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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 and biophysics of membrane proteins. Guest Editor: Christine Winterbourn.

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 have complex oligomeric structures that are influenced by the redox state of the protein [5–7]. This combined 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-

Abbreviations: AMS, 4′-4-acetamido-4′-((iodoacetyl)amino)stilbene-2,2′-disulfonic acid; Cys, cysteine; IAM, iodoacetamide; NEM, N-ethylmaleimide; Prx, peroxiredoxin; Srx, sulfiredoxin

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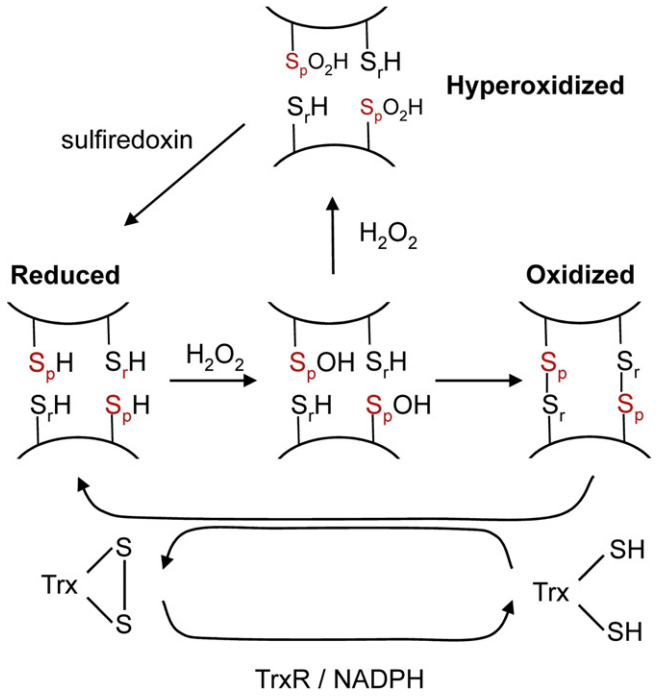

Fig. 1. Redox interconversions of typical 2-Cys Prxs. The peroxidatic cysteine (S_pH) reacts with hydrogen peroxide to form a sulfenic acid. The oxidized cysteine can then condense with the resolving cysteine (S_rH) on the opposing Prx subunit to form an intermolecular disulfide bond that is reduced 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.

covalent homodimers arranged in a head-to-tail formation, and disulfide 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 obtained with Prx mutants confirmed a role for this arginine. A critical role was also revealed for a second arginine, which 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

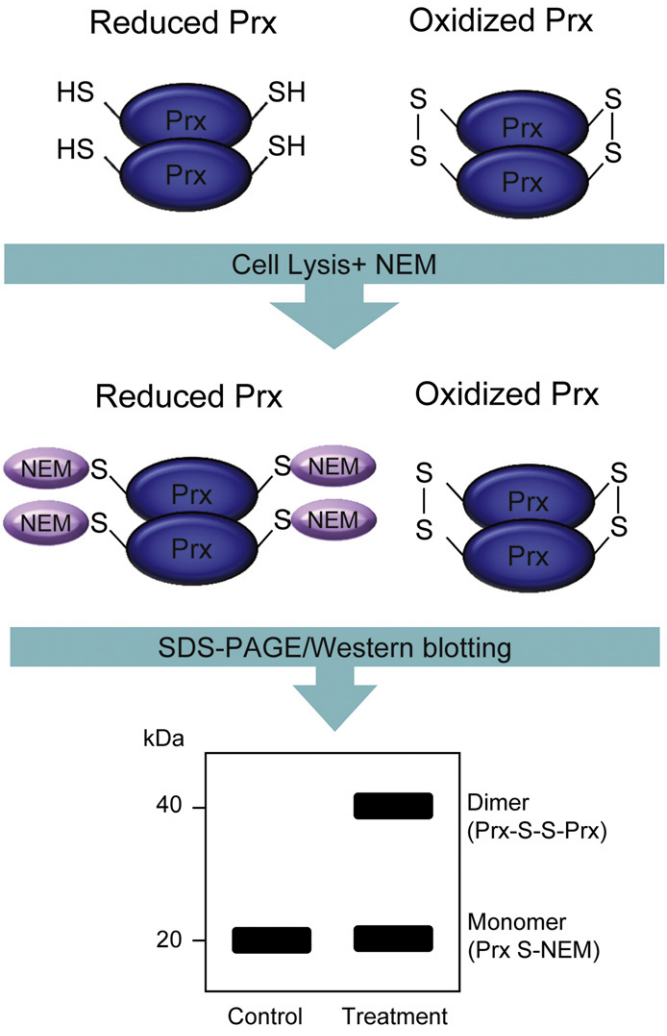

Fig. 2. Measurement of oxidized dimers of 2-Cys Prxs. Cells are lysed in the presence of an alkylating agent such as NEM, trapping the reduced monomer. The monomer and dimer forms are separated by non-reducing SDS-PAGE and visualised by Western blotting with specific Prx antibodies.

factors, and the modification had a measurable effect on peroxidase activity [27]. While a variety of proteins have been shown to interact with cellular Prxs, little is known about their ability to influence the redox status of Prxs.

It was initially believed that Prx hyperoxidation was irreversible and that new synthesis was required to reconstitute Prx activity. However, Prx recovery was observed in cells placed under oxidative stress that had been treated with protein synthesis inhibitors [28]. The protein sulfiredoxin (Srx) was discovered to bind hyperoxidized Prxs [28] and reduce them using Mg^{2+} and ATP as co-factors [29,30]. This reduction is very slow, and hyperoxidized Prxs can be present for several hours after exposure to oxidative stress [31], depending on the level of Srx present. Srx is known to be upregulated in response to various environmental factors [32].

Prxs are highly abundant proteins, constituting up to 1% of the total protein in some organisms and cell types. The combination of abundance and reactivity ensures the Prxs are major targets of cellular hydroperoxides [9]. Indeed, 90% of the hydrogen peroxide generated within mitochondria has been predicted to react with reduced Prx 3 [33], assuming that reduction of the Prx disulfide is sufficiently rapid to maintain a pool of reduced protein. The abundance of these proteins has contributed to the appearance of Prxs in a number of proteomic studies, either by altered expression in response to oxidative stress, or as proteins that undergo redox changes in cells or tissues exposed to oxidative stress. We identified Prxs as redox-sensitive proteins in a proteomic screen of oxidative modifications in Jurkat T-lymphoma cells treated with a sub-lethal dose of hydrogen peroxide (20 μM) [34]. Thiol proteins were labelled with 5-iodoacetamidofluorescein and separated by 2D SDS-PAGE [35]. While over 50 proteins were identified following exposure to 200 μM hydrogen peroxide, only two major proteins were oxidized at 20 μM: GAPDH and Prx 2. These proteins are not necessarily the most sensitive to oxidation, but their abundance and positioning on a 2D gel made them stand-out targets.

While Prxs are able to react with a broad spectrum of hydroperoxides, the peroxidatic cysteine is more resistant to alkylation by N-ethylmaleimide (NEM) than other thiols, and while hypochlorous acid and chloramines are able to react with Prxs, they also do so relatively slowly [11,36]. This is consistent with the models invoking hydrogen bonding between active site arginine residues and the oxygen atoms of the hydroperoxide, and it provides a degree of selectivity when Prxs are assessed as biomarkers.

3. Prxs as markers of oxidative stress

The oxidized dimer of the typical 2-Cys Prxs accumulates during the breakdown of hydroperoxides, both because the rate of oxidation is faster than the rate of reduction and that Prxs are present at higher concentrations in cells than the major reductant thioredoxin. The extent of the bottleneck will vary depending on cell type and metabolic status, but monitoring the redox status of the 2-Cys Prxs could reveal subtle alterations in the rate of hydroperoxide generation, or impairment of the reductive pathways required for Prx turnover under basal conditions. Prx hyperoxidation occurs during increased exposure to hydrogen peroxide, and this species also accumulates because reduction is the rate-limiting step. Methodology exists to measure both Prx oxidation and

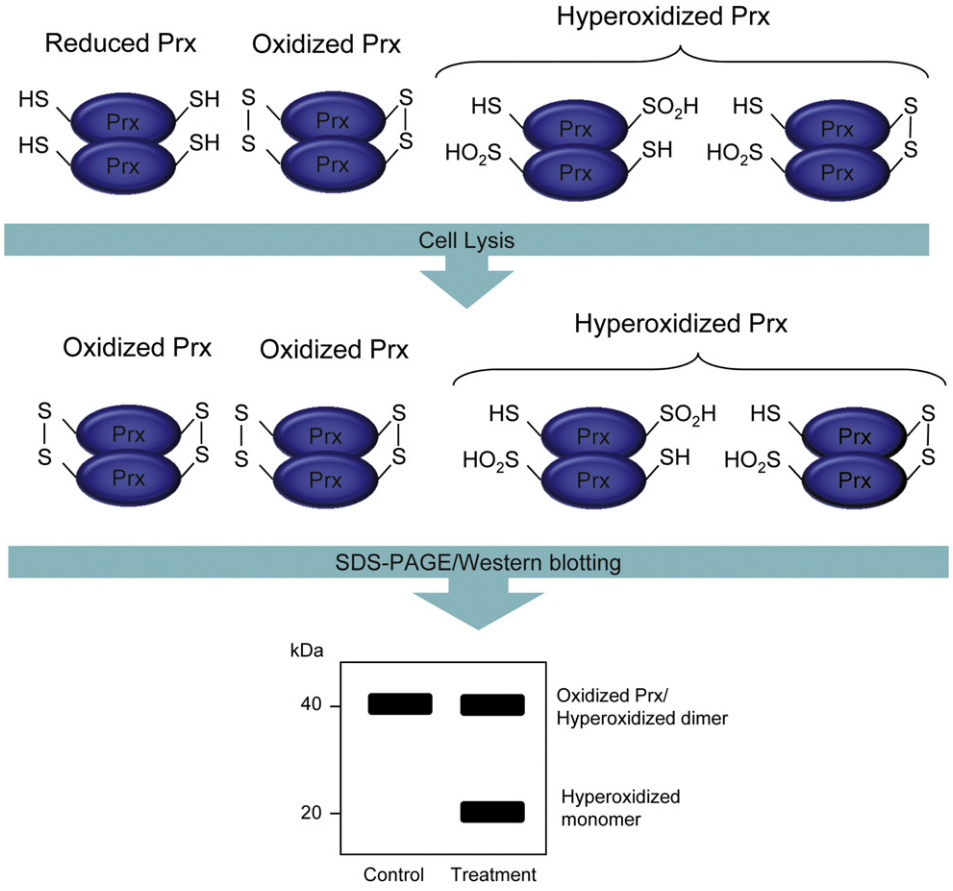

Fig. 3. Measurement of hyperoxidized 2-Cys Prxs. Cells are lysed in the absence of an alkylating agent. Trace amounts of hydrogen peroxide in the lysis buffers oxidize any reduced Prxs, while hyperoxidized protein remains as a monomer. The monomer and dimer forms are separated by non-reducing SDS-PAGE and visualised by Western blotting with specific Prx antibodies.

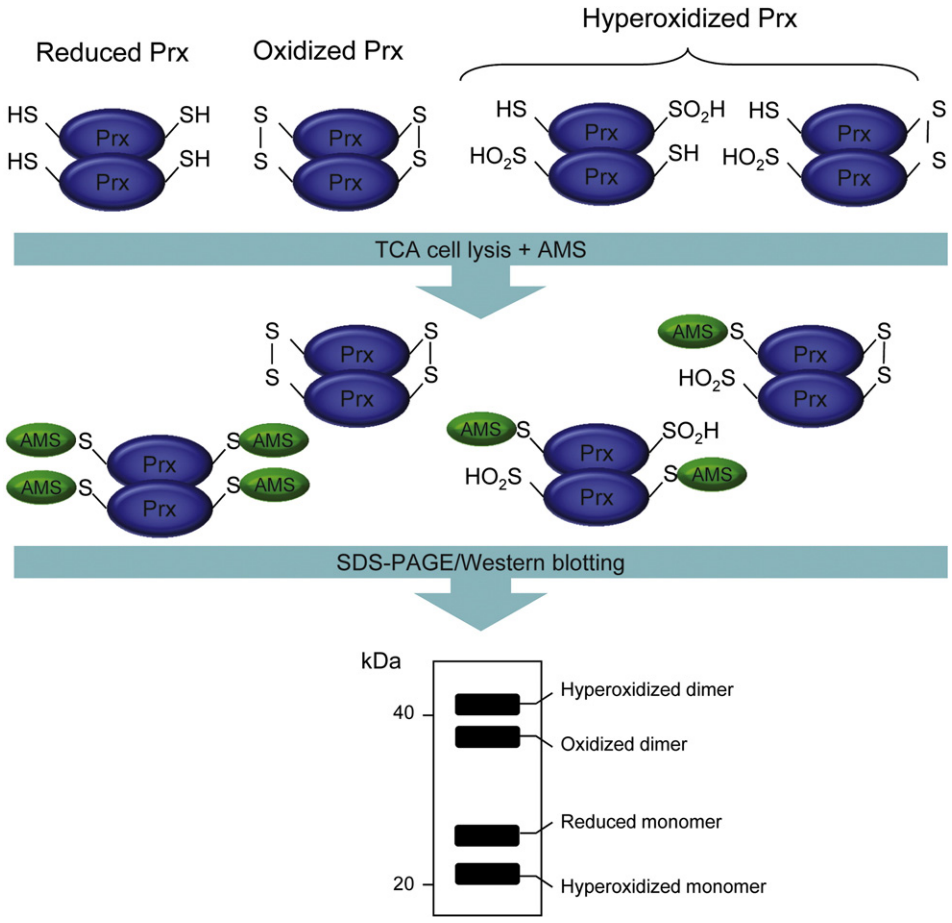

Fig. 4. Measurement of Prx redox states with AMS. Cells are lysed using TCA to protonate the peroxidatic cysteine and denature the protein. Precipitated proteins are resuspended in an AMS buffer to alkylate free thiols. The four redox states of the Prx can be separated by SDS-PAGE under non-reducing conditions or by mass spectrometry.

hyperoxidation, and this has been applied to a variety of experimental models.

3.1. Measurement of oxidized Prx dimers

Oxidized Prx dimers (~40 kD) can be distinguished from reduced Prx monomers (~20 kD) by non-reducing SDS-PAGE (Fig. 2). Antibodies are commercially available to monitor selected Prxs in biological samples by Western blotting. The key step in this method is to block all reduced Prxs prior to cell lysis to prevent subsequent artefactual oxidation. The Prxs are exquisitely sensitive to trace hydroperoxides present in buffers, and it is very difficult to maintain them in a reduced form in vitro. Protein denaturation and acidification both disrupt reactivity of the peroxidatic cysteine, thereby limiting artefactual oxidation, however we have found that the most effective method is to alkylate the peroxidatic cysteine prior to cell lysis. Sample

Table 1
Summary of methods for measuring Prx oxidation.

Marker	Assays	Advantages	Limitations
Oxidized	WB (Prxs) of non-reducing SDS-PAGE	– Sensitive marker – Examines individual Prxs – High-throughput – Quantifies proportion of oxidized dimer	– Susceptible to artefactual oxidation – Requires optimisation of alkylation
Hyperoxidized	WB (Prxs) of 2D PAGE	– Examines individual Prxs – Quantifies proportion of hyperoxidized protein	– Low-throughput – Gel-to-gel variation – PI shifts independent of hyperoxidation
	WB ($SO_{2/3}H$) of reducing SDS-PAGE	– Examines individual Prxs – High-throughput	– Higher affinity to sulfonic acid – Does not differentiate Prxs – Qualitative
	WB (Prxs) of non-reducing SDS-PAGE	– Examines individual Prxs – High-throughput – Differentiates between Prxs – Quantifies proportion of hyperoxidized Prx monomer	– Underestimates total hyperoxidation by ignoring hyperoxidized dimers
Oxidized and hyperoxidized	Mass spectrometry	– Fully quantitative – Examines all redox species – Examines individual Prxs	– Requires optimisation of protein alkylation – Requires specialised equipment

WB = Western blotting.

alkylation has been optimised for cell culture, with variations depending on the use of suspension or adherent cells [37], and tissue samples [38]. Methyl methanethiosulfonate has recently been found to be a very efficient blocking agent [39].

Monitoring of oxidized Prxs has proved valuable for measuring oxidative stress in erythrocytes. Prx 2 is the third most abundant protein in these cells, and exposure to low micromolar concentrations of hydrogen peroxide results in immediate oxidation [40]. It takes 20–30 min after consumption of the peroxide for Prx 2 to be converted back to its reduced form. This slow rate of reduction is due to the extremely low thioredoxin reductase activity in these cells. The biological significance of this phenomenon is unclear, but it significantly increases the sensitivity of Prx 2 as a marker of oxidative stress in these cells. The use of 2, 4-dinitrobenzene to inhibit the thiol regeneration systems that are present resulted in the accumulation of oxidized Prx 2 without the addition of exogenous oxidants [40]. Haemoglobin autoxidation was identified as the major source of endogenous hydrogen peroxide responsible for Prx 2 oxidation. More recently, co-culture of erythrocytes with activated neutrophils resulted in the accumulation of oxidized Prx 2, even with phagocytic neutrophils where hydrogen peroxide generation is localized to intracellular phagosomes [41]. Oxidized Prx 2 was also detected in erythrocytes isolated from the peripheral circulation of mice exposed to endotoxin [41]. The abundance of Prx 2 in erythrocytes means that the assay can be performed on a single drop of blood.

Prx oxidation has also been observed in cultured cells exposed to compounds that induce oxidative stress or trigger apoptosis [39,42–48], and ex vivo hearts undergoing ischaemia and reperfusion [38,49]. In the ischaemia/reperfusion and apoptosis models the stress was localized to mitochondrial Prx 3, without observable oxidation of cytoplasmic Prxs, thereby providing insight into the site of redox disruption. In contrast, we were unable to detect Prx oxidation during EGF signalling in A431 cells, a system in which NOX-dependent redox changes are proposed to play a signal transduction [50]. This may be due to the compartmentalization of redox changes, or Prx reduction pathways may be more robust in the A431 cells. There is likely to be variability in the reductive capacity of cells; as described earlier oxidized Prxs accumulate easily in erythrocytes, and in our experience Jurkat T-lymphoma cells also display significant accumulation oxidized Prxs compared to some other cell types. Erythrocyte sensitivity can be explained by their very low thioredoxin reductase activity, but a more comprehensive profiling of the sensitivity of other cell types and their reductive capacity would be valuable.

Two redox disruptive agents, auranofin and phenethyl isothiocyanate, required a functional respiratory chain to trigger mitochondrial Prx 3 oxidation [45]. The accumulation of oxidized Prxs does not necessarily mean that there has been increased hydrogen peroxide from the respiratory chain; impairment of Prx turnover through inhibition of the thioredoxin system will have the same net effect. Indeed, auranofin is a well-characterised inhibitor of thioredoxin reductase [51]. However, all redox probes will be subject to this limitation as increased signal could be due to inhibition of the endogenous enzymes responsible for removing the reactive species being monitored rather than increased generation.

3.2. Measurement of Prx hyperoxidation

Prx hyperoxidation was first measured by 2D PAGE, with an acidic shift due to sulfinylation or sulfonylation of the peroxidatic cysteine [3,28,52]. Immunoblotting of the gels indicates the proportion of each Prx that is hyperoxidized, and with this method it was shown that Prxs 1 and 2 were completely converted to the hyperoxidized form in Jurkat cells treated with 200 μM hydrogen peroxide, but only 25% of Prx 3 was modified [53]. The 2D technique has been applied to animal tissues. Bae et al. exposed mice to hyperoxia conditions (95% O_2 and 5% N_2) and hyperoxidized Prx 3 was shown to accumulate in the lung tissue [54]. When the redox state of Prx 3 was examined in the liver of old (28 month) and adult (12 month) rats accumulation of hyperoxidized Prxs was only observed in the older rats [55]. A disadvantage of the 2D method is the need to run a gel for each sample, limiting throughput and increasing variability. The method is also limited by the potential for pI shifts that occur independently of hyperoxidation, such as protein phosphorylation. Indeed, we have observed acidic species that are not labelled by antibodies to hyperoxidized Prxs (unpublished). However, it is still a valuable for validating results obtained with other techniques method.

The most common method to measure Prx hyperoxidation is immunoblotting with an anti-hyperoxidized Prx antibody. This commercially available antibody recognises a 9 amino acid sequence at the active site (DFTVCPTEI) in which the peroxidatic cysteine has been modified to either the sulfinic (SO_2H) or sulfonic (SO_3H) acid [28]. In our experience the anti-hyperoxidized Prx antibody appears to have a higher affinity for the sulfonic acid. This was revealed by observations of increased antibody binding in blots probed for a second time, which we interpreted as generation of the sulfonic acid following exposure to hydrogen peroxide during chemiluminescence detection (unpublished).

Since each Prx homodimer has two active sites, hyperoxidized Prxs can be present in both monomers and disulfide-linked dimers. Samples are usually analysed by reducing SDS-PAGE so both species migrate in a single band. The active site is conserved between Prxs 1 and 4, so the anti-hyperoxidized Prx antibody does not differentiate between these Prx family members. There are slight differences in the apparent molecular weight of the Prxs, which makes it possible to achieve some degree of separation i.e. Prxs 3 and 4 run at a higher MW than Prxs 1 and 2. Mitochondrial purification can be adopted to enable visualisation of Prx 3 [56]. However, the cytoplasmic Prxs are too close to separate. This is an important consideration since differential hyperoxidation does occur. For example, in the livers of ethanol-fed mice approximately 30% of Prx 1 was measured in the hyperoxidized state, while Prx 2 was unaffected [57].

Immunoblotting has been applied to blood and tissue samples. Peripheral blood mononuclear cells taken from asthma patients showed increased levels of hyperoxidized Prxs 1, 2 and 3 compared to control donors, and oxidation was linked to disease severity [58]. Hyperoxidized Prx 3 was observed in the adrenal cortex of healthy wild-type mice, and a link was established between Prx 3 hyperoxidation, p38 MAPK activation, and decreased StAR synthesis and steroid production in the adrenal cortex [59]. In this system, a circadian variation in Prx 3 hyperoxidation was reported. This followed the remarkable report of oscillating levels of hyperoxidized Prx 2 in erythrocytes [60]. Other studies have used the antibody to hyperoxidized Prxs for immunohistochemistry [61,62]. One group found higher levels of total hyperoxidized Prxs in the older age groups in cells of the gerbil hippocampus [62]. While this method does not offer any information on which Prx is oxidized or the extent of hyperoxidation, it is valuable for identifying sites of increased oxidative stress.

An alternate method for measuring Prx hyperoxidation takes advantage of the fact that upon cell lysis in the absence of an alkylating agent any reduced Prx will immediately dimerize, while hyperoxidized protein will run as a monomer (Fig. 3). The samples are analysed by Western blotting with antibodies to each specific Prx. This enables estimation of the proportion of hyperoxidized protein for each Prx, which cannot be determined by use of the anti-hyperoxidized Prx antibody [37]. This method has been used to show the accumulation of hyperoxidized Prx 3 in the ovaries of rats from the offspring of dams undernourished during pregnancy and/or lactation in comparison to those fed control diets [63]. The method was also used to indicate hyperoxidation of a barley 2-Cys Prx in seedlings exposed to environmental stress [64], and compare oxidative stress in wild-type Jurkat cells compared to those overexpressing the anti-apoptotic oncogene bcl-2 [65]. In the latter system, Prx 2 hyperoxidation was seen 10 min after exposure to 5 μM hydrogen peroxide, much lower doses than

those required for detection of protein carbonyl formation or lipid peroxidation. Monitoring the transition from oxidized dimer to hyperoxidized monomer is simpler than the 2D electrophoresis method, and in contrast to the use of the anti-hyperoxidized Prx antibody, it enables better identification of which Prx is hyperoxidized and the proportion of the protein hyperoxidized. The disadvantage of this method is that it underestimates hyperoxidation by not measuring hyperoxidized dimers.

An alternate method for measuring hyperoxidation involves alkylation of cysteine residues with NEM, IAM or 4-acetamido-4′-((iodoacetyl)amino)stilbene-2,2′-disulfonic acid (AMS). Reduced proteins are fully alkylated, and display greater retardation of electrophoretic migration [66]. AMS is particularly effective because it adds approximately 500 Da per reduced cysteine (Fig. 4). To preserve the cellular oxidation state trichloroacetic acid is used to lyse the cells, denature protein and protonate peroxidatic cysteines, and the samples are treated with AMS. The molecular weight shift can be used to separate dimer, hyperoxidized dimer, reduced and hyperoxidized monomers by SDS-PAGE and Western blotting. Using this method the Prx from *Schizosaccharomyces pombe*, TPx 1, was shown to be hyperoxidized upon exposure to hydrogen peroxide [66]. As with the method for measuring protein dimerization, the disadvantage of this method is that trapping and derivatisation is required before artefactual oxidation occurs.

Recently, using a similar labelling approach, we have developed a mass spectrometry method that allows quantification of the redox states of recombinant Prxs [22]. NEM was used to add 125 Da to reduced cysteines, under conditions that limited non-specific alkylation of amine groups. There is the potential for this method to be developed for more complex samples, where cell lysate and tissue homogenate undergo proteolytic digestion, followed by quantification of reduced and oxidized peptides containing the peroxidatic cysteine.

4. Summary

Several features of the Prxs make measurement of their redox state a valuable biomarker of oxidative stress. They are abundant and highly reactive proteins, and oxidation has been observed where few if any other redox modifications are detectable. This enables detection of more subtle disturbances in redox homeostasis that could occur during signalling events, although sensitivity will vary between cell types depending on the robustness of their reduction systems. The varied intracellular expression pattern of different Prxs enables exploration of redox disruption in different cellular compartments. For example, Prx 3 oxidation has been observed without changes to cytoplasmic Prxs, indicating specific disruption of mitochondrial redox homeostasis.

Two specific redox states of the 2-Cys Prxs are proving useful as biomarkers: intermolecular disulfide formation and hyperoxidation. Their respective advantages and limitations are summarized in Table 1. Measurement of disulfide formation requires a blocking step to limit artefactual oxidation, which has to occur at the time of harvesting. It would be difficult to prevent Prx oxidation in the material collected and stored without alkylation. Measurement of hyperoxidation is simpler, and has been more widely utilized, but accumulation of hyperoxidized forms requires a greater degree of oxidative stress and is therefore less sensitive. The majority of methods depend on the use of specific antibodies, which are commercially available, but this does limit quantification. The most robust assay is disulfide formation, where the ratio of reduced monomers and oxidized dimers can be calculated within the same lane of a gel, removing the requirement to have equal protein loading between samples. The development of quantitative mass spectrometry techniques would have major benefits.

Most research to date has focussed on the two major cytoplasmic family members Prxs 1 and 2, and mitochondrial Prx 3. In contrast, considerably less is known about redox changes to Prxs 4–6. Prx 4 has an additional cysteine that forms covalent disulfides between homodimer units, stabilizing higher-order structures but complicating gel-based analytical techniques. Monitoring the reduced to oxidized transition by gel electrophoresis is difficult with Prx 5, which is an atypical 2-Cys that forms an intramolecular disulfide upon oxidation, and impossible in Prx 6, which is a 1-Cys Prx. Mass spectrometry methods would enable selective measurement of all Prxs and their oxidized forms.

The monitoring of an endogenous marker avoids the problems associated with the delivery of chemical probes or expression of reporter proteins, and any unanticipated effects they may have on the experimental system. Sobotta et al. measured Prx 2 oxidation in HEK293 cells expressing the roGFP-Orp1 engineered protein [39]. Prx 2 was more sensitive than the fluorescent protein at detecting oxidation in response to exogenous hydrogen peroxide, but it was possible to measure responses at the single cell level with the roGFP-Orp1. An antibody that specifically recognised the disulfide-linked Prxs would be a valuable advance. Both Prx oxidative modifications are reversible, albeit at different rates. As a consequence, measurement of Prx oxidation allows monitoring of transient oxidative stress and the timing associated with return to homeostasis. This is an advantage over redox-sensitive probes that undergo irreversible modification and only report on cumulative effects. However, the Prx methods require destructive processing of the sample, meaning that multiple samples are required for time-course studies. Fluorescent reporter proteins, in contrast, can provide real time imaging.

In summary, the Prxs are prominent antioxidant proteins that operate under normal physiological conditions. Methodology exists to visualise modified forms that accumulate during disruption of redox homeostasis. It is speculated that Nature utilizes Prxs as redox sensors, and the system is tuned to allow accumulation of oxidized forms. Further research is required to explore this hypothesis, but for now we are able to utilize the Prxs as sensitive biomarkers of oxidative stress.

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