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Down Regulation of Superoxide Dismutases and Glutathione Peroxidase by Reactive Oxygen and Nitrogen Species

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The levels of antioxidative enzymes are regulated by gene expressions as well as by post-translational modifications. Although their functions are to scavenge reactive oxygen (ROS) and nitrogen species (RNS), they may also be targets of various oxidants. When ROS and RNS modify the functions of antioxidative enzymes, especially glutathione peroxidase, they may induce apoptotic cell death in susceptible cells. It is conceivable, therefore, that at least a part of the apoptotic pathways mediated by ROS and RNS may be associated with modification of the redox regulation of cellular functions due to elevations of such substances. In this article we review recent findings about the effects of various oxidative conditions associated with alteration of these antioxidative enzymes and the concomitant cellular damage induced.

Keywords: Apoptosis, diabetes, glycation, peroxide

INTRODUCTION

Reactive oxygen (ROS) and nitrogen species (RNS) are implicated as causes of various disease conditions such as inflammation, cancer, diabetes, and aging.[1] Production of superoxide occurs by a one electron reduction of molecular oxygen followed by a reaction chain producing harmful ROS. Antioxidative enzymes participate in detoxification of these harmful compounds. Suppression of their gene expression and dysfunction of these enzymatic activities cause serious damage to cells by augmenting intracellular oxidative state. While Mn-superoxide dismutase (Mn-SOD) can be induced by various stimuli such as inflammatory cytokines, phorbol ester, and ROS, Cu,Zn-SOD is constitutively expressed in most cells.[2] The expression of catalase is restricted to certain cells. Members of the glutathione peroxidase (GPx) family occur in specific cellular locations and are expressed in a tissue-specific manner. Their transcription and translation are regulated by the trace element, selenium, because selenocysteine (Sec) is essential for their activity. A novel enzyme family with thioredoxin-dependent peroxidase activities has been found and

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called peroxiredoxin.[3] Five of these enzymes have been cloned[4] from a single mammalian species and although they exhibit structural similarity to GPx, their catalytic center contains Cys instead of Sec. Various biological effects other than peroxidase activity have also been reported. Our discussion will focus on the different mechanisms of regulation of SODs and GPxs under a variety of conditions.

GENETIC DEFECT IN ANTIOXIDATIVE ENZYME GENES: SOD1 MUTATION IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (FALS)

Cu,Zn-SOD exists at micromolar levels in most cells and defects in the SOD gene have been identified in patients with the motor neuron disease, familial amyotrophic lateral sclerosis (FALS).[5] At present more than fifty mutations have been found to be linked to FALS. SOD activities in erythrocytes isolated from FALS patients are significantly lower than those in normal and sporadic ALS patients.[6] A decrease in SOD activity could lead to enhanced levels of hydroxyl radicals, and consequent neuronal cell damage. However, mutant SODs produced in heterogenous systems such as insect[7] and monkey cells[8] exhibit almost the same or only slightly lower activities. Both inhibition of SOD activity[9] and overexpression of mutant Cu, Zn-SOD in transgenic mice[10] induce neuronal cell death, suggesting that the mechanism by which FALS is caused is not simply due to decreased SOD activity. Acquisition of some additional function by the SOD activity is presumed to be the actual cause of this fatal disease.

Several groups showed by electron spin resonance (ESR) using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap reagent that mutant SODs exhibit enhanced hydroxyl radical production via their reaction with hydrogen peroxide.[11,12] Although wild-type enzyme also has this free radical producing activity, some mutant SODs show decreased K_m values but maintain the same apparent V_{max} as the wild-type. However, recent opposing data has been reported and seem to be more convincing.[13] Crow *et al.*[14] showed that the binding affinity for Zn ion is lowered in mutant SODs. We have found that at least seven mutant SODs are less stable than the wild-type enzyme and some show only weak binding for Cu (unpublished data). Since free Cu can mediate a Fenton-type reaction to produce hydroxyl radicals, the released metal ions, but not enzyme activity, may be a direct cause of the disease. In terms of cellular histology, Lewy body-like inclusions are often seen in degenerating motor neurons and mutant SODs are also detected in them, suggesting that mutant SOD protein may undergo aggregation within the neurons. Supporting evidence for this hypothesis is provided by using transgenic mice, which exhibit mutant SOD-containing inclusions like those of patients.[15] Although several other possible mechanisms have been proposed,[16] this appear to be the most attractive one.

ALTERATION OF ANTIOXIDATIVE ENZYME ACTIVITIES THROUGH GENE EXPRESSION

Activities of some antioxidative enzymes are regulated at the stage of gene expression. The expression of the antioxidative enzymes, Mn-SOD, Cu,Zn-SOD, catalase, and glutathione-*S*-transferase subunits 1 and 2 in isolated hepatocytes[17] are suppressed by TGF-β1. A lowered amount of Mn-SOD has been demonstrated in hepatoma where TGF-β1 is expressed.[18] Since TGF-β1 induces apoptosis in hepatocytes, suppression of these antioxidative enzyme genes may trigger apoptosis due to the accumulation of intracellular peroxides.

Animals fed Se-deficient diets suffer from disorders in several organs such as liver, heart, and kidney. A deficiency of this trace element causes defects in the syntheses of Sec-containing

proteins such as GPx and thioredoxin reductase.[19] Cells cultured under Se-deficient conditions are more sensitive to H_2O_2 and/or organic peroxides than those cultured in conventional medium. Despite maintaining other antioxidative enzyme levels constant, Se-deficient cells after treatment with H_2O_2 exhibit internucleosomal DNA fragmentation characteristic of apoptosis.[20] Cells transfected with GPx cDNA become resistant to apoptosis following withdrawal of growth factors.[21] GPx can compensate for the hypersensitivity to oxidant stress of Cu,Zn-SOD-overproducing cells and suppress the induction of apoptotic cell death.[22] Mitochondria may be an important target of ROS because overexpression of Mn-SOD[23] or of phospholipid hydroperoxide GPx[24] protected cells from ROS. TGF-β decreases GPx expression and concomitantly increases susceptibility of pancreatic β-cell lines to oxidative stress-induced apoptosis, supporting a cytoprotective role for this enzyme.[25]

ROLES OF ROS AND RNS IN DESTRUCTION OF ANTIOXIDATIVE ENZYMES

In addition to genetic defects and transcriptional suppression of antioxidative enzymes, post-translational modifications also lower enzymatic activities. ROS and RNS are involved in oxidative modification of cellular components and cause various disorders including neuronal, cardiovascular, and inflammatory disease. Insulin-dependent diabetes mellitus (IDDM) is mediated by an autoimmune mechanism or inflammatory process that is characterized by destruction of pancreatic β-cells. Interleukin-1β (IL-1β) has been proposed to play an important role in generating these conditions. Since IL-1β stimulates the induction of NOS II in pancreatic β-cells, NO is implicated as an effector molecule for glucose-induced insulin secretion as well as cellular injury. We examined effects of NO on rat pancreatic islet cells and β-cell-derived HIT cells and found that both exogenous NO released from a NO donor, *S*-nitro-*N*-acetyl-D,L-penicillamine (SNAP), and endogenously generated NO from NOS II by treatment with IL-1β brought about apoptosis in these cells.[26]

Peroxynitrite, a molecule with strong oxidant activity that is formed by interaction of NO with superoxide, also causes apoptosis in several types of cells. Thus it is conceivable that NO induces apoptosis in certain cells through formation of peroxynitrite. Many molecules possessing essential Fe–S complexes, heme, or free thiol groups are potent targets for NO and several mechanisms are reported for this type of apoptosis. Contrariwise accumulating evidence suggests that NO also functions as a mediator for anti-apoptotic effects. Several hypotheses have been proposed to explain this.[27] Cells such as neurons and pancreatic β-cells, which are sensitive to NO-mediated apoptosis, have rather poor redox capacities. However, some cancer cells and hepatocytes in which NO suppresses apoptosis have stronger redox capacities than the former cells (Figure 1). Hence, redox potentials of a cell may be a major determinant for NO to function as an apoptotic or an anti-apoptotic agent.

Marked induction of NOS II, an inducible form, and concomitant suppression of SODs and GPx activities are found in the inflammatory lesions of experimental colitis in rats.[28] Since both NO and superoxide are produced in colitis tissues, the formation of peroxynitrite is possible.[29] Although GPx can detoxify peroxynitrite,[30] the NO donor, SNAP, selectively inhibits GPx activity, resulting in an increased cellular peroxide level.[31] Since GPx is inactivated specifically by SNAP, nitrosation and oxidation of a specific residue in the catalytic center is a likely cause. GPx contains a rare amino acid Sec, which is more reactive than thiols and is essential for GPx activity. Thus the target for RNS in this case is the active center Sec.[32] This process would be mediated by a two step reaction (Figure 2). The first step would be the nitrosation of a selenium and is reversed by a reductant such as dithiothreitol. The second

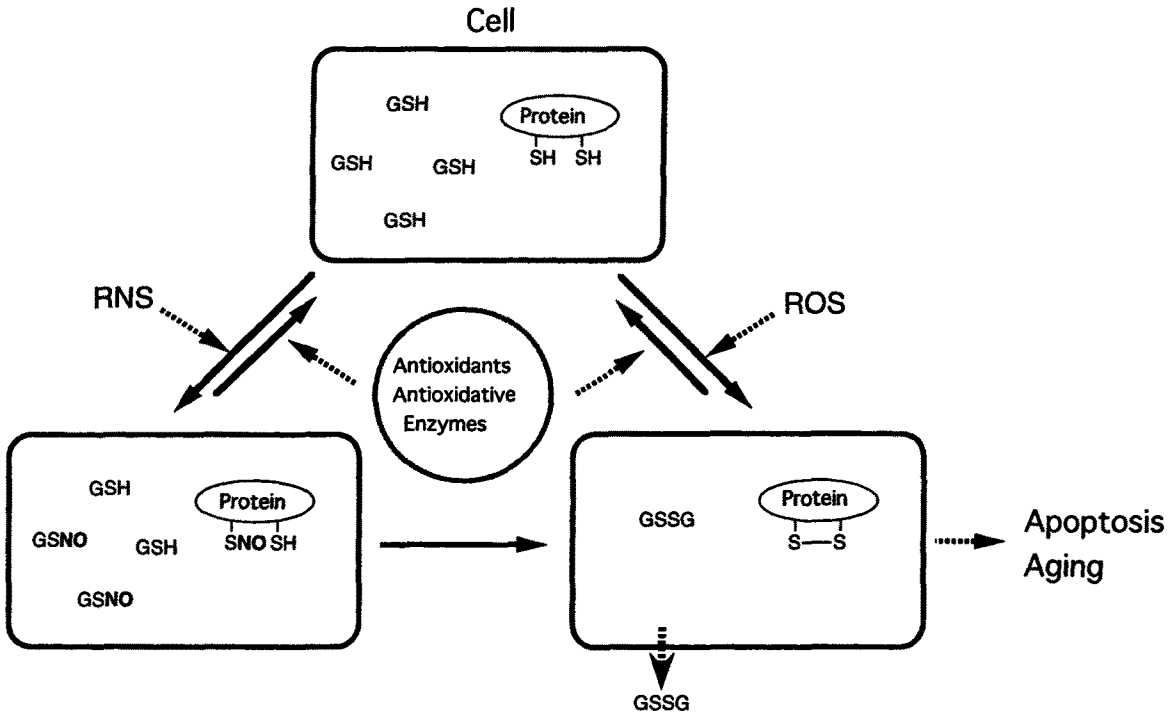

FIGURE 1 ROS and RNS induce oxidative modification and nitrosation of free thiol in cells. A portion of oxidized glutathione (GSSG) is pumped out by a specific transporter in plasma membrane.

reaction is related to formation of a sulfur-seleno bridge between Cys91 and Sec45 in bovine GPx and is not reversed by reducing agents. Since 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1), a precursor of peroxynitrite, as well as synthetic peroxynitrite inactivate GPx irreversibly, the latter process would be caused by peroxynitrite. As a consequence, inactivation of GPx would increase peroxide levels followed by a ROS-mediated chain reaction, leading to various harmful cellular effects.

INVOLVEMENT OF THE GLYCATION REACTION IN THE DYSFUNCTION OF ANTIOXIDATIVE ENZYMES

The carbonyl groups of reducing sugars interact with free amino groups in proteins, lipids, and nucleic acids to form adducts, which are converted to glycation end products (AGE). ROSs are produced during this process. In diabetic conditions, the enhanced glucose levels lead to the accumulation of glycated proteins. The Cu,Zn-SOD of erythrocytes is glycated and inactivated under diabetic conditions.[33] The ROS produced from the Amadori product cause site-specific cleavage of Cu,Zn-SOD followed by random fragmentation through the Cu-mediated Fenton-type reactions.[34] Methylglyoxal and 3-deoxyglucosone, intermediates of the glycation reaction, increase levels of intracellular ROS and induce cellular injury.[35] Thus in addition to the cross-linking of proteins, increases in reactive intermediates such as ROS and dicarbonyl compounds may cause age-related diseases as well as diabetic complications.

While glucose is the major reducing sugar in our body, fructose produced by the polyol pathway has stronger glycating capacity than glucose because the amount of linear, non-furanose form, is about one order of magnitude higher than that

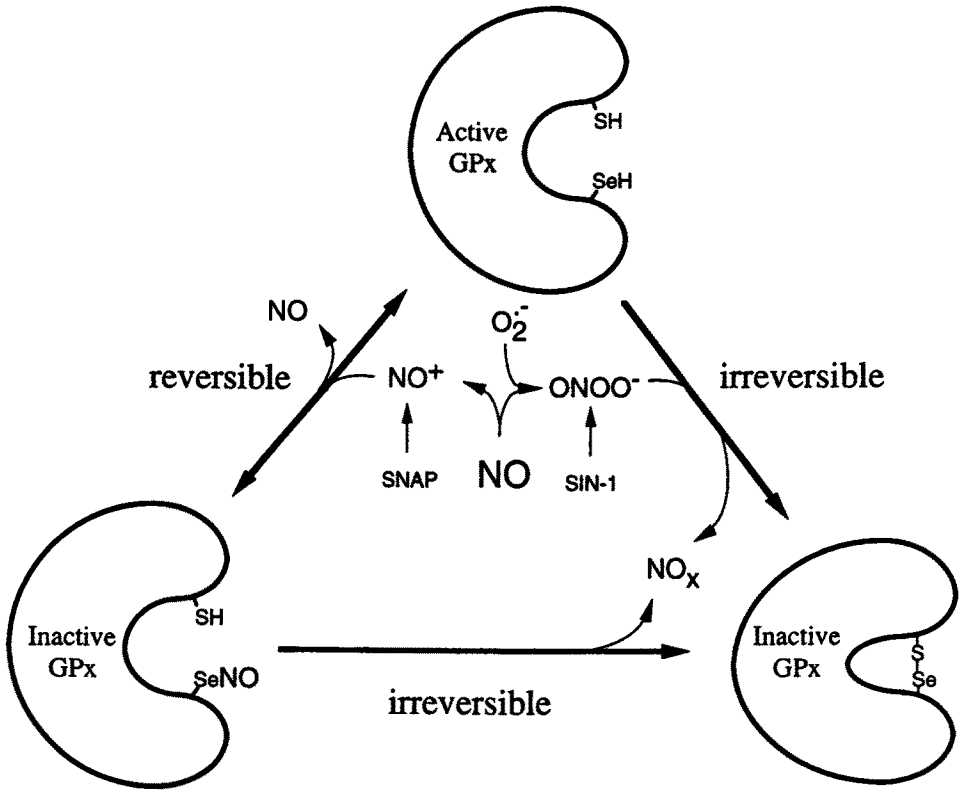

FIGURE 2 A hypothetical pathway for NO- and peroxynitrite-mediated GPx inactivation.

of glucose at a physiological pH. Fructose and ribose have been found to induce apoptosis more rapidly than glucose in a pancreatic β-cell line.[36] The levels of intracellular peroxides, protein carbonyls, and malondialdehyde are increased in the presence of fructose. The adduct of fructose with an amino group, called the Heyns product, is structurally different from the glucose Amadori derivative. It is possible to distinguish them by using specific antibodies capable of recognizing the Amadori[37] and Heyns products.[38] We have utilized these antibodies for evaluation of the extent of glycation reactions in lens tissues.[39] It is apparent that not only reducing sugars but also other compounds with aldehyde or ketone groups may undergo reactions similar to those engendered by glucose and fructose.

Cells have a scavenging system for removing harmful carbonyl compounds, such as methylglyoxal and 3-deoxyglucosone, based on NADPH-dependent enzymes. We have identified aldehyde reductase, a member of the aldo-keto reductases, as an entity.[40] Aldose reductase, another member of the aldo-keto reductase, also belongs to this enzyme family. The cytotoxic effects of 3-deoxyglucosone and glyceraldehyde are enhanced by treatment of cells with an aldose reductase inhibitors.[41] It should be remembered that aldose reductase together with sorbitol dehydrogenase also constitutes a metabolic polyol pathway, which converts glucose to fructose. Thus aldose reductase acts as a biphasic enzyme depending on the substrates. Carbonyl compounds can be eliminated by cells to some extent through actions of aldo-keto reductases. However, prolonged exposure to them would affect NADPH levels and trigger redox imbalances (Figure 3). Since redox systems are involved in

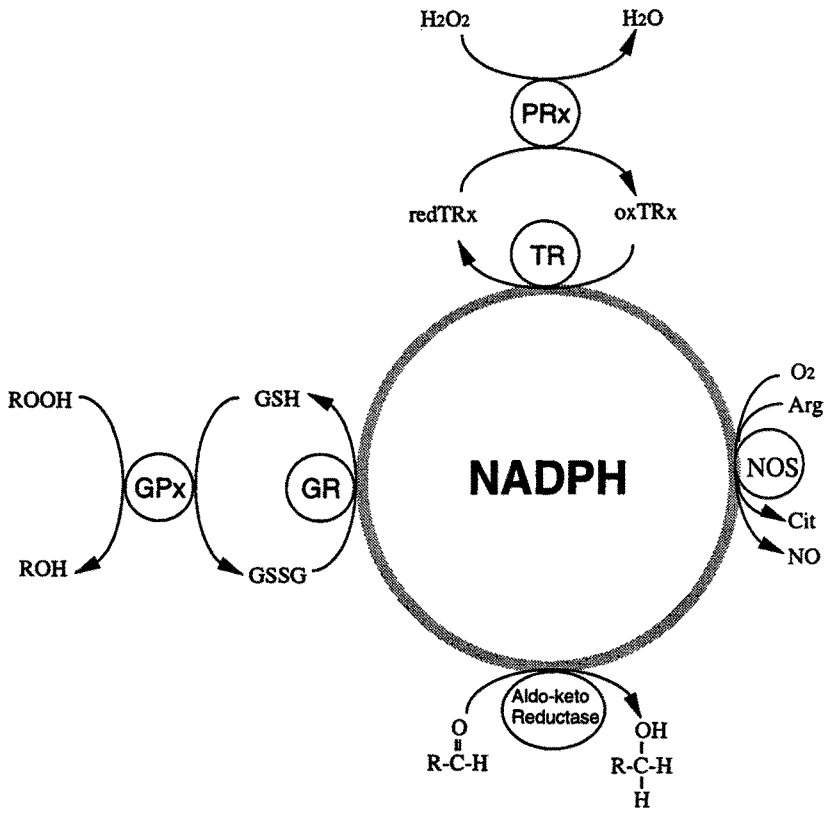

FIGURE 3 Interrelationships among NO synthases, aldo-keto reductases and redox systems through NADPH. GPx, glutathione peroxidase; GR, glutathione reductase; PRx, peroxiredoxin; TRx, thioredoxin; TR, thioredoxin reductase; NOS, NO synthase.

many processes such as gene regulation, cell growth, and suppression of apoptosis,[42] defect in them would also lead to cellular damage.

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