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The Chemistry of Peroxiredoxins

by

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Abbreviations

Cys	cysteine
DTT	dithiothreitol
HBP23	heme-binding protein, 23 kDa
hPAG	human proliferation-associated gene product
hORF6	human open reading frame 6; human Prx VI, a 1-cys Prx
NADPH	reduced nicotinamide adenine dinucleotide phosphate
PLA_2	phospholipase A_2
Prx	peroxiredoxin
ROOH	alkyl hydroperoxide
ROS	reactive oxygen species
SOH	sulfenic acid
TSA	thiol specific antioxidant
TPx	thioredoxin peroxidase
TrxR	thioredoxin reductase
Trx	thioredoxin
WT	wild type
yTAS	yeast thiol specific antioxidant

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Abstract

Peroxiredoxins (Prx) are a relatively recently described and important family of antioxidant proteins. Prx proteins are peroxidases, catalyzing the degradation of peroxides to either water or alcohol, depending on the substrate. Structurally, all Prx proteins have a thioredoxin fold with a conserved Cys residue present in their N-terminal region. Peroxiredoxins belong to one of two major groups, the 2-Cys and 1-Cys Prx. The former possess an additional Cys in their C-terminal domain, while the latter have only the single Cys. This C-term domain forms a disulfide bond with the oxidized catalytic Cys of another 2-Cys Prx molecule, which can be reduced by Trx. 1-Cys Prx do not form disulfide bonds with other Prx, and are not reduced by Trx. The physiologic reductant for 1-Cys Prx is unknown. Prx exhibit a number of redox-related enzymatic activities, which make them obvious candidates for signal transduction components or modulators.

Introduction

Peroxiredoxins comprise a large group of related proteins, the function of which is to catalyze the degradation of ROOH and H_2O_2 [1]. In excess of 40 gene sequences have been identified as possessing homology with the first identified member of the family, yeast TSA, although not all have been assigned a function as yet [1]. Yeast TSA, now known as Prx I, was first identified as a 25 kDa protein capable of preventing the oxidant-induced inactivation of glutamine synthetase [2]. In that original report, the authors demonstrated that they had isolated a factor that exerted its protective effect towards glutamine synthetase in the presence of a thiol reductant, DTT, but not in combination with a non-thiol reducing agent, ascorbate [2]. In subsequent work, Prx I was shown to in fact be a peroxidase, capable of reducing both ROOH and H_2O_2 [3]. Perhaps even more interesting than the discovery of a novel family of peroxidases was the finding that at least some of them used Trx as their immediate electron donor, which was the first reported example of Trx supplying reducing equivalents to a peroxidase [3].

That the Prx family of proteins is an important class of oxidant scavenging enzymes was first made obvious by the observation that aerobically grown yeast express Prx I to such a degree that as much as 0.7% of their total cellular protein consists of this enzyme [4]. Since that first report, the existence of numerous Prx homologues in a wide range of organisms and tissues has been demonstrated [1]. Proposed roles for Prx range from scavenging of H_2O_2 and repair of membrane lipids to signal transduction and modulation of apoptosis induction [3, 5-7]. Given the now appreciated role of ROS in physiology, the need to study this ubiquitous and versatile family of antioxidant proteins should be well evident, and to that end this review is offered.

Structure and Properties

The peroxiredoxins are a family of relatively small (ca. 25 kDa) proteins that share as their common structural feature a domain known as the thioredoxin fold, which consists of five twisted beta strands, running parallel to two alpha helices. Within the thioredoxin fold of the Prx protein is a single conserved cysteine, unlike thioredoxin itself, which has two [8]. Two types of Prx are defined by most authorities, the 2-Cys and 1-Cys, examples of which are shown below in Figure 1 [8].

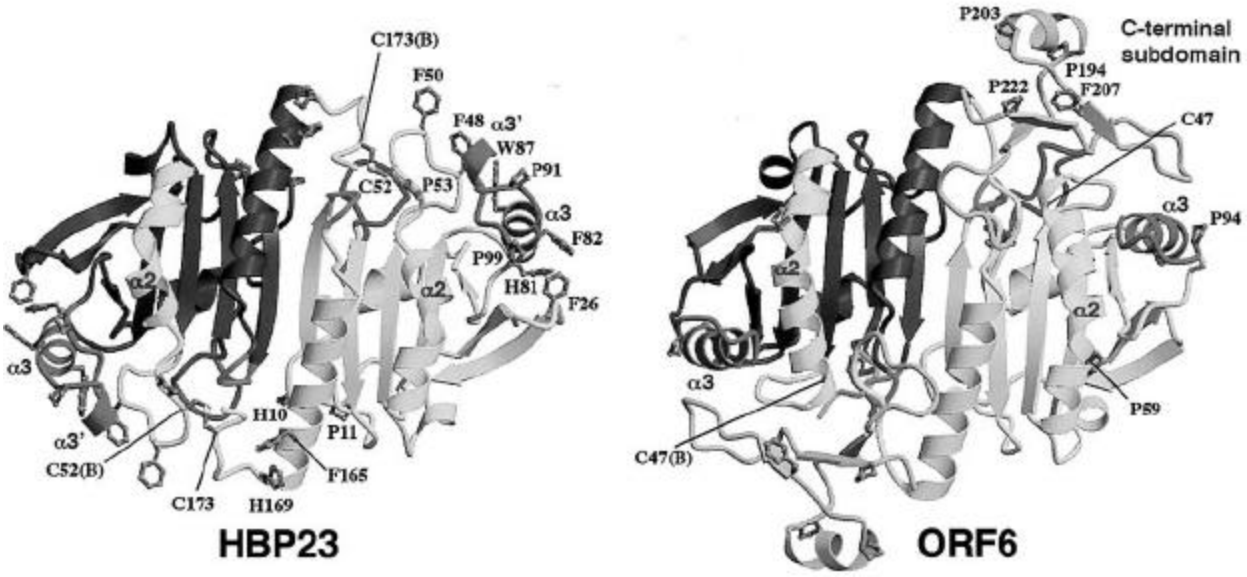

Figure 1. Structures of Representative Peroxiredoxins. On the left is a homodimer of a representative 2-Cys peroxiredoxin, the rat homologue of hPAG/yTAS (Prx I). On the right is the homodimeric form of the human 1-Cys Prx, hORF6 (Prx VI). The conserved redox active N-terminal cysteines are noted at C52 (HBP23) and C47 (hORF6). Adapted from [8].

terminal fashion [9]. In addition to the single conserved N-terminal cysteine residue found in all peroxiredoxins (C47 and C52, Figure 1), 2-Cys peroxiredoxins have a C-terminal Cys residue that forms an intermolecular disulfide bond with the active site Cys during the redox cycle [8, 9]. Another notable difference between the structures of 2-Cys Prx and the 1-Cys varieties is that the active site Cys of the 1-Cys Prx is relatively solvent inaccessible compared to the 2-Cys [8].

This solvent accessibility may play a role in the observed redox mechanisms of the two classes, as will be discussed below.

Catalytic Mechanisms

The N-terminal Cys residue is thought to be involved in the peroxidase activity of both types of Prx [1, 3, 10]. As shown below in Figure 2., H_2O_2 first oxidizes the N-terminal Cys residue (either Cys^{52} or Cys^{47}, in the example given) to a sulfenic acid (–SOH) [1, 3]. For a 2-Cys Prx, the next step is condensation with the C-terminal Cys residue of another 2-Cys Prx to form an intermolecular disulfide, followed by Trx-dependent reduction to regenerate the reduced active site thiol [1, 3]. The reaction mechanism of the 1-Cys Prx has been found to be somewhat different. Obviously, since the 1-Cys peroxiredoxins lack the C-terminal Cys residue necessary for the formation of the intermolecular disulfide seen in the oxidation of the 2-Cys peroxiredoxins, the initially formed SOH intermediate is free to react with some other SH group [10]. What the identity of that group might be is unknown, as is the identity of the electron donor that may reduce the SOH intermediate (depicted below as XH_2) [9, 10].

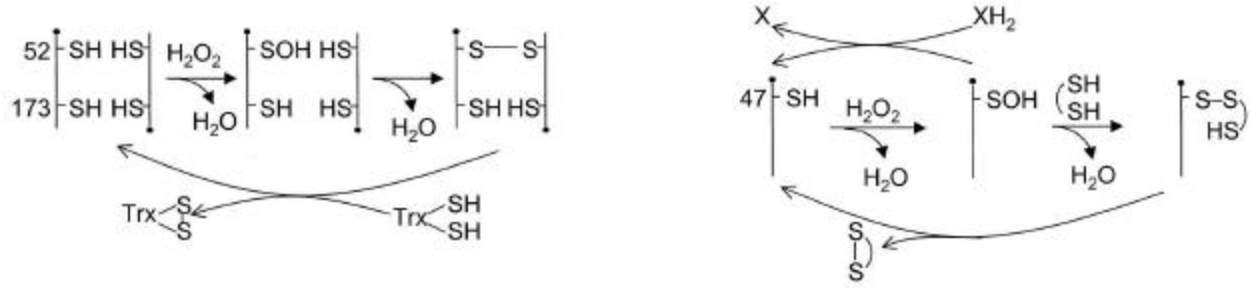

Figure 2. Reaction Mechanisms of Representative Peroxiredoxins. On the left is a representative human 2-Cys peroxiredoxin, hPAG (Prx I). On the right is the human 1-Cys Prx, hORF6 (Prx VI). Adapted from [9].

Analytical Methods

Historically, the most commonly used measure of Prx activity has been the glutamine synthetase protection assay [2, 11]. This method is based upon the fact that oxygen radicals inactivate glutamine synthetase, and that Prx can prevent this by scavenging H_2O_2 [2, 11]. Briefly, assays are performed in a mixture containing glutamine synthetase, iron, DTT, and a source of Prx [11]. The reaction is initiated by the addition of DTT and Fe^{3+} to the other components, followed by a 10-minute incubation at 30°C [11]. After this 10-minute inactivation reaction is complete, the residual glutamine synthetase activity is measured by adding L-glutamine and hydroxylamine to the mixture, followed by the more Fe^{3+} [11]. The product of the enzymatic reaction plus Fe^{3+} is ?-glutamyl hydroxamate-Fe^{3+}, which can be measured spectrophotometrically at 540 nm [11]. Because this assay is based upon an enzymatic activity it is inherently sensitive [11]. However, owing to the fact that oxidant-induced glutamine synthetase inactivation is not linear with respect to H_2O_2 concentration, this method cannot be considered truly quantitative [11].

An alternative method for measuring Prx uses the enzymatically coupled oxidation of NADPH as an indicator of Prx activity. This assay takes advantage of the fact that the reduction of H_2O_2 by 2-Cys peroxiredoxins is accomplished through the donation of electrons from Trx, which in turn is reduced by NADPH *via* Trx reductase (TrxR). The reaction mixture consists of NADPH, Trx, TrxR, H_2O_2, and the Prx sample. The progress of the reaction can be followed at 340 nm, and is considered to be linear with respect to time, provided all components save the Prx are used in excess. Known problems with this assay include the irreversible higher order oxidation of the active site Cys to sulfinic or sulfenic acids at high H_2O_2 concentrations, and the inability to measure the activity of 1-Cys Prx enzymes.

Biochemistry

The primary activity associated with peroxiredoxins is that of the reduction of H_2O_2 and ROOH to water or ROH. As shown in Figure 3, the Trx-dependent oxidation of NADPH in the presence of H_2O_2 indicates that Prx I, II, and III have peroxidase activity. Furthermore, this reductive activity could not be supported by other thiols, as a combination of glutaredoxin, GSH, and GSH reductase did not lead to NADPH oxidation.

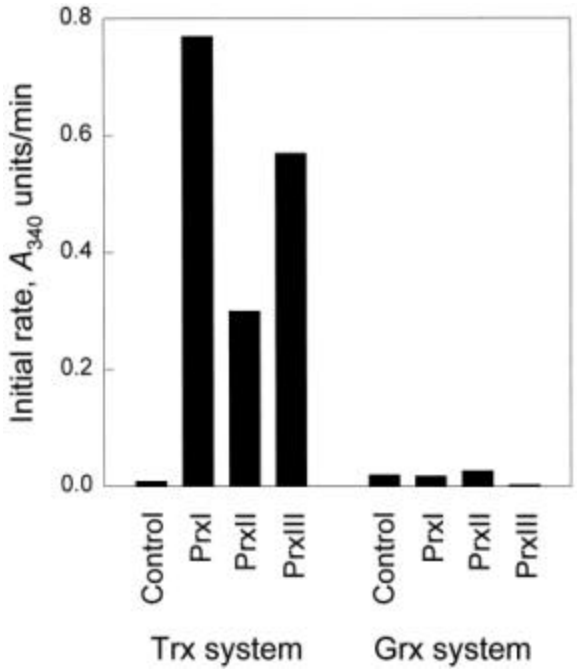

Figure 3. The rate of H_2O_2 reduction catalyzed by Prx enzymes in the presence of the Trx or Grx systems. NADPH oxidation was monitored spectrophotometrically in the absence (control) or presence of Prx (Prx I, II, or III) at 37°C. The reaction mixture contained NADPH, Prx protein, and either TR and Trx (Trx system) or GR, Grx, and glutathione (Grx system). The reaction was initiated by the addition of 0.5 mM H_2O_2. Adapted from [10].

In addition to peroxidase activity directed towards H_2O_2, some Prx isoforms have been reported to have alkyl or phospholipid hydroperoxide peroxidase activity [1, 3, 12]. Perhaps most intriguing of all is the reported bifunctional, or "moonlighting" enzyme, 1-Cys Prx (Prx

VI), which has been reported to have phospholipase A_2 activity in addition to its peroxidase function [5, 10]. Figure 4 shows the PLA_2 activity of NIH 3T3 cells transfected with 1-Cys Prx [10]. Note that transfection with a mutant Prx known to be peroxidase-inactive (C? S mutants) increases PLA_2 activity by as much as WT, which is indicative of separate active sites [5, 10].

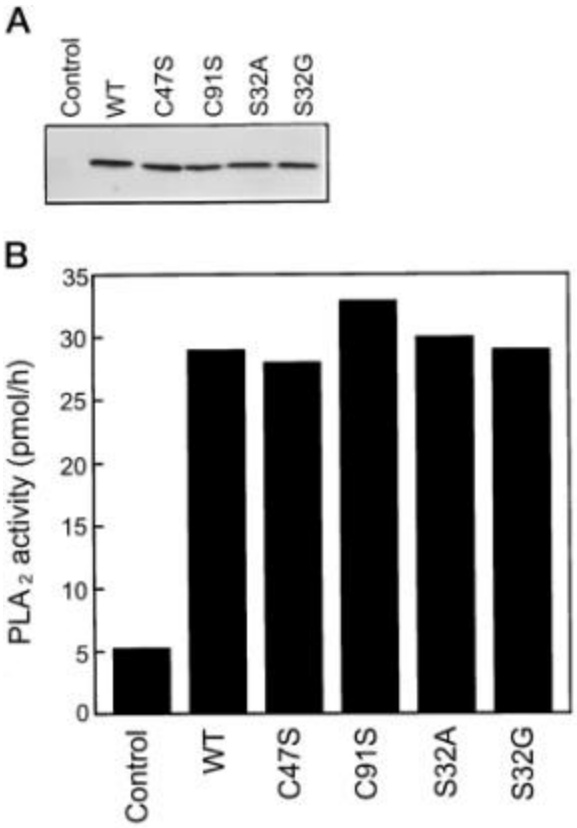

Figure 4. PLA_2 activity catalyzed by transfected Prx enzymes. PLA2 activity in transfected cells was measured in cellular lysates by reacting lysate with ^3H-labeled phospholipid and measuring the appearance of radiolabeled product by TLC. Panel A shows the level of Prx protein in transfected cells. Panel B compares the PLA_2 activity of mutants designed to knock out redox activity (C→S) or the putative site of PLA_2 activity (S→A,G). Adapted from [10].

Summary

Peroxiredoxins are an evolutionarily conserved and ubiquitous group of antioxidant proteins. Prx proteins have a conserved Cys residue present in their N-terminal region; this is the redox active residue responsible for peroxidase activity. Prx proteins belong to one of two groups, the 2-Cys and 1-Cys Prx. The former possess, in addition to their catalytic Cys residue,

another Cys in their C-terminal domain. This C-term domain forms an intermolecular disulfide bond with the oxidized catalytic Cys of another 2-Cys Prx molecule, which can be reduced by Trx. 1-Cys Prx do not form disulfide-bonded dimers, and are not reduced by Trx; the identity of the electron donor for 1-Cys Prx reduction is unknown. Prx exhibit a number of enzymatic activities, most notably redox-related, which make them attractive candidates for signal transduction components or modulators.

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