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Review

Peroxiredoxins as biomarkers of oxidative stress ☆

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ABSTRACT

Background: Peroxiredoxins (Prxs) are a class of abundant thiol peroxidases that degrade hydroperoxides to water. Prxs are sensitive to oxidation, and it is hypothesized that they also act as redox sensors. The accumulation of oxidized Prxs may indicate disruption of cellular redox homeostasis.
Scope of review: This review discusses the biochemical properties of the Prxs that make them suitable as endogenous biomarkers of oxidative stress, and describes the methodology available for measuring Prx oxidation in biological systems.
Major conclusions: Two Prx oxidation products accumulate in cells under increased oxidative stress: an intermolecular disulfide and a hyperoxidized form. Methodologies are available for measuring both of these redox states, and oxidation has been reported in cells and tissues under oxidative stress from external or internal sources.
General significance: Monitoring the oxidation state of Prxs provides insight into disturbances of cellular redox homeostasis, and complements the use of exogenous probes of oxidative stress. This article is part of a Special Issue entitled Current methods to study reactive oxygen species — pros and cons.
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1. Introduction

Peroxiredoxins (Prxs) are a class of thiol peroxidases that degrade hydroperoxides to water [1,2]. Catalase and glutathione peroxidases also remove hydroperoxides, and these enzymes were considered to be the major enzymes responsible for protecting cells against hydroperoxides. However, recent data on the reactivity and abundance of the Prxs has revealed them to also be prominent members of the antioxidant defence network. There are indications that the role of Prxs in antioxidant defence is more complex than the removal of hydroperoxides. Prxs can be easily inactivated by hydrogen peroxide, disabling peroxidase activity and thereby limiting their ability to act as antioxidants [3]. Eukaryote Prxs are considerably more susceptible to oxidative inactivation than prokaryote family members, suggesting a gain-of-function that has been selected by evolution [4]. Prxs also have complex oligomeric structures that are influenced by the redox state of the protein [5–7]. This knowledge has led to the hypothesis that Prxs act as redox sensors, regulating signal transduction pathways upon oxidation [8,9].

Redox sensors detect disturbances in redox homeostasis, and as such, they are ideal entities to monitor for signs of oxidative stress. Many redox signalling models invoke transient and localized generation of reactive oxygen species. Markers of global protein and lipid oxidation associated with oxidative damage are insufficiently sensitive for detecting subtle modulations of redox homeostasis. To aid this endeavour, an array of redox reporter probes has become available for experimental use. An alternate approach is to specifically monitor endogenous redox-sensitive proteins. This review discusses the biochemical properties of the Prxs that make them suitable as sensitive biomarkers of oxidative stress, describes current methodology for measuring Prx oxidation in biological samples, and highlights studies where oxidation has been reported.

2. Biochemical properties of the Prxs

Prxs are highly conserved proteins that have been identified in all phyla. Many species contain more than one Prx; mammals have six different Prxs, with Prxs 1, 2 and 6 located in the cytosol, Prx 3 in the mitochondrial matrix, Prx 4 in the endoplasmic reticulum and Prx 5 in mitochondria, peroxisomes and the cytosol. Prx activity is characterised by a peroxidatic cysteine that is oxidized to a sulfenic acid by hydroperoxides including hydrogen peroxide, organic peroxides, peptide and protein hydroperoxides, and peroxynitrite [10–14] (Fig. 1). In 1-Cys Prxs, the sulfenic acid is reduced by low molecular weight thiols or ascorbate. In 2-Cys Prxs, the sulfenic acid reacts with an adjacent resolving cysteine to form a disulfide. Typically, the 2-Cys Prxs are present as non-covalent homodimers arranged in a head-to-tail formation, and disulfide

Abbreviations: AMS, 4′-acetamide-4′maleimidylstilbene-2,2′-disulfonic acid; Cys, cysteine; IAM, iodoacetamide; NEM, N-ethylmaleimide; Prx, peroxiredoxin; Srx, sulfiredoxin

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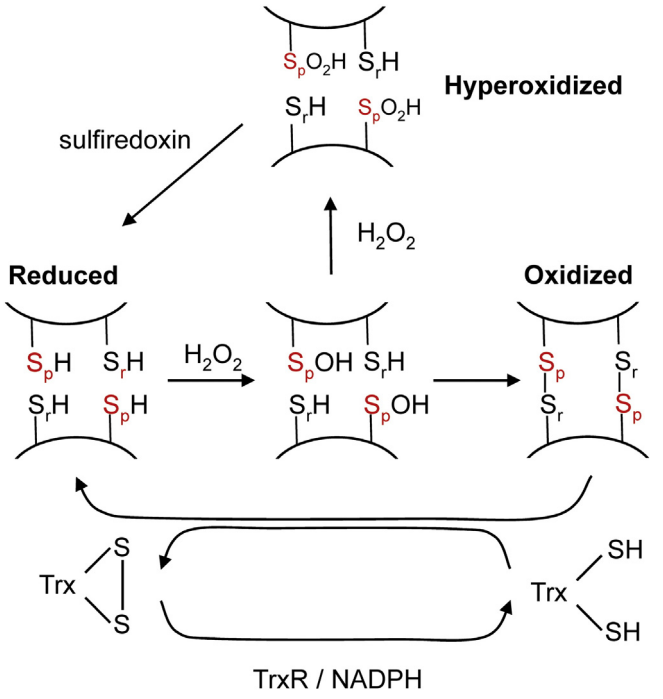

Fig. 1. Redox interconversions of typical 2-Cys Prxs. The peroxidatic cysteine (S_pH) reacts with hydrogen peroxide to form a sulfenic acid. The oxidized cysteine can then condense with the resolving cysteine (S_rH) on the opposing Prx subunit to form an intermolecular disulfide bond that is reduced by the Trx/TrxR/NADPH system. The sulfenic peroxidatic cysteine (S_pOH) can also react with hydrogen peroxide molecule to form the hyperoxidized sulfinic form (S_pO_2H). The hyperoxidized protein has no peroxidase function, but can be recycled back to the reduced form by sulfiredoxin (Srx). The Prx homodimer has two active sites; the scheme shows both being oxidized in the same way, but it is possible to get mixed oxidation products with a dimer at one end and hyperoxidation at the other.

formation occurs between the peroxidatic cysteine of one monomer with the resolving cysteine of another monomer. The homodimers of oxidized Prxs are therefore linked through an intermolecular disulfide that can be distinguished by non-reducing SDS-PAGE. The thioredoxin system is responsible for reduction of the 2-Cys Prxs. Both thioredoxin and thioredoxin reductase are present at lower concentrations in cells than the Prxs, and in vitro studies indicate that the reduction of the disulfide is the rate-limiting step in the Prx catalytic cycle [6]. As such, the transient accumulation of oxidized Prxs is predicted in cells placed under oxidative stress.

Early experiments measuring the reaction of yeast, trypanosome and prokaryotic Prxs with hydroperoxides estimated rate constants in the range of 10^4–10^5 M^{-1} s^{-1} [15,16]. In systems with significant catalase or glutathione peroxidase expression, which have rate constants in the order of 10^7–10^8 M^{-1} s^{-1}, the Prxs would therefore not be considered as significant antioxidants. However, in these experiments measurement of steady state Prx activity was coupled to NADPH oxidation in the presence of thioredoxin and thioredoxin reductase, and was subsequently shown to be limited by the rate of reduction of the oxidized Prx [6]. Direct measurement of the reaction of hydrogen peroxide with reduced Prxs has revealed rate constants in the order of 10^7 M^{-1} s^{-1} [10,11,17,18]. Recent experiments provide insight into the dramatic increase in reactivity of the peroxidatic cysteine. Cysteine nucleophilicity is increased through lowering of its pK_a, and there is an arginine residue appropriately positioned in the active site to perform this function. However, changes in pK_a are insufficient to explain the increased reactivity, and structural models reveal that the arginine also lowers the activation energy of the reaction through hydrogen bonding to the reacting oxygen of the hydroperoxide [19]. Experimental data with Prx site-directed mutants confirmed a role for this arginine, and also revealed a critical role for a second arginine that is proposed to hydrogen bond with the leaving oxygen atom [20].

The peroxidatic and resolving cysteines of 2-Cys Prxs are approximately 13 Å apart [21], and disulfide bond formation requires local unfolding and movement of the peroxidatic sulfenic acid to the resolving cysteine. While the peroxidatic cysteine is present in the active site as a sulfenic acid it is able to react with a second molecule of hydrogen peroxide, producing a sulfinic acid (Fig. 1). This hyperoxidation (or overoxidation) reaction inactivates the peroxidase activity of the protein. Intriguingly, hyperoxidation is predominantly restricted to eukaryotic Prxs, and is associated with a C-terminal extension that slows unfolding [4]. Recently, the rate constant for the hyperoxidation reaction of Prxs 2 and 3 was reported to be 1.2×10^4 M^{-1} s^{-1} [22]. While the rates of Prx 2 and 3 hyperoxidation were identical, Prx 3 is more resistant to hyperoxidation than Prx 2 because it has a faster rate of dimerization [22].

Post-translational modification and intracellular protein–protein interactions have the potential to influence Prx catalytic activity and susceptibility to hyperoxidation. Phosphorylation, glutathionylation and S-nitrosylation are all reported to directly influence the peroxidase activity and/or oligomeric structure of Prxs [23–26]. In one example, phosphorylation of Tyr194 of Prx 1, which is within 9 Å of the peroxidatic cysteine, was reported in cells stimulated with growth

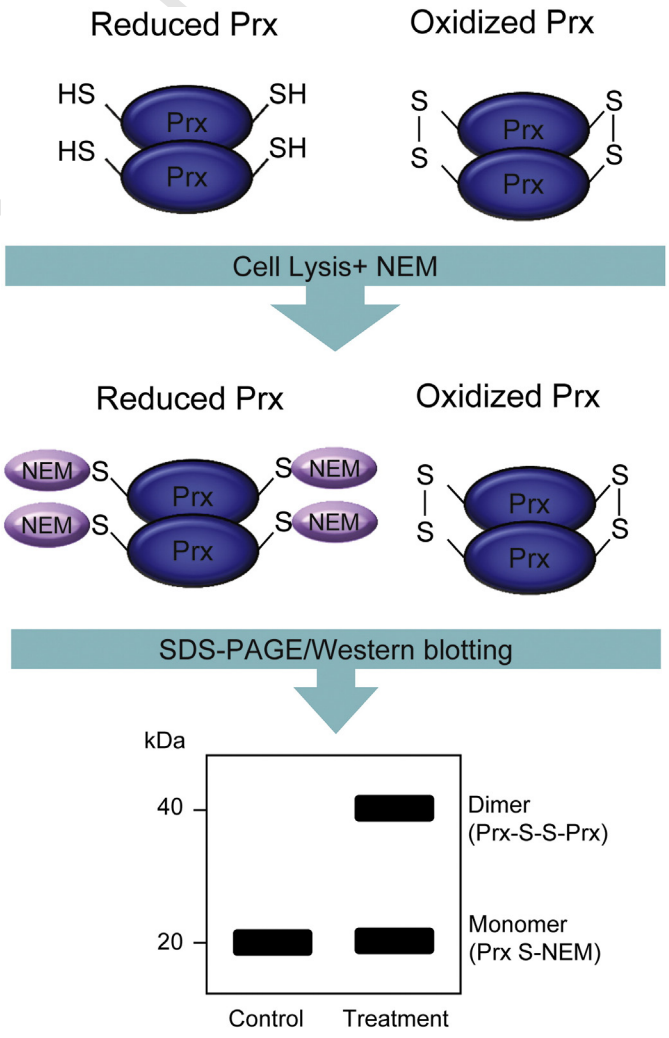

Fig. 2. Measurement of oxidized dimers of 2-Cys Prxs. Cells are lysed in the presence of an alkylating agent such as NEM, trapping the reduced monomer. The monomer and dimer forms are separated by non-reducing SDS-PAGE and visualised by Western blotting with specific Prx antibodies.

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