Selenoproteins*

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Selenium is an essential micronutrient for man and animals. The role of selenium has been attributed largely to its presence in selenoproteins as the 21st amino acid, selenocysteine (Sec, U). Sec is encoded by TGA in DNA. A unique mechanism is used to decode the UGA codon in mRNA to co-translationally incorporate Sec into the growing polypeptide because there is no free pool of Sec. In the human genome, 25 genes for selenoproteins have been identified. Selenoproteins such as glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases are involved in redox reactions, and Sec is an active-site residue essential for catalytic activity. Selenoproteins have biological functions in oxidoreductions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses. They thus possess a strong correlation with human diseases such as cancer, Keshan disease, virus infections, male infertility, and abnormalities in immune responses and thyroid hormone function.

Selenium was discovered by the Swedish chemist Jöns Jacob Berzelius in 1817 and has been recognized as an essential trace element for many life forms including man since 1957 (1, 2). The main form of selenium in mammalian proteins is selenocysteine encoded by the TGA codon. Sec is co-translationally incorporated within the growing polypeptide chain by an unusually complex machinery first described in Escherichia coli (3) and also well characterized in mammalian cells (1, 2). Sec differs from Cys by a single atom (selenium and also well characterized in mammalian cells (1, 2). Sec is encoded by TGA in DNA. A unique mechanism is used to decode the UGA codon in mRNA to co-translationally incorporate Sec into the growing polypeptide because there is no free pool of Sec. In the human genome, 25 genes for selenoproteins have been identified. Selenoproteins such as glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases are involved in redox reactions, and Sec is an active-site residue essential for catalytic activity. Selenoproteins have biological functions in oxidoreductions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses. They thus possess a strong correlation with human diseases such as cancer, Keshan disease, virus infections, male infertility, and abnormalities in immune responses and thyroid hormone function.

Selenoproteins exist in archaea, bacteria, and eukaryotes with Sec as a key catalytic group, but not in all species in these kingdoms. Yeast and higher plants do not have selenoproteins and components of the selenoprotein synthesis machinery; instead, they express cysteine-containing homologues (7, 8). Mammalian selenoproteins can be classified mainly into two groups according to the location of Sec (Fig. 1) (9). One group of selenoproteins possesses Sec in a site very close to the C terminus of protein, such as TrxRs and selenoproteins S, R, O, I, and K. The other group (including GPxs; DIOs; selenoproteins H, M, N, T, V, and W; SPS2; and Sep15) has Sec in the N-terminal part and in most cases possesses thioredoxin fold structure, and some selenoproteins contain a CXXU motif, corresponding to the thioredoxin active-site CXXC motif (10–13). From their structure, it is apparent that the functions of most selenoproteins should be involved in the redox-related reactions. In fact, the transcription of several selenoproteins such as TrxR1 and GPx2 is regulated by the redox-sensitive transcription factor Nrf2/Keap1 system (14, 15). Moreover, severe oxidative stress induces nuclear accumulation of SBP2 and blocks Sec incorporation (2, 16). The two main protein thiol reduction systems, thioredoxin and glutathione-glutaredoxin, are potential electron donors for oxidized SBP2 and may thus protect the cells by regulating selenoprotein synthesis via controlling SBP2 redox state and trafficking (16). The pre-
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Selenoproteins can be divided into two groups according to the location of Sec. Sec is located very closely to the C terminus of one group of selenoproteins such as TrxRs, SelS, SelR, SelO, and SelL. The other group has Sec in the N terminus and normally possesses the Trx fold structure. In most of these selenoproteins, Sec is located in the pocket Trx fold of the substrates (Fig. 2).

Thioredoxin Reductases—TrxRs in mammalian cells are members of the pyridine nucleotide-disulfide oxidoreductase family, and three TrxRs have been identified in mammals: TrxR1 in the cytosol/nucleus (17, 18), TrxR2 in mitochondria (19, 20), and thioredoxin glutathione reductase in the testis (21), with the latter also possessing glutathione and glutaredoxin reductase activity (Fig. 1) (21, 22). The three-dimensional crystal structure of TrxR is similar to that of glutathione reductase with FAD- and NADPH-binding domains and an interface domain (23). Besides the N-terminal -Cys-Val-Asn-Val-Gly-Cys-dithiol/disulfide, mammalian TrxRs contain a 16-residue C-terminal elongation with the conserved active-site sequence -Gly-Cys-Sec-Gly-OH (24). The two subunits in active homodimeric TrxR have a head-to-tail arrangement, and the N-terminal redox-active dithiol in one subunit and the C-terminal selenothiol active site of the adjacent subunit form a redox-active center (23). The proposed mechanism of mammalian TrxR involves the conserved active-site sequence -Gly-Cys-Sec-Gly-OH, which is essential for DNA synthesis by converting ribonucleotide to deoxyribonucleotides. The exact role of Trx and glutaredoxin systems for ribonucleotide reduction in mammalian tissues is yet unknown. Trx can also reduce methionine-sulfoxide reductase and thioredoxin peroxidase (peroxiredoxin) and thus is involved in the repair of methionine sulfide-oxidized proteins or redox signaling via hydrogen peroxide (26, 27). In addition, the Trx system participates in many cellular signaling pathways by controlling the activity of transcription factors containing critical cysteines in their DNA-binding domains, such as NF-kB, AP-1, p53, and the glucocorticoid receptor (28). It is also known that reduced Trx can bind to and inhibit ASK1 (apoptosis signal-regulating kinase 1), whereas the oxidation of Trx results in the activation of ASK1 and the induction of ASK1-dependent apoptosis (28). Therefore, TrxRs are involved in the control of cellular proliferation, viability, and apoptosis through the control of Trx activity and redox state. The discovery of mammalian TrxR as a selenoprotein (17, 18) occurred after its identification as a selenite-reducing activity (5, 6) and was a big surprise because bacterial and plant TrxRs are smaller specific Cys-containing enzymes of entirely different structures and functions (28).

Glutathione Peroxidases—GPxs are well known to be the major components of human antioxidant defense. In humans, there are now five Sec-containing GPxs: the ubiquitous cytosolic GPx (GPx1), the gastrointestinal-specific GPx (GPx2), the plasma GPx (GPx3), the ubiquitous phospholipid hydroperoxidase GPx (GPx4), and the olfactory epithelium- and embryonic tissue-specific GPx (GPx6) (9). GPx1–3 catalyze the reduction of hydrogen peroxide and organic hydroperoxides, whereas GPx4 can directly reduce phospholipid and cholesterol hydroperoxides. GPx4 is also involved in sperm maturation and male fertility because it has been found to be a main structural component of the sperm mitochondrial capsule in mature spermatozoa as an enzymatically inactive, oxidatively cross-linked, insoluble protein (29).

GPx1–3 are homotetrameric proteins with a subunit molecular mass of ~22–25 kDa, whereas GPx4 is a 20–22-kDa monomeric enzyme. All known GPx subunit structures exhibit the typical structure motif of the Trx fold (30–32). The core structure of the GPx subunits thus consists of central β-strands surrounded by several α-helices (Fig. 1). One
helix connects an antiparallel β-strand to a neighboring β-strand and forms a βββ-substructure. The catalytically active Sec is normally located at the N-terminal end of the helix. The conserved catalytic triad of all these GPxs contains Sec, Gln, and Trp. The catalytic redox cycle involves the oxidation of Sec to selenenic acid by hydrogen peroxide and organic hydroperoxides and reduction to the selenolate anion form by the GSH system (Fig. 2B) (33).

Iodothyronine Deiodinases—Three DIOs have been identified with a tissue and subcellular localization (34). DIO1 is found primarily in the liver, kidney, and thyroid; DIO2 is in the brain, pituitary, thyroid, skeletal muscle, and brown adipose tissue; and DIO3 is found in the cerebral cortex and skin and is expressed at a very high level in the placenta and pregnant uterus. DIO1 and DIO2 catalyze the deiodination of T4, the major thyroid hormone secreted by the thyroid gland, into the active hormone T3; DIO3 converts T3 into reverse T3 and also T3 into 3,3′-diidothyronine. DIO1 and DIO2 can also convert reverse T3 into 3,3′-diidothyronine (34, 35).

All three DIOs are integral membrane protein of 29–33 kDa and share significant structural similarity; they belong to the Trx fold protein family and contain a glycoside hydrolase clan A-like structure (Fig. 1). DIO1 and DIO3 are localized at the plasma membrane, whereas DIO2 resides in the ER membrane. A common striking feature is the presence of an active center in the pocket Trx fold βββ-motif (Fig. 1). The proposed deiodination mechanism involved is the generation of an oxidized DIO-Sel intermediate, and thus, the intermediate is reduced by thiol-containing reductants and releases iodide (36). The identity of the DIO reductant is currently unknown, with the Trx and glutaredoxin systems as prime candidates.

Some of the other selenoproteins have been cloned and characterized. For example, Sep15 is also a member of the Trx-like fold superfamily of proteins, highly homologous to the ER protein-disulfide isomerase and containing a surface-accessible, Sec-containing, redox-active motif (-CXX-). Sep15 has been proposed to be involved in the folding of the ER, with a similar role as protein-disulfide isomerase. SelR (MsrB1 (methionine-R-sulfoxide reductase B1)) catalyzes the reduction of oxidized Met residues (methionine sulfoxides). The oxidation of protein methionine occurs under oxidative stress and can lead to protein damage. SelR was shown to specifically reduce methionine R-sulfoxides, and the Sec residue is crucial for enzymatic activity (37). Selenoprotein P is a selenoprotein with multiple Sec residues per protein subunit, 10 Sec residues in all, and is present in human plasma. The main role of SelP is the transport and
Physiological and Pathophysiological Functions of Selenoproteins

The physiological and pathological effects of selenoproteins are closely related to selenium status. Selenium deficiency leads to a dramatic loss of activity of selenoproteins, including TrxRs, GPxs, and DIOs. However, the expression of selenoproteins exhibits a hierarchical style during selenium deprivation and repletion; the significance of specific selenoproteins in the specific tissues may determine the priority of the mRNA level and protein expression (39). For example, under selenium-deficient conditions, the activities of most selenoproteins in the liver, kidney, and lung decrease, whereas selenoprotein activities in the brain remain at a level similar to that during normal selenium supplementation. Whereas selenium deficiency leads to a rapid decrease of GPx1 mRNA and protein levels in the liver, phospholipid hydroperoxide GPx and TrxR mRNAs are kept at higher levels (39).

One example of severe selenium deficiency causing a human disease is Keshan disease, a potentially fatal form of cardiomyopathy that was first found in northeast China (1). The disease occurs upon selenium deficiency combined with infection by coxsackie B virus and has been prevented by selenium supplementation. A cardiomyopathy that resembles Keshan disease occurs when GPx1 knock-out mice are infected with a benign coxsackievirus, suggesting that GPx1 is closely associated with protection against virus infection (40).

Another good example for the purpose of viewing the overall selenoprotein function in man is the SBP2 mutation (41). The defect in SBP2 induces a global decrease in selenoprotein activity and results in abnormal thyroid hormone metabolism. The amino acid substitution Rs40Q resulted from a homozygous missense mutation and produced an abnormal thyroid phenotype associated with a reduction in DIO2 enzymatic activity and prepubertal growth retardation (41). This mutation led to elevated total T4, free T4, and reverse T3 metabolites but low total and free T3 in three children of a Bedouin Saudi family. Another child from an unrelated family exhibits similar abnormal thyroid metabolism phenotypes and has a genetically prematurely terminated SBP2 protein lacking the C-terminal domain (41).

More specific roles of selenoproteins have been revealed by recent investigations using gene knock-out techniques and by several mutant selenoprotein human disease cases. Mutations of selenoprotein N cause rigid spine muscular dystrophy and the classical phenotype of multiminicore disease (42, 43). One promoter genetic variation in selenoprotein S impairs SelS expression and influences the production of inflammatory cytokines such as tumor necrosis factor-α, interleukin-6, and interleukin-1β, suggesting that the selenoprotein SelS is involved in regulating inflammation (44). At least three selenoproteins TrxR1, TrxR2, and GPx4 are involved in embryogenesis because deletions of the genes for these proteins in mice result in embryonic death. All GPx1, DIO1, and DIO2 knock-out mice grow, develop, and reproduce normally under laboratory conditions (40). However, GPx1 knock-out mice are more sensitive to paraquat- and H2O2-induced oxidative stress.

DIO2 knock-out mice have impaired auditory function and thermogenesis as well as mild brain function defects and temporary growth retardation (40). DIO1 knock-out mice have abnormal excretion patterns of thyroid hormone metabolites, including iodide. The DIO3 knock-out model exhibits reduced viability, significant growth retardation, impaired fertility, and hypothyroid symptoms with significantly reduced T3 and increased T4 levels (40). These studies reveal that selenoproteins play critical roles in antioxidant defense, fertility, thyroid hormone metabolism, immune responses, and muscle development and functions.

Selenoproteins have been believed to be closely linked with cancer and carcinogenesis because there are numerous epidemiological reports on an inverse correlation between selenium intake and occurrence of cancer risk (for review, see Ref. 2). However, the exact mechanism of how selenium intake protects against cancer is unknown. Large-scale clinical trials with supplementation against prostate cancer are under way (2). As described above, many selenoproteins are involved in the antioxidant reaction and can thus participate in the protection of normal cells against oxidative stress. On the other hand, when a normal cell turns into a tumor cell, selenoproteins in the tumor switch their role to protect the malignant phenotype. It is therefore not surprising that TrxR and Trx have been found to be overexpressed in many aggressive tumors. Moreover, the tumor cell may require enough electrons from the Trx system for ribonucleotide reductase to keep up a constant DNA synthesis. Thus, the Sec-containing mammalian TrxRs have emerged as new targets for anti-cancer drug development (45, 46). Selenol has a low pKa value, and Sec in the open C-terminal -Gly-Cys-Sec-Gly active site of TrxR makes the C terminus easily susceptible to attack by electrophilic agents. Many anti-cancer compounds, including clinical drugs such as the alkylating agents cisplatin, cyclophosphamide, and arsenic trioxide, have been shown to be strong inhibitors of mammalian TrxR (46, 47), and other various potent anti-cancer compounds such as curcumin, myricetin, and quercetin strongly and irreversibly inhibit mammalian TrxR (48, 49). The elucidation of TrxR and Trx roles in cancer biology may yield some promising anti-cancer paradigms for new cancer therapeutic agent development.

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doi: 10.1074/jbc.R800045200 originally published online August 29, 2008

Access the most updated version of this article at doi: 10.1074/jbc.R800045200

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