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Review

Peroxiredoxin 2 and Peroxide Metabolism in the Erythrocyte

Felicia M. Low, Mark B. Hampton, and Christine C. Winterbourn

Abstract

Peroxiredoxin 2 (Prx2) is an antioxidant enzyme that uses cysteine residues to decompose peroxides. Prx2 is the third most abundant protein in erythrocytes, and competes effectively with catalase and glutathione peroxidase to scavenge low levels of hydrogen peroxide, including that derived from hemoglobin autoxidation. Low thioredoxin reductase activity in the erythrocyte is able to keep up with this basal oxidation and maintain the Prx2 in its reduced form, but exposure to exogenous hydrogen peroxide causes accumulation of the disulfide-linked dimer. The high cellular concentration means that although turnover is slow, erythrocyte Prx2 can act as a noncatalytic scavenger of hydrogen peroxide and a sink for hydrogen peroxide before turnover becomes limiting. The consequences of Prx2 oxidation for the erythrocyte are not well characterized, but mice deficient in this protein develop severe hemolytic anemia associated with Heinz body formation. Prx2, also known as calpromotin, regulates ion transport by associating with the membrane and activating the Gárdos channel. How Prx2 redox transformations are linked to membrane association and channel activation is yet to be established. In this review, we discuss the functional properties of Prx2 and its role as a major component of the erythrocyte antioxidant system. *Antioxid. Redox Signal*. 10, 1621–1629.

Antioxidant Protection in the Erythrocyte

ERYTHROCYTES ARE UNDER CONSTANT OXIDATIVE STRESS. Their physiological role as oxygen transporters results in a unique cellular environment rich in oxygen and heme-containing hemoglobin (Hb). During reversible oxygen binding, iron is maintained in its ferrous state. However, occasional spontaneous conformational fluctuations in the heme pocket of oxyHb enable water or a small anion to enter, resulting in transfer of an electron from the iron to oxygen to produce metHb and superoxide radicals (98, 100). This autoxidation occurs at a rate of 2–3% of total Hb a day (9, 36). The dismutation of superoxide to hydrogen peroxide by superoxide dismutase (SOD) makes autoxidation the major source of hydrogen peroxide in the erythrocyte. In addition to detoxifying endogenously-generated oxidants, erythrocytes are able to act as a sink for extracellular hydrogen peroxide (101). They are also exposed to oxidants generated via the interaction of redox-active xenobiotics with Hb.

As anucleated cells, erythrocytes are unable to synthesize new proteins or lipids. They rely on cellular constituents developed prior to cell maturation for protection and lifespan preservation (37). Erythrocytes have a broad repertoire of antioxidants to counter the potentially detrimental effects of oxidative insult (Fig. 1). SOD facilitates the conversion of superoxide to hydrogen peroxide, and the hydrogen peroxide is decomposed by catalase, cytoplasmic glutathione peroxidase (GPx1), and the peroxiredoxins (Prxs). The latter two are regenerated by the glutathione/glutathione reductase and thioredoxin (Trx)/thioredoxin reductase (TrxR) systems respectively, both of which derive reducing equivalents from NADPH. Glutathione is present at 2 m*M* in erythrocytes (48, 70), and maintenance of a high GSH:GSSG ratio also requires NADPH. The pentose phosphate pathway, via glucose-6-phosphate dehydrogenase activity, is responsible for metabolizing glucose to produce NADPH.

Prxs were discovered relatively recently, and little attention has been given to their antioxidant role in erythrocytes. Catalase and GPx1 were considered to constitute the erythrocyte's major defense system against hydrogen peroxide,

Free Radical Research Group, Department of Pathology, University of Otago, Christchurch, New Zealand.

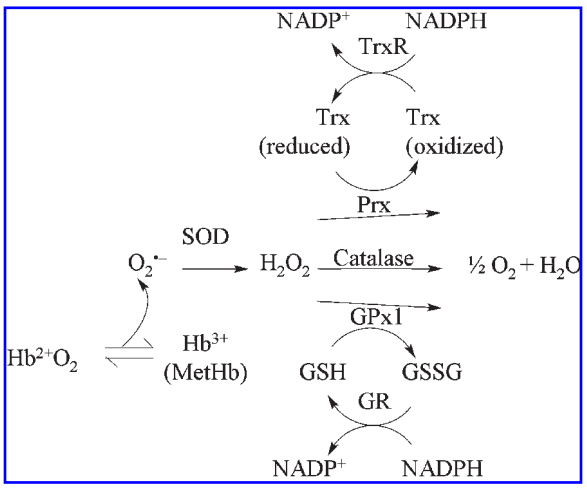

FIG. 1. Antioxidant systems that protect the erythrocyte from hydrogen peroxide. The erythrocyte antioxidant strategy combines direct elimination of oxidants and restoration of reducing equivalents. NADPH is central to antioxidant activity and is generated from glucose by the pentose phosphate shunt. GPx1, glutathione peroxidase 1; GR, glutathione reductase; MetHb, methemoglobin; Prx, peroxiredoxin; SOD, superoxide dismutase; Trx, thioredoxin; TrxR, thioredoxin reductase.

and there has been ongoing debate about which of these was the more significant (16, 27, 28, 61, 70, 89). A widely held opinion is that GPx1 is responsible for eliminating low concentrations of peroxide, whereas catalase scavenges hydrogen peroxide efficiently at higher concentrations (39,46). This is often (wrongly) attributed to catalase having a higher K_m for hydrogen peroxide, but as pointed out by Flohé (26), neither enzyme saturates with hydrogen peroxide. Instead, GPx can become relatively less efficient at high hydrogen peroxide concentrations due to GSH recycling becoming rate limiting. Recent studies by Johnson and coworkers (46) indicate that erythrocyte peroxide metabolism cannot be explained by these two enzymes alone. Using mathematical modeling, they showed that inclusion of Prx2 was required to explain their data for consumption of hydrogen peroxide generated by Hb autoxidation in GPx1-deficient compared with normal erythrocytes. This suggests that the significance of Prx2 in peroxide consumption may have been overlooked thus far.

Prx2 has been isolated from erythrocytes and its structure and peroxidase activity have been well studied. It has also been known for nearly 40 years under other names before this activity was recognized. This review brings together information from both these areas to address the function of Prx2 in the erythrocyte and its importance in antioxidant defense.

Peroxiredoxin 2

The Prxs constitute a family of peroxidases that reduce hydrogen peroxide, organic hydroperoxides, and peroxynitrite (40, 80, 83, 104). Prxs are ubiquitously distributed, having been identified in bacteria, plants, protozoa, yeast, and mammals (104), and even anaerobes (44, 47). There are six known mammalian isoforms, with Prx2 being the major erythrocyte Prx. Enzyme-linked immunosorbent assays have estimated the concentration of Prx2 to be 5.6 mg/ml of packed erythrocytes (67), equivalent to 15 million copies per cell. This makes it the third most abundant protein in the erythrocyte cytosol after Hb and carbonic anhydrase. Erythrocytes also possess Prx1 and Prx6, but with both present in much lower quantities than Prx2 (6, 59, 94).

Erythrocyte Prx2 has been studied under various names based on its different structural and functional properties. Early structural studies described a toroidal-shaped protein from erythrocyte membranes that formed hollow cylinders. Prior to being named Prx2 it was known as torin (33, 34). Prx2 has also been called calpromotin due to observations that calcium induces association of high molecular mass forms at the erythrocyte membrane, where it regulates K^+ efflux via the Gárdos ion channel (66, 67, 79). Erythrocyte Prx2 has additionally been described as thiol-specific antioxidant/protector protein, band-8, or natural killer enhancing factor-B (55, 63, 90). Use of recombinant protein later showed that the natural killer enhancing ability was in fact specific to Prx1 (86). In other cell types, Prx2 has also been called thioredoxin peroxidase II (108).

Prx2 is an obligate homodimer, and its subunits are orientated in a head-to-tail antiparallel manner (87). The catalytic activity of Prx2 is reliant on two highly conserved Cys residues. Cys51, also known as the peroxidatic cysteine, reacts with hydrogen peroxide to generate a sulfenic acid. Arginine 127 provides a positive charge to lower the pK_a of Cys51 and therefore enhance its reactivity with hydrogen peroxide (84). Its essential role in facilitating catalysis, like that of Cys51 (14), has been demonstrated by the loss of catalytic activity when subject to mutation (64). The sulfenic acid that forms at Cys51 then reacts with Cys172, the resolving cysteine located ~13 Å away on the second subunit of Prx2, to give a disulfide bond. Reduction of the disulfide is accomplished by Trx. Neither glutathione nor glutaredoxin is able to substitute as a reductant (13), although under *in vitro* conditions Prx2 is reduced by dithiothreitol (14). Trx is in turn regenerated by TrxR, which derives its reducing equivalents from NADPH (12) (Fig. 2).

Peroxiredoxins were first recognized on the basis of their peroxide scavenging ability (83). In initial kinetic studies of 2-Cys Prxs from bacteria, yeast, and trypanosomes, catalytic efficiencies (equivalent to second order rate constants) for their reaction with hydrogen peroxide of ~10^4–10^5 M^{-1} s^{-1} were obtained (3, 40, 71). These were noted (40) to be catalytically inefficient compared to catalase (k_{H2O2} ~10^7 M^{-1} s^{-1} (22)) and GPx1 (k_{H2O2} ~10^8 M^{-1} s^{-1} (26)). Kinetic studies also suggested lower values for mammalian Prx1 and Prx2 (13) and (106), although it is now apparent that these are likely to reflect the rate of regeneration of Prx by Trx in the Trx/TrxR/NADPH-coupled assay used (76). Saturation with low micromolar hydrogen peroxide was observed, but it is unclear how this would relate to the situation in the erythrocyte where the Prx2 concentration is likely to exceed that of the peroxide. More recent rate measurements for several 2-Cys Prxs have since shown that the initial studies considerably underestimated their catalytic efficiency. Rate constants have been measured for bacterial AhpC and yeast Tpx I and II that are approximately two orders of magnitude higher than previously thought (74, 76). Using a competitive kinetic approach, a similar value of 1.3×10^7 M^{-1} s^{-1} has been obtained for human Prx2 reacting with hydrogen peroxide

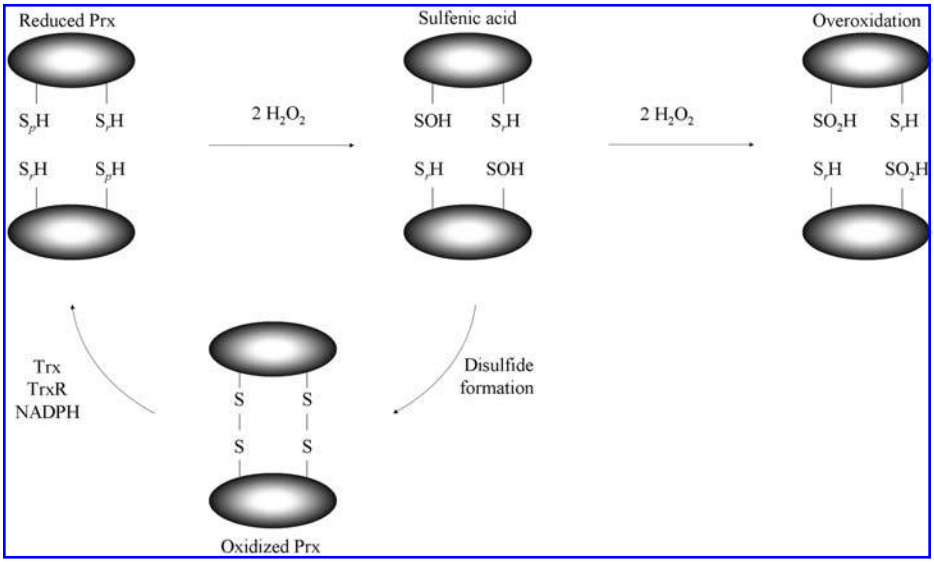

FIG. 2. Prx2 catalytic mechanism. The reaction cycle starts with the oxidation of the Prx2 peroxidatic Cys to a sulfenic acid. The sulfenic acids condense with the resolving cysteines on the opposing subunits, yielding a disulfide-linked dimer that is reduced by the Trx system. The sulfenic acid can also react with hydrogen peroxide to produce the overoxidized sulfinic acid. S_pH, peroxidatic Cys 51; S_rH, resolving Cys 172.

(77). As this is comparable to that for catalase, Prx2 would be expected to be competitive in the erythrocyte.

In addition to hydrogen peroxide, Prx2 is able to rapidly decompose organic peroxides including lipid hydroperoxides and peroxynitrite (17, 77). The rate constant for reaction of Prx2 with peroxynitrite is high (10^6 M^{-1} s^{-1}) (85), but still lower than that for hydrogen peroxide, which is unusual for a thiol protein. Nevertheless, these authors proposed that erythrocytes could act as a sink for plasma peroxynitrite. Prx2 also reacts with hypochlorous acid and chloramines, but much more slowly than with hydrogen peroxide (77). This contrasts with other thiol compounds that typically react ~10^3 times more rapidly with chloramines than with hydrogen peroxide (78), and highlights the selectivity of Prx2 for peroxides.

Oligomerization and Chaperone Activity of PRX2

Biochemical, biophysical, and crystallographic studies on the purified proteins have revealed a wide array of quaternary structures adopted by Prxs from mammals, bacteria, and protozoa, including human Prx2 (41, 51, 87, 102, 104). The most commonly reported and well-characterized oligomeric state of Prx2, as determined from crystal structure analyses, is the decamer (pentamer of dimers) (Fig. 3). As shown by analytical ultracentrifugation and size exclusion chromatography of other 2-Cys Prxs (76, 102), there is an equilibrium between the decameric and dimeric forms. The evidence points towards the reduced form existing primarily as a decamer, which upon oxidation dissociates into dimeric units.

Human Prx2 is also able to assemble in higher oligomeric forms. Early observations of hollow cylindrical structures (33, 34) have since been extended to show that these comprise stacks of decamers aligned on their edges (35). Prx2 even forms a 12-decamer dodecahedron "cage" under certain crystallization conditions (60). However, the peroxidase activities of these structures and their presence *in vivo* have not yet been determined.

Recently, human Prx2 and yeast cytosolic Prxs1 and 2 were found to exhibit chaperone activity by protecting against protein denaturation or cell death by heat shock (43, 65). Exposure to heat or high hydrogen peroxide resulted in oligomerization of Prx subunits into high molecular mass structures, coincident with abolishment of peroxidase activity. The finding that high peroxide treatment caused oligomerization as well as overoxidation of the peroxidatic Cys (described below) led to the proposal that overoxidation switches the protein to a form that protects the cell via a mechanism not involving hydrogen peroxide scavenging (65). The oxidation state of Prx2 as a heat shock chaperone was not examined. Thr90 phosphorylation of Prx1 also stimulates oligomerization and chaperone activity, even though this is unlikely to involve oxidation (42). Thus, oligomerization could occur via various mechanisms and the role of chaperone activity of Prx2 requires further elucidation.

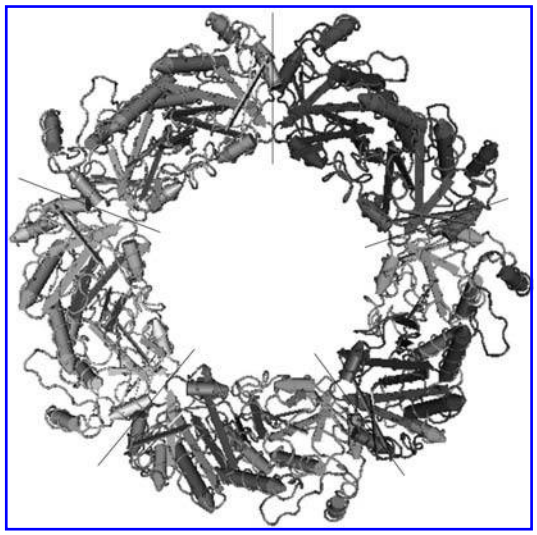

FIG. 3. Crystal structure of decameric human erythrocyte Prx2. Ribbon diagram showing decameric human Prx2 (purified from erythrocytes as the overoxidized form). The *lines* delineate the five dimeric units. Structure rendered on Cn3D v 4.1 using data deposited in the Protein Data Bank (87) (accession number 1QMV).

Peroxiredoxin 2 Reactivity in Erythrocytes

Although Prxs have been closely studied in various cell lines and organisms, only recently has the redox behavior of Prx2 been examined within the erythrocyte. It was shown (56) to be remarkably sensitive to oxidation when erythrocytes were exposed to micromolar concentrations of hydrogen peroxide (56). By tracking the oxidation state of Prx2 using nonreducing immunoblots (Fig. 4), the product was identified as the disulfide-linked dimer. Overoxidation was almost undetectable even at high peroxide concentrations. Prx2 was oxidized despite the presence of active catalase and GPx1, and was recycled only slowly, taking many minutes to reverse once the peroxide had been consumed. Inhibition by dinitrochlorobenzene implicated thioredoxin reductase as the reductant. Thioredoxin reductase has been reported as being present (10) or undetectable (32) in erythrocytes, but our analyses showed that they contain very low, but consistently detectable levels (56). This would explain the slow turnover and accumulation of the Prx2 dimer. Inhibition of thioredoxin reductase led to the accumulation of dimerized Prx2 in incubated erythrocytes, presumably due to endogenously generated hydrogen peroxide. Inhibition by carbon monoxide indicated that Hb autoxidation was a major contributor and these findings imply that, in the circulating erythrocyte, Prx2 is continually being oxidized by the products of Hb autoxidation. The low thioredoxin reductase activity is able to keep up with this slow oxidation rate but with increased hydrogen peroxide generation the Prx2 would become trapped as the dimer and its antioxidant activity would be compromised due to the low turnover rate.

The Prx2 dimerization seen in the erythrocyte is in distinct contrast to what is observed in other cell types (*e.g.*, Jurkat cells) exposed to hydrogen peroxide. Studies analyzing human 2-Cys Prxs by 2D gel electrophoresis found that these were converted to low isoelectric point forms (62, 82, 97), which could also be observed as prevention of dimerization (4, 18). This is a result of the sulfenic acid on the peroxidative Cys reacting with a second molecule of hydrogen peroxide to become a sulfinic acid (82, 106). Overoxidation, as this phenomenon is known, was observed with purified Prx1 but only when undergoing redox cycling with the Trx regeneration system, with just 0.07% of the sulfenic acid becoming overoxidized per redox cycle (see Fig. 2) under steady-state hydrogen peroxide levels (106). Even so, substantial amounts of overoxidized Prx2 can be detected in Jurkat cells treated with low concentrations of hydrogen peroxide (4, 18), suggesting that overoxidation is particularly favored in intact cells. Prx overoxidation has been linked to cell signaling through the "floodgate model," in which Prxs consume low levels of endogenous peroxides, but increased generation leads to overoxidation and a dramatic increase in the hydrogen peroxide available to act as a second messenger (103). It was queried why erythrocytes would contain a Prx sensitive to overoxidation because they would be unlikely to utilize the floodgate mechanism for signal transduction (29). Our findings that overoxidation does not occur in the erythrocyte indicate that this is probably the case (56). This can be explained, at least in part, by slow Prx2 turnover in the erythrocyte due to their much lower thioredoxin activity (~2% of that in Jurkat cells).

An acidic form of Prx2 has been reported in erythrocytes isolated from healthy individuals (107). This constituted up to 30% of the protein in some controls, and increased to 50% in some Alzheimer's patients. Whereas the acidic form was interpreted as overoxidized Prx2, no direct evidence was provided. These findings contrast with the lack of overoxidized Prx detectable in erythrocytes with a specific antibody and further characterization of the acidic form is needed.

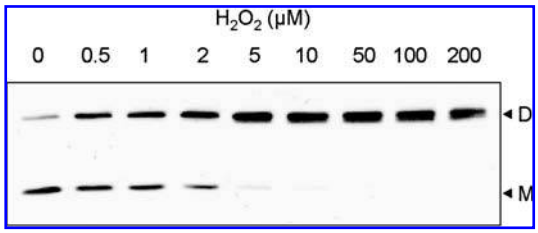

FIG. 4. The effect of exogenous hydrogen peroxide on the redox state of Prx2 in erythrocytes. Erythrocytes (5×10^6/ml) were treated with the indicated concentrations of hydrogen peroxide for 10 min before the cells were lysed in the presence of *N*-ethylmaleimide to prevent artefactual oxidation. Nonreducing SDS-PAGE following by immunoblotting was performed to allow detection of Prx2 monomers and oxidized dimers. This research was originally published in *Blood*. Low *et al*. Peroxiredoxin 2 functions as a noncatalytic scavenger of low level hydrogen peroxide in the erythrocyte. *Blood* 109: 2611–2617, 2007. © the American Society of Hematology.

Membrane Association and Calpromotin Activity

Prx2 is able to associate with the plasma membrane of erythrocytes. Erythrocyte Prx2 is mostly cytosolic, but an estimated 0.05% (~3 μg/ml packed cells) is bound to the membrane under native conditions (57, 67). Many of the membrane association studies were undertaken before the antioxidant properties of Prx2 were known, but the ability of Prx2 to reduce lipid hydroperoxides (11) may give this association a physiological role in protecting the membrane against oxidative damage.

Before its peroxidase activity was recognized, Prx2 (as calpromotin) was identified as a protein required by erythrocytes for the Ca^{2+}-mediated activation of K^+ efflux through the Gárdos channel (66, 67, 79). This appears to involve membrane localization of Prx2, which is stimulated by influx of Ca^{2+} into the cells and by inhibition of calpain, a Ca^{2+}-dependent protease (2, 79). Membrane association is possibly mediated via its C-terminal extension (11), and reversal of calcium-induced localization may be accomplished by truncation of the Prx2 C-terminal tail by calpain (88). Activation of the Gárdos channel results in KCl loss and the formation of dehydrated dense erythrocytes (8, 58). This may be relevant in the pathology of sickle cell anemia, in which vaso-occlusive events are associated with higher levels of dense cells (25). K^+ efflux has been linked to the formation of dense sickle cells (52) in which Prx2 is found at unusually high levels (69). Several other diseases, including favism, are associated with high intracellular Ca^{2+} in the erythrocyte (19, 23). Ca^{2+}-dependent cell shrinkage also occurs during erythrocyte apoptosis (eryptosis) and the Gárdos channel has been implicated (8, 49). The role of Prx2 was not considered in these studies, but it has been reported in an abstract that

phosphatidylserine scrambling in the erythrocyte membrane normally seen on adding Ca^{2+} is increased in cells from Prx2-deficient mice (21). This is counterintuitive to Prx2 promoting eryptosis, but highlights the need for further investigation of its role in clearance mechanisms. Oxidants have been shown to affect Gárdos channel activity (30), but we do not know how they affect Prx2 binding to the membrane or its ability to activate the channel. Deciphering these relationships and their links with peroxide metabolism by Prx2 may provide further insight into its role in the erythrocyte.

Peroxiredoxin 2 Knockout Studies in Animals

The physiological importance of erythrocyte antioxidant proteins has been investigated through observations of clinical symptoms in human cases of deficiency, and more recently, the study of gene knockout models. Prx2 knockout mice have been produced (50). They developed severe hemolytic anemia, showing symptoms of lowered Hb content and hematocrit (indicating hemolysis), increased reticulocyte count (suggesting erythropoietic compensation to maintain hematologic homeostasis), and splenomegaly (which points to destruction of many abnormal erythrocytes). Furthermore, their erythrocytes contained Heinz bodies, which are inclusions composed of oxidatively denatured Hb. There was an increased proportion of a dehydrated rigid subpopulation of dense cells, reported on the basis of autofluorescence to be severely oxidized (21). Basal levels of metHb in Prx2 knockout mice were equivalent to those in wild-type erythrocytes, but there was increased metHb formation on adding hydrogen peroxide. A subtle increase in membrane thiol oxidation was also observed. This, plus the Heinz body formation, could trigger increased erythrocyte removal from circulation and eventuate as hemolytic anemia. The mice expressed normal levels of functional catalase and GPx1. The inability of the two classical peroxide-removing enzymes to substitute for Prx2 highlights the nonredundancy of Prx2 and the symptoms imply some form of protection against oxidative damage.

One human patient has been documented as having decreased erythrocyte membrane levels of Prx2 (75). The individual suffered from congenital dyserythropoietic anemia, and the erythrocytes showed stomatocytosis. Stomatin is a membrane protein thought to regulate monovalent cation transport (91), but although stomatocytes typically suggest increased permeability to Na^+ and K^+, erythrocyte Na^+/K^+ content was normal. Stomatin and Prx2 have been shown to bind to each other (68), so the relevance of the co-deficiency requires further investigation. Total Prx2 was not measured in this patient and no cases of complete deficiency in humans have been reported. On the basis of the mouse phenotype, it would be expected to present as Heinz body hemolytic anemia. There are individuals with this condition where the cause has not been established and Prx2 deficiency should be tested as a possible diagnosis.

Mice lacking Prx1, Prx6, and Prx3 have also been created. Prx1 shares 91% homology with Prx2. It is also cytosolic, has the same catalytic mechanism, and similar reaction kinetics (13). Prx1 knockout mice developed malignant tumors and also a Heinz body hemolytic anemia, although not until after 9 months of age compared with 5 weeks in mice lacking Prx2 (73). It was suggested that this late onset reflects an initial compensation by other antioxidant systems that become less effective with age. However, in another study, detectable oxidant production in the erythrocytes was age independent (24). There appears to be some overlap in the effects of Prx1 and Prx2 deficiencies, but it is clear from the mouse studies that the two Prxs are unable to compensate for each other. *In vitro* studies have shown that purified Prx6 prevents spontaneous and ascorbate-induced Hb oxidation (92) and macrophages from Prx6 knockout mice showed greater susceptibility than wild type to peroxide or paraquat-induced oxidative stress (99). However, no erythrocyte abnormalities were reported. Mitochondrially expressed Prx3 is not present in mature erythrocytes, but a deficiency does impair the differentiation of mouse and human erythroleukemia cells (72, 105). Despite this, Prx3 knockout mice do not present with hematological abnormalities (54).

So how does Prx2 deficiency in the erythrocyte relate to deficiencies in other peroxide-metabolizing enzymes? Heinz body hemolytic anemia was first recognized in individuals with glucose-6-phosphate dehydrogenase deficiency, where the cause was established as an inability to generate sufficient NADPH under conditions of oxidative stress (5). This led to the discovery of glutathione peroxidase and the recognition of the glutathione peroxidase/glutathione reductase/NADPH pathway in hydrogen peroxide removal (16, 61). GPx1 is widely considered to be important in protecting against Heinz body formation. The fact that it is a selenoenzyme and hemolysis occurs in selenium-deficient mice support this view (7). However, several groups have developed different Gpx1 knockout mice (15, 20, 38, 45, 53), and although these showed a range of symptoms and adverse responses to oxidative stress, their red cells did not develop Heinz bodies or signs of oxidant sensitivity. NADPH is also required to maintain the activity of catalase, and on this basis it has been argued that compromised catalase activity is the critical factor in G6PD deficiency (27, 28, 89). However, humans with congenital acatalasemia do not develop hemolytic diseases (1, 31, 93), and mice generated to be completely catalase deficient have normal hematological profiles (39). This could mean there is redundancy between the two NADPH-dependent enzyme systems. With Prx2 now recognized as a component of erythrocyte antioxidant defense, the impact of NADPH depletion on its action in G6PD deficiency also needs to be considered.

It is not entirely clear why a deficiency of Prx2, but not the other peroxide-metabolizing systems, causes Heinz body hemolytic anemia. One possibility is that Prx2 is important for protecting against low levels of endogenous hydrogen peroxide that could cause Hb denaturation over the 120-day lifespan of an erythrocyte. Because of slow recycling, it would be less effective than GPx1 and catalase when peroxide generation is higher, as in drug-induced hemolysis. Prx2 may also help prevent iron-induced oxidative damage during erythropoiesis. It is highly expressed in proerythroblasts (81), where there are large quantities of iron and heme are present for Hb synthesis. Alternatively, the protective effect of Prx2 may involve more than just hydrogen peroxide removal. One possibility is that it has a broader substrate preference than catalase and GPx1, which react only with hydrogen peroxide and water-soluble organic peroxides (96). Although not specifically investigated for Prx2, this appears to be the case for other 2-Cys peroxiredoxins (95). Perhaps

Prx2 is able to break down membrane lipid peroxides and thus protect against damage by membrane-associated hemoglobin. GPx4, which also shows this activity, is not detectable in erythrocytes. Another possibility is that Prx2 exhibits chaperone-like activity and prevents Hb denaturation and/or precipitation. In support of this, we observed that adding purified Prx2 to hemolysate decreased the level of Hb precipitation upon exposure to Heinz body inducers but not the rate of Hb oxidation (unpublished observations).

Conclusions

Prx2 appears to play an important role in the metabolism of low-level hydrogen peroxide in the erythrocyte. This includes scavenging hydrogen peroxide derived from Hb autoxidation. Low thioredoxin reductase activity in the erythrocyte is able to keep up with this basal oxidation and maintain the Prx2 in its reduced form, but exposure to even low concentrations of hydrogen peroxide causes it to accumulate as a disulfide-linked dimer. The high cellular concentration means that, although turnover is slow, erythrocyte Prx2 can act as a noncatalytic scavenger of hydrogen peroxide and effectively act as a sink for up to 250 μM hydrogen peroxide before turnover becomes limiting. In addition to its antioxidant function, Prx2 regulates ion transport by associating with the membrane and activating the Gárdos channel. How its redox transformations are linked to membrane association and channel activation are yet to be established, and the mechanism whereby Prx2 deficiency leads to hemolytic anemia is not fully understood. Further studies with normal and Prx2 deficient erythrocytes should help decipher these interactions and clarify the role of Prx2 alongside GPx1 and catalase in the erythrocyte's antioxidant defense. Studies on the erythrocyte may also shed light on how Prx2 functions in other cells.

Acknowledgments

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Abbreviations

G6PD, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; Grx, glutaredoxin; GR, glutathione reductase; Hb, hemoglobin; MetHb, methemoglobin; Prxs, peroxiredoxins; Prx2, peroxiredoxin 2; SOD, superoxide dismutase; Trx, thioredoxin; TrxR, thioredoxin reductase.

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Address reprint requests to:
Christine Winterbourn
Free Radical Research Group
Department of Pathology
University of Otago
PO Box 4345
Christchurch, New Zealand

E-mail: christine.winterbourn@otago.ac.nz

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