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Selenium and selenoproteins: an overview on different biological systems

Running title: Selenoproteins in biological systems

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Abstract

Selenium (Se) is an essential trace element for humans, plants and microorganisms. Inorganic selenium is present in nature in four oxidation states: selenate, selenite, elemental Se and selenide in decreasing order of redox status. These forms are converted by all biological systems into more bioavailable organic forms, mainly as the two seleno-amino acids selenocysteine and selenomethionine. Humans, plants and microorganisms are able to fix these amino acids into proteins originating Se-containing proteins by a simple replacement of methionine with selenomethionine, or "true" selenoproteins if the insertion of selenocysteine is genetically encoded by a specific UGA codon. Selenocysteine is usually present in the active site of enzymes, being essential for their catalytic activity. This review will focus on the strategies adopted by the different biological systems for selenium incorporation into proteins and on the importance of this element for the physiological functions of living organisms. The most known selenoproteins of humans and microorganisms will be listed highlighting the importance of this element and the problems connected with its deficiency.

KEYWORDS: glutathione peroxidase, human selenoproteome, selenium, selenocysteine, selenomethionine, selenoproteins

1. Selenium: history, natural forms, toxicity and functions

Selenium (Se) is an element belonging to the group VIA of the Periodic Table; it is naturally present 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 it is readily oxidized to Se^0 [1].

Selenium was discovered in 1818 and its unusual electric and photoelectric properties immediately attracted the scientific community. The toxicity of Se started to be considered after a report dealing with the death of patients that received overdoses of sodium selenate [2]. Furthermore, a few years later, forage crops containing high Se-levels were identified as responsible for the death of thousands of sheep and cattle in some regions of the USA characterized by a naturally high selenium content. In 1943, the element itself was claimed to be carcinogenic causing the end of all further therapeutic uses of selenium [3]. Several years later Se was still referred to as 'the essential poison' or 'toxin' [4], and was treated with great suspicion, even if it was proven to be a nutritionally essential trace element [5]. Nowadays, more than fifthy years after the discovery of its nutritional essentiality, its early negative history still influences current opinions toward it.

It is still not well established the selenium amount necessary for the optimal maintenance of a good health state, and whether its supplementation should be recommended to the general public [6]. The recommended levels of selenium intake are 60 μg/day for men and 53 μg/day for women [7]. In contrast to many other micronutrients, its intake varies hugely worldwide, ranging from deficient (associated with selenium-deficiency diseases) to toxic concentrations that can cause garlic breath, hair and nail loss, disorders of the nervous system and skin, poor dental health and paralysis [8]. Most of the Se intake derives from the diet and Se-content of any food system is a consequence of its presence in the soil. Most soils contain 0.1-2 mg Se/kg [9, 10] but some areas of the world (*i.e.* Denmark, Finland, New Zealand, eastern and central Siberia (Russia) and some parts of China) are known to have poor selenium soils and, as consequence, low selenium amounts in their food systems [11]. In contrast, other areas (*i.e.* the Great Plains of the USA and Canada and parts of Ireland, Colombia, Venezuela and China) are seleniferous [11].

The bioavailable Se amount for plants depends on pH, redox conditions, presence of competing ionic species (such as sulfate), microbial activity, soil temperature and other climatic variables [12, 13]. Furthermore also the form of selenium is important: selenate is more mobile soluble and less-well adsorbed than selenite, thus alkaline conditions, that increase selenate amount, improve Se bioavailability, while reducing conditions has an opposite effect [11].

Most of the reports of chronic human exposure to hazardous levels of Se regards occupational exposures, *i.e.* workers in Cu smelters or Se-rectifier plants, due to the inhalation of Se-containing aerosols. Other cases have involved the accidental oral consumption of various inorganic Se compounds [14, 15, 16]. An acute exposure to Se high levels may result in hypotension, respiratory distress and a garlic-like smell of the breath.

A report of chronic selenosis was described in the 60s in the Hubei Province of China, apparently resulting from exceedingly high concentrations of Se in the local food supplies and in all the local environments [17, 18, 19, 20, 21]. The results of this chronic exposure were mainly the losses of hair and nails and only rarely more severe manifestations such as skin lesions, hepatomegaly, polyneuritis and gastrointestinal disturbances.

The importance of Se to biological systems is underlined by the fact that it is the only trace element to be specified in the genetic code. It is specified as selenocysteine (SeCys), now recognized as the 21st amino acid, as it has its own codon and selenocysteine-specific biosynthetic and insertion machinery [22, 23]. Se as selenocysteine in proteins,

scores over its analogue, S, in existing as an anion (selenolate) at biological pH (acid dissociation constant: selenocysteine 5.2, cysteine 8.5). This property enables it to carry out biological redox reactions [22, 24] and is the reason of its presence in the catalytic sites of redox enzymes like glutathione peroxidase, thioredoxin reductase and formate dehydrogenase.

2. Effects of selenium deficiency in humans

It has been widely accepted that selenium deficiency may not cause illness by itself. The only two pathologies for which Se has been actually identified as the only cause are the Kaschin-Beck and the Keshan diseases which occur only in rural areas of China and Russia, mainly eastern Siberia, characterized by food systems with exceedingly low selenium amounts [25]. On the other hand it has been proved the role of selenium deficiency in the occurrence of several human diseases [26, 27]. In this section a summary of the pathological states correlated to selenium deficiency will be described.

Kaschin-Beck disease: Kaschin-Beck disease is a chondrodystrophy affecting the epiphyseal and articular cartilage and the epiphyseal growth plates of growing bones. It is characterized by enlarged joints, shortened fingers, toes and extremities and, in severe cases, dwarfism [28, 11]. It is still not clear if selenium deficiency is the primary cause of Kaschin-Beck disease; it is more likely that an endemic Se deficiency is a pre-disposing factor to the pathogenic effects of some other agents [11].

Keshan disease: Keshan disease is a multifocal myocarditis occurring primarily in children aged 2-10 years and, to a lesser extent, in women of child-bearing age [29, 30]. Its main manifestations are insufficiencies of cardiac function, cardiac enlargement, arrhythmias, and electrocardiographic and radiographic abnormalities. It was first described in China more than 100 years ago and in 1935, the involvement of selenium deficiency in its etiology was proved [28]. However, selenium is of little or no therapeutic value after the occurrence of the pathology. It has been suggested that Keshan disease may be caused by RNA-viruses, whose pathogenicity may be increased by severe deficiencies of Se and other antioxidant compounds [31, 32].

Ageing: Ageing is defined as the lifelong accumulation of molecular damage to cells and tissues in response to exposure to stress associated with lifestyle. Such damage results in loss of functions and an increased vulnerability to diseases, ultimately leading to death. Nutrition and lifestyle are key environmental factors that can modify the rate of damage accumulation in cells [33]. Trace elements, and especially Se, may act on the ageing process mainly through the modulation of oxidative damage and DNA repair capacity. The ability of Se to attenuate oxidative damage or enhance repair capacity depends on its action as essential amino acid (SeCys) for anti-oxidant enzymes such as the different types of glutathione peroxidases. It has been hypothesized that GPx which scavenges H_2O_2 and prevents the initiation of free radical chain reactions, may extend life span and prevent age-related functional disorders [34]. The enhanced repair capacity induced by Se was proved in human leukocytes, where bleomycin-induced DNA damages are repaired more efficiently in presence of Se in the form of selenomethionine (SeMet) [35]. Similarly it was proved that SeMet could also induce a DNA repair response in normal human fibroblasts *in vitro*, protecting cells from DNA damage [36]. Trace elements, among which Se, have the capacity to modulate the rate at which damage is accumulating over time. In this context Se has the potential to influence the prevalence of chronic disease thanks to its implication in the healthy immune response, the regulation of inflammatory pathways, the protection against some forms of cancer and the reduction of cardiovascular disease-related mortality [37]. Several papers deal with an increased mortality and

prevalence of chronic diseases correlated with low Se intake. Additionally, as for many other micronutrients, Se inadequacy is common in elder people and it was proved that Se status declines in an age-dependent manner [38].

Viral infections: Selenium deficiency can have a deep impact on the genome of RNA viruses; in particular it may contribute to the emergence of new viral strains capable of promoting epidemics [39]. It was described a non-virulent Coxsackie virus that, when passing through a Se-deficient host, may acquire more virulent features leading to cardiac damages to the host [31]. The enhanced virulence depends on modifications to the nucleotide sequence resulting in phenotypical changes [40] that were maintained also after subsequent passages through animals with normal selenium status [41]. It was also proved a correlation between Se deficiency and a decreased survival in HIV-infected patients [42]: Se-deficient HIV-infected patients are 19,9 times more likely to die from HIV-related causes than those with adequate Se levels [43]. This may depend on a protective role that Se displays against HIV infections: it is, in fact, a strong inhibitor of HIV replication *in vitro* [44].

Thyroid function: The thyroid has the highest selenium concentration of all tissues [45]: its importance in this gland is suggested by the fact that the deiodinases (Type I, II and III) are all Se-enzymes [46]. In particular the selenium-dependent iodothyronine deiodinases produce the active thyroid hormone, triiodothyronine T3, from its inactive precursor, thyroxine T4. It was demonstrated that a relatively mild selenium depletion (<0.9 μmol/l), in both infants and elderly subjects, may induce changes in the ratio T3/T4 [47]. Furthermore selenium, in the form of GPx3, protects thyroid cells from the hydrogen peroxide, avoiding its use by thyroid peroxidase to produce triiodothyronine or thyroxine [45]. Selenium also displayed to positively affect the autoimmune hyperthyroidism, known as Graves' disease [48].

Heart disease: The benefits induced by selenium on the cardiovascular system depend on the ability of selenoproteins to prevent oxidative modifications of lipids, inhibit platelet aggregation and reduce inflammation [42]. Se-deficiency is correlated with an increased production of radical oxygen species that may lead to the oxidation of low density lipoprotein (LDL), giving rise to the beginning of atherogenesis in heart diseases [49]. However, in epidemiological studies, clear evidences are still missing between low Se status and an increased risk of heart diseases [50]. Early supportive evidences in the USA suggested that a higher mortality of heart diseases was linked to Se-deficient areas [51]; on the contrary epidemiological studies from other countries, namely Finland, a country of low Se status, gave contradictory results [52].

Cancer: Epidemiological studies showed an inverse association between blood Se concentration and the risk of several cancers, primarily in men [53]. Prospective studies have provided some evidence for a beneficial effect of selenium on the risk of lung, bladder, colorectal, liver, esophageal, thyroid and prostate cancers [54]. Generally, it is recognized that selenium may be considered as an anticarcinogenic agent [55] whose effects depend on its chemical form and dosage. Several metabolite pools seem to be of particular importance, such as selenite, selenodiglutathione, methylselenol, selenomethionine and Se-methylselenocysteine [56], whose molecular action deals with alterations in the metabolism of endo- or exogenous carcinogens [57]. However, the exact mechanisms of the anticarcinogenic effect of Se remain still unclear.

3. Fixation strategies of selenium into living organisms

Inorganic forms of Se, namely selenate and selenite, can be efficiently reduced in living organisms and then incorporated into proteins. Se-containing proteins can be divided into three main groups according to their mechanisms

of insertion/interaction of Se: i) post-translational binding as a cofactor; ii) non-specific incorporation (SeMet: selenomethionine); iii) specifically incorporated during translation as SeCys (selenocysteine) [58]. As just mentioned, only two selenium-amino acids are known to be present into proteins: SeMet and SeCys. This incorporation occurs because of the strong relationship between Se and S. So, what happens in mammals, but also in prokaryotes, is that methionine (Met) and SeMet are treated in the same way. This is due to the fact that in nature there is no evidence of the presence of a specific $tRNA^{SeMet}$ in any organism. This means that the incorporation of SeMet into proteins is an unselective event in which a substitution of Met for SeMet happens in $tRNA^{Met}$ [59]. In mammals SeMet can substitute Met in the total body protein pool in a manner that is dependent on the nutritional supplied selenomethionine.

Unlike SeMet, the incorporation of SeCys is a co-translational specific event that employs a complex machinery of proteins and is genetically encoded; indeed the incorporation of free SeCys could not occur since the abundance of cysteine in the cell inhibits this phenomenon avoiding an improper loading of SeCys onto $tRNA^{Cys}$ [60]. SeCys is therefore described as the 21st amino acid identified as the major biological form of Se present in enzymes and proteins belonging to bacteria, archaea and eukaryotes [61]. The insertion mechanism of SeCys presents several common aspects among the different living kingdoms. SeCys is always encoded by an in-frame triplet TGA (in DNA) or UGA (in mRNA), that normally functions as a stop codon, but that is recognized as the specific codon for SeCys if a series of other conditions occurs. The first one is the presence of a secondary mRNA structure characterized by a stem-loop, called SECIS (SelenoCysteine Insertion Sequence) element. This secondary structure necessary for decoding UGA codon differs among phyla in consensus sequence, position in the gene and shape. The first SECIS element was identified in bacteria (*Escherichia coli*) immediately downstream of the UGA [62], while in eukarya it is present in the 3' UTR region and its elimination completely inhibits selenoproteins synthesis [63, 64]. The identification of conserved features in bacterial SECIS is very difficult; moreover putative SECIS elements identified in several bacterial species seem to have no similarity to each other or to the *E. coli* one [65]. Furthermore there is no certainty of the presence of SECIS elements in all bacterial selenoprotein genes.

Another necessary condition is the presence of a specific tRNA called $tRNA^{SerSec}$. This particular tRNA differs for sequence between eubacteria and eukarya, but in both cases it is not charged with SeCys but with serine by a seryl-tRNA synthetase [66, 67, 68]. The conversion of seryl-$tRNA^{SerSec}$ into selenocysteyl-tRNA is then achieved by a selenocysteine synthase that employs selenophosphate, the "activated form" of Se, as selenium donor. In bacteria selenocysteine synthase is a 500 kDa protein [69]. In mammals another step is necessary for the transformation of seryl-$tRNA^{SerSec}$ to selenocysteyl-$tRNA^{SerSec}$: a specific phosphorylation at the serine –OH group [70]. $tRNA^{SerSec}$ performs other tasks: it must recognize specific translation factors, SelB in bacteria and SBP-2 and mSelB in eukarya, avoiding the recognition of canonical elongation factors.

Finally a correct translation requires the specific recognition of SECIS by SelB in bacteria and SBP-2 and mSelB in eukarya. Bacterial SelB possesses several binding sites: for the $tRNA^{SerSec}$, the SECIS element, GTP and the ribosome. This protein has a N-terminal region homologous to EF-Tu that is normally responsible for protein synthesis; while its C-terminus is able to bind the SECIS element [71, 72]. SelB is specifically able to bind selenocysteyl-$tRNA^{SerSec}$, while it does not recognize the seryl-form. This characteristic binding induces the enhancement of its affinity with SECIS. In eukaryotic cells this function is performed by two distinct proteins: SBP-2 and mSelB. SBP-2 shows no homology to bacterial SelB and does not specifically recognize $tRNA^{SerSec}$ [73], while mSelB is an elongation factor that specifically binds $tRNA^{SerSec}$ and that shows homology to the bacterial counterpart.

To summarize (Figure 1), four gene products are involved in SeCys biosynthesis and insertion in bacteria: SelA (selenocysteine synthase), SelB (a special translation factor that recognizes selenocystyl-$tRNA^{SerSec}$), SelC (SeCys-

specific tRNA) and SelD (selenophosphate synthase) [74]. In eukarya, this process is comparable even if with some differences already described and so any model is still speculative.

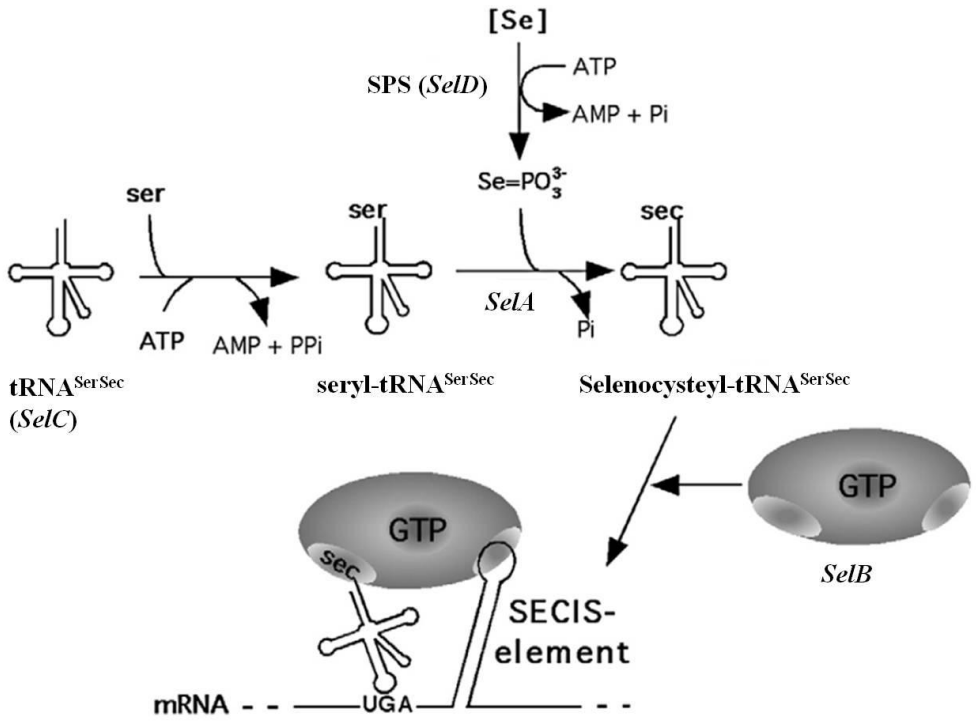

Figure 1: Selenocysteine insertion mechanism in *Escherichia coli* pathway requires a specific tRNA^{SerSec}, obtained by the *selC* gene, that is originally charged with serine. Selenium is converted by a selenophosphate synthetase (the *selD* gene product) into the "activated form" selenophosphate, used by selenocysteine synthase (*selA* gene) to produce the selenocysteyl-tRNA^{SerSec}. Finally is necessary the *selB* gene product, a translation factor, able to bind GTP, tRNA^{SerSec} and the SECIS element, a mRNA secondary structure present immediately downstream of the UGA codon (figure adapted from Stock *et al.* [124]).

Even if selenoproteins are present in all the three domains of life (bacteria, eukarya and archaea), some organisms, such as yeasts and higher plants, does not use SeCys and seem to have lost the SeCys insertion machinery during evolution [75].

For what concerns plants, Se is not an essential element even if some beneficial effects on plants growth have already been demonstrated [76]. Plants can be divided into Se non-accumulators and Se hyperaccumulators: the first are able to discriminate against selenate to take up sulfate, the latter preferentially absorb Se over sulfur [77]. This can suggest that, in non accumulator plants, selenate is taken up by the same transporters as sulfate, while hyperaccumulators could express additional specific transporters. Though no selenoprotein exists in plants, SeCys is a fundamental intermediate used by plants to produce other seleno-amino acids and also volatile Se-metabolites. Plants are able to take up from the soil both selenate and selenite [78]. Selenate can be easily reduced to selenide; two different pathways are possible: i) *via* non-enzymatic reactions and glutathione reductase; ii) *via* APS (adenosine 5'-phosphosulfate) reductase and sulfite reductase [79]. Then selenide can be converted to SeCys that can follow two pathways: in the first one, it can enter the Met pathway in order to synthesize SeMet *via* selenocystathionine and selenohomocysteine [80]. The second possibility consists in the methylation of Cys with the formation of methylselenocysteine (MeSeCys) used for the biosynthesis of γ-glutamylmethylselenocysteine [81].

4. Selenoproteins: roles and metabolism

Once ingested with the diet, SeMet and other organic Se-compounds can be non-specifically incorporated into human proteins such as albumin or hemoglobin in substitution of Met. SeMet can also be trans-selenated to SeCys. It must be underlined that SeCys ingested with the diet or derived from SeMet cannot be used in its original form to synthesize human selenoproteins in our bodies. SeCys has to be prior converted into H_2Se (hydrogen selenide) by the action of a β-lyase and then phosphorylated to selenophosphate by a selenophosphate synthetase [7]. Selenophosphate will then follow the pathway already described in the previous paragraph.

Se-containing proteins formed in this way constitute the human selenoproteome that includes 25 proteins comprising five glutathione peroxidase (GPx), three iodothyronine deiodinase (DIO), three thioredoxin reductase (TRxR), selenoprotein SelP and other proteins listed in Table 1 together with the relative functions and the related disorders.

SELENOPROTEIN NAME	FUNCTION	SELENIUM EFFECTS AND RELATED DISORDERS
GPx1: cytoplasmic glutathione peroxidase	Oxidative stress	Sensitive to Se status from the diet Cardiovascular diseases
GPx2: gastrointestinal glutathione peroxidase	Oxidative stress	Relatively resistant to Se changes Intestinal cancer
GPx3: plasma glutathione peroxidase	Antioxidant	Sensitive to Se status Cardiovascular protection
GPx4: phospholipid hydroperoxide glutathione peroxidase	Antioxidant and structural protein in sperm	Relatively resistant to Se changes Immune disorders, HIV
GPx6: olfactory glutathione peroxidase	Unknown	Unknown
DIO1: deiodinase Type I	Thyroid hormone production and level at systemic level	Thyroid dysfunctions and Kaschin-Beck disease
DIO2: deiodinase Type II	Thyroid hormone production and level at local level	Stable expression under low Se status Thyroid dysfunctions and Kaschin-Beck disease
DIO3: deiodinase Type III	Inactivates thyroid hormone	Thyroid dysfunctions and Kaschin-Beck disease
TrxR1: thioredoxin reductase Type I	Oxidative stress, present in the cytosol	Enhanced activity under high Se status Overexpression in several cancer
TrxR2: thioredoxin reductase Type II	Oxidative stress, mitochondrial protein	Subject to dietary Se changes
TrxR3: thioredoxin reductase Type III	Oxidative stress, specific expression in testes	-

Selenoprotein H	Transcription factor, present in nucleus, important in oxidative stress	-
Selenoprotein I	Phospholipid biosynthesis	-
Selenoprotein K	Localized in the endoplasmic reticulum (ER) at transmembrane level	-
Selenoprotein M (also called Sep 15)	ER localization, oxidoreductase	-
Selenoprotein N	ER localization, Calcium signaling, role in early muscle formation	Multiminicore diseases, muscular dystrophy
Selenoprotein O	ER localization, probable redox function	-
Selenoprotein P	Secreted and cytoplasmic, very abundant in plasma, Se transport, oxidative stress, metal detoxification	Cancer, neurodegenerative diseases (Alzheimer's disease)
Selenoprotein R	Cytoplasmic protein, reduction of methyl sulphoxy groups	-
Selenoprotein S	ER localization, removal of misfolded proteins	Inflammation responses
Selenoprotein T	ER localization, Calcium mobilization	-
Selenoprotein V	Expression testes-specific	-
Selenoprotein W	Oxidative stress	Highly dependent on dietary Se levels and SelP levels
SPS2: selenophosphate synthetase	Selenoproteins synthesis	Thyroid dysfunction

Table **1**: List of human selenoproteins with their functions and related disorders.

Human selenoproteins possess several functions ranging from protection against oxidative stress, to Se storage and transport, from SeCys synthesis to redox signaling [75, 82].

The evolutionary advantage related to the use of selenoproteins resides in the high performance of SeCys in catalysis [83]: the Se atom confers different properties rendering SeCys a stronger nucleophile than the less reactive Cys. For this reason selenoproteins can have a higher reaction rate with electrophilic substrates [84].

Glutathione peroxidase 1 (GPx1) was the first selenoprotein to be identified in humans [85], and, subsequently, the GPx family became one of the best characterized groups of selenoproteins. Today this family includes five isoenzymes in humans [58]. GPx family possesses a wide spectrum of antioxidant protection that depends on dietary Se levels, thanks to the variability in the physiological localization and substrate specificity of each member [86]. Apparently all of them share the catalytic mechanism of the GPx1 enzyme and the factors controlling or modulating their activity are not

known with certainty, even if all of them seem to share the same catalytic mechanism involving three conserved amino acids: selenocysteine, tryptophan (Trp157) and glutamine (Gln83) [87]. A mechanism of action has been proposed by Prabhakar and coworkers [88] and is described in figure 2. In the first step, hydrogen peroxide reduction is coupled with the oxidation of the selenolate anion (or selenol) to the selenenic acid. During the second reaction, the selenenic acid reacts with the substrate GSH to produce the selenosulfide adduct (E-Se-SG). In the third step, a second molecule of GSH attacks the seleno-sulfide adduct originating the disulfide GS-SG and regenerating the active form of the enzyme. GPx1 is one of the most abundant and ubiquitously expressed selenoproteins; it is a homotetrameric cytosolic enzyme very abundant in liver [58, 89, 90]. It is the most sensitive to changes in Se status: low Se concentrations can cause a decrease in its mRNA and protein levels [91]. GPx2 is principally present in the gastrointestinal tract, even if it is also detectable in liver [92]; its main role is to protect intestinal epithelium from oxidative stress. Another important member of this family is GPx3 that is the only one to be secreted constituting about 20% of the total Se in the plasma [93], even though this number may change depending on Se status [94]; for this reason it is considered a bioindicator of Se status correlated with nutrition. Disorders linked to this family of selenoproteins are cardiovascular diseases, cancer and epilepsy [95, 96, 97].

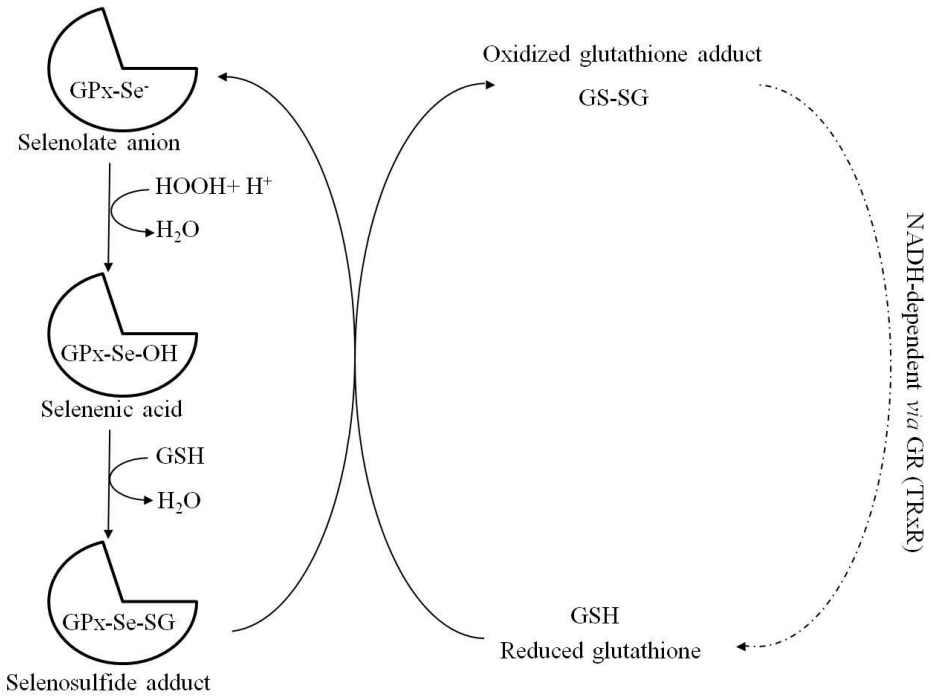

Figure 2: Simplified catalytic mechanism of the glutathione peroxidase: in the first step, SeCys is oxidixed by hydrogen peroxide to form a selenenic acid, which is then reduced again by glutathione.

Another important selenoprotein family is represented by thioredoxin reductase that use NADPH to catalyze the reduction of oxidized thioredoxin. TrxR belongs to a family of homodimeric pyridine nucleotide disulfide reductase [58]. In humans are known three TrxR: TrxR1 is present at cytoplasmic/nuclear level, TrxR2 is a mitochondrial protein with highest levels in the prostate, liver and small intestine, while TrxR3 is a thioredoxin-glutathione reductase testes-specific. TrxR1 and 2 are distributed in several tissues [98]. It has been reported that TrxR system is overexpressed in several tumors and cancer cell lines [99].

Also the deiodinase family consists of three isoforms: DIO1, DIO2 and DIO3. They are all membrane-bound enzymes sharing sequence homology and catalytic properties [100]. All three members are expressed in a number of fetal and adult tissues, where they may control the concentration of active thyroid hormone available to specific tissues or cell types at certain stages of development [101]. Disorders in this protein family are also correlated to Kaschin-Beck disease and may clearly lead to thyroid dysfunction, since the correct functionality of this gland depends on two trace elements, iodine and selenium, that are commonly low in Western diets. [102, 103].

A peculiar member of the human selenoproteome is selenoprotein P. SelP is unique since is the only member of the selenoprotein family containing multiple SeCys residues in its sequence per protein molecule. In humans it contains 10 SeCys residues. This is exactly the feature that suggests a role in selenium transport [97]. SelP represents the major selenoprotein in plasma, also providing more than 50% of the total plasma selenium [104]. Se derived from the diet is transported to the liver and used for SelP synthesis. SelP is then secreted into the plasma and delivered to target tissues where it enters the cells *via* a receptor-mediated mechanism. Within the cell, the degradation of SelP and of its 10 SeCys liberates free Se that can be subsequently employed for the synthesis of novel selenoproteins [105]. In conclusion, the bioavailability of Se for humans is not only related to the adequate amount ingested with the diet, but strongly depends on the correct action of SelP. Several studies have reported that SelP displays additional functions as highlighted by the presence of different domains having glutathione peroxidase activity, heparin and heavy metal ion binding capacity [106]. For example the first SeCys present at the N-terminus has been suggested to supply antioxidant capacity to cells [107]. SelP has been linked to several human diseases: in Alzheimer's disease, a co-localization of SelP with amyloid plaques was demonstrated [107]. This observation coupled with lower circulating SelP during inflammatory conditions like sepsis and Crohn's disease [108, 109] may have important implications for potential links between Se status, inflammation and neurological disorders.

The study of SeCys incorporation into bacterial selenoproteins is very complex and the knowledge of the evolution of Se utilization traits is still poor. In a study by Zhang and coworkers [110], 25 known bacterial selenoprotein families, comprising 285 selenoprotein sequences, were analyzed. The results indicated that FdhA (formate dehydrogenase) and SelD (selenophosphate synthetase) were the most widespread Se-proteins, indeed, at least one of these two proteins was present in each selenoprotein-containing microorganism. Moreover, in most selenoprotein families, SeCys-containing proteins are less represented than the corresponding Cys-containing homologs, suggesting a close evolutionary relationship between these proteins, even if, nowadays, is still not clear whether SeCys evolved from Cys or *viceversa*. In the same study, Deltaproteobacteria, Firmicutes/Clostridia and Actinobacteria resulted to be the three phyla containing the highest number of selenoprotein families: 22, 16 and 12 respectively. A common feature of the selenoprotein-rich organisms belonging to these three phyla is that they are mainly anaerobic or hyperthermophiles.

The first bacterial selenoproteins were described and analyzed in *E. coli*. These proteins are three molybdo-selenoprotein formate dehydrogenase: FdhH (associated with hydrogen production), FdhN (induced in the presence of nitrate) and FdhO (also detected during aerobic growth) [111]. They are all membrane-associated; FdhN and FdhO are highly related at both the amino acid sequence and functional levels. SeCys role is fundamental: it coordinates Molybden present in the pterin cofactor participating in the catalysis [112]. As already described, the formate dehydrogenase family is highly represented, being present in 24 different bacterial species [113]. In many of these microorganisms, Fdh was the only selenoprotein and its gene often flanked genes involved in SeCys insertion. Fdh catalyzes the oxidation of formate to CO_2 and is involved in energy metabolism.

Many other selenoproteins are present in bacteria; an example is the glycine reductase (GR) system. It is present mainly in clostridia where it catalyzes the reductive deamination of glycine to acetylphosphate and ammonia [114]. This system

has been studied in two anaerobic Gram positive bacteria, *Clostridium sticklandii* and *Eubacterium acidaminophilum* and in the Gram negative *Treponema denticola* [115]. The GR system is composed by three proteins called protein A, B and C [116]. Protein A is a 17-kDa protein containing a SeCys and encoded by *grdA* [117]; protein B derives from *grdB* encoding a 47-kDa SeCys-containing protein [118] and *grdE* which encodes a pro-protein of 48 kDa that will be post-translationally processed into two proteins of 25 and 22 kDa respectively [119]. Last, protein C is encoded by *grdC* (54 kDa) and *grdD* (40 kDa) [120]. Interestingly in most organisms, the genes involved in GR system form an operon that includes genes of the thioredoxin system. Another selenoprotein involved in this complex system is formate dehydrogenase, indeed formate represses the oxidation of glycine in favor of GR [121].

In strictly anaerobic bacteria several enzymes involved in antioxidant defense are present. Methionine sulfoxide reductase (Msr), as an example, reduces oxidized Met present in proteins. MrsA acts specifically on *S*-form of methionine sulfoxide, while MrsB on the *R*-form [122]. Interestingly both MsrA and MsrB can be or not selenoproteins, depending on the microorganism [123].

Selenophosphate synthetase (SPS) is a SeCys-containing protein identified in several bacterial species both Gram positive and negative. In *E. acidaminophilum* SPS was experimentally shown to possess a SeCys residue, even if the exact role of SeCys during the catalysis (synthesis of selenophosphate) is still not clear [124].

In a recent paper [125], the ability of a *Lactobacillus reuteri* strain, a probiotic lactic acid bacterium, to synthesize SeCys-containing proteins was demonstrated. The authors proved the selective insertion of SeCys residues within specific proteins (glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase, phosphoketolase, 6-phosphogluconate dehydrogenase, arginine deiminase, ornithine carbamoyltransferase and ribonucleoside hydrolase RihC), although it has not been elucidated if the insertion is genetically encoded. The same authors, in a previous work, revealed the expression of a selenocysteine lyase, involved in the metabolism of SeCys, suggesting once more the ability of the strain to metabolize Se introducing the element into proteins [126]. None of these proteins has been previously described as selenoprotein in other bacteria, opening new perspectives on the study of the “selenoprotein world” in probiotic Gram positive bacteria.

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