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The glutathione peroxidases

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Abstract. There are several proteins in mammalian cells that can metabolize hydrogen peroxide and lipid hydroperoxides. These proteins include four selenium-containing glutathione peroxidases that are found in different cell fractions and tissues of the body. This review considers the structure and distribution of the selenoperoxidases and how this relates to their biological function. The functions of the selenoperoxidases were originally studied in systems where their activity was manipulated by changing dietary selenium levels. More recently, molecular techniques have allowed overexpression of selenoperoxidases in cell lines and animals. Additionally, cellular glutathione peroxidase knockout mice have been used to investigate the functions of this protein. From this work it is clear that the selenoperoxidases are involved in cell antioxidant systems. However, they also have more subtle functions in ensuring the regulation and formation of arachadonic acid metabolites that are derived from hydroperoxide intermediates. The range of biological processes, which are potentially dependent on optimal selenoperoxidase activity in mammals, emphasises the importance of achieving adequate selenium intake in the diet.

Key words. Selenium; glutathione peroxidase; peroxides; overexpression; knockouts.

Introduction

Since 1973, when Rotruck et al. [1] suggested that selenium was a component of glutathione peroxidase and Flohe and co-workers [2] showed that the purified enzyme contained one selenium per subunit, there have been many thousands of publications which have included information on glutathione peroxidases (GPXs). For example, a search of databases from 1981 to 1999 generates 4700 'hits' with the search term 'glutathione peroxidase'. Many of these papers include the measurement of GPX activity in different disease states or as a measure of response to selenium supplementation. In addition, many of the papers report GPX activities as components of antioxidant systems. It is not my intention in this review to address such work, as it is often specific to unique biological problems. I intend to review the structure and properties of the different GPXs and studies into the control of expression and functions of the different enzymes.

Types of selenium-containing GPX

There are four known GPXs which contain selenocysteine at the active site. Additionally, there are at least two other proteins with over 40% sequence identity to cytosolic GPX, but which do not contain selenocysteine. Proteins similar to GPX also occur in some microorganisms and parasitic worms. Two types of malarial parasites contain GPX activity that can be regulated by selenium supply, in a similar way to many mammalian systems [3]. This review will, however, concentrate on the mammalian forms of GPX and their biological functions [4]. In addition to the selenium-containing GPXs and the proteins which contain cysteine and have similar structures to the GPXs, there are many selenium-independent GPX activities in mammalian systems. These activities are mainly associated with the glutathione-*S*-transferases a family of enzymes with the activity directed mainly towards organic hydroperoxides and very little activity against hydrogen peroxide [5, 6].

Mills first described GPX activity in 1957 [7], and its function was hypothesized to be protection of red blood cells against haemolysis by oxidation. This enzyme, originally called glutathione peroxidase, has been called classical GPX and is now generally called GPX-1 [EC 1.11.1.9]. In some publications this enzyme has also been called cytosolic or cellular GPX. GPX-1 can metabolize hydrogen peroxide and a range of organic peroxides, including cholesterol and long-chain fatty acid peroxides [4]. However, unless accompanied by phospholipase A2 activity to release the fatty acids, GPX-1 cannot metabolize fatty acid hydroperoxides in phospholipids [8]. GPX-1 can interact with a wide range of organic hydroperoxides as well as hydrogen peroxide. Despite this range of peroxide substrates, it is very specific for glutathione as reducing substrate. Thus GPX-1 activity is often discussed in parallel with glutathione reductase activity, which maintains a constant supply of GSH from GSSG for enzyme activity.

GPX-1 has been sequenced, either directly or through complementary DNA (cDNA) cloning in a range of species including human, rat, mouse, rabbit, cattle and sheep [9]. In all species examined GPX-1 is a tetrameric protein with four identical subunits, each of which contains one selenocysteine residue in a total molecular weight of 22–23 kDa. The three-dimensional structure of bovine GPX-1 shows that it contains four spherical subunits, each with a selenocysteine residue in a depression in the surface [10]. The proximity of each selenocysteine to an adjacent subunit suggests that the active site of the enzyme relies on both subunits. Initially, chemical derivatization was used to show that selenocysteine is at the active site of the enzyme. In common with other selenium-containing proteins, replacement of selenocysteine with cysteine at the active site of glutathione peroxidase causes a large decrease in enzyme activity [11]. This is consistent with selenocysteine being a much more efficient redox catalyst than cysteine at physiological pH.

A second form of GPX which occurs in the cytosol and is tetrameric has been called gastrointestinal or GPXG1. This protein is now called GPX-2 and has approximately 65% amino acid sequence identity and 60% nucleotide sequence identity with GPX-1 [12]. Both GPX-1 and GPX-2 have similar substrate specificity in that they reduce hydrogen peroxide or fatty acid hydroperoxides rapidly but not phospholipid hydroperoxides. In the rat, GPX-2 messenger RNA (mRNA) is found mainly in the gastrointestinal tract; however, in humans the mRNA can be found in liver and large intestine but not in other organs [12].

After the discovery of GPX-1 it was considered for many years that GPX activity in plasma was due to leakage of the enzyme from the liver and different organs. Furthermore, plasma GPX activity increased with increasing selenium status and decreased with lower selenium status, consistent with the liver being postulated as the major source of the activity. Additionally, the plasma GPX activity had similar substrate specificities to GPX-1. However, plasma GPX (GPX-3) did not react with antibodies to GPX-1 originally purified from red blood cells [13] that would precipitate red blood cell or liver GPX-1. This led to attempts to purify GPX-3 from human plasma, work which showed that the protein is an isozyme of GPX with distinct properties. GPX-3 is a glycoprotein consistent with an extracellular function [14]. The partial sequencing of the protein and then its cDNA confirmed that GPX-3 is distinct from GPX-1. The cDNA of GPX-3 has been sequenced for many species, including human, rat, mouse and bovine. These studies predict subunit molecular weights of approximately 23–25 kDa with between 40 and 50% homology with human GPX-1 [15]. The mRNA for GPX-3 is found predominantly in kidney, in particular the epithelial cells of the proximal tubules [16]. A number of other cell types have been proposed to produce GPX-3 based on the presence of mRNA or immunological identification of the protein. These include heart, placenta, lung, gastrointestinal cells and thyroid [17–20]. In addition, the presence of GPX-3 in milk suggests its synthesis within mammary cells. Many cell lines, including HepG2 cells and a human adenocarcinoma line, Caco-2, synthesize GPX-3, and this originally led to a hypothesis that it originated in liver [21, 22]. Since GPX-3 requires millimolar concentrations of glutathione for activity, there have been questions as to whether it could function as a peroxidase in plasma (glutathione concentrations of ~ 0.5 μm). However, thioredoxin reductase, the thioredoxin system or glutoredoxin can also act as electron donors for human GPX-3 at concentrations which are relevant to plasma [23]. This increases the potential of the enzyme to act as an extracellular antioxidant.

GPX-4 or phospholipid hydroperoxide GPX is the fourth selenium-containing GPX which has been characterized. This is a 20–22 kDa protein that contrasts with GPX-1 in that it is a monomer that can react with phospholipid hydroperoxide as substrate. GPX-4 was first purified in 1982 by Ursini and co-workers and described as a 'peroxidation-inhibiting protein' [24]. The protein was unlike GPX-1 in it could use phospholipid hydroperoxides as substrates. However, it was not until 1985 that this protein was confirmed to contain selenium [25]. There are many differences between GPX-4 and the other GPXs. The major structural difference is that GPX-4 is a monomer in contrast to the tetrameric structure of the other GPXs [26].

As well as phospholipid hydroperoxides, GPX-4 can use hydrogen peroxide as substrate as well as a wide range of other lipid hydroperoxides. The fact that GPX-

4 is a monomer may allow it to bind with the wider range of substrates than the tetrameric GPXs [27]. In contrast to GPX-1 and GPX-2, GPX-4 can also use a wide range of reducing substrates as well as glutathione [28]. Some authors have argued, therefore, that GPX-4 is not a 'glutathione' peroxidase. However, all the GPXs have a selenocysteine at the active site, which is successively oxidized and then reduced during catalytic cycles. Additionally, there are tryptophan and glutamine residues that are conserved in all the GPXs [29]. Thus, they are clearly a family of similar enzymes. Like GPX-1, replacement of the selenocysteine at the active site of GPX-4 with cysteine dramatically decreases catalytic activity [30]. This catalytic activity with phospholipid hydroperoxides is stimulated by addition of detergents, which has led to belief that the enzyme is membrane associated in vivo [25].

Cloning GPX-4 showed it to be a polypeptide of 170 amino acids with a theoretical molecular weight of approximately 19 kDa. The sequence identity of GPX-4 is between 30 and 40% of GPX-1, dependent on the species being compared. Like the other GPXs in the 3′ untranslated region of the mRNA, there is a selenocysteine insertion sequence (SECIS) element which directs the incorporation of selenium into the protein at the UGA triplet within the coding region. Within the amino acid sequence of GPX-4 there is a tyrosine which is thought to be phosphorylated, but the role of this in control of enzyme activity is not known. In the full-length GPX-4 gene there are 7 exons and several hormone-responsive elements in the 5′ untranslated region which may be related to the function of PHGPX in the testis [31–33].

Some studies in which GPX-4 was purified from soluble and membrane fractions of testis indicated that there may be two forms of the enzyme [34]. The full-length cDNA clone of GPX-4 has alternative start sites, which can code for proteins of 197 or 170 amino acids. The differences between the two potential forms are 27 amino acids at the N terminal region. These 27 amino acids are a mitochondrial targeting sequence [35]. This would indicate the protein functions within the mitochondria to prevent damage to phospholipid molecules in the oxidizing environment.

GPX-like proteins

Using molecular biological techniques, some proteins which are very similar to the selenoglutathione peroxidases have been identified. An epididymis-specific GPX (GPX-5) has been demonstrated in rats, mice, pigs, monkeys and humans. Cloning these proteins shows them to be distinct from the other GPXs, with ~ 60–70% identity to the GPX-1 coding region. A major difference is that cysteine is retained at the active site of GPX-5 [36–41]. The purified protein contains no selenium, and its GPX activity towards hydrogen peroxide or organic peroxides is less than 0.1% of that of GPX-1. This low activity, coupled with very low glutathione concentrations in the epididymal fluids has led to the suggestion that this protein protects sperm by binding organic hydroperoxide which would otherwise induce an acrosome reaction. However, expression of GPX-5 may be very low in human sperm due to incorrect splicing of transcripts [40]. The human sperm with very low levels of GPX-5 were not prone to a premature acrosome reaction; therefore, the function of this protein must remain a matter for debate.

An odourant metabolising protein (GPX-6) has been sequenced and has a 40% amino acid sequence identity to GPX-1, with cysteine in place of selenocysteine. It is found in the Bowman's gland of the olfactory system. There is still uncertainty as to its biological function [42].

Non-selenium-dependent GPX

The family of glutathione-*S*-transferases has many members which have GPX activity. This activity is against organic hydroperoxides, and thus when hydrogen peroxide is used as substrate, the activity is not measured. Although the K_m's for hydroperoxide substrates of glutathione-*S*-transferases are higher than those for the GPXs, the transferases may still play an important role in protection against oxidative stress by mechanisms involving conjugation of aldehydes and other potentially oxidative compounds. These properties of the GSTs have been comprehensively discussed in a recent review [6].

Functions of the GPXs

Many of the pathological consequences of selenium deficiency were originally associated with what was hypothesized to be oxidative damage to tissues. Thus, it was considered that loss of GPX-1 activity, particularly when vitamin E levels were low, would result in peroxide-induced membrane damage and pathogenesis [43]. Although this hypothesis was credible, much subsequent work has shown that the functions of the GPXs are much more subtle than a blanket protection against oxidative stress [44–47]. The existence of four selenoGPXs in different cell compartments immediately implies specific functions for each of the isozymes. After the discovery that GPX-1 was a selenoenzyme in 1973, several studies showed that in very severe selenium deficiency, where tissue levels of GPX in experimental animals fell to less than 1% of control, there was no

evidence of any oxidative tissue damage or pathology [45, 46, 48]. These findings should always be remembered when epidemiological studies report very small percentage changes in blood GPX activities in patient groups and then extrapolate this to an oxidative mechanism for disease pathogenesis. The recognition that large changes in tissue GPX activity were not always associated with oxidative damage in selenium deficiency was also very important in indicating the need to recognize and identify other selenoproteins and functions for selenium.

Manipulation of selenium status of experimental animals by dietary means and association of biochemical changes with changes in GPX activity has, however, provided some insights into potential metabolic roles of selenium [49, 50]. For instance, in selenium-deficient rats there is evidence for increased concentrations of isoprostane F2α, a product of peroxidative degeneration of eicosanoids [51]. In addition, by manipulating GPX activities there is abnormal production of several eicosanoid metabolites which have been associated with inappropriate survival of peroxide intermediates in the formation of the compounds [52–54]. In some cases the compounds formed in selenium-deficient animals would favour the onset of tissue damage associated with heart disease. The study of selenium deficiency and regulation of the expression of the GPXs has also provided useful insights into the control of selenoprotein expression. In particular, differences between tissues have provided further ideas for potential functions for GPXs [50]. In the brain and some endocrine tissues during selenium deficiency, GPX (GPX-1 and GPX-4) activities are much less affected than are GPX activities in liver, kidney, muscle and so on [50, 55, 56]. This indicates that the GPXs may have a more important function in neural and endocrine tissues due to preservation of activity during low selenium status. Despite such hypotheses, failure to associate very large changes in GPX-1 activity in tissues of experimental animals with any pathology has led some authors to conclude that GPX-1 may be more important as a buffer or storage form of selenium than as a glutathione peroxide [44, 57].

In the light of evidence from dietary studies, the suggestion that GPX-1 was a storage form of selenium was quite plausible. However, more recently molecular techniques have provided insights into the functions of GPX-1 and GPX-4 and highlighted the specificity of their antioxidant functions. These molecular techniques have included overexpression of GPX-1 in cell lines and in mice and the production of GPX-1 knockout mice. Some workers have also examined the effects of overexpression of GPX-4 in cell lines. Similar data are as yet unavailable on GPX-2 and GPX-3, although studies on the location of expression of mRNAs for these GPXs and the control of such expression have provided insights into their function. These studies which provide new perspectives into the functions of the seleno-GPXs are discussed below.

Over-expression of GPX-1 in cells and animals

Within the normal nutritional range of selenium intake, GPX-1 activity in cells and mammalian tissues is very closely regulated. The mechanisms of this regulation have been reviewed and are generally thought to involve the instability of GPX mRNA at low selenium intakes [50, 58]. However, as selenium intake increases, GPX-1 activity plateaus and does not increase further. A problem in assigning biological function to GPX-1 by analyzing effects of low selenium intake is that this also compromises the other GPXs and activities of other selenoproteins. One approach to overcome these problems was to use transfection of cell lines with constructs, which express GPX-1 and thus specifically overproduce the protein. Many of these studies show that overexpression of GPX-1 can protect cells against reactive oxidant species, for example, damage caused by hydrogen peroxide and lipid hydroperoxides and redox cycling drugs such as paraquat [59, 60]. However, overexpression of GPX-1 can also inhibit hydrogen peroxide-induced apotosis in cell lines. Use of a construct to increase GPX-1 expression by four- to fivefold in human T cells has also been proposed as a model for examining the effects of selenium in human immunodeficiency virus (HIV) infection [61]. A drawback in many of these overexpression studies is that GPX-1 activity may be 10–100 times greater than in the parent cell lines [62]. Whereas this treatment may confer protection against peroxide-induced damage, this process is unlikely to be physiologically relevant and may have deleterious effects on other cell systems. Cellular reduced glutathione could be depleted by the excess demands of increased GPX-1 activity, with consequent disruption of other biochemical pathways. However, as well as increased resistance to cell damage, overexpression of GPX-1 decreases hydrogen peroxide or tumour necrosis factor-α induced NFκB activation in human T47D cell transfectants [63]. The effect is associated with lower reactive oxygen species levels and not by changes in NFκB subunits P65 and P50 or the inhibitory subunit IκB. The effects of overexpression of GPX-1 on NFκB are reversed by selenium deficiency in the cell line. These studies emphasize the importance of GPX-1 in modulation of reactive oxygen species and thus transcription factor activation. Hence, activation of GPX-1 by treatment with fatty acids may be important in redox regulation of transcription factors in cells [64].

In general, overexpression of GPX-1 in cell lines has been interpreted as having beneficial effects on cells, particularly when they are subjected to reactive oxygen species. However, overexpression of GPX-1 (and GPX-3) in animals cannot be said to be beneficial. Transgenic mice which overexpress either GPX-1 or GPX-3 by 1.3–4-fold in different tissues are less able to produce heat shock protein 70 in response to exposure to 40 °C environmental temperature [65]. In addition, the prostaglandin PGE2 was not decreased in the brain of the transgenic animals in response to increases in body temperature. Thus, if hyperthermia leads to oxidative stress in regions of the brains in normal animals, the transgenic animals overexpressing GPX-1 or GPX-3 do not have the normal responses, which include induction of cellular protective systems. GPX-1 may therefore be involved in regulation of normal levels of peroxides within mammalian cells, and disruption of this system by creating levels of enzyme activity that are normally encountered may have deleterious effects [65]. Another deleterious effect of high expression of GPX is enhanced skin carcinogenesis. Transgenic mice with a 100% increase in the expression of GPX-1 have an increased carcinogenic response to initiation by 7,12-dimethylbenz[a]anthracene (DMBA) followed by promotion with a phorbol acetate. The transgenic mice had 2.5 times the number of tumours of control mice, with a 100 vs. 83% tumour incidence [66]. After phorbol ester application was stopped, regression of tumours occurred more rapidly in the control mice and overall was twice that of the transgenic mice. Again, overexpression of peroxide-metabolizing GPX-1 may have decreased cell peroxide levels and thus impaired the resistance to the carcinogenic challenge. In another study mice overexpressing either human GPX-1 or GPX-3 were challenged with acetaminophen (paracetamol). Overexpression of GPX-3 increased resistance to toxicity of the drug, an effect which was mirrored by intravenous injection of GPX in nontransgenic mice. In contrast, however, mice which overexpress GPX-1 in the liver were more susceptible to acetaminophen toxicity compared with nontransgenic animals [67]. This effect was hypothesized to be an inability of the transgenic animals to replace glutathione that was depleted due to the drug toxicity. The work emphasizes that the cellular and tissue location of the different GPXs are critical for their biological functions. The apparently deleterious effects of overexpression of GPX-1 should not be surprising, given that reactive oxygen species are involved in cell-signalling mechanisms, and therefore GPXs are likely to be essential for normal regulation of the process.

These adverse effects of GPX overexpression are apparent in clearly defined circumstances. Other work on overexpression indicates no deleterious effects, and in some cases advantage to the animal. Mice overexpressing GPX-1 have been fed diets containing different levels of selenium and then compared with control animals. The transgenic mice expressed between 1 and 6 times more GPX-1 activity than the control animals at high or low dietary selenium intakes in all tissues with the exception of liver, when differences in activity were only apparent in selenium-deficient animals [68]. Significantly, the GPX-1 overexpressing mice have similar GPX-3 and GPX-4 activities to the control animals [68]. This shows that in experiments using such animals any effects can be attributed to changes in GPX-1 activity. However, although this may give clues as to the function of GPX-1 under specific circumstances, in selenium deficiency all selenoprotein activities may change with consequent modulation of the effects of alterations in GPX-1 [50]. The ability to induce GPX-1 may be important in the resistance to some stresses. In transgenic mice in which GPX-1 activity is under the control of a metallothionein IIA promoter, no significant changes occur in skin GPX activity. However, when the animals are treated with the tumour promoter phorbol ester, GPX-1 activity does not decrease in the skin as it does in control mice [69]. This model, in which there is an increase in potential for induction of GPX-1, may provide a more realistic means of assessing the biological function of the protein than direct overexpression of the protein. Although overexpression of GPX-1 may prevent some beneficial effects of reactive oxygen species, in other situations the damaging effect of these molecules is prevented. GPX-1 overexpression protects mouse brain from a model of cerebral ischaemia. This protection included a 48% decrease in infarction size and a 33% decrease in brain oedema [70]. These effects were postulated to be due to direct scavenging of reactive oxygen species. Similarly, GPX-1 overexpression protects neurons from impairment of energy metabolism caused by malonate, an inhibitor of succinate dehydrogenase. Thus the transgenic animals were protected against loss of brain dopamine after infusion of malonate [71]. Over-expression of human GPX-1 can also protect mice against the neurotoxin 6-hydroxydopamine. Loss of dopaminergic neuron being decreased from 52.4 to 20.5% and dopamine decreased from 71.2 to 56.5% by the transgenic manipulation. Cell damage due to ischaemia in the heart is also decreased in mice which overexpress GPX-1. In this case a fourfold increase in heart GPX protected against 30 min of ischaemia with 20 min of reperfusion. In this system the transgenic hearts had better recovery of contractile force and greater contraction compared with controls [72]. In addition, infarct sizes were decreased and release of creatine kinase (a muscle enzyme) was significantly decreased.

All these overexpression studies stressed the importance of GPX-1 in the metabolism of reactive oxygen species, whether this is beneficial or deleterious to the animal.

GPX-1 'knockout' models

The gene knockout technique has been used to explore the effects of loss of GPX-1 activity to elucidate the function of the protein. Again this model has a caveat that the complete loss of GPX-1 activity may not be comparable with that which occurs in low-selenium status. In low-selenium status other selenoprotein activities will also decrease. Thus, the loss of the other peroxidase activities may well also compromise metabolic systems which are dependent on GPX-1. In the knockout animal these other GPXs may be able to compensate for the loss of the GPX-1. Thus, mice made GPX-1 deficient using knockout technology were healthy and showed a normal phenotype in the absence of stress [73]. When these animals were subjected to hyperoxia, they had no increased levels of products of lipid or protein oxidation when compared with normal, GPX-1-expressing mice. The platelets from the knockout mice were, however, hypothesized to have decreased ability to metabolize 12-hydroperoxyeicosatetraenoic acid produced from arachidonic acid. In another study, knockout of GPX-1 also had no effect on body weight or health of mice. In addition, there was no effect on susceptibility to dietary vitamin E or selenium deficiency [74]. Therefore, as in overexpression of GPX-1, there was no influence of knockout technology on expression of GPX-3 or GPX-4. This suggests a very protein-specific nature of selenoprotein metabolism distinct from changes caused by variation in dietary selenium.

Although the GPX-1 knockout mouse had no obvious deleterious phenotype, the animals have increased susceptibility to different oxidative stresses when compared with normal mice. Hearts from the knockout mice were more susceptible to myocardial ischaemia reperfusion injury, the opposite to the protective effect of GPX-1 overexpression. After 30 min of ischaemia followed by 2 h of reperfusion, hearts from knockout mice had lower developed contraction force than normal controls. Less of the muscle enzyme creatine kinase was released into perfusates from the control hearts than those of the knockouts [75]. Expression of GPX-1 therefore seems to be important in protection against ischaemia reperfusion injury. Similarly, like selenium-deficient mice, GPX-1 knockout mice are susceptible to the stress caused by very high levels of paraquat. Whereas GPX-1 overexpression causes some protection against the redox cycling caused by the herbicide, the lethality of paraquat increases in two different GPX-1 knockouts. Additionally, another herbicide, diquat, which also causes superoxide formation from oxygen, is also lethal in GPX-1 knockouts [76, 77]. These experiments are difficult to reconcile with work with selenium-deficient rats, which showed that protection against diquat occurred after small doses of selenium which did not apparently change GPX-1 activity [78, 79]. This again emphasizes that knockout animals may not be entirely comparable with selenium-deficient animals, as a single enzyme change is being compared with multiple enzyme modulations. The effects of paraquat have been further investigated in GPX-1 knockouts wherein the major deleterious effects are oxidation of NADPH, NADH, lipids and proteins [80]. In knockouts or selenium-deficient mice, paraquat-induced liver peroxidation and protein oxidation in liver and lungs peaked 1 h after treatment, with no effects in control animals. Clearly, GPX-1 has the potential to protect against oxidative damage to a wide range of tissues.

GPX-1 knockout mice are also susceptible to other challenges which cause problems to selenium-deficient animals. Thus, when infected with the benign strain of coxsackie virus B3, GPX-1 knockout mice develop a myocarditis. There were no effects on control mice, and when the viruses were characterized from the infected GPX-1 knockout mice, there were seven changes in the viral genome [81]. These changes were consistent with oxidation. Thus, GPX-1 may protect animals against viral problems which may occur due to mutations caused by oxidation. Lenses from eyes of knockout mice challenged with a 4-h photochemical stress showed increased damage compared with control animals. This was again attributed to the loss of GPX-1 compromising the ability to deal with oxidative stress [82]. GPX-1 knockout mice also have increased susceptibility to galactosamine/endotoxin treatment, which is used as a model of acute liver failure. This model involves neutrophil-mediated damage to liver cells. The increased damage in the knockout mice was thought to be due to oxidant molecules diffusing from neutrophils into the hepatocytes with consequent cell death [83].

Thus, both GPX-1 overexpression and knockout models point to a role of the enzyme in protecting against oxidative attack of tissues. Whether the enzyme has a more subtle role in regulation of tissue peroxide levels remains to be elucidated. Furthermore, the effects of GPX-1 appear to be specific and cannot be replaced by excess dietary vitamin E acting as a general antioxidant.

GPX-2 functions

There is much less information on the functions of GPX-2 in mammals. However, its unusual distribution in the epithelium of the gastrointestinal tract suggests a

specific function in metabolizing the ingested lipid hydroperoxides [84]. Regulation of the mRNA for GPX-2 in cell lines in comparison with other GPXs indicates that it is one of the proteins that is well preserved in selenium deficiency. Under some conditions it is one of the few selenoprotein mRNAs that increase in selenium deficiency, the other being type I iodothyronine deiodinase [50, 85]. The GPX-2 gene is inducible by retinoic acid, unlike the GPX-1 gene [86]. The functions of GPX-2 are clearly very specialized as well as protection against hydroperoxides in food. Brigelius-Flohe has suggested that GPX-2 may metabolize peroxides built up as a byproduct of oxidative metabolism of xenobiotics by the liver [50]. The enzyme may be particularly important in protection against cancer-promoting changes in the gastrointestinal tract.

GPX-3 functions

As mentioned above, GPX-3 is effective in protection against acetominophen toxicity, although its overexpression may also cause deleterious effects [65]. Otherwise, use of knockouts and overexpression technology in the study of GPX-3 has been quite limited. Most of the information regarding the biological function of the protein has come from studies of its tissue expression and of the effects of disease on synthesis of the protein. Since most GPX-3 is found in plasma and extracellular fluids and is glycosylated to improve stability, it is thought to act as an antioxidant, perhaps protecting cell membranes. In contrast to GPX-1, GPX-3 has activity against phospholipid hydroperoxides, thus perhaps giving it a more direct role in protection of membranes [87]. Although GPX-3 is synthesized in a range of tissues, the major source for plasma is in the kidney. After cloning the cDNA for GPX-3 from a placental library, the mRNA was not found in human liver [15]. Subsequently, the cDNA from mouse and rat allowed the screening of various tissues, and in these rodents the kidney had the highest content of mRNA. GPX-3 mRNA was also found in heart, lung and other tissues [23, 88, 89]. In humans, the kidney has the highest levels of GPX mRNA followed by other organs [25]. The importance of kidney as a source of extracellular GPX has been emphasized by the localization of its mRNA in the epithelial cells of the proximal tubules in humans, rats and mice [23]. Anephric humans and rats have very low plasma GPX-3 activity. Patients with renal diseases also have very low plasma GPX activity, including those undergoing renal dialysis. These decreases in GPX-3 in plasma are not associated with selenium deficiency in the patients [90]. Because of the synthesis and secretion of GPX-3 in the kidney, it has been hypothesized that this is the site of function of the protein [16]. Thus, it may protect the proximal tubules of the kidney from localized peroxide-induced damage. GPX-3 synthesis has been detected in mammary tissue, gastrointestinal tract, lung, amniotic fluid, thyroid and a number of other cell lines [17, 20, 22, 91]. This has led to the suggestion of a localized role for GPX-3 in metabolism of peroxides in these different tissues. Additionally, in the thyroid gland GPX-3 may control levels of hydrogen peroxide necessary for thyroid hormone synthesis [20].

GPX-4 functions

The functions of GPX-4 are clearly associated with its ability to metabolize phospholipid hydroperoxides. In addition, GPX-4 represents at least 50% of the capsule material that embeds the helix of sperm mitochondria [92]. The protein is enzymatically inactive and has been cross-linked by an oxidative process. This renders the protein insoluble and implies a novel structural role for GPX-4. Though selenium status in animals has been associated with low male fertility, which was always assumed to be caused by increased oxidative damage to sperm or sperm production, it is now likely that sperm require GPX-4 as a structural protein [92]. Thus, the characteristic sperm damage seen in selenium-deficient animals may result from impaired production of GPX-4. At the early stages of sperm development, however, GPX-4 still exists in an enzymatically active form, so it may still have a function in the prevention of oxidative damage.

Interactions between selenium deficiency and vitamin E deficiency have been recognized for many years. For example, myopathies in sheep and cattle are caused by a combined selenium and vitamin E deficiency. However, it was difficult to reconcile loss of GPX-1 in one cell compartment and decreased vitamin E in cell membranes with a plausible mechanism for onset of disease. In selenium deficiency alone there is no evidence of increased lipid peroxidation in membranes which would be exacerbated by a concurrent vitamin E deficiency to cause disease. However, since the discovery of membrane-associated GPX-4, synergistic effects of the selenoprotein and vitamin E in preventing lipid peroxidation have been demonstrated [93]. The basis for this interaction is likely to be the ability of GPX-4 to remove phospholipid hydroperoxides, which have the potential to cause damage in the membrane. GPX-1 cannot react directly with such hydroperoxides unless they are first metabolized by phospholipase A2 [8]. Thus, clearly GPX-4 is responsible for protection of membranes against oxidative damage; however, it is also certain that the protein has many more subtle functions in controlling the metabolism of lipid perox-

ides. The role of GPX-4 in control of cell function is apparent from overexpression experiments with cell lines. Overexpression of GPX-4 in ECV304 cells requires the cotransfection of selenophosphate synthetase (selD) to provide selenophosphate for selenoprotein synthesis. When these cells were incubated in the presence of selenite, interleukin (IL)-1-induced NFκB activation, was inhibited compared with wild-type cells. The overexpression of GPX-4 was suggested to inhibit the IL-1 activation of the transcription factor by removing a fatty acid hydroperoxide [94]. In another cell line (RBL-2H3) that overexpressed GPX-4 approximately threefold, the conversion of arachidonic acid to intermediates in the lipoxygenase pathway was inhibited eightfold (leukotriene C_4 and leukotriene B_4). Normal rates of leukotriene formation were restored in the transfected cells by treatment with the inhibitor diethylmalate, which decreases GPX-4 activity [95]. In the transfected cells the pattern of product formation from arachidonic acid was consistent with inactivation of the 5-lipoxygenase pathways. The authors concluded that GPX-4 deactivated 5-lipoxygenase via decreases in levels of the hydroperoxides required for activation of the enzyme.

Overexpression of GPX-4 in mitochondria RBL2H3 cells was compared with control cells expressing nonmitochondrial GPX-4. In the cells overexpressing mitochondrial GPX-4, apoptosis induced by 2-deoxyglucose was delayed. The overexpression of mitochondrial GPX-4 did not alter levels of other proteins involved in apoptosis. Less hydroperoxide was produced in the cells overproducing mitochondrial GPX-4, and this was assumed to be a major factor in preventing apoptosis [96]. The regulation of hydroperoxide metabolism and control of leukotriene metabolism are clearly very important functions of GPX-4 located in both mitochondria and the cytosol. In addition, in some circulating granulocytes there is evidence that GPX-1 also has important functions in the control of leukotriene metabolism [97].

Conclusions

The selenium-containing GPXs represent a cell-specific and tissue-specific mechanism for prevention of oxidative damage in mammalian systems. However, the manipulation of GPX activities by changing selenium levels in diet and by molecular overexpression or knockout techniques indicate they have more subtle functions. GPX-4 has a novel structural function in sperm as well as an involvement in the appropriate synthesis of leukotriene metabolites from arachidonic acid. Care must be exercised when implying GPX function using overexpression or knockout technology. When selenoperoxidase activity is manipulated by dietary means there is coordinate regulation of several other selenium-containing proteins, notably the iodothyronine deiodinases and the thioredoxin reductases. These other selenoproteins can have a profound effect upon metabolism and will greatly alter the context in which changes in selenoperoxidase activity are expressed. Thus, when finally assigning biological effects to changes in selenoperoxidase activity, there is no substitute for studying these in the environment of changes in other selenoperoxidase and selenoprotein activities induced by selenium. Only in very specialized circumstances such as interleukin or essential fatty acid stimulation is there specific induction of certain selenoperoxidases in the absence of changes in other selenoproteins.

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