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The role of peroxiredoxin 1 in redox sensing and transducing

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ABSTRACT

Peroxiredoxin 1 is a member of the ubiquitous peroxiredoxin family of thiol peroxidases that catalyse the reduction of peroxides. In recent years eukaryotic peroxiredoxins have emerged as a critical component of cellular redox signalling, particularly in response to alterations in production of hydrogen peroxide. Peroxiredoxins are exquisitely sensitive to oxidation by hydrogen peroxide making them key peroxide sensing enzymes within cells. Evidence gathered over the last decade suggests that in addition to sensing the redox signal, peroxiredoxins have a major role in transducing this signal to downstream signalling proteins, ultimately contributing to regulation of diverse cellular processes including proliferation, differentiation and apoptosis. In this review we present the three current models for the sensing and signal transducing roles of peroxiredoxins, with a specific focus on mammalian peroxiredoxin 1. The evidence for each mechanism is discussed and areas for future work are identified.

Changes in the redox state of cells are intimately involved in regulating a multitude of cell processes. Key questions revolve around how changes in the redox state are sensed, and then how this information is transduced into a specific change in cell function. A critical molecule in redox signalling is the mild oxidant hydrogen peroxide (H_2O_2). H_2O_2 is a strong candidate for a second messenger in redox signalling because its production in cells can be regulated, it is relatively stable compared to many cellular oxidants, and it has a preference of oxidation of cysteine thiols in proteins [1–3]. Reversible thiol oxidation has emerged as an important and widespread mechanism of controlling protein function [4,5]. The use of thiol labelling combined with quantitative proteomics by mass spectrometry is leading the way in identifying signalling proteins with cysteine residues subject to reversible oxidation [6–9]. However there remains a major gap in our knowledge to understand how specific protein thiols become preferentially oxidised in the context of a signal transduction pathway. Based purely on reaction kinetics, H_2O_2 will be reduced by professional peroxidases and other proteins will not be able to compete with this interaction [10,11]. Nevertheless, in cells thiol residues in non-peroxidase proteins do become reversibly oxidised. Thus there must be regulated and highly specific mechanisms that enable this to occur, analogous to the multiple layers in regulation of signal transduction by phosphorylation and dephosphorylation.

When considering the relationship between H_2O_2-mediated thiol oxidation, and redox sensing and signalling, members of the peroxiredoxin enzyme family are key players. These ubiquitous enzymes are found in almost all forms of life, and use cysteine thiols to catalyse reduction of peroxides to water. The mammalian peroxiredoxins were first identified due to their involvement in a wide range of cellular processes including proliferation, differentiation and inflammation. These enzymes were subsequently found to be homologues of the yeast thiol specific antioxidant, with a primary catalytic function of thiol-dependent reduction of peroxides. In the ensuing ~20 years since the discovery of peroxiredoxins, we have gained a detailed understanding of their enzymology and structure. However despite early indications of the involvement of peroxiredoxins in signal transduction [12], there is still ongoing debate regarding the precise role(s) of peroxiredoxins in redox sensing and signalling.

The enzymology and structural biology of peroxiredoxins has been extensively studied and recently reviewed [13,14]. Here we provide a brief overview of this information as it pertains to peroxiredoxin 1 (Prdx1), and then present the current understanding of the role(s) of mammalian Prdx1 in signal transduction and discuss future research directions. Prdx1, along with other peroxiredoxins, has been implicated in numerous disease states [15]. Therefore understanding how these enzymes function in both physiological and pathological settings is of broad interest.

1. Enzymology of peroxiredoxin 1

All peroxiredoxins use thiol residues to reduce peroxides. The

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PeroxiRedoxin classification indEX (PREX) database categorises peroxiredoxins into six subfamilies [13,16]. In mammalian species there are six peroxiredoxins, Prdx1-4 (Ahp/Prdx1 subfamily), Prdx5 (Prdx5 subfamily) and Prdx6 (Prdx6 subfamily). Prdx1-4 utilise a typical 2-Cys mechanism, whereas Prdx5 uses an atypical 2-Cys mechanism and Prdx6 has a 1-Cys mechanism [17]. These Prdx1 family members differ in subcellular location with Prdx1 and 2 primarily cytosolic, Prdx3 mitochondrial and Prdx4 present in the endoplasmic reticulum lumen. Expression levels of each vary across different cell types [17,18].

Mammalian Prdx1 is a typical 2-Cys peroxiredoxin and is estimated to be present at 15–60 μM in the cytosol of mammalian cells [19,20]. In a mechanism common to all 2-Cys peroxiredoxins, Prdx1 functions as a head-to-tail homodimer, with each monomer containing a peroxidatic Cys (C_P) and a resolving Cys (C_R) (residues 52 and 173 respectively in Fig. 1A&B). The C_P thiol has a low p*K*a and thus is deprotonated at physiological pH. The thiolate (C_PS^-) reacts with peroxide to form a sulfenic acid (C_PSOH), which condenses with C_RSH on the other monomer to form a disulfide bond (Fig. 1B). Thus each active homodimer has the capacity to form two disulfide bonds and reduce 4 molecules of peroxide. The disulfide is then reduced in a disulfide exchange reaction originally postulated to be catalysed by thioredoxins [21]. The second order rate constant for reduction of peroxide has not been reported for a mammalian Prdx1 but, based on the homology of the active site with other mammalian 2-Cys peroxiredoxins (Fig. 2), is expected to be in the range of 10^7-10^8 M^{-1} s^{-1} [22,23]. As an alternative to disulfide formation, the C_PSOH can further react with peroxide to form sulfinic (C_PSO_2H) and sulfonic (C_PSO_3H) acids, commonly referred to as over- or hyper-oxidation [13,24,25]. These forms are catalytically inactive, with C_PSO_2H slowly reduced by sulfiredoxin in an ATP-dependent reaction [26,27] and C_PSO_3H regarded as irreversible. An ongoing question in the field has been given an antioxidant role of peroxiredoxins, why should they be so susceptible to inactivation by their own substrate?

In addition to inactivation by hyperoxidation, the activity of Prdx1 is regulated by other post-translational modifications. The best understood of these is phosphorylation, which can both inhibit [28–32] and activate [33] the peroxidase activity of Prdx1. Prdx1 activity is also inhibited by S-nitrosylation [34]. Prdx1 can be glutathionylated at three of its cysteine residues: C_P52, C83, and C_R173 *in vitro* and in response to H_2O_2 treatment in cultured cells [35]. The significance of glutathionylation of Prdx1 in redox signalling is as yet unknown, but may represent a possible regulatory mechanism or an alternative recycling mechanism as proposed for Prdx2 [36]. High throughput mass spectrometry approaches as indexed by the PhosphoSitePlus resource [37] indicate modification of Prdx1 by ubiquitylation, sumoylation, acetylation and succinylation, although the physiological roles of these modifications await further investigation.

2. Tertiary and quaternary structure of peroxiredoxin 1

Structures of five mammalian Prdx1 have been reported; oxidised rat C83S Prdx1 (PDB accession code 1QQ2) [38], rat C52S Prdx1 (PDB accession code 2Z9S) [39], oxidised human C83S Prdx1 (PDB accession code 4XCS), human C71S, C83E, C173S Prdx1 in complex with sulfiredoxin (PDB accession code 2RII) [40] and human C52D, C71S, C83E, A86E, C173S, K185C Prdx1 in complex with sulfiredoxin, ATP and Mg^{2+} (PDB accession code 3HY2) [41]. In common with other members of the Ahp/Prdx1 subfamily, reduced mammalian Prdx1 exists in equilibrium between fully folded (FF) and locally unfolded (LU) states (Fig. 1A&B). The structure of the active site pocket in the FF state of the protein optimises H_2O_2

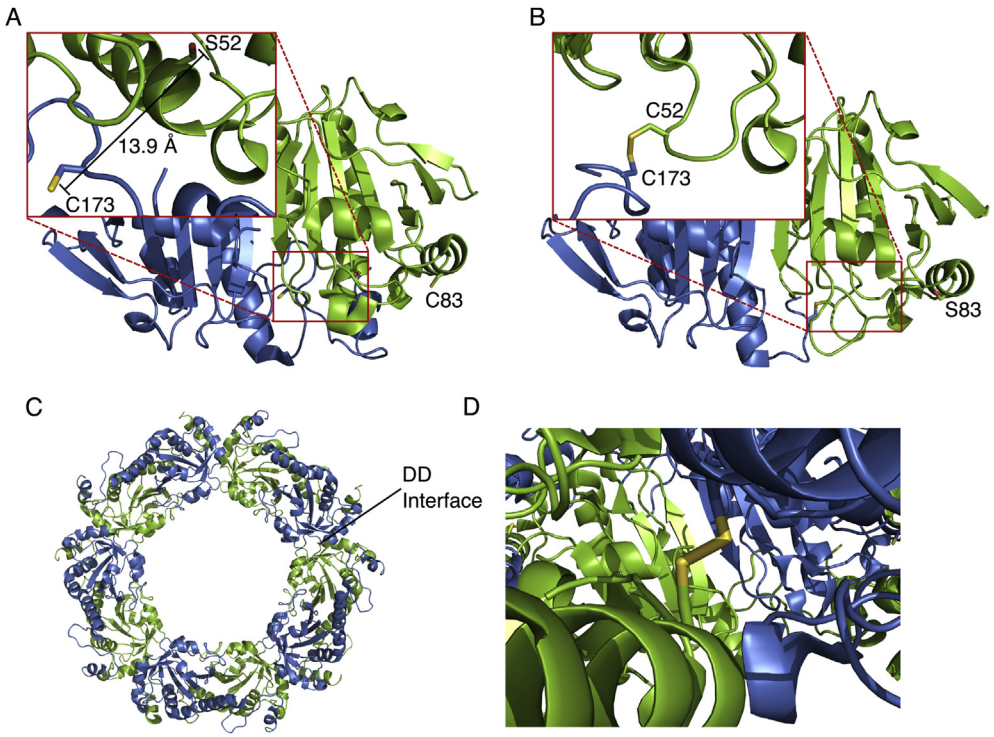

Fig. 1. Structural features of Prdx1. **A**. Dimeric rat C52S Prdx1 (PDB accession code 2Z9S). The Prdx1 homodimer contains two centrally located active sites with C83 at the periphery. The C173 C_R (inset) is 13.9 Å from the catalytic mutant C_P, S52 that exists in a fully folded (FF) helix. **B**. Dimeric rat C83S Prdx1 (PDB accession code 1QQ2). The homodimer exists with active site cysteines oxidised as a disulfide bond (inset), with the C_P (C52) now occupying a locally unfolded (LU) loop at the conclusion of the catalytic cycle. **C**. Decameric rat C52S Prdx1 (PDB accession code 2Z9S). The decamer exists in a toroidal $(\alpha_2)_5$ configuration, with each dimer shown as a blue and green monomer pair. **D**. C83-C83 disulfide bond at dimer-dimer (DD) interface of decameric rat C52S Prdx1 (PDB accession code 2Z9S). Colouring as in Fig. 1C and disulfide bonding C83 sulfurs shown in yellow.

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