An unexplored role for Peroxiredoxin in exercise-induced redox signalling?

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Mini Review

An unexplored role for Peroxiredoxin in exercise-induced redox signalling?

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ABSTRACT

Peroxiredoxin (PRDX) is a ubiquitous oxidoreductase protein with a conserved ionised thiol that permits catalysis of hydrogen peroxide (H₂O₂) up to a million times faster than any thiol-containing signalling protein. The increased production of H₂O₂ within active tissues during exercise is thought to oxidise conserved cysteine thiols, which may in turn facilitate a wide variety of physiological adaptations. The precise mechanisms linking H₂O₂ with the oxidation of signalling thiol proteins (phosphates, kinases and transcription factors) are unclear due to these proteins’ low reactivity with H₂O₂ relative to abundant thiol peroxidases such as PRDX. Recent work has shown that following exposure to H₂O₂ in vitro, the sulfenic acid of the PRDX cysteine can form mixed disulphides with transcription factors associated with cell survival. This implicates PRDX as an ‘active’ redox relay in transmitting the oxidising equivalent of H₂O₂ to downstream proteins. Furthermore, under oxidative stress, PRDX can form stable oxidised dimers that can be secreted into the extracellular space, potentially acting as an extracellular ‘stress’ signal. There is extensive literature assessing non-specific markers of oxidative stress in response to exercise, however the PRDX catalytic cycle may offer a more robust approach for measuring changes in redox balance following exercise. This review discusses studies assessing PRDX-mediated cellular signalling and integrates the recent advances in redox biology with investigations that have examined the role of PRDX during exercise in humans and animals. Future studies should explore the role of PRDX as a key regulator of peroxide mediated-signal transduction during exercise in humans.

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1. Introduction

Reactive oxygen and nitrogen species (RONS) are known to mediate a range of signalling processes within mammalian tissues, with their production, interaction and removal by antioxidants all
critical to cell function. The acute production of RONS in response to exercise has been an active area of research over the last 20 years. The oxidising properties of RONS have been implicated with mediating skeletal muscle excitation-contraction coupling [1,2] (via regulation of calcium signalling [3]), enzyme release [4,5], as well as modulating post-exercise mitochondrial biogenesis [6] and cytoprotective gene expression (e.g. heat shock [7,8] and antioxidant proteins [8,9]). A range of cell types can produce RONS during exercise, including skeletal muscle, immune and endothelial cells [10,11]. The enzymes nicotinamide adenine dinucleotide phosphate- (NADPH) oxidase and nitric oxide synthase (NOS) [12,13] produce superoxide (O2•−) and nitric oxide (NO•) respectively [14,15], with secondary oxidants such as peroxynitrite (ONOO−) formed from the favourable reaction between O2•− and NO• [16,17], and hydrogen peroxide (H2O2), by the conversion of O2•− to H2O2 by the antioxidant enzyme superoxide dismutase (SOD) or spontaneous dismutation [18]. The role of RONS in redox-mediated exercise adaptation are far from understood, but evidence strongly implicates the reversible oxidation of conserved cysteine residues within various proteins [19,20]. The aim of this review is to discuss the role of H2O2 as a cellular signal, paying particular attention to the role of Peroxiredoxin (PRDX) in transmitting H2O2 signals via various thiol-mediated mechanisms. Given the growing body of evidence that point to H2O2 in mediating thiol oxidation in skeletal muscle, and how it may have received a great deal of attention with regards to their high abundance and catalytic turnover of H2O2 [33]. Briefly, post-translational modifications (i.e. serine and threonine phosphorylation [52], glutathionylation [53], tyrosine nitration [54], acetylation [55] or s-nitrosylation [56]) on non-catalytic amino acids sites of PRDX or over-oxidation of the active site thiol [57] can...
drive the accumulation of H$_2$O$_2$ in specific cellular domains, depending on the active PRDX isoform [58]. These modifications can inactivate PRDX thiol activity and conceivably redirect the initial H$_2$O$_2$ signal to elicit a change in cell function via oxidation of signalling thiol proteins such as PTP1B, PTEN or ATM [26]. This would render PRDX as a negative regulator of H$_2$O$_2$ signalling in a mechanism known as the ‘floodgate model’ [59]. However, a recent paper by Sobotta et al. [22] eloquently demonstrated that PRDX has a ‘direct’ role in translating the oxidizing equivalent of H$_2$O$_2$ to the cysteine rich transcription factor STAT3. Following H$_2$O$_2$ exposure, the –SOH intermediate of PRDX-2 was shown to form a mixed disulphide with STAT3 directly, initiating its translocation to the nucleus. This implies that PRDX may act as an active ‘redox relay’ with respect to increased cellular H$_2$O$_2$ levels. The interplay between PRDX floodgate signalling and active redox relays are unclear, however the mechanisms are likely not mutually exclusive [60].

5. Peroxiredoxin: an abundant and highly active peroxidase

An array of factors can influence the capacity of a given redox active cysteine to reduce H$_2$O$_2$ by nucleophilic attack (Fig. 2). Accessibility of H$_2$O$_2$ to the catalytic thiol/thiolate motif and structural factors (e.g. adjacent neighbouring amino acids on the polypeptide chain) that affect electron density can alter the midpoint potential ($E_m$) of the thiol. Cysteine residues that have a lower $E_m$ are more readily oxidised [24,61] and even alternative isoforms of the same redox active protein may contain cysteine residues with differing $E_m$ and thus alternative propensities for oxidation [62]. The sensitivity of a redox active cysteine to oxidation is also, in part, determined by pH, which relates to the solution and microenvironment in which that cysteine resides. Acid residues adjacent to a cysteine have been shown to alter sensitivity of that cysteine to oxidation presumably via thiol protonation (–S$^-$ to –SH) [63]. Whether a redox cysteine is protonated at physiological pH is determined by the $pK_a$ of the thiol group [24]. All of these factors explain the broad range of sensitivities exhibited by thiol-based proteins to fluctuations in H$_2$O$_2$ concentrations. In particular, PRDX has a turnover of H$_2$O$_2$ up to a million times greater than protein phosphatases such as PTP1B and cdc25b in vitro [23,33].

PRDX is a ubiquitously expressed oxidoreductase protein (160–220 amino acids) located in the cytosolic (isoforms I, II and VI), endoplasmic reticulum (isoform IV) and mitochondrial (isoforms III and V) domains of the cell [64]. The nascent form of most PRDX’s is the decamer form (I–IV), with PRDX V and VI unable to form oligomers. The ‘catalytic’ cysteine of all PRDXs (–S$^-$) can convert H$_2$O$_2$ [65], ONOO$^-$ [66] and other peroxide substrates [65] to H$_2$O via the oxidation of its conserved thiolate to a –SOH intermediate, before reacting with a ‘resolving’ cysteine thiol (–SH). In this regard, the –S$^-$ form acts as the ‘redox-sensor’ via nucleophilic attack and is the target for oxidation, whereas the –SH form is resolving in nature [67,68]. The mechanism of –SOH re-solution determines the sub-classes of the PRDX family. These include typical-2 cysteine PRDX (I–IV), atypical-2 cysteine PRDX (V) or 1-cysteine (VI) PRDX, whereby mixed disulphides are formed through inter-molecular bonding with a neighbouring thiol (PRDX molecule or thiol-based protein), intra-molecular bonding with a native thiol or inter-molecular bonding with GSH [69] respectively. These disulphide bonds are reduced by the antioxidants TRX (I–IV), GSH-S-Transferase (V) and GSH (VI) in bioenergetically favoured reactions [69,70].

Other antioxidant enzymes such as catalase, GSH and GPx have prominent and defined roles in H$_2$O$_2$ catalysis [71]. Importantly, these enzymes likely work in synergy with PRDX to modulate the
overall peroxide signal [72]. Catalase is primarily located to peroxisomes where H₂O₂ is formed from the breakdown of various substrates by flavoprotein oxidases [71], and therefore has no role in the transfer of cytosolic to nucleic peroxide signals. GSH is a low molecular weight antioxidant with a relatively low turnover of H₂O₂ [33], and unlikely acts a direct ‘sensor’ for translating the peroxide signal for transcriptional activation. GPx has a comparable rate constant to PRDX for H₂O₂ catalysis [33]; however the transient and highly reductive selenenic acid (Se⁻) intermediate that is formed following GPx reaction with H₂O₂ rapidly resolves with a native amide group to form an intramolecular sulfenamide [73]. Therefore GPx does not act as a dimerising peroxide ‘sensor’ protein. It is the structure and high affinity that PRDX has for H₂O₂ to favourably form –SOH intermediates and resolving dimers that may give this antioxidant protein a unique role in actively transducing peroxide signals into a dynamic biological response. Ubiquitous PRDX expression has been estimated to be far more abundant than both GPx and catalase [74] and the decamer conformation of typical 2-cysteine PRDXs can stabilise the active site and increase peroxidase activity [75]. Further, structural analysis of PRDX has revealed a hydrogen-bonding network surrounding the active site that favours peroxide substrate binding and catalysis [76,77]. A deprotonated catalytic thiolate (due to a low pKa value) coupled with favoured polarisation of the peroxide bond (O=O) has led to estimates that PRDX reacts with up to 99% of cytosolic H₂O₂ [78,79].

6. Is PRDX a peroxide sensing protein during and following exercise?

The available evidence suggests that H₂O₂ plays a crucial role in mediating tissue function during exercise [2], as well as modulating post-exercise metabolic adaption [49] via redox-sensitive pathways. Despite this, the direct reactivity of H₂O₂ with signalling protein thiols is known to be very low. Given PRDXs high abundance and turnover of cytosolic H₂O₂, PRDX would likely favour the formation of –SOH intermediates in response to heightened H₂O₂ production during exercise. Indeed, accumulating evidence suggests that cytosolic, rather than mitochondrial sources of RONS predominate during exercise [80,81]. Oxidation of PRDX may therefore act as a physiologically conserved mechanism for translating contractile signals from exercise-induced oxidants to downstream transcription factors, thus facilitating the adaptive response to exercise.

The targets of PRDX –SOH intermediates are to date, largely unclear, with no studies exploring this in the context of exercise. It has been demonstrated in vitro that transcription factors associated with exercise adaptation, namely, STAT3 [22] and p38 [82] are responsive to this signalling pathway. Following H₂O₂...
H2O2 that permits oxidation of other signalling thiol proteins, for in response to acute exercise in humans is extremely limited, with environmental (i.e. higher pH).

AP-1 [57], eliciting changes in cell function in a different microenvironment (I–IV) disulphide [93]. As well as increased peroxide levels during exercise, these changes may also relate, in part, to reductions in cellular pH that might reduce the sensitivity of the TRX cysteine to oxidation. As introduced earlier, this ‘floodgate model’ mechanism (Fig. 1) may allow accumulation of H2O2 that permits oxidation of other signalling thiols, for example PTPIP, which has a much lower pKa value than TRX [33]. Moreover, in vitro evidence suggests that TRX may then redirect its reducing power to transcription factors such as NF-κB [94] and AP-1 [57], eliciting changes in cell function in a different microenvironment (i.e. higher pH).

The research assessing the interplay between H2O2 and PRDX in response to acute exercise in humans is extremely limited, with descriptive changes in PRDX expression and oxidation only previously monitored [93,87,95]. Mechanistic approaches in knockout mouse models have highlighted a clear and prominent role for mitochondrial PRDX-3 in controlling skeletal muscle force production [96] and mitochondrial homeostasis [97]. For example, absence of PRDX-3 caused deregulation of mitochondrial membrane potential and a faster rate of muscle fatigue in the extensor digitorum longus and soleus muscles of mice, possibly as a result of elevated peroxide concentrations [96]. A study by Kil et al., 2012 provided evidence to suggest that PRDX-III hyperoxidation has an important role in modulating steroid hormone production following physiological stress in mice [26], which will undoubtedly be important in an exercise context whereby steroidogenesis is elevated post-exercise [98]. These studies do not provide insight into the specific sources of RONS that mediate PRDX cysteine oxidation, nor the associated downstream signalling mechanisms. The only study to assess changes in PRDX following regular exercise in humans, reported an increase in erythrocyte PRDX-2 expression in overweight males after 3-months of aerobic exercise training (3 sessions per week at 75% of maximum heart rate; progressive increase in session duration (25–50 min) over the 3 month period) [95]. Increased PRDX expression following exercise indicates an important role for PRDX in cellular remodelling following exercise training, which likely occurs via the redox-sensitive transcription factor NF-κB (as recently demonstrated in vitro [72]). Interestingly, in a non-exercise context, PRDX has also been shown to have an active role in the progression of mitosis through selective phosphorylation of PRDX-1 during anaphase [99]. Phosphorylated and inactivated PRDX-1 allows the transient and localised accumulation of H2O2 that oxidises centrosome-bound phosphatases, thus permitting cell proliferation. This highlights a complex interplay between thiol modifications and cellular phosphorylation in mediating cell growth, which may have applications to tissue remodelling (i.e. skeletal muscle) in response to exercise.

7. Future Perspectives

The redox environment within cells is a complex network of highly transient RONS that work in strict cooperation with cellular and dietary antioxidants. Despite rapid progression in our understanding of many aspects of redox signalling, it is clear that the analytical techniques currently available to monitor thiol modifications following exercise in humans are limited. Technologies for the evaluation of −SOH formation in vitro and in vivo have been developed, which include dimered based reagents and conjugates [100]. These reagents ‘trap’ −SOH formation in real time, which can successfully be detected by mass-spectrometry [101,102]. The use of such reagents has real potential in the field of exercise physiology, particularly regarding the delineation of redox sensing and/or signalling pathways where PRDX and H2O2 are implicated. The evidence presented in this article highlights three primary functions of PRDX in managing H2O2 gradients: (1) an antioxidant with a very high affinity for H2O2 [2] an intracellular signal that transfers the oxidising equivalent of H2O2 to cysteine rich target proteins via its −SOH intermediate and (3) an extra-cellularly secreted protein that may permit local signalling. These signalling properties may well underpin a fundamental aspect of redox communication in response to exercise (Fig. 3).

At present, it is unclear how H2O2 acts as an intracellular signal in vivo and importantly, how widespread H2O2 ‘sensor’ proteins are within cells. A recent review by Cobley et al., [103] suggested that H2O2 contained to the cellular domain of production during exercise (by proteins such as PRDX) likely permits its actions as an intracellular signal, whereas distal diffusion permits non-specific exposure, PRDX-2 can form mixed disulphides with STAT3 directly [22], whereas PRDX-1 resolves with ASK-1, subsequently increasing p38 phosphorylation [82]. It is well documented that STAT3 [83] and p38 [84] activation are linked with increased muscle anabolism and mitochondrial biogenesis following exercise respectively. It is important to note that PRDX has been shown to interact with various other signalling complexes (e.g. platelet-derived growth factors [85], receptor tyrosine kinases [52] and lipid phosphatases [37]) that are undoubtedly involved with signalling pathways following exercise. The translocation of PRDX to these receptors has been previously regarded as a mechanism to prevent localised protein oxidation via its reduced thiolate. However, advances in our understanding of how PRDX −SOH resolve with signalling proteins may question this viewpoint. Given that there is no known receptor for H2O2 binding, highly reactive and specific cysteine targets such as PRDX may offer a control point for managing H2O2 gradients with targeted precision in response to a bout of exercise.

Increased levels of oxidised PRDX (I–IV) dimers [86] and over-oxidised monomers [70,87,88] have been reported in immune cells and erythrocytes following exercise. Under cellular oxidative stress, the PRDX decamer can expose an oxidised cysteine that resolves with a neighbouring PRDX thiol to form a stable oxidised dimer. This state is known to be favoured following PRDX oxidation [89] and levels have been shown to increase following a single bout of ultra-endurance exercise (174 km, 30 h) in isolated PBMCs from well-trained male participants [87]. Recent work by Salzano et al. [53] has indicated that glutathionylation of non-catalytic cysteine residues may facilitate extracellular secretion of dimerised PRDX from immune cells, suggesting that this may act as an extracellular ‘stress’ signal. Interestingly, there is also evidence that skeletal muscle cells can also increase their secretion of PRDX in response to injury [50]. In this regard, extracellular PRDX may act in a paracrine or hormonal manner between cells under redox stress during exercise. Furthermore, PRDX-2 has been shown to bind to toll-like receptor-4 in immune cells, increasing inflammatory cytokine transcription via NF-kB (i.e. IL-1beta) [91]. This indicates an additional aspect of extracellular communication under cellular redox stress, with immune cells known to target, infiltrate and repair skeletal muscle following exercise.

A unique feature of the PRDX catalytic cysteine is the capacity of the thiolate to react with a second and third H2O2 molecule, leading to sulfonic (SO3H) acid oxidation states [92]. This ‘over-oxidation’ occurs at a rate too quickly for thiol ‘resolution’ and leads to the formation of over-oxidised monomers. Over-oxidised PRDX monomers have been reported during and following exercise in human peripheral blood mononuclear cells (PBMCs) [93,87] and erythrocytes respectively [88]. Formation of over-oxidised PRDX in PBMCs (I–IV isoforms) has been shown to be dependent on the intensity of exercise, with heightened peroxide concentrations during high intensity exercise (80% maximal oxygen consumption [VO2max] vs. 60% VO2max) likely exceeding the reduction power of TRX, the exclusive reductant of the PRDX (I–IV) disulphide [93]. As well as increased peroxide levels during exercise, these changes may also relate, in part, to reductions in cellular pH that might reduce the sensitivity of the TRX cysteine to oxidation. As introduced earlier, this ‘floodgate model’ mechanism (Fig. 1) may allow accumulation of H2O2 that permits oxidation of other signalling thiols, for example PTPIP, which has a much lower pKa value than TRX [33]. Moreover, in vitro evidence suggests that TRX may then redirect its reducing power to transcription factors such as NF-κB [94] and AP-1 [57], eliciting changes in cell function in a different microenvironment (i.e. higher pH).
oxidation of biomolecules through reactions with free iron. At this stage, we cannot be certain that H₂O₂ acts solely as an intracellular signal. Indeed, H₂O₂ is a freely diffusible, uncharged molecule that may signal in an autocrine, paracrine or hormonal manner between cells during exercise [104]. This may also be spatially favoured given that membrane-bound NADPH oxidases are likely a source of O₂•− during exercise [105]. Similarly, the function of decamer PRDX, a product of high levels of peroxides such as H₂O₂, needs further exploration as a possible extracellular redox signal.

The role of other 2-electron oxidants (i.e. ONOO−) and even free radical species (i.e. 1-electron oxidants such as O₂•− and NO•) in thiol-mediated redox signalling following exercise must not be discounted; particularly if the source of RONS is localised to the target protein [23]. Furthermore, other thiol modifications (i.e. s-nitrosylation and s-glutathionylation) may be responsible for mediating the adaptive response to exercise, with the role of reactive sulphur species (i.e. H₂SS−) in forming mixed persulphides emerging as a post-translational modification likely governing physiological effects [106].

8. Conclusion

PRDX is an abundant cellular protein with a diverse range of functions in mammalian cells, above and beyond its fundamental function as a thiol peroxidase. This review has drawn on a series of excellent advances in redox biology research to highlight the need to dissect the molecular mechanisms underpinning redox-mediated signal transduction in response to exercise. Future studies need to elucidate the precise role of PRDX and other peroxide sensors in transmitting H₂O₂ signals into dynamic biological responses during and following exercise.

References

[3] P. Donoso, C. Hidalgo, G. Sánchez, Posttranslational modifications of cardiac ryanodine receptors/calcium release channels by reactive oxygen species