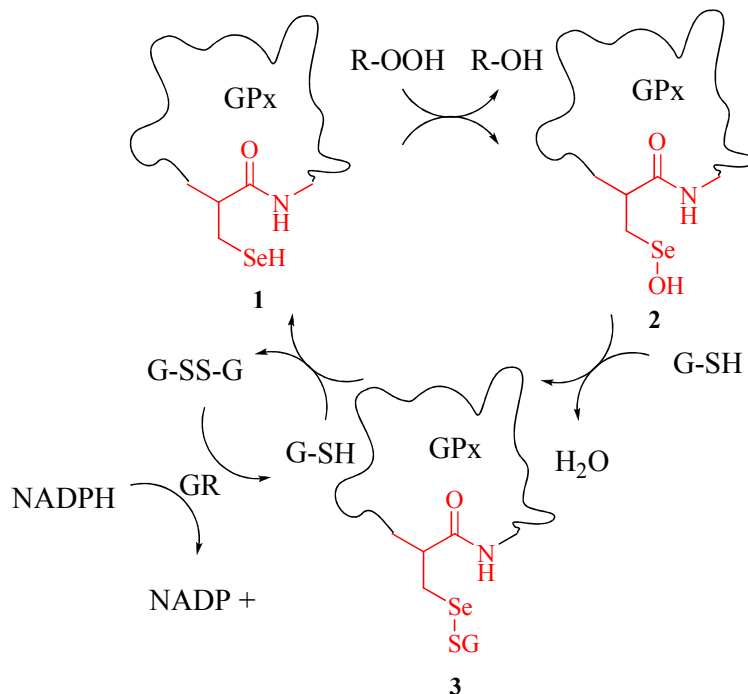
**Scheme 1** Air Oxidation of selenocysteine

The main mammalian selenium containing enzymes are: the antioxidant glutathione peroxidase (GPx), the deiodinating enzyme iodothyronine deiodinase (ID) and the redox enzyme thyoredoxin reductase (TrxR). Recently diselenide bonds were identified in natural proteins even if their role in redox regulation is not clear.

## 2. The Glutathione Peroxidase (GPx)

GPx is a mammalian antioxidant selenoenzyme which protects biomembranes and other cellular components from oxidative damages by catalyzing the reduction of a variety of reactive oxygen species (ROS) like hydrogen peroxides and almost every hydroperoxide including lipid hydroperoxides by using glutathione (GSH) as well as other reducing co-factors. [6]

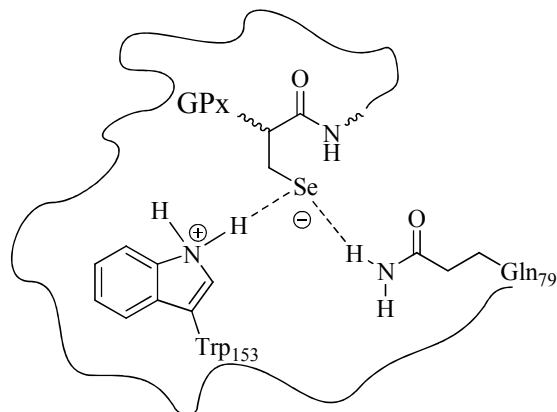
The catalytic mechanism proceeds through the reaction of the selenol moiety (**1**) with the peroxide and the subsequent formation of an unstable selenenic acid (**2**) which immediately reacts with GSH to generate a selenenyl sulfide intermediate (**3**). A second GSH molecule attacks the Se-S bond regenerating the active selenol site and producing a molecule of oxidized glutathione (GS-SG) that is enzymatically reduced back to GSH by the glutathione reductase (GR)-NADPH system (Scheme 2).



**Scheme 2** Catalytic reduction of peroxides mediated by GPx

Only the selenol moiety in the enzyme and not the thiol reacts with the peroxides as a consequence of the involvement of the Sec residue in a “catalytic triad” with a tryptophan (Trp)

and a glutamine (Gln). The stability and the reactivity of the selenol in Sec towards the hydroperoxide are increased by an hydrogen bond with the imino group of the Trp-153 residue and the amido group of the Gln-79 (Figure 1). [7]



**Figure 1**

Some theoretical investigations at the atomic level of the GPx catalytic mechanism in the reduction of hydrogen peroxide [8] and in the peroxynitrite reductase activity [9] have been performed using density functional calculations.

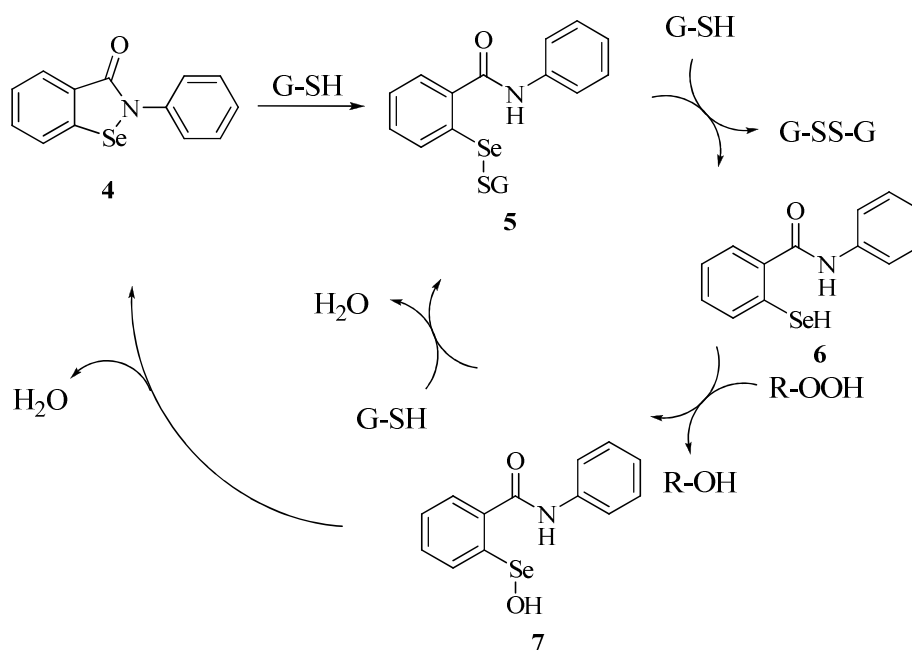
Jacob and co-workers demonstrated that GPx catalytically oxidizes metallothionein and releases zinc in the presence of *t*-BuOOH suggesting that this type of redox chemistry may be employed in biology for the control of metal metabolism. [10]

After the observation that ebselen (**4**) mimics the catalytic action of GPx [11] special attention has been paid by several research groups to identify efficient and simple GPx-mimics in order to develop new drugs able to protect cell membranes and other cellular components against oxidative stress.

Several modification of the basic ebselen structure have been reviewed by Singh [12] and Mugesh [13] observing a considerable influence of the substituents on the reactivity. Considering the central role of the selenol in the seleno-enzyme mechanism the ebselen-like derivatives could be considered as pre-catalysts and presumably the catalytic cycle involves the reaction with two molecules of GSH leading first to a selenenyl sulfide (**5**) and then to the actual catalytically active selenol (**6**) (Scheme 3).

Recently Mugesh and coworkers revises the mechanism that accounts for the GPX-like catalytic activity of the ebselen. They demonstrated that the reaction of ebselen with H<sub>2</sub>O<sub>2</sub> yields selenenic acid (**7**) as the only oxidized product, in which the selenium atom is involved in a noncovalent interaction with the carbonyl oxygen atom.

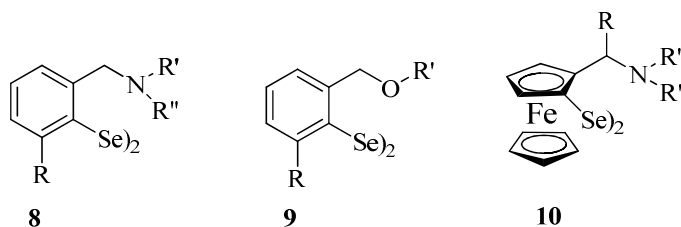
A large excess of thiols leads directly to the formation of the corresponding selenenyl-sulfide (**5**) that undergoes a disproportionation in the presence of the peroxide affording a diselenide that can be subsequently oxidized to a mixture of selenenic and seleninic acid. This oxidation can be reversed to selenenyl-sulfide by the addition of thiol. If the thiol concentration is low the selenenic acid undergoes a cyclization to produce ebselen, protecting the selenium moiety from irreversible inactivation. [14]



**Scheme 3** Mechanism for the GPx like activity of ebselen

More recently a series of ebselen analogues were prepared. These compounds were tested as GPx-like catalytic antioxidant using several peroxides as ROS and several thiols as co-substrates. The studies, supported by DFT calculations and <sup>77</sup>Se-NMR analyses, demonstrated that the presence of a phenyl substituent on the nitrogen atom is important and suggest that the strength of the Se—N covalent bond as well as the nature of the peroxide do not have a significant effect on the antioxidant activity. However the catalytic efficiency is strongly reduced by the strength of the noncovalent interaction between oxygen and selenium in the selenenyl sulfide intermediate and is strongly correlated to the structure of the thiol. [15]

Also aryldiselenides (**8-10**) can act as GPx mimics and it was demonstrated that is necessary an activating *ortho* chelating group. [16][17] (Figure 2)



**Figure 2**

The antioxidant activities of amino-substituted GPx mimics can be enhanced by the introduction of methoxy substituents *ortho* to selenium. This makes the basicity of the amino group perfect for the catalysis, increasing the zwitterionic character of selenols and protecting the selenium in the selenenic acid intermediates from overoxidation. The presence of the methoxy group reduces the strength of the Se—N interaction in the selenenyl sulfide intermediate and enhances the nucleophilic attack of the incoming thiols at sulfur. [18]

Five different classes of selenium containing GPx have been classified: cytosolic (GPx-1), gastrointestinal (GPx-2), extracellular (GPx-3), phospholipid peroxide (GPx-4) and the recently identified GPx-6. [19]

GPx-1 is one of the most abundant and ubiquitous mammalian selenoproteins. Several factors can influence its expression: the protein synthesis is dramatically decreased under circumstances of low selenium as well as deeply affected by pathological conditions. In general, oxidative stress has been shown to suppress GPx-1 expression [20] but at the same time, the protein rapidly reappears after the damage; [21] this suggests a role in the overall recovery of the cells. Some studies have established a relationship between asthma and GPx-1 level, which decreases in lung during inflammation; however, these data have not been confirmed by other research groups. [22] Whereas, it is strongly supported the link with cancer by various *in vitro* and *in vivo* models, [23] that underline how GPx-1 expression can be decreased or increased in different form of malignant and non- malignant tumours.[24] GPx-1 is also involved in neurodegenerative disturbs like Parkinson's disease and dementia with Lewy bodies, where high levels of the protein in microglia seem to play a protective role. Finally, transgenic mouse models have been used for further investigations: GPx-1 knockout mice has been found to be healthy but very susceptible to mortality in presence of oxidative stress-inducing agents like paraquat and hydrogen peroxide. [25] Recent studies described the development of insulin resistance and obesity in GPx-1 overexpressing mice.[26]

The expression of GPx-2 is restricted to the gastrointestinal tract where it is highest in the crypt grounds and decreases toward the luminal surface; [27] this suggests a role in proliferation, in addition to a general protective activity in the whole intestinal epithelium from oxidative stress. Substrate specificity of GPx-2 is similar to that of GPx-1 but the expression under conditions of

low Se intake is much more stable. [28] A GPx-1/GPx-2 double knockout mice model has been used to support the relationship between GPx-2 expression and intestinal cancer and inflammation: the mice have developed first ileocolitis and then tumour.[29] These findings suggest that GPx-2 could be a good target for cancer therapy.

GPx-3 is the only member secreted into the blood; it is produced mainly by the kidney, in the cells of the proximal tubular epithelium and of Bowman's capsule.[30] However, it is also expressed in other organs, like the heart and the thyroid gland, where it carries out a protective activity against the extracellular oxidant stress. [31] Several studies have demonstrated the involvement of GPx-3 in cardiovascular disorders: the deficiency of this selenoprotein in plasma leads to the increase of peroxides and subsequently to NO inactivation; this results in the inhibition of normal platelet function that predisposes to thrombosis and stroke.[32] These data are consistent with the notion that GPx-3 is the major plasma selenoprotein, together with Sel P. Promoter polymorphism in GPx-3 gene has been investigated as another potential risk factor but this finding still needs to be confirmed.[33] Finally, GPx-3 expression has been linked to obesity: it has been found to be reduced in several obese animal models and increased after weight loss; recent studies also describe the importance of GPx-3 as mediator of the effects of peroxisome proliferator-activated receptor  $\gamma$  in skeletal muscle cells [34] and of estrogen receptor in white adipose tissue. [35]

GPx-4 is widely distributed in several tissues and reduces phospholipid hydroperoxides in biomembranes, providing protection against oxidative damage; it is also involved in lipid metabolism [36] and in transduction of cell death signalling.[37] GPx-4 expression is believed to be essential in early embryo stages for a normal development, as described in mouse models.[38] This evidence has been confirmed by the deletion of GPx-4 gene that resulted in embryonic lethality and in retardation of brain development.[39] However, the expression of this selenoprotein is important also for adult brain: GPx-4 has been linked to neurodegeneration, in particular to PD and Alzheimer's disease. The relationship with PD has been suggested by the finding that alteration of DJ-1, an antioxidant protein with a pathogenetic important role, is associated with higher GPx-4 levels. [40] As regards AD, a recent study based on GPx-4+/- mice, has demonstrated the contribute to amyloidogenesis of lipid peroxidation, which causes the overexpression of BACE1 ( $\beta$ -site amyloid precursor protein cleavage enzyme 1). [41] GPx-4 has also an important role in the protection against cardiovascular disorders: the inhibition of atherosclerotic development can be gained increasing its peroxide scavenger activity through up-regulation. [42] Finally, it is interesting to note the contribute of low levels of GPx-4 to male

infertility and the dual role as enzyme and structural protein during spermatogenesis, that distinguishes this selenoprotein from the other members of GPx family. [43]

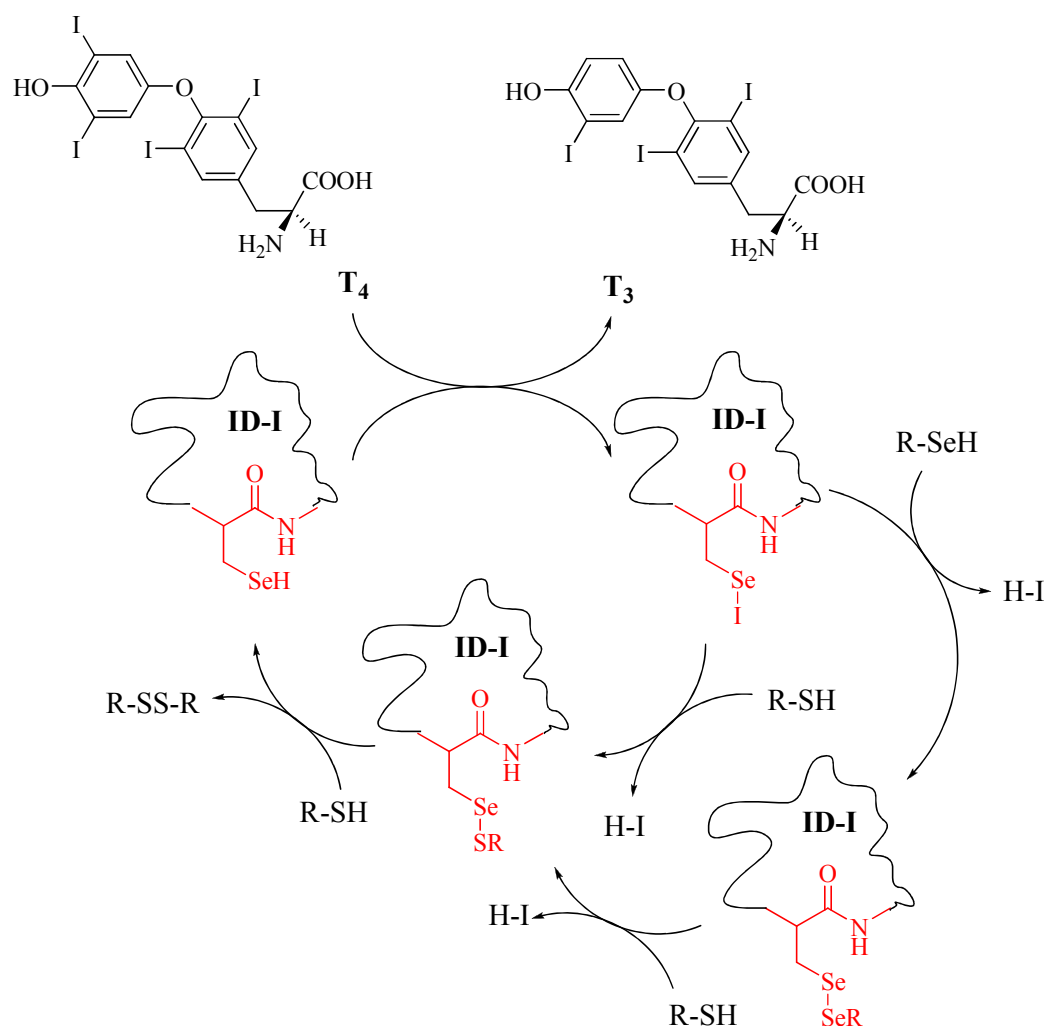
GPx-6 appears to be present only in developing embryo and in olfactory epithelium in adults.[44]

The biological role is still unclear so further investigations are necessary.

### 3. The Thyronine Deiodinase (ID)

Thyronine deiodinase of type I (ID-I) is the enzyme central to the deiodination of thyroxine (T4) which produces the active hormone tri-iodothyronine (T3) in the thyroid gland.

The first model for the mechanism was proposed by Reglinsky *et al.* studying the deiodination of aryl phenols by benzene-selenol and by theoretical calculations. [45]



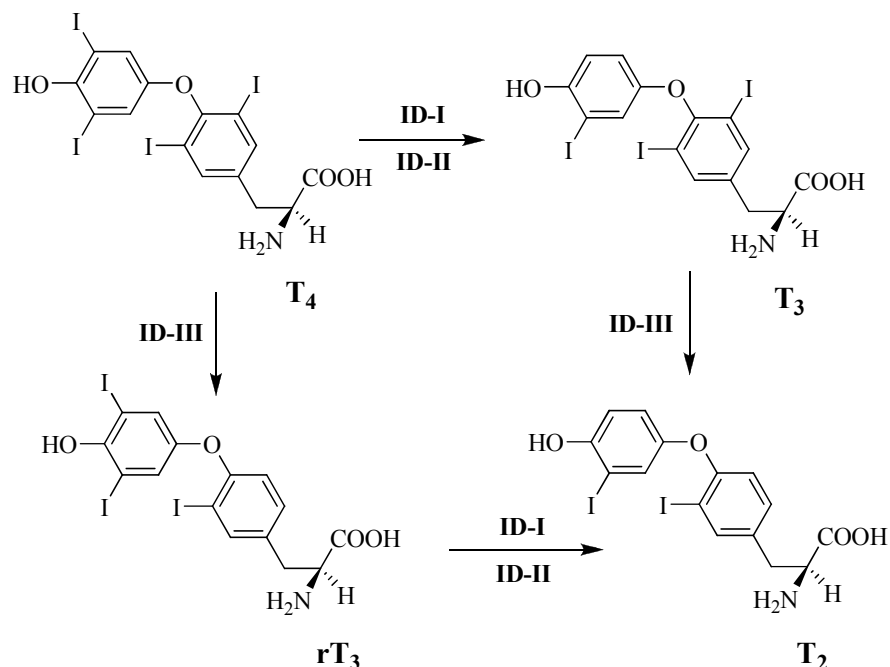
**Scheme 4.** Catalytic mechanism proposed for ID-I

The 5' deiodination catalyzed by ID-1 has been proposed as a ping-pong bisubstrate reaction in which the selenolate reacts with T4 leading to a selenenyl iodide (E-Se-I) and the active hormone

T3. [46] The enzymatic active site is subsequently regenerated through a reduction of the selenenyl iodide promoted by a cytoplasmatic thiol. (Scheme 4)

Mugesh and co-workers [47] studied the mechanism of this reduction using an internally chelated arenselenenyl iodide suggesting that if the reductant is a thiol a selenenyl sulfide intermediate is formed.

The same authors also demonstrated that in the absence of important sterical hindrance, the selenenyl sulfide is able to react with a selenol leading to a diselenide as intermediate. The latter can be reduced in a second step by two equivalents of thiol to regenerate the active site on the enzyme. Other two deiodinase of type II (ID-II) and type III (ID-III) have been identified as responsible of the catalytic selective deiodination of T4 to T3 and to the inactive rT3 and T2 (Scheme 5). [46b]



**Scheme 5.** Deiodination reactions promoted by ID-I, ID-II and ID-III

ID-I and ID-II knockout mice have been generated in order to achieve a full comprehension of the functional aspects of these selenoenzymes.

It results in relatively healthy mice with quite normal level of serum T<sub>3</sub>. [48] This suggests that ID-I and ID-II are not essential for thyroid hormone metabolism but they play important roles in the homeostasis of T<sub>3</sub> for local use in specific tissues such as pituitary, brown fat, brain (ID-II) but also in thyroid gland and peripheral tissues (ID-I).

#### 4. The Thioredoxin Reductase (TrxR)

The thioredoxin reductase (TrxR) catalyzes the NADPH-dependent reduction of a disulfide bridge in oxidized thiredoxin (Trx). The reduced form of Trx is involved in several cellular

processes: it is used as a cofactor by the antioxidant enzyme Trx peroxidase and by ribonucleotide reductase, which reduces ribonucleotides to deoxyribonucleotides for DNA synthesis; it also plays important roles in regulating gene transcription, cell growth and inhibiting apoptosis.

These evidences suggest that TrxR could be a good target for anti-tumor therapy. [49]

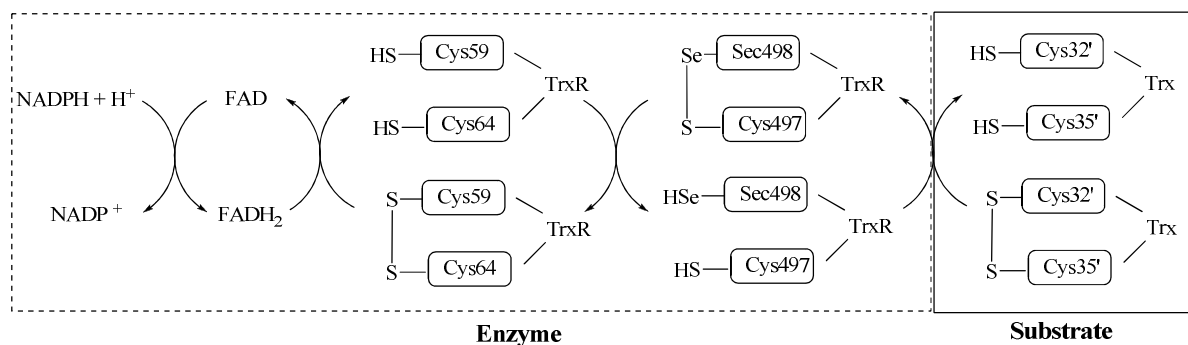
Three different mammalian selenocysteine containing Trxr-enzymes were described: cytosolic (TrxR1), mitochondrial (TrxR2) and testis specific thioredoxin glutathione reductase (TrxR3).

TrxR1 is the best characterized among these isoenzymes and is widely distributed throughout a variety of tissues. TrxR1 and TrxR2 are both housekeeping proteins, constitutively expressed by the cells. [50] Studies in mice have demonstrated that both the enzymes are essential for life; in fact, genetic deletion of either results in embryonic lethality. [51]

Several diseases have been associated with TrxR activity. Recently, TrxR expression has been shown to be decreased in HIV-1-infected Jurkat T cells. [52] Trx1/TrxR1 system is also implicated in cancer: [53] both proteins are highly overexpressed in aggressive tumors and the reduction of their level results in limiting tumor progression as emerged by in vitro and in vivo studies. [54] These data support the protective role it plays. Finally, it is also important to note that Se levels regulate the expression of both TrxR1 and 2 proteins: it is higher when Se intake increases. [55] However, not only Se levels but also other conditions like oxidative stress and response to growth factors can induce the expression of TrxRs. [56]

Recently the presence of a selenocysteine in human TrxR has been demonstrated and it has been reported that this aminoacid plays an important role in the catalytic mechanism. [57]

The mechanism starts with the hydride transfer from NADPH to FAD and subsequently to the active site centre of the enzyme that, in the cytoplasmatic isoform, is a disulfide bond between Cys59 and Cys64. The reduced thiols are now able to reduce a Se-S bond to produce a thiol and a selenol which act as hydrogen donors to the TrX or to other different substrates (Figure 3).



**Figure 3**

Brandt [58] and co-workers described for this class of enzymes a new catalytic triad between selenocysteine 498, histidine 472 and a glutamate 477. This is essential to support the proton transfer from selenol to histidine, necessary to stabilize the dissociate form of the first enabling it to react with the sulfur-sulfur bond of thioredoxine.

By means of DFT calculations they demonstrated that, even if a simple proton transfer from selenocysteine to the histidine is thermodynamically disfavoured it becomes favoured when stabilized by the acid group of a glutamate (Figure 4). The presence of a selenocysteine instead of a cysteine gives a  $10^3$  rate acceleration to the process.

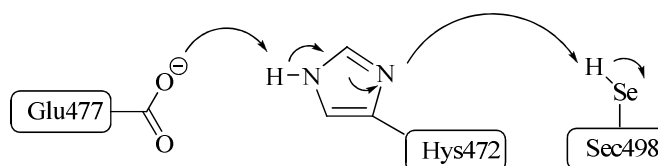


Figure 4

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