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Forum Review

Selenium-Dependent Enzymes in Endothelial Cell Function

REGINA BRIGELIUS-FLOHÉ, ANTJE BANNING, and KERSTIN SCHNURR

ABSTRACT

Glutathione peroxidases and thioredoxin reductases are the main selenoproteins expressed by endothelial cells. These enzymes reduce hydroperoxides, their role in endothelial cell physiology, however, by far exceeds prevention of oxidative damage. Reactive oxygen and nitrogen species, especially superoxide, hydroperoxides, and nitric oxide, are crucial signaling molecules in endothelial cells. Their production is regulated by vascular NAD(P)H oxidases and the endothelial nitric oxide synthase. Their metabolism and physiological functions are coordinated by glutathione peroxidases and the thioredoxin/thioredoxin reductase system. Endothelial selenoproteins are involved in the regulation of the vascular tone by maintaining the superoxide anion/nitric oxide balance, of cell adhesion by controlling cell adhesion molecule expression, of apoptosis via inhibition/activation of apoptosis signal-regulating kinase-1, and of eicosanoid production by controlling the activity of cyclooxygenases and lipoxygenases. Accordingly, they regulate inflammatory processes and atherogenesis. The underlying mechanisms are various and differ between individual selenoproteins. Scavenging of hydroperoxides not only prevents oxidative damage, but also interferes with signaling cascades and enzymes involved. Modulation of proteins by hydroperoxide-driven thiol/disulfide exchange is a novel mechanism that needs to be further investigated. A better understanding of the complex interplay of selenoproteins in regulating endothelial cell functions will help to develop a rationale for an improvement of health by an optimum selenium supply. *Antioxid. Redox Signal.* 5, 205–215.

INTRODUCTION

THE ROLE OF SELENOPROTEINS in the context of endothelial function is commonly seen in their ability to prevent oxidative cell damage. Selenoproteins such as glutathione peroxidases (GPx) and thioredoxin reductases (TR) directly or indirectly reduce hydroperoxides (3, 15) and should thereby prevent oxidative events that are believed to initiate pathogenic processes ultimately leading to atherosclerosis (81, 98). This seemingly trivial conclusion merits reconsideration in view of more topical data. It disregards both the diversified functions of reactive oxygen species (ROS) and the diversity of the selenoproteins (17). Over the past decades, evidence accumulated indicating that ROS and in particular hydroperoxides are involved in the fine-tuning of cellular responses to various stimuli and only become toxic if produced in excess. Accordingly, it became untenable to explain the essentiality of the trace element selenium by its "antioxidative" potential. In fact, selenium is incorporated as catalytically active selenocysteine residue into a large number of proteins. Only a few of them can reasonably be implicated in the metabolism of ROS (34, 56).

This article focuses on the role of individual selenoproteins in the endothelium. Beyond the concept of mere antioxidant defense by GPx, the potential impact of selenoproteins on endothelial cell physiology, in particular on the redox regulation of signaling cascades, is emphasized.

Department of Vitamins and Atherosclerosis, German Institute of Human Nutrition, D-14558 Bergholz-Rehbrücke, Germany.

SELENIUM STATUS AND CARDIOVASCULAR DISEASE

Animal studies

Selenium deficiency syndromes known for long from lifestock and experimental animals (26), in part at least, point to a pivotal role of selenium in the endothelium. In poultry, severe deficiency causes exsudative diathesis, a disease characterized by severe subcutaneous edema and hemorrhages associated with low plasma protein, anemia, and decreased growth (26). As the syndrome could be mimicked by mercaptosuccinate, a dead-end inhibitor of the cytosolic GPx (cGPx), it might result from shortage of this particular selenoprotein (32). Vitamin E- and selenium-deficient pigs develop a severe cardiomyopathy with widespread epicardial and myocardial hemorrhages, which led to the characteristic name "mulberry heart disease" (26). The mechanism of disease development is not known nor have the responsible selenoproteins been identified yet. The cause of the fulminant liver necrosis observed in vitamin E- and selenium-deficient rodents has not been clarified either, but similar liver failure is commonly observed in septic syndromes, which are due primarily to disturbances of the microcirculation.

Human selenium-deficiency syndromes

Overt selenium deficiency syndromes in humans are rare and hardly a problem in developed countries where food from different areas guarantee micronutrient supply. Even in rural areas extremely poor in selenium, disease manifestation depends on complicating factors. Keshan disease, a cardiomyopathy endemic in China, is apparently due to selenium-dependent aggravation of viral virulence. In Kashin–Beck disease, a disabling chondronecrosis with a similar geographic distribution, selenium shortage is complicated by iodine deficiency and likely by exposure to mycotoxins. Myxedematous cretinism in Congo results from combined iodine and selenium deficiency. Chondronecrosis and cretinism are obviously unrelated to endothelial dysfunction. Keshan disease may be subsumed under the term cardiovascular disease, but the primary site of damage is not likely the endothelium either. The myocytes and in particular the conductive system of the heart appear to be affected, which leads to arrhythmias and heart dilation (for review, see 56).

Selenium intakes and cardiovascular disease risk

Subacute selenium deficiency due to a sustained suboptimum selenium intake has for long been discussed as a risk factor of atherogenesis. Early epidemiological studies revealed a higher mortality from cardiovascular diseases in selenium-deficient areas of the United States (86). Attempts to verify such relationship by prospective studies remained equivocal. Whereas Salonen *et al.* found an increase in fatal or nonfatal myocardial infarction in individuals with serum selenium concentration below 45 μg/L (85), no such associations with selenium concentrations above and below that threshold were detected by Virtamo's group except for stroke mortality (94). In a multicenter case-control study (EURAMIC), a correlation between a low toenail selenium concentration and the risk of myocardial infarction was shown only for the center with the lowest selenium level (Berlin, Germany) (53). Other studies did not show a clear association between a low selenium status and cardiovascular diseases either (for reviews, see 50, 69, 78). Compiling available data, one can conclude that only individuals with a low selenium status might profit from supplementation.

SELENOPROTEINS RELEVANT TO ENDOTHELIAL FUNCTION

Mammals are believed to express 30–50 distinct selenoproteins. So far, 10 of those have been identified by sequence and function, and eight more by sequence only; the existence of the remaining ones is inferred from pulse-labeling experiments with ^{75}Se. Labeling of human umbilical vein endothelial cells (HUVEC) with [^{75}Se]selenite revealed bands at molecular masses of about 72, 64, 58, 24–26, 21.7, 15.1, and 14.6 kDa, a pattern that markedly differed from that observed in HepG2 cells or thyrocytes. The 58-kDa protein was the most prominent and was identified as TR (1). Other highly expressed selenoproteins were a 24–26-kDa protein, most probably cGPx, and a 21.7-kDa protein, identified as phospholipid hydroperoxide GPx (PHGPx) (see below). The other bands were less prominent and have not been identified. Umbilical cord vein endothelial cells did not express and release extracellular selenoproteins, like plasma GPx (pGPx) (1, 4) or selenoprotein P (SelP) (1), whereas SelP mRNA has been detected in bovine arterial endothelial cells (43). Thus, the major selenoproteins in endothelial cells are TR and the two intracellular GPxs, cGPx and PHGPx. Beyond, the extracellular selenoproteins, pGPx and SelP, may be considered to interact with the endothelial layer.

Glutathione peroxidases

GPx form a widespread family of phylogenetically related proteins that are characterized by a catalytic triad composed of (seleno)cysteine, glutamine, and tryptophan (66). They reduce a large spectrum of hydroperoxides at the expense of thiols, typically GSH. In mammals, four selenium-containing GPx were described: the classical or cytosolic GPx (cGPx, GPx-1), the extracellular plasma GPx (pGPx, GPx-3), the phospholipid hydroperoxide GPx (PHGPx, GPx-4), and the gastrointestinal GPx (GI-GPx, GPx-2) (for reviews, see 15, 17). All four catalyze the reduction of hydroperoxides by means of GSH (18). Yet the extracellular GPx reacts equally well with glutaredoxin and thioredoxin (Trx), (10), and the PHGPx also accepts protein thiols as reducing substrates (39, 82, 93).

Reduction of lipid hydroperoxides present in oxidized low-density lipoprotein (LDL) or inhibition of the formation thereof is considered to be the beneficial effect of the GPx in preventing atherogenesis. However, LDL is oxidized extracellularly, whereas PHGPx, the only GPx that can effectively reduce complex hydroperoxides even within oxidized LDL, is present intracellularly only. cGPx cannot act with complex lipid hydroperoxides at all. The only GPx that is in contact with oxidized LDL in plasma is pGPx. It might also remove soluble hydroperoxides that initiate LDL oxidation.

Thioredoxin reductases

TR are FAD-containing homodimeric enzymes belonging to the family of pyridine nucleotide-disulfide oxidoreductases. In mammals, all TR are selenoproteins. Electrons are transferred from NADPH via FAD to the active-site disulfide of Trx. Trx in turn supplies reducing equivalents to enzymes such as ribonucleotide reductase (58) or peroxiredoxin-type Trx peroxidases (47). As a dithiol–disulfide exchange system, Trx also regulates the DNA binding of certain transcription factors including nuclear factor-κB (NFκB), p53, and the glucocorticoid receptor (for review, see 75). In this way, the Trx/TR system is associated with the regulation of cell growth, differentiation, and apoptosis. *In vitro*, TR can further reduce lipid hydroperoxides (11), dehydroascorbic acid, and the ascorbyl free radical (67), functions that led to the questionable classification of TR as an antioxidative enzyme.

Selenoprotein P

SelP is the most unusual selenoprotein in containing nine (human) to 17 (zebrafish) selenocysteines. It further contains one or two histidine clusters, a lysine-rich domain, and several glycosylation sites (48, 89). The primary source of SelP appears to be the liver from which it is released into the plasma guided by the N-terminal leader sequence.

An enzymatic activity of SelP has not been identified yet, although its multiple selenocysteine residues have suggested the ability to catalyze redox functions. It has indeed been postulated that SelP can act as an extracellular GPx (83). An alternative role of SelP may be selenium transport and storage (19). Despite its still poorly defined function, SelP is discussed to protect the endothelium. Two histidine clusters with a lysine-rich domain in between render rat SelP able to bind to cell-surface glucosaminoglycans (48), which explains its association with vascular endothelial layers in liver, kidney, and brain of rats (20). Acidic pH, as present at sites of inflammation, favors binding of SelP to heparan proteoglycans, which might be a mechanism by which SelP is recruited to the endothelium at areas of inflammation. Endothelial membranes should thereby be shielded from attack of peroxynitrite ($ONOO^-$) produced from superoxide anion ($O_2^{\cdot -}$) and nitric oxide ($NO^{\cdot}$) in inflammatory situations.

SUBSTRATES, PRODUCTS, AND EFFECTORS OF SELENOPROTEINS IN THE ENDOTHELIUM

Hydrogen peroxide (H_2O_2) and lipid hydroperoxides in signaling cascades

The endothelium is exposed to ROS derived from phagocytes or the endothelial cells themselves. The term ROS, however, is too imprecise to be satisfactory. In fact, the most reactive ones, $^{\cdot}OH$ or $RO^{\cdot}$, would be too promiscuous to meet any requirement for specificity. They also tend to modify proteins irreversibly and thus are rather relevant to oxidative tissue damage. However, there is growing evidence that ROS are not generally harmful, but exert vital functions. ROS suitable for mediating signaling have to be produced in a regulated and compartmentalized manner. They should also allow reversible modifications of proteins. ROS best fulfilling these requirements are H_2O_2 and other hydroperoxides. Needless to state that their steady state may be regulated by GPx or peroxiredoxins, thus depending on the selenium status irrespective of their preferred metabolic route.

The most relevant and best investigated source of ROS is certainly the NADPH oxidase system of phagocytes (5). More recently, however, it has become evident that a number of growth factors, cytokines, and hormones trigger the rapid production of intracellular ROS. Via binding to their receptors, they activate cyclooxygenases, lipoxygenases, or NAD(P)H oxidases as potential hydroperoxide or superoxide generating systems. A ligand-activated NAD(P)H oxidase has been detected in a variety of nonphagocytic cells, including smooth muscle cells (41) and endothelial cells (60, 61). In contrast to the phagocyte NADPH oxidase, the $gp91^{phox}$ and $p22^{phox}$ subunit of the endothelial enzyme are predominantly intracellular and located in the vicinity of the endoplasmic reticulum (7). This clearly shows that an intracellular production of $O_2^{\cdot -}$ with subsequent formation of H_2O_2 is possible in endothelial cells. Receptor-mediated activation of NADPH oxidase includes the activation of phosphatidylinositol 3-kinase and the small GTPase Rac-1. Rac-1 is a component of the NADPH oxidase complex that supports recruitment of $p67^{phox}$ to the membrane, thereby activating NADPH oxidase (40). However, the activation of lipoxygenases by Rac-1 also has been reported (73).

Hydroperoxides influence signaling cascades by activating protein kinases, inhibiting protein phosphatases, and modulating transcription factor activities (for reviews, see 30, 31, 42). The mechanisms by which they exert these functions are not well understood. Targets being modified by hydroperoxides are thiol groups in proteins. Particular thiols can either be directly oxidized by ROS to sulfenic acids, as has been shown for protein phosphatases (42), or become *S*-thiolated, *e.g.,* by glutathione according to Eqs. 1 and 2.

$$\text{Prot-S}^- + \text{ROOH} + \text{H}^+ \rightarrow \text{Prot} - \text{SOH} + \text{ROH} \qquad (1)$$

$$\text{Prot-SOH} + \text{R}'\text{SH} \rightarrow \text{Prot-S-SR}' + \text{H}_2\text{O} \qquad (2)$$

Alternatively, glutathionylation takes place by thiol–disulfide exchange between protein thiols and oxidized glutathione (GSSG) (13, 14):

$$\text{Prot-S}^- + \text{GSSG} \leftrightarrows \text{Prot-S-SG} + \text{GS}^- \qquad (3)$$

Finally, it could be envisaged that glutathionylation is catalyzed by GPx, especially PHGPx, as outlined in Fig. 1. Proteins whose activity is regulated by glutathionylation include transcription factors like c-Jun (55), NFκB (74), glyceraldehyde-3-phosphate dehydrogenase (27), phosphotyrosine phosphatase 1B (6) and 2A (77), creatine kinase (79), actin (95), and Trx (23). By redox proteomics, 24 additional glutathionylated proteins have been identified (36).

Hydroperoxides also deserve interest as mediators of a key step in the inflammatory response that is adhesion of leukocytes to endothelial cells and subsequent extravasation of leukocytes into affected tissue (22). Adherence is mediated by endothelial and leukocyte surface molecules. Typically, endothelial cells are

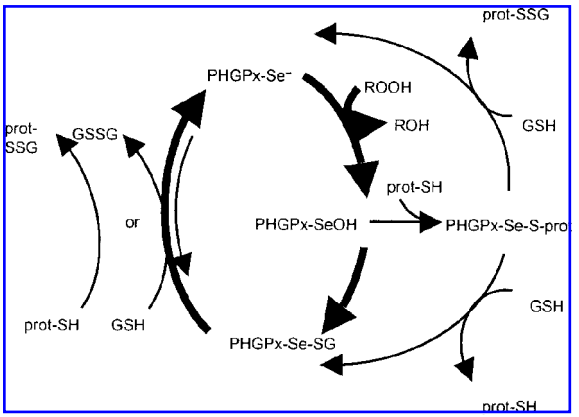

FIG. 1. Catalytic cycle of PHGPx (central part in bold) and possible side reactions leading to protein modification by hydroperoxides, GSH, and PHGPx. In a reaction with hydroperoxides, selenium in PHGPx is oxidized to selenenic acid. PHGPx-SeOH might react with protein thiols instead of GSH, forming a PHGPx-Se-S-prot. This can be reduced by GSH leading to ground-state PHGPx and a glutathionylated protein. A reaction of PHGPx-Se-S-prot with GSH leads to deliberation of the reduced protein (prot-SH), whereas PHGPx enters back into the cycle as selenodisulfide. Also, from this state, glutathionylation of proteins may be possible by the reduction of PHGPx-Se-SG with prot-SH instead of GSH. Although this scheme awaits a direct proof, it has been clearly shown that PHGPx can react with protein thiols (39, 82, 93). Interestingly, the reaction of PHGPx with GSSG with concomitant release of GSH has also been shown in a bioelectrocatalytical system (59). All these reactions show that PHGPx might be involved in thiol/disulfide exchange rather than act as a mere antioxidant device.

stimulated by inflammatory cytokines such as interleukin-1 (IL-1) or tumor necrosis factor-α (TNFα) to express E-selectin, the vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1). The cytokines can, however, be substituted by hydroperoxides. H_2O_2 stimulated ICAM-1 but not VCAM-1 or E-selectin expression in HUVEC (12, 37, 62, 80). In contrast, induction of ICAM-1 was not observed in human dermal microvascular endothelial cells (91). Fatty acid hydroperoxides, such as 13-hydroperoxyoctadecadienoic acid (13-HPODE), enhanced TNFα-induced VCAM-1 expression (54). A direct effect on ICAM-1 but not VCAM-1 or E-selectin expression was shown for 13-HPODE, its reduction product 13-hydroxyoctadecadienoic acid (13-HODE), and phosphatidylcholine hydroperoxide (37), whereas 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and 12-hydroxyeicosatetraenoic acid (12-HETE) induced ICAM-1, VCAM-1, and E-selectin (90).

The induction of cell adhesion molecules by hydroperoxides is explained by the redox sensitivity of the NFκB activating system. NFκB is a transcription factor primarily activated by IL-1 and TNFα that regulates i.a. a wide range of genes related to inflammation. NFκB is a heterodimer consisting of subunits such as p50 and p65, and is retained in the cytosol by an inhibitory molecule IκB. Upon phosphorylation and ubiquitinylation, IκB is degraded and the remaining NFκB complex is translocated into the nucleus (Fig. 2). Oxidative processes in the cytosol facilitate phosphorylation of IκB (57) and subsequent translocation of NFκB into the nucleus. In contrast to the oxidative processes in the cytosol, reducing conditions are required in the nucleus for DNA-binding activity of NFκB (for review, see 33). Maintenance of the critical Cys^{62} in p50 in the reduced form is achieved by Trx (45). Expression of E-selectin, VCAM-1, and ICAM-1 then results from binding of functional NFκB to responsive elements in the pertinent genes. These recognition sites are necessary, but not sufficient for maximum cytokine-induced gene expression as determined by deletion and mutation experiments. The promoter of E-selectin contains three closely spaced NFκB sites, the VCAM-1 promoter has two adjacent NFκB sites, and the ICAM-1 promoter apparently has only one NFκB site (for review, see 25). These differences in cell adhesion molecule promoters may account for the differential responses mentioned above.

Redox regulation by GPx

Because of the overlapping substrate specificities of selenoperoxidases and peroxiredoxins, it is difficult to analyze which of these enzymes is most relevant in regulating hydroperoxide-sensitive signaling cascades. Overexpression of cGPx was reported to dampen TNFα-induced NFκB activation (57), as did overexpression of PHGPx in case of IL-1-induced NFκB activation (16). Analogous effects were seen with peroxiredoxin (for review, see 47), and also the GPx mimic BXT-51072 inhibited expression of VCAM-1, ICAM-1, and P- and E-selectin (28, 68). Not surprisingly, incubation of endothelial cells with varying amounts of sodium selenite inhibited the TNFα-induced mRNA and surface expression of ICAM-1, VCAM-1, and E-selectin in a dose-dependent manner (99). Accordingly, selenium deficiency, as evidenced by a reduced GPx activity, led to enhanced cytokine- and H_2O_2-induced neutrophil adhesion to endothelial cells via ICAM-1 and E-selectin (65). None of these findings, however, enables us to discriminate between the contributions of the individual selenium-containing or selenium-dependent peroxidases. Nonetheless, a particular regulatory role of PHGPx is suggested by the following experiment. In ECV cells overexpressing PHGPx, IL-1-induced NFκB activation was completely abrogated (16). Interestingly, large variations of cGPx activity achieved by selenium deprivation/repletion only marginally affected IL-1-induced NFκB activation, whereas a moderate change in PHGPx activity had dramatic effects. Whether PHGPx is more relevant to NFκB activation in general remains to be demonstrated. It further remains to be investigated whether PHGPx regulates NFκB activation by scavenging particular hydroperoxides involved in activation cascades, by catalyzing glutathionylation of essential thiols, or by acting as a thiol modifying agent itself as suggested in Figs. 1 and 2.

Redox regulation by the Trx system

Regulation of signaling cascades by reduction of hydroperoxides can in principle also be achieved by peroxiredoxins, which like the glutathione peroxidases are broad-spectrum peroxidases (47). Of the six different types of mammalian peroxiredoxins, types I–III are Trx peroxidases and thus depend on TR and thus on selenium for supply of reduction equiva-

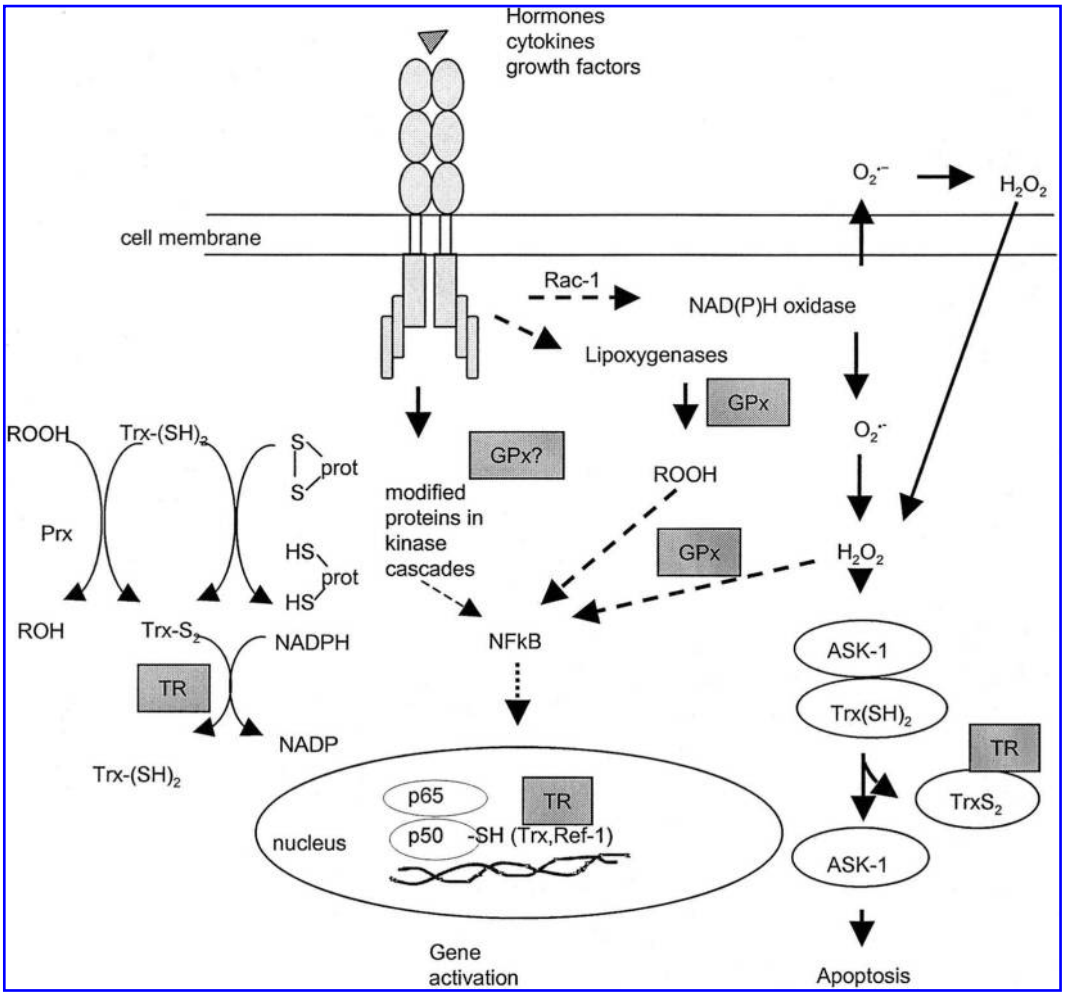

FIG. 2. Redox events in cell signaling influenced by TR and/or GPx. Binding of ligands to their receptors leads to stimulation of kinase cascades and activation of cell type-specific NAD(P)H oxidases and/or lipoxygenases. Produced hydroperoxides facilitate signaling by increasing the phosphorylation state of kinases and the activation of transcription factors by yet unknown mechanisms. GPxs regulate lipoxygenase activities via the cellular peroxide tone and activation of NFκB by scavenging hydroperoxides and may modify thiols of proteins involved in the signaling cascade. The Trx/TR system reduces oxidized proteins, *e.g.,* p38 MAP kinase in the cytosol, and the p50 subunit of NFκB and AP-1 via the redox factor-1 (Ref-1) in the nucleus. ASK-1 is bound to reduced Trx [Trx(SH)$_2$], which is dissociated upon oxidation, enabling ASK-1 to oligomerize and become active. Oxidized Trx (TrxS$_2$) is reduced by TR.

lents. Although their efficiency, which depends on sulfur catalysis, cannot compete with that of the selenoperoxidases, they deserve particular interest in the context of redox regulation. Some of them were shown to substitute for GPx in suppressing NFκB activation when being overexpressed. Further, they tend to associate with regulatory proteins such as cyclophilin and often proved to be up-regulated upon specific cell stimulation (for review, see 47). Their donor substrate specificities have to be worked out, but types I–III appear to be specialized for the reaction of proteins with CXXC motifs like Trx. They must therefore be considered whenever a regulatory process involves the redox state of Trx.

The Trx/TR system modulates signaling by regulating the redox state of a variety of enzymes essential for promoting the signaling cascades including mitogen-activated protein (MAP) kinases and transcription factors (see Fig. 2; for review, see 70). A particularly interesting case is the induction of apoptosis by the apoptosis signal-regulating kinase-1 (ASK-1) (Fig. 2). ASK-1 is kept inactive by complexing with reduced Trx. Exposure to H_2O_2 oxidizes Trx, which leads to its dissociation and induction of apoptosis by the thus activated ASK-1 (84). As reduced Trx does not readily react with H_2O_2, a catalytic oxidation must be inferred. As none of the intracellular GPx accepts Trx, a peroxiredoxin is considered a likely candidate to promote the apoptotic process, whereas TR will stop it by regenerating reduced Trx.

The superoxide, nitroxide, and ONOO$^-$ balance

Vascular endothelial cells generate NO$^{\cdot}$ from arginine catalyzed by the constitutively expressed isoform of nitric oxide synthase (NOS), eNOS. NO$^{\cdot}$ is the most potent endogenous

vasodilator. It further inhibits smooth muscle cell proliferation and migration, adhesion of leukocytes to the endothelium, and platelet aggregation. Thus, a balanced turnover of $NO^{\cdot}$ is crucial for endothelial function. $NO^{\cdot}$ can interact with $O_2^{\cdot -}$ resulting in the production of the more reactive $ONOO^-$ (8). An overproduction of $O_2^{\cdot -}$, as present in inflammatory processes, lowers and inactivates locally produced $NO^{\cdot}$ (Fig. 3). This imbalance of the $O_2^{\cdot -}/NO^{\cdot}$ ratio in favor of $O_2^{\cdot -}$ leads to a clinical syndrome known as endothelial dysfunction, characterized by increased endothelial permeability and increased expression of adhesion molecules facilitating leukocyte extravasation (for review, see 64). The delicate balance between $O_2^{\cdot -}$ and $NO^{\cdot}$ was shown to be modulated by selenium (63). How this is being achieved is not straightforward, because neither $NO^{\cdot}$ nor $O_2^{\cdot -}$ is a substrate of any of the known selenoproteins.

Excessive production of endogenous $NO^{\cdot}$ is toxic to a number of cells, including vascular endothelial cells (72). $NO^{\cdot}$ causes inhibition of cGPx (2), even of eNOS activity itself, and down-regulates Trx and TR (100). Possible mechanisms include *S*-nitrosylation by $NO^{\cdot}$ or oxidation by $ONOO^-$ to sulfenic/selenenic acids of cysteine/selenocysteine residues and disulfide/selenodisulfide formation. Whereas modification of cGPx by $NO^{\cdot}$ was reversible by thiols, $ONOO^-$ caused irreversible inhibition of cGPx (2).

$ONOO^-$ also inactivated TR within 1 h in HUVEC. Surprisingly, the activity was completely restored within 24 h, an effect accompanied by up-regulation of TR mRNA and protein, indicating that destructive mechanisms of $ONOO^-$ can somehow lead to transcriptional activation (71). $NO^{\cdot}$-induced loss of eNOS activity was best restored by the Trx/TR couple (72). Overexpression of Trx prevented inactivation of eNOS in porcine pulmonary artery endothelial cells (100). The enhancement of relaxation of aortic rings observed upon selenium supplementation (63) might, therefore, have been achieved by increasing the expression of TR, which so far is the only selenoprotein shown to respond to supranutritional selenium supply (9).

Recent evidence points to a pivotal role of cGPx in maintaining an appropriate $NO^{\cdot}$ tonus. First hints were provided by experiments on $NO^{\cdot}$ depletion due to homocysteine-induced oxidative stress. Overexpression of cGPx normalized endothelium-dependent vasodilator response in hyperhomocysteinemic mice and restored $NO^{\cdot}$ release from homocysteine-treated bovine aortic endothelial cells. It was concluded that cGPx prevents oxidative inactivation of NOS (96) either by scavenging hydroperoxides or by its postulated role in detoxifying $ONOO^-$ (87). Even more convincingly, the key role of cGPx in the regulation of $NO^{\cdot}$ bioavailability was demonstrated by studies with cGPx knockout mice (35). Mesenteric arterioles of $cGPx^{-/-}$ mice responded to bradykinin with vasoconstriction instead of vasodilatation, an effect that was associated with low $NO^{\cdot}$ levels and could be reversed by $NO^{\cdot}$ donors. Likely, the cGPx deficiency, due to a permanently elevated peroxide tone, causes depletion of bioavailable $NO^{\cdot}$ by inhibition of NOS (35). This interpretation complies with the view that cGPx among the selenoproteins is most relevant in balancing a systemic oxidative stress. Although $cGPx^{-/-}$ mice did not show any obvious phenotype as long as unchallenged (46), they proved to be highly sensitive to redox-cycling herbicides (24, 29) and exposure to lipopolysaccharide (LPS) mimicking septicemia (52). The endothelial dysfunction now demonstrated in $cGPx^{-/-}$ mice might certainly contribute to the high lethality by aggravating the disturbance of the microcirculation in the LPS-shocked animals. The impairment of $NO^{\cdot}$ production appears to be prominent in the endothelium that depends on constitutive eNOS, whereas macrophages equipped with inducible NOS (iNOS) respond differently. LPS/interferon γ-induced $NO^{\cdot}$ production in macrophages from $cGPx^{-/-}$ mice was increased (38), as was LPS-mediated expression of iNOS in selenium-deficient macrophages (76).

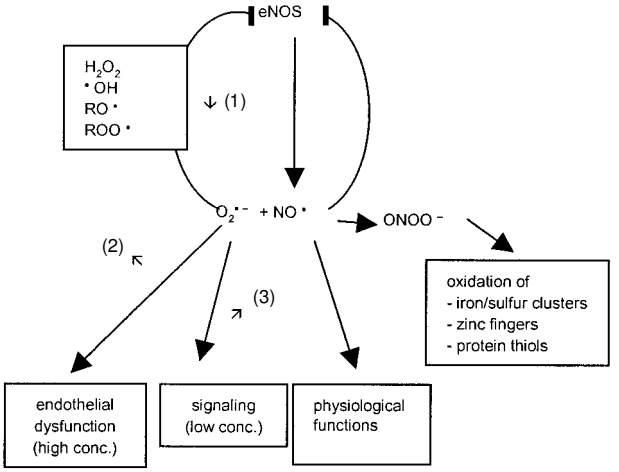

FIG. 3. The $NO^{\cdot}/O_2^{\cdot -}$ balance and the possible interference of GPx and the Trx/TR system. (1) GPx scavenges ROOH and prevents eNOS inactivation. Trx/TR reduces oxidized cysteine residues in eNOS and restores activity. (2) GPxs prevent oxidative damage and loss of bioactive $NO^{\cdot}$. (3) GPxs modulate signaling. Trx/TR reduces protein thiols involved in signaling cascades (see Fig. 2).

GPx and eicosanoids

Endothelium regulates vascular tone and also platelet function via the release of $NO^{\cdot}$ and prostacyclin (PGI_2) causing both dilatation and inhibition of platelet aggregation. On the other hand, platelets synthesize and release thromboxane (TXA_2) and thereby promote aggregation and vasoconstriction. PGI_2 and TXA_2 are derived from the same primary cyclooxygenase product prostaglandin G_2. This is converted to the final products by prostacyclin synthase and thromboxane synthase, respectively. The extracellular peroxide tone has long been implicated in the activation of prostaglandin biosynthesis (88). Also lipoxygenases, the key enzymes in leukotriene biosynthesis, require a certain peroxide tone to be active (44). Under inflammatory conditions, extracellular H_2O_2 increases due to the oxidative burst produced by activated macrophages. This stimulates both prostaglandin and leukotriene production. High peroxide levels, however, also interfere with prostaglandin synthesis by inactivation of key enzymes from which prostacyclin synthase is most susceptible. Consequently, the production of PGI_2 decreases, leading to an increased platelet aggregation due to overwhelming TXA_2 levels.

Without any balancing system, any oxidative burst reaction would be amplified by the activation of lipoxygenases. A broad-spectrum peroxidase like pGPx could prevent such signal amplification, thereby dampening undue responses to irrelevant inflammatory stimuli. This dampening system, however, would easily be overcome if the initial oxidative burst is pronounced enough to exhaust the limited reduction capacity in the extracellular space. In other words, pGPx being a highly reactive peroxidase, yet supported by a tiny capacity of reduction equivalents, may be considered as the ideal redox buffer to discriminate between relevant and irrelevant inflammatory stimuli. pGPx responds fast to selenium depletion/repletion. Accordingly, selenium deficiency not only inhibited PGI_2 synthesis, but also increased TXA_2 levels in cultured endothelial cells (21) and in rat plasma (49). Certainly, the pGPx-controlled peroxide tone relates eicosanoid synthesis only to the redox state of the cellular environment. The kind and levels of intracellular peroxides appear equally important. As a rule, the lipoxygenases are more easily activated by lipid hydroperoxides than by H_2O_2. Not surprisingly, PHGPx with its preference for lipophilic hydroperoxides proved to be the more relevant enzyme in regulating leukotriene biosynthesis (51, 97).

The potential role of pGPx in the regulation of eicosanoid metabolism, if valid, could also be of outstanding importance with respect to atherogenesis. A permanent overactivation of lipoxygenases due to pGPx deficiency could certainly lead to oxidation of LDL by primary lipoxygenase products, which themselves are lipid peroxides. Such induction of LDL oxidation can only be prevented by an antioxidant device present in the extracellular space. Only pGPx and SelP meet this criterion. Lipid hydroperoxides in oxidized LDL are estimated to be in the nanomolar range (92), and the micromolar extracellular glutathione level would theoretically be sufficient to support pGPx in keeping them reduced. In antagonizing oxidizing LDL-induced atherogenesis, however, pGPx does not necessarily have to reduce hydroperoxy groups of the oxidized lipoprotein. The dampening of inflammatory responses by pGPx, as discussed above, would inevitably lower the steady-state peroxide tone that initiates lipid peroxidation in LDL.

CONCLUSIONS

Selenoproteins definitely modulate endothelial function. The individual roles of the selenoproteins are not easily discriminated, because they display overlapping substrate specificity. Further, their individual actions cannot be independent, because they all compete for the same pool of reduction equivalents, NADPH. A clear, though indirect role in preventing endothelial dysfunction could be attributed to cGPx by inverse genetics. Analogous knockout models for pGPx, peroxiredoxins, and TR have not yet been performed. Related stringent evidence for PHGPx and Trx can no longer be expected, because the knockouts proved to be lethal.

The common denominator of the regulatory phenomena that are catalyzed by selenoproteins in the endothelium is the reduction of hydroperoxides. The mechanisms, however, differ markedly. They comprise scavenging hydroperoxides needed for direct enzyme activation, prevention of oxidative inactivation of enzymes, modulation of protein phosphorylation by thioylation, release of Trx from complexes with kinases, and probably hydroperoxide-dependent protein/protein interaction. The few phenomena that could so far be clarified at the molecular level underscore the diversity of regulatory principles and promise future surprises.

In physiological terms, it can be stated that the endothelial selenoproteins are relevant to vascular tone, cell adhesion, and apoptosis, and accordingly to inflammatory processes and atherogenesis. A better understanding of the most complex interplay of the selenium proteins might help in the rational design of future trials aiming at an improvement of human health by selenium supplementation.

ACKNOWLEDGMENTS

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ABBREVIATIONS

ASK-1, apoptosis signal-regulating kinase-1; cGPx, cytosolic glutathione peroxidase; eNOS, endothelial nitric oxide synthase; GPx, glutathione peroxidases; H_2O_2, hydrogen peroxide; 13-HPODE, 13-hydroperoxyoctadecadienoic acid; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MAP, mitogen-activated protein; NFκB, nuclear factor-κB; $NO^{\cdot}$, nitric oxide; NOS, nitric oxide synthase; $O_2^{\cdot -}$, superoxide anion; $ONOO^-$, peroxynitrite; PGI_2, prostacyclin; pGPx, plasma glutathione peroxidase; PHGPx, phospholipid hydroperoxide glutathione peroxidase; ROS, reactive oxygen species; SelP, selenoprotein P; TNFα, tumor necrosis factor-α; TR, thioredoxin reductases; Trx, thioredoxin; TXA_2, thromboxane A_2; VCAM-1, vascular cell adhesion molecule-1.

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Address reprint requests to:
Prof. Dr. Regina Brigelius-Flohé
Department of Vitamins and Atherosclerosis
German Institute of Human Nutrition
Arthur-Scheunert-Allee 114–116
D-14558 Bergholz-Rehbrücke
Germany

E-mail: flohe@mail.dife.de

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