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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Peroxiredoxins

By

Sarita Menon

B-180 Med Labs

Free Radical and Radiation Biology

The University of Iowa

Iowa City, IA 52242-1181

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Abbreviations

GR: Glutathione disulfide reductase

GSH: Glutathione

Prx: Peroxiredoxin

ROS: Reactive oxygen species

Trx: Thioredoxin

TR: Thioredoxin reductase

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Abstract

Reactive oxygen species produced by intracellular biochemical reactions as well as by external agents like ionizing radiation can cause serious damage to cellular components. The antioxidant defense system of the cell protects them from harmful effects of ROS. A new family of peroxidases has been recently identified that are seen to be present in organisms from all kingdoms. This enzyme was first discovered in yeast as a 25-kDa enzyme that protects cells against oxidative damage [1]. As of now there are six isoforms of Prx identified in mammalian cells, and a lot of its properties and functions are yet to be studied. This review will focus on the mammalian Prx family, its structure, classification and mechanism of action.

Introduction

Organisms that respire aerobically are constantly being exposed to intracellularly as well as extracellularly generated, reactive oxygen species. These include superoxide anion, hydroxyl radical and hydrogen peroxide. Antioxidant enzymes like superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GPx) scavenge the reactive oxygen species and protect cells from the deleterious effects of oxidative stress. A new family of proteins has been identified in most organisms, which is also shown to possess antioxidative function. Initially named thioredoxin peroxidases (TPx), these are now commonly known as peroxiredoxins (Prx). So far six isoforms have been identified in mammals. Prx enzymes with molecular size of 20-30 kDa, contain a reactive Cys in the N-terminal conserved domain. The three-dimensional crystal structures of Prx from many organisms have been determined [2,3]. Figure 1 shows the crystal structure of Prx5 indicating the positions of the critical cysteine residues. The catalytic Cys (Cys47) forms a sulfenic acid reaction intermediate during peroxide reduction reactions.

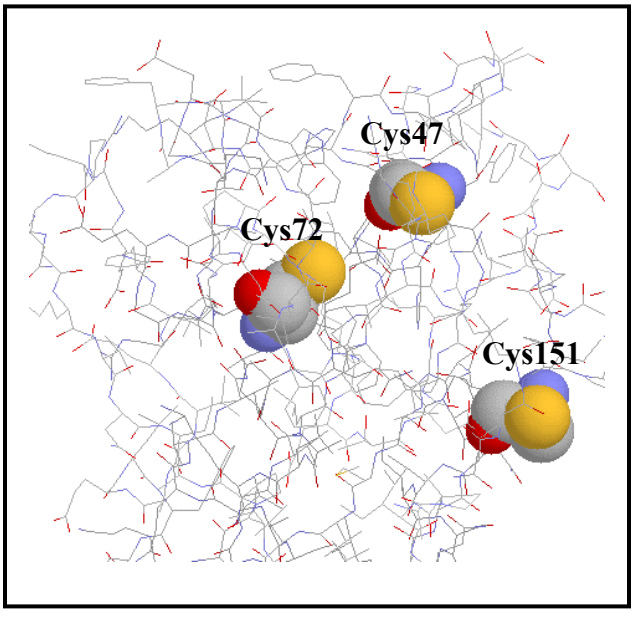

Figure 1. The 1.5 °A resolution crystal structure of human Prx5 in its reduced form. Positions of the N-terminal (Cys47) and C-terminal (Cys151) cysteines are shown. Prx5 does not form a dimer following its reductase activity, but instead an intermolecular disulfide bond is formed between residues Cys47 and Cys151. Due to a 13.8 °A distance between the two cysteines, the disulfide bond formation would require major conformational changes. Adapted from [3].

Classification

All the six isoforms of Prx share striking amino acid sequence homology and their peroxidase activity all depend on thioredoxin (Trx) and/or glutathione (GSH). Inspite of high sequence homology, each is unique, in that they have different catalytic mechanisms, different expression patterns and also are expressed in different subcellular compartments of the cell. Crystallographic analyses have shown that almost all of them possess Trx-like domains. Figure 2 shows a schematic representation of all six members of the Prx family seen in mammalian cells, including their intracellular localization, and associated electron donor.

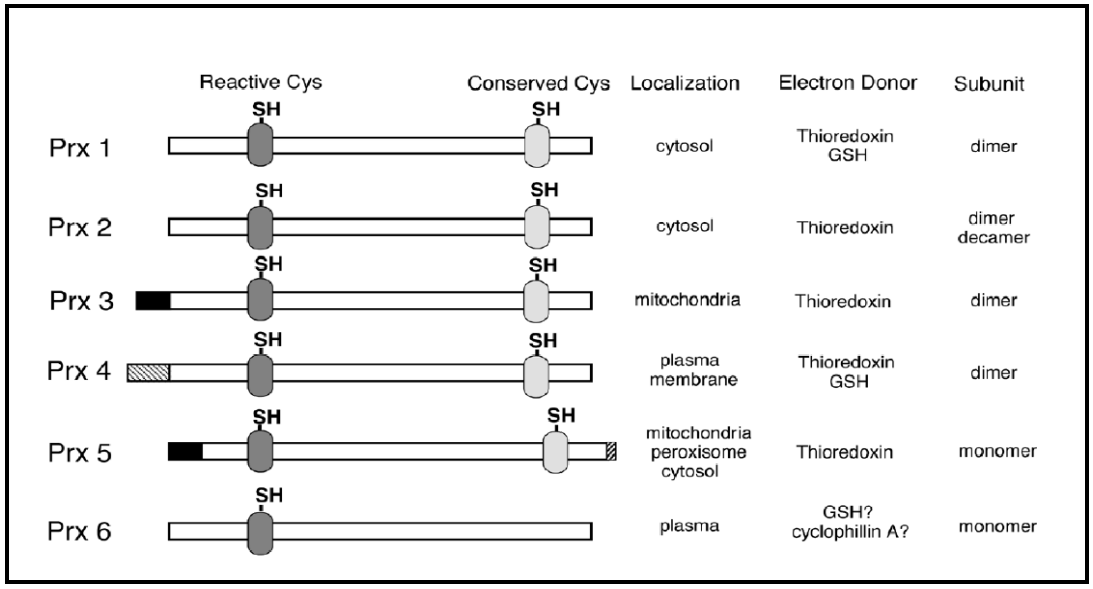

Figure 2. Schematic representation of the mammalian Prx family. Position of the critical catalytic cysteine is shown, in addition to localization in the cellular compartments, and the subunit association [4].

All six isoforms have a Cys residue in its active site near the N-terminus. Prx1 to Prx4 contain another Cys in the C-terminus. The C-terminal Cys in Prx5 is located more in the C-terminal region of the protein, while Prx6 contains only one critical Cys in its N-

teminal region. As seen from the subcellular distribution of the various isoforms, Prx is seen in all locations of ROS production.

Mechanism of Action

One of the main functions of the Prx family is the reduction of peroxides including H_2O_2 (K_m < 20 μM) [6]. Initially, it was thought that the peroxidases reaction was specifically Trx-dependant. Prx I, II, and III proteins each catalyzed the H_2O_2-dependent oxidation of NADPH in the presence of the Trx system (Trx, TR and NADPH) but showed no H_2O_2-dependant NADPH oxidation when the Grx system (Grx, GSH, GR and NADPH) was the electron donor [6]. However, it was later found that GSH functions as an electron donor for Prx6 (K_m (app) = 180 μM) [7] and substitution with Trx system made Prx6 functionally inactive. Thus there seems to be specificity for the electron donor within different members of the Prx family.

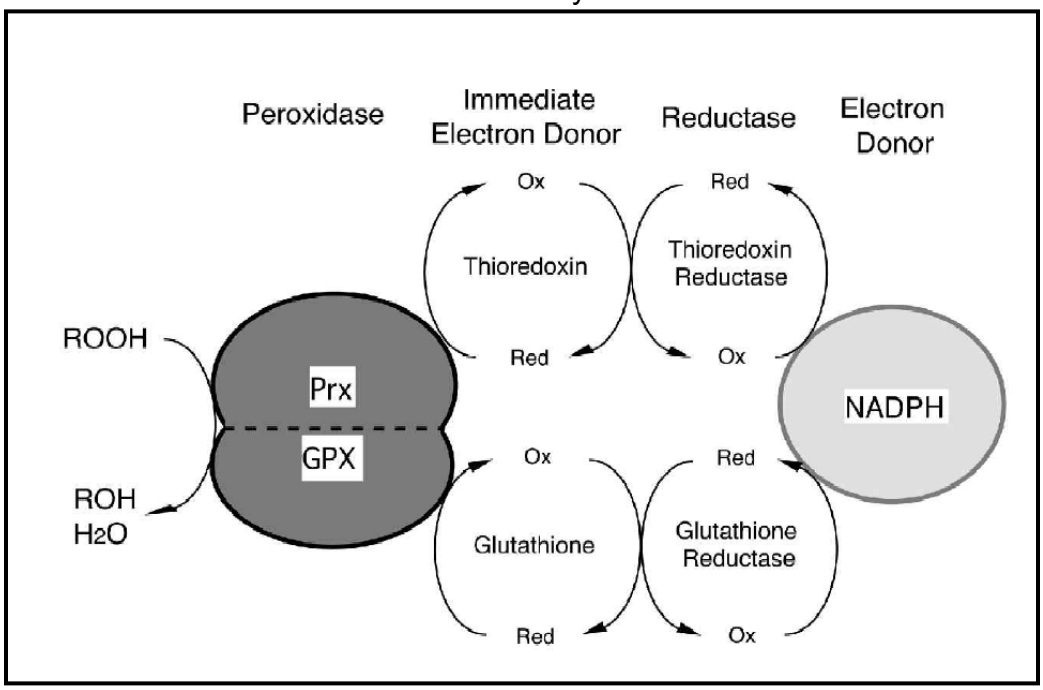

Figure 3. The reaction mechanism of Prx. As seen, both GSH as well as Trx can function as electron donors, however while Trx system functions as electron donor for Prx1 to Prx3 isoforms, the Grx system seems to be the electron donor for Prx6 [4].

Since active site Cys is the main reaction center for peroxidase reaction, different reactions have been proposed based on the presence of the number of Cys in the enzyme. Prx1 to Prx4 contain two critical cysteines and form a homodimer with a head to tail association. Figure 4 represents the reaction mechanism proposed for this group of Prx enzymes. The N-terminal Cys forms sulfenic acid intermediate after reacting with peroxides. It then reacts with the C-terminal Cys and forms an intermolecular disulfide bond.

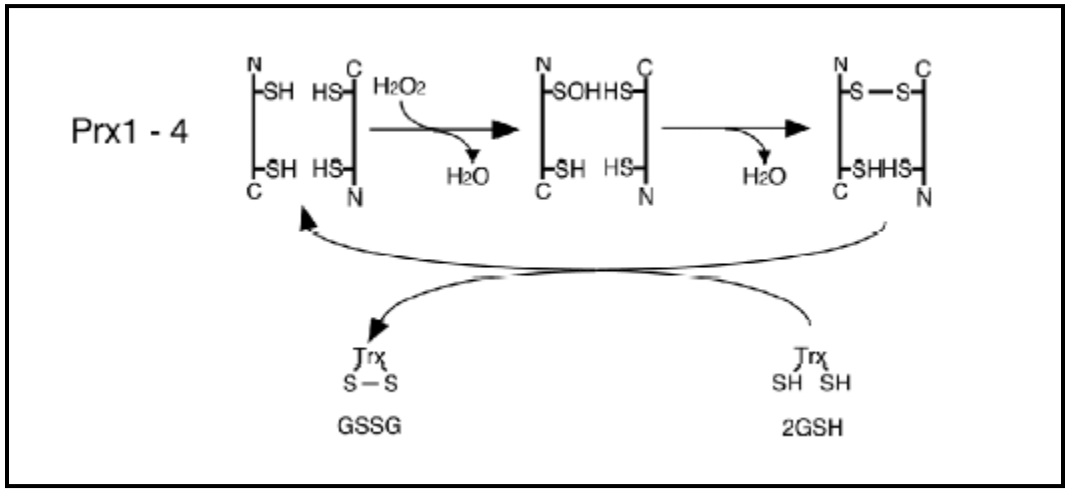

Figure 4. Reaction mechanism of Prx 1 to Prx4 family members with peroxides. The catalytic N-terminal Cys reacts with peroxides and forms Cys sulfenic acid (-SOH), which reacts with the C-terminal cysteine of the other protein and forms an inter-molecular disulfide bond. The Trx system provides the electrons and reduces the cysteines (-SH) [4].

Prx5 is present as a monomer and hence its reaction mechanism is proposed to be slightly different. After formation of the sulfenic acid intermediate, the N terminal Cys-SOH reacts with the C-terminal Cys of the same protein, forming an intramolecular disulfide bond (Figure 5).

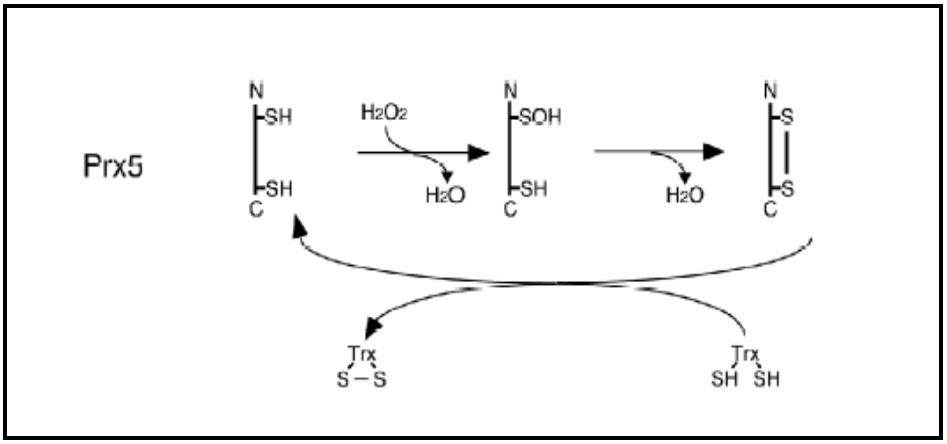

Figure 5. Reaction mechanism for Prx5. Reaction with H_2O_2 .The sulfenic acid intermediate of N-terminal Cys forms an intramolecular disulfide bond with the C-terminal end of the same protein as against the Prx1-4 proteins where the disulfide bond formation occurs between two Prx proteins forming the dimer. Trx reduces the disulfide bond. [4].

Still another mechanism is proposed for the 1-Cys containing isoform, Prx6 (Figure 6). Prx6 is also present as a monomer, and hence cannot form inter or intra molecular disulfide bonds. Although the exact electron donor to oxidized Prx6 is not yet identified some reports have shown GSH as the donor electron [7]. Protein overlay assays have identified a 20-kDa Prx6 binding protein as Cyclophilin A [8].

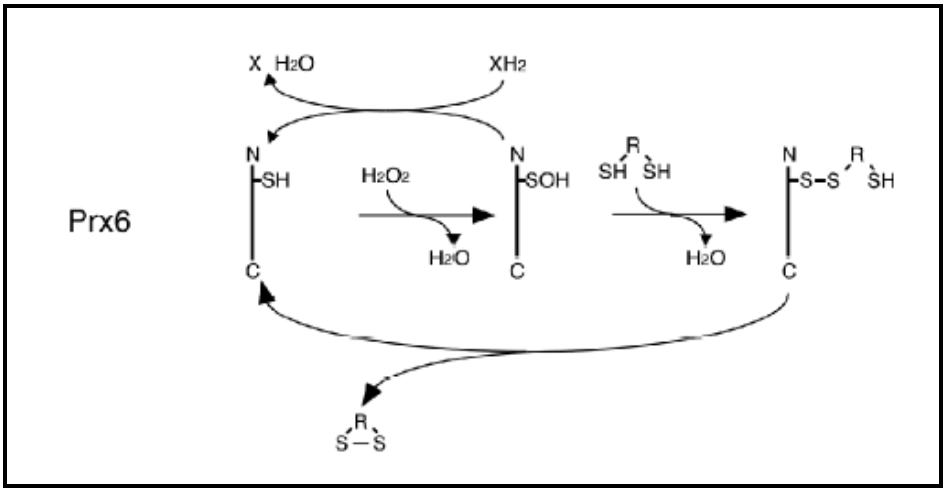

Figure 6. Reaction mechanism of Prx 6. This enzyme contains only one conserved catalytic cysteine in the N-terminal region. Moreover this protein exists as a monomer, and hence the above mechanism has been proposed for this enzyme. The Cys-SOH reaction intermediate could form interprotein disulfide linkages with other proteins [4].

Distribution of Prx in tissues

Like most proteins, peroxiredoxins can also be detected using immunoblot analysis. Isoform-specific antibodies can be used for detecting all the different members of the family. Table 1, shows distribution of the Prx isoforms in various rat tissues. This enzyme seems to be present in almost all tissues at high levels, indicating that this enzyme also plays an important role as an antioxidant [5]. The only isoform not detected is the Prx4, and this is because Prx4 is a secreted protein and found in plasma.

	2-Cys Prx				1-Cys Prx
Tissues	Prx I	Prx II	Prx III	Prx V	Prx VI
Placenta	0.2	0.7	<0.3	0.7	0.2
Thymus	0.3	0.7	0.3	0.2	0.03
Testicle	0.7	1.0	0.3	0.2	1.0
Thyroid	0.7	0.7	0.5	0.3	0.2
Pancreas	N.D.[a]	N.D.[a]	0.3	0.2	0.03
Adrenal	>2.0	2.0	>4.0	0.3	0.3
Brain	1.3	1.3	0.5	1.0	1.7
Hypothalamus	1.3	1.3	0.5	0.7	0.7
Spleen	0.7	2.0	0.3	0.3	0.03
Lung	1.0	1.3	0.3	0.3	1.7
Kidney	2.0	0.7	0.7	1.3	0.3
Liver	0.7	0.5	0.7	0.7	0.3
Heart	1.3	2.0	3.3	0.5	0.3

[a]N.D., not detectable.
Data are expressed as micrograms of Prx per milligram of soluble protein.

Table 1. Tissue distribution of Prx isoforms, in rat. Table illustrates the amounts of various Prx isoforms in the indicated tissues that show a wide expression pattern of all isoforms [5].

Peroxiredoxin IV

Unlike other members of the family Prx4 is the only isoform that exists uniquely in the plasma, even though it shares almost 70% homology with other forms of Prx. Prx4 has an N-terminal signal sequence, which is cleavable and this sequence allows it to be secreted into the plasma. It has been seen that with the intact N-terminal signal sequence, Prx4 can bind to the membrane through heparan sulfate interaction, but the cleaved form cannot [9]. Some speculate that once oxidized, conformational changes

within the enzyme mask binding site of heparan sulfate. Prx4 serves as an antioxidant enzyme in the blood, as is seen from Figure 6. Although the secretable form of Prx4 is enzymatically active and the membrane-associated form is inactive, it is possible that the bound form could serve other functions [10].

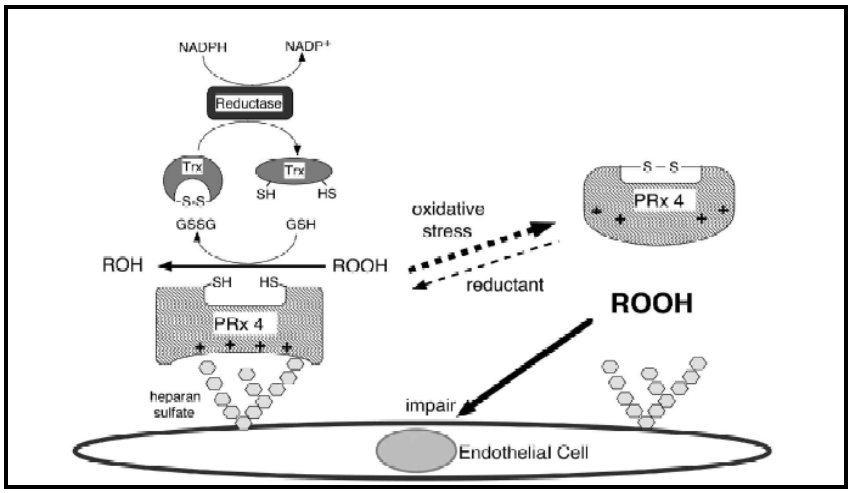

Figure 6. Reaction of Prx4 and its interconversion between bound and secreted forms. Once the redox dependant formation of intermolecular disulfide bond takes place, Prx4 can no longer anchor to the membrane and is secreted into the plasma [4].

Summary

Peroxiredoxins are a relatively new family of antioxidant enzymes found in all species. Some isoforms of Prx like mammalian Prx6 have also been shown to possess additional phospholipase A_2 activity [11]. The main function of peroxiredoxin is to regulate the intracellular levels of hydrogen peroxide and thereby protects cells from H_2O_2 mediated cellular responses like apoptosis [12]. This regulation of H_2O_2 also has an impact on cell proliferation. Likewise it has recently been shown that Thr (90) on Prx1 can be phosphorylated by cell cycle dependant kinases (CDK). The phosphorylation inactivates Prx1 and thereby results in accumulation of H_2O_2 that seems to be necessary for cell cycle progression [13]. Moreover, almost all the Prx isoforms are seen to be overexpressed during tumorigenesis [14]. Hence, due to their ubiquitous expressions, peroxiredoxins could play a more important role during

oxidative stress, however more studies need to be done to define the exact roles and reaction mechanisms of the Prx members

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