Human peroxiredoxin 5 is a peroxynitrite reductase

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Abstract Peroxiredoxins are an ubiquitous family of peroxidases widely distributed among prokaryotes and eukaryotes. Peroxiredoxin 5, which is the last discovered mammalian member, was previously shown to reduce peroxides with the use of reducing equivalents derived from thioredoxin. We report here that human peroxiredoxin 5 is also a peroxynitrite reductase. Analysis of peroxiredoxin 5 mutants, in which each of the cysteine residues was mutated, suggests that the nucleophilic attack on the O–O bond of peroxynitrite is performed by the N-terminal peroxidatic Cys^{47}. Moreover, with the use of pulse radiolysis, we show that human peroxiredoxin 5 reduces peroxynitrite with an unequalled high rate constant of $(7 \pm 3) \times 10^7\ M^{-1}\,s^{-1}$.

Keywords: Peroxiredoxin; Peroxynitrite; Nitrooxidative stress; Mitochondrion; Peroxisome; Cytosol; Nucleus

1. Introduction

Along the mitochondrial electron-transfer chain, the reaction between nitrogen monoxide and superoxide is highly favorable and produces peroxynitrite [1], which can not only damage mitochondrial components but also the cell by promoting lipid peroxidation as well as DNA and protein oxidation leading to cell death [2]. Considering the implication of peroxynitrite in numerous pathological processes [3–5], it is surprising that defense mechanisms against this toxic nitrooxidant are still poorly understood.

Peroxiredoxins (PRDXs) have been identified as a large family of peroxidases able to reduce H_2O_2 and alkyl hydroperoxides [6–10]. These enzymes are widely distributed among prokaryotes and eukaryotes [9]. It is now becoming clear that PRDXs are part of the enzymatic antioxidant system, collaborating in cells with well-characterized catalase, superoxide dismutases and selenium glutathione peroxidases [11]. In mammals, there are six PRDXs encoded by six distinct genes [10,12]. PRDX5, also named PrxV, AOEB166, PMP20 or ACR1, is the last discovered mammalian PRDX [12–16]. Human PRDX5 can be intracellularly localized to mitochondria, peroxisomes, the cytosol and, to a lesser extent, the nucleus [16]. Mitochondrial localization of PRDX5 seems to be essential as peptidic sequence alignment of the human protein with its orthologues in other animal species from invertebrates to vertebrates reveals the conservation of a predicted mitochondrial targeting presequence (Fig. 1).

Recently, alkyl hydroperoxide reductase subunit C (AhpC), a bacterial PRDX homologue of human PRDX5, was reported to possess a peroxynitrite reductase activity [17]. In the view of this, we hypothesized that human PRDX5 could also afford protection against peroxynitrite.[1] Results presented here show that PRDX5 is indeed a peroxynitrite reductase and that it could play a major protective role in animal cells against this toxic nitrooxidant.

2. Materials and methods

2.1. Expression and purification of recombinant proteins

Human PRDX5 (GenBank Accession No. NM_012094) [13] and human thioredoxin 2 (TXN2, GenBank Accession No. U78678) [18] were expressed without their mitochondrial presequence in *Escherichia coli* strain M15 (pRep4) as 6×His-tagged proteins using pQE-30 expression vector (Qiagen) and purified as described previously [15,16]. Human PRDX5 mutants, C47S, C72S and C151S (amino acid numbering refers to mature protein, see Fig. 1), were generated by PCR-mediated site-directed mutagenesis using complementary primers containing base mismatch that converted the codon for Cys to a codon for Ser. Lactate dehydrogenase (LDH) from *Streptococcus thermophilus* was expressed in *E. coli* strain TOP10 (Invitrogen) as a 6×His-tagged protein using pBAD/His expression vector (Invitrogen) and purified as for PRDX5 and TXN2.

2.2. Assay of peroxynitrite-mediated oxidation of dihydrorhodamine 123 (DHR 123)

Peroxynitrite-mediated oxidation of DHR 123 was followed as described [19] using a fluoroscan Ascent FL spectrophotometer (Labsystems) with excitation and emission wavelengths of 515 and 555 mn, respectively. Peroxynitrite was synthesized as described by Koppenol et al. [20]. Peroxynitrite (600 nM) was added to 750 nM DHR 123 (Molecular Probes) and to different concentrations of reduced or oxidized PRDX5. DHR 123 was dissolved in phosphate buffer, 100 mM (pH 7.4), chelexed and supplemented with 100 μM diethylenetriamine

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Abbreviations: AhpC, alkyl hydroperoxide reductase subunit C; DHR 123, dihydrorhodamine 123; DTT, dithiothreitol; GPX, glutathione peroxidase; Mn-SOD, manganese-superoxide dismutase; $ONOO^-$/ONOOH, peroxynitrite; ONO^-/ONOH, nitrite; PBS, phosphate-buffered saline; PRDX, peroxiredoxin; RDH 123, rhodamine 123; TXN, thioredoxin; TXNRD, thioredoxin reductase

[1] In the present work, we use the term peroxynitrite for peroxynitrite anion and peroxynitrous acid without any distinction.

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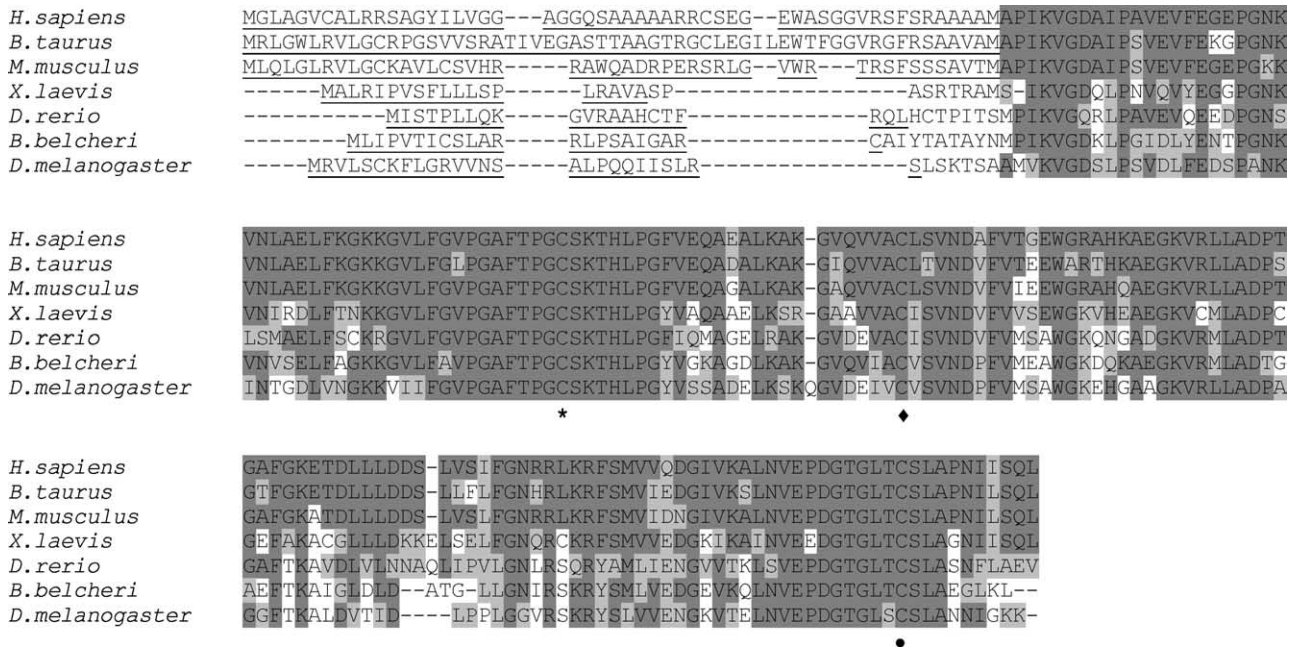

Fig. 1. Amino acid sequence alignment of human PRDX5 and orthologues in vertebrates and invertebrates. The alignment was generated by CLUSTAL W 1.7 [40]. Residues conserved between mature human PRDX5 and its orthologues are highlighted in dark gray and conservative residues are highlighted in light gray. Confirmed (*Homo sapiens*) and predicted mitochondrial presequences are underlined. Mitochondrial presequences were predicted with TargetV1.0 (http://www.cbs.dtu.dk/services/TargetP/; [41]). Alignment of cysteines corresponding to human peroxydatic Cys47 (*), Cys72 (◆) and resolving Cys151 (●) is indicated. GenBank Accession Nos. are NM_012094 (*Homo sapiens*), NM_012021 (*Mus musculus*), NM_174749 (*Bos taurus*), BJ053922 (*Xenopus laevis*), CN508467 (*Danio rerio*), AF498232 (*Branchiostoma belcheri*), and NM_176512 (*Drosophila melanogaster*).

pentaacetic acid. Reduced PRDX5 was obtained after incubation for 30 min at 37 °C with 10 mM dithiothreitol (DTT) in phosphate-buffered saline (PBS) and then passed through a PD10 desalting column (Amersham) to remove DTT. Oxidized PRDX5 was prepared by the addition of 10 mM H_2O_2 during 30 min at 37 °C in PBS and passed through a PD10 column.

2.3. TXN-dependent peroxidase activity of PRDX5

Peroxidase reaction was performed as previously described [21] with minor modifications. Briefly, peroxidase reaction was performed in a 75 μl reaction mixture containing 250 μM NADPH (Sigma), 0.2 μM recombinant rat thioredoxin reductase 1 (TXNRD1, IMCO), 2.2 μM TXN2 and 1.5 μM PRDX5 in PBS (pH 7.4). The reaction was initiated by the addition of 0.5 mM H_2O_2. The initial rate of NADPH oxidation was monitored by measurement of absorbance decrease at 340 nm in the presence of PRDX5 at 37 °C.

2.4. Measurement of nitrite and nitrate

Nitrite and nitrate concentrations were measured by anion chromatography with conductometric detection as described earlier [22].

2.5. Stopped-flow spectrophotometry

The reaction was initiated by mixing a peroxynitrite solution (4 μM final concentration) in 0.01 M sodium hydroxide with protein solubilized in 0.14 M PBS (pH 7.4) with a ratio of 1:11. Proteins were first reduced or oxidized with 10 mM DTT or H_2O_2 and were subsequently passed through a PD10 desalting column. Stopped-flow observations were carried out at 300 nm (25 °C) with an Applied Photophysics SX 17 MV device.

2.6. Pulse radiolysis

Experiments were carried out with Febetron 705 2 MeV accelerator (Titan Corp.) with a pulse width of 50 ns and doses between 20 and 60 Gy. A 75 W Xe-Arc lamp was used as light source. Irradiations were carried out in a 1 cm Suprasil Quartz cell (Hellma). Peroxynitrite was produced in situ with formate and nitrite as previously described [23], except that the concentration of formate was decreased to a final concentration of 10 mM. Nitrite concentration was 2 mM. pH was set to 7.8. Protein was used at a final concentration of 50 μM. Peroxynitrite was followed at 300 nm. As the energy of the 2 MeV electrons is absorbed by water, there was no radiation damage to the solutes.

3. Results

3.1. Human PRDX5 protects against DHR 123 oxidation caused by peroxynitrite

Reduced wild-type recombinant PRDX5 caused a pronounced inhibition of DHR 123 oxidation into rhodamine 123 (RDH 123) (Fig. 2A). When wild-type PRDX5 was first oxidized by H_2O_2, this inhibition disappeared. As a negative control, reduced 6×His-LDH had no protective effect (Fig. 2A).

3.2. Cys^{47} of PRDX5 is necessary for protection against DHR 123 oxidation caused by peroxynitrite

In order to investigate the role of the three cysteines of PRDX5, we tested the inhibition of DHR 123 oxidation by peroxynitrite conferred by the three PRDX5 mutants: C47S, C72S and C151S. PRDX5 mutants were reduced or oxidized. Among the reduced mutants, only C72S and C151S retained their activity, which was similar to that of the reduced wild-type enzyme (Fig. 2B). Reduced C47S mutant was totally ineffective. No protection was detected with any of the oxidized mutants. These results show that Cys^{47} is essential for protection by PRDX5 against peroxynitrite-mediated oxidation of DHR 123 and that the reduced status of Cys^{47} is also required.

3.3. PRDX5 is a peroxynitrite reductase

We then investigated whether PRDX5 oxidized by peroxynitrite could be reduced enzymatically by the TXN system

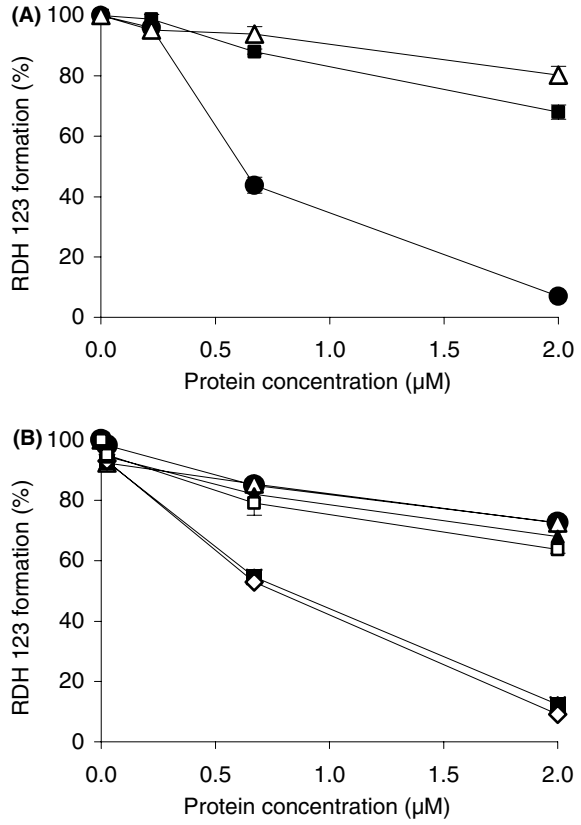

Fig. 2. (A) Protection by different concentrations of PRDX5 against DHR 123 oxidation into RDH 123 caused by peroxynitrite. Peroxynitrite was added to wild-type PRDX5 (reduced, *closed circles*; oxidized, *closed squares*) or reduced LDH (*open triangles*) (mean ± S.E.M., $n = 3$). (B) Protection by PRDX5 mutants against DHR 123 oxidation caused by peroxynitrite. Mutants C47S (reduced, *open triangles*; oxidized, *closed circles*), C72S (reduced, *open diamonds*; oxidized, *closed triangles*) or C151S (reduced, *closed squares*; oxidized, *open squares*) were added at different concentrations to the reaction mixture. Peroxynitrite was added to initiate the reaction (mean ± S.E.M., $n = 3$).

and thus whether oxidation of PRDX5 by peroxynitrite is reversible as required for peroxynitrite reductase activity. To demonstrate this, 20 μM wild-type PRDX5 as well as C47S, C72S and C151S mutants were first treated with 40 μM peroxynitrite. Reductase activity of PRDX5 oxidized by peroxynitrite was then measured with the TXN system containing NADPH, TXNRD1, TXN2 and H_2O_2 (Fig. 3A). Results showed that wild-type PRDX5 as well as C72S mutant oxidized by peroxynitrite can be reduced by the TXN system and that this oxidation does not alter their functionality. As expected from previous biochemical analyses [14], C47S and C151S mutants were devoid of TXN-dependent peroxidase activity. We then measured the formation of nitrite and nitrate from peroxynitrite decay in the presence or absence of either reduced wild-type PRDX5 or C47S mutant. As the spontaneous decay of peroxynitrite generates mostly nitrate at physiological pH [22], the increase in nitrite in the presence of protein is a measure of peroxynitrite reduction. As shown in Fig. 3B, the spontaneous decomposition of peroxynitrite yields 80% nitrate and 20% nitrite. The addition of reduced wild-type PRDX5 reversed that ratio as the proportion of nitrate and nitrite was then 29% and 71%, respectively, indicating a successful competition with the spontaneous isomerization of peroxynitrite to nitrate.

3.4. PRDX5 reacts with peroxynitrite with a very high rate constant

Initially, we used stopped-flow spectrophotometry to study the kinetics of reaction of PRDX5 with peroxynitrite. Results showed that only reduced wild-type PRDX5 reacted rapidly and completely with peroxynitrite (data not shown). The disappearance of peroxynitrite could not be recorded because all peroxynitrite had been consumed during the mixing time of 1.5 ms. As expected, the reduced or oxidized C47S mutant did not significantly affect the rate of peroxynitrite decay. We then used pulse radiolysis in order to observe the fast kinetics of the reaction of wild-type PRDX5 with peroxynitrite. When the protein was added, all peroxynitrite disappeared extremely rapidly (Fig. 4). From eight replicates of peroxynitrite decay

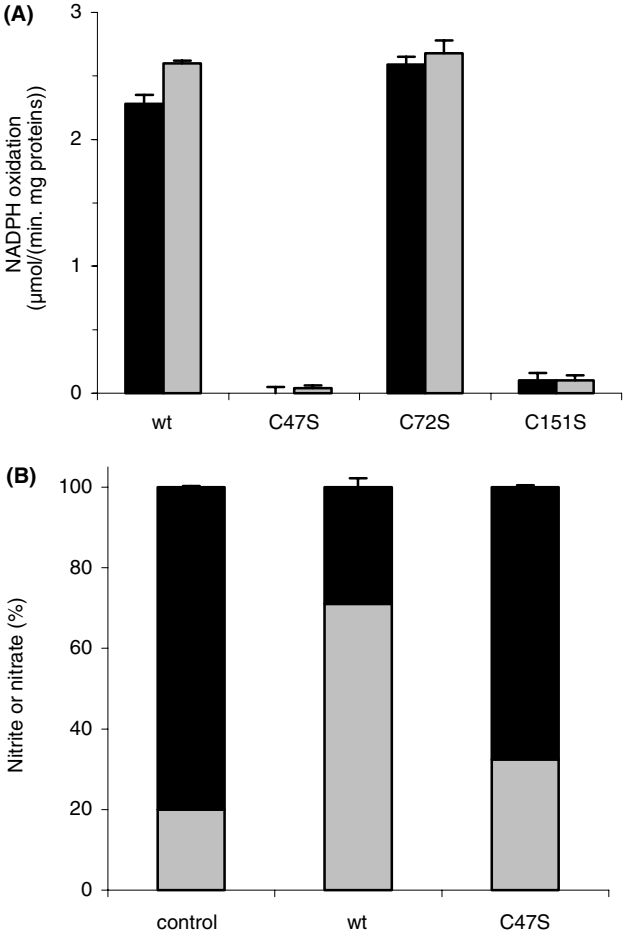

Fig. 3. (A) TXN-dependent peroxidase activity of wild-type PRDX5 (wt) and mutants after oxidation by peroxynitrite. Wild-type PRDX5 or mutants (20 μM) were pretreated either with peroxynitrite (40 μM) (gray columns) or buffer alone (black columns) during 30 min at 37 °C, after which they were passed through a desalting column and TXN-dependent peroxidase activity was measured in the presence of NADPH, TXNRD1, TXN2 and H_2O_2 (mean ± S.E.M., $n = 3$). (B) Measurement of nitrite and nitrate levels after peroxynitrite degradation, alone or in the presence of either reduced wild-type PRDX5 (wt) or C47S mutant. Results are expressed in percents of the total content of nitrite and nitrate in the reaction mixture (black, nitrate; gray, nitrite; mean ± S.E.M., $n = 3$).

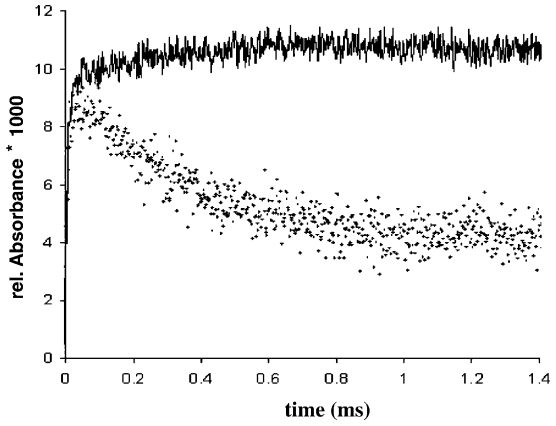

Fig. 4. Formation of peroxynitrite from $O_2^{\cdot-}$ and ·NO by pulse radiolysis in the presence (dots) and absence (line) of 50 μM reduced wild-type PRDX5. The traces were recorded at 300 nm. Air saturated solutions of 2 mM nitrite and 10 mM formate at pH 7.8 were irradiated.

kinetics, we calculated that the rate constant of reduced wild-type PRDX5 with peroxynitrite is $(7 \pm 3) \times 10^7$ M^{-1} s^{-1}.

4. Discussion

We demonstrate here that human PRDX5 reduces peroxynitrite extremely rapidly and acts as a peroxynitrite reductase. The obligatory presence of reduced Cys^{47} was established for this protein to reduce peroxynitrite, suggesting that PRDX5 reaction towards peroxynitrite is similar to the one previously proposed for AhpC and bovine PRDX6 [17,24]. Cys^{47} may be oxidized to a sulfenic acid after a nucleophilic attack on the O–O bond of peroxynitrite, with the concomitant formation of nitrite. In the case of PRDX5, following the peroxidatic pathway, the cysteine sulfenic acid (Cys^{47}-SOH) would react with resolving Cys^{151} (Cys^{151}-SH) to form an intramolecular disulfide intermediate, which could then be reduced by a physiological electron donor such as cytosolic TXN1 or mitochondrial TXN2 (Fig. 5). Accordingly, preincubation experiment of PRDX5 with peroxynitrite and assay for peroxidatic activity with the TXN system shows that Cys^{47} oxidized by peroxynitrite and subsequent intramolecular disulfide intermediate can indeed be reduced by the TXN system, including mitochondrial TXN2, demonstrating that this reaction is catalytic and that the enzyme therefore exhibits true peroxynitrite reductase activity. This peroxynitrite reductase activity is further confirmed by the measurements of the ratio of nitrite and nitrate formed in the presence of reduced PRDX5.

The rate constant of PRDX5 towards peroxynitrite, $(7 \pm 3) \times 10^7$ M^{-1} s^{-1}, is remarkably high. This rate constant is at least two times higher than that of peroxynitrite with Mn(III) 5,10,15,20-tetrakis(*N*-methylpyridyl)porphyrin (1.9×10^7 M^{-1} s^{-1}) [25], 20 times that with ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one, 2×10^6 M^{-1} s^{-1}) [26], and 5 times that with reduced glutathione peroxidase 1 (GPX1, 8×10^6 M^{-1} s^{-1}) [27]. Moreover, it is at least 25 times higher than that reported for PRDX5 bacterial homologue, AhpC (1.5×10^6 M^{-1} s^{-1}) [17]. Even if cellular concentration and turnover of PRDX5 must be considered, this high rate constant allows one to speculate that PRDX5 at the micromolar concentration level competes effectively for peroxynitrite with carbon dioxide [28] at a physiological concentration of 1 mM. A physiological protective role against peroxynitrite is therefore conceivable and is further supported by in vivo data, suggesting that PRDX5 could protect from reactive nitrogen species generated in alcohol-treated *Xenopus* embryos [29]. Similarly, as reported recently, mitochondrial PRDX3 could also function in vivo as a protective peroxynitrite reductase [30].

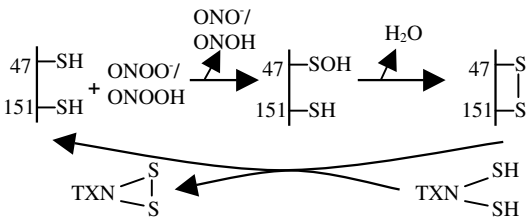

Fig. 5. Proposed catalytic mechanism of PRDX5 in the reduction of peroxynitrite to nitrite.

The lack of inactivation of PRDX5 catalytic activity by peroxynitrite in our experimental conditions underlines even more the fine adaptation of this enzyme to react with peroxynitrite. Many proteins or enzymes are indeed inactivated by peroxynitrite. Mitochondrial manganese-superoxide dismutase (Mn-SOD) is inactivated by nitration of its Tyr^{34} when exposed to peroxynitrite [31]. Peroxynitrite also inactivates GPX1 [32,33]. Interestingly, there is no Tyr residue in mature human PRDX5 (Fig. 1). This could explain its lower susceptibility to deleterious peroxynitrite attacks and may confer an advantage of PRDX5 over other candidate peroxynitrite reductase enzymes in mammalian cells such as GPX1 [34], PRDX3 [30] and PRDX6 [24]. This absence of tyrosines in PRDX5 could be a characteristic acquired later during evolution, at least in mammals, as the bacterial AhpC is prone to nitration of its tyrosines by peroxynitrite [17].

Recently, different PRDXs were shown to be endowed with peroxynitrite reductase activity, such as the yeast peroxiredoxin Ttsa2p [35], the mammalian PRDX6 [24] and the bacterial AhpC [17,36]. However, it has also been demonstrated that peroxynitrite reductase activity could not be generalized to all PRDXs as bacterial Tpx or Bcp was not active to detoxify peroxynitrite [37].

Although PRDX5 has been detected in the peroxisomes, in the cytosol and in the nucleus of mammalian cells, it appeared that in many cell types, PRDX5 is primarily addressed to mitochondria [13,14,38,39]. This mitochondrial localization, which appears to be highly conserved throughout evolution, probably reflects the physiological protective role of PRDX5 in these organelles, which are major sources and targets for oxidative or nitrosative injuries. The peroxynitrite reductase activity of PRDX5 could have important implications for the understanding of mitochondrial protective mechanisms against peroxynitrite-mediated damages in animal cells.

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References

[1] Nauser, T. and Koppenol, W.H. (2002) J. Phys. Chem. A. 106, 4084–4086.
[2] Radi, R., Cassina, A., Hodara, R., Quijano, C. and Castro, L. (2002) Free Radic. Biol. Med. 33, 1451–1464.
[3] Wiseman, H. and Halliwell, B. (1996) Biochem. J. 313, 17–29.
[4] Heales, S.J., Bolanos, J.P., Stewart, V.C., Brookes, P.S., Lan, J.M. and Clark, J.B. (1999) Biochim. Biophys. Acta 1410, 215–218.
[5] Beal, M.F. (2002) Free Radic. Biol. Med. 32, 797–803.
[6] Chae, H.Z., Robison, K., Poole, L.B., Church, G., Storz, G. and Rhee, S.G. (1994) Proc. Natl. Acad. Sci. USA 91, 7017–7021.
[7] Rhee, S.G., Kang, S.W., Chang, T.S., Jeong, W. and Kim, K. (2001) IUBMB Life 52, 35–41.
[8] Fujii, J. and Ikeda, Y. (2002) Redox Rep. 7, 123–130.
[9] Hofmann, B., Hecht, H.J. and Flohe, L. (2002) Biol. Chem. 383, 347–364.
[10] Wood, Z.A., Schroder, E., Robin Harris, J. and Poole, L.B. (2003) Trends Biochem. Sci. 28, 32–34.
[11] Henkle-Duhrsen, K. and Kampkotter, A. (2001) Mol. Biochem. Parasitol. 114, 129–142.
[12] Leyens, G., Donnay, I. and Knoops, B. (2003) Comp. Biochem. Physiol. B 136, 943–955.
[13] Knoops, B., Clippe, A., Bogard, C., Arsalane, K., Wattiez, R., Hermans, C., Duconseille, E., Falmagne, P. and Bernard, A. (1999) J. Biol. Chem. 274, 30451–30458.
[14] Seo, M.S., Kang, S.W., Kim, K., Baines, I.C., Lee, T.H. and Rhee, S.G. (2000) J. Biol. Chem. 275, 20346–20354.
[15] Declercq, J.P., Evrard, C., Clippe, A., Vander Stricht, D., Bernard, A. and Knoops, B. (2001) J. Mol. Biol. 311, 751–759.
[16] Banmeyer, I., Marchand, C., Verhaeghe, C., Vucic, B., Rees, J.-F. and Knoops, B. (2004) Free Radic. Biol. Med. 36, 65–77.
[17] Bryk, R., Griffin, P. and Nathan, C. (2000) Nature 407, 211–215.
[18] Damdimopoulos, A.E., Miranda-Vizuete, A., Pelto-Huikko, M., Gustafsson, J.A. and Spyrou, G. (2002) J. Biol. Chem. 277, 33249–33257.
[19] Glebska, J. and Koppenol, W.H. (2003) Free Radic. Biol. Med. 35, 676–682.
[20] Koppenol, W.H., Kissner, R. and Beckman, J.S. (1996) Methods Enzymol. 269, 296–302.
[21] Chae, H.Z., Kang, S.W. and Rhee, S.G. (1999) Methods Enzymol. 300, 219–226.
[22] Kissner, R. and Koppenol, W.H. (2002) J. Am. Chem. Soc. 124, 234–239.
[23] Goldstein, S. and Czapski, G. (1995) Free Radic. Biol. Med. 19, 505–510.
[24] Peshenko, I.V. and Shichi, H. (2001) Free Radic. Biol. Med. 31, 292–303.
[25] Ferrer-Sueta, G., Batinic-Haberle, I., Spasojevic, I., Fridovich, I. and Radi, R. (1999) Chem. Res. Toxicol. 12, 442–449.
[26] Masumoto, H., Kissner, R., Koppenol, W.H. and Sies, H. (1996) FEBS Lett. 398, 179–182.
[27] Briviba, K., Kissner, R., Koppenol, W.H. and Sies, H. (1998) Chem. Res. Toxicol. 11, 1398–1401.
[28] Lymar, S.V. and Hurst, J.K. (1996) Chem. Res. Toxicol. 9, 845–850.
[29] Peng, Y., Yang, P.H., Guo, Y., Ng, S.S., Liu, J., Fung, P.C., Tay, D., Ge, J., He, M.L., Kung, H.F. and Lin, M.C. (2004) Invest. Ophtalmol. Vis. Sci. 45, 23–29.
[30] Hattori, F., Murayama, N., Noshita, T. and Oikawa, S. (2003) J. Neurochem. 86, 860–868.
[31] Yamakura, F., Taka, H., Fujimura, T. and Murayam, K. (1998) J. Biol. Chem. 273, 14085–14089.
[32] Asahi, M., Fujii, J., Suzuki, K., Seo, H.G., Kuzuya, T., Hori, M., Tada, M., Fujii, S. and Taniguchi, N. (1995) J. Biol. Chem. 270, 21035–21039.
[33] Padjama, S., Squadrito, G.L. and Pryor, W.A. (1998) Arch. Biochem. Biophys. 349, 1–6.
[34] Sies, H. and Arteel, G.E. (2000) Free Radic. Biol. Med. 28, 1451–1455.
[35] Wong, C.M., Zhou, Y., Raymond, W.M.Ng., Kung, H. and Jin, D.Y. (2002) J. Biol. Chem. 277, 5385–5394.
[36] Chen, L., Xie, Q.W. and Nathan, C. (1998) Mol. Cell. 1, 195–805.
[37] Comtois, S.L., Gildey, M.D. and Kelly, D.J. (2003) Microbiology 149, 121–129.
[38] Oberley, T.D., Verwiebe, E., Zhong, W., Kang, S.W. and Rhee, S.G. (2001) Free Radic. Biol. Med. 30, 412–424.
[39] Leyens, G., Knoops, B. and Donnay, I. (2004) Mol. Reprod. Dev., in press.
[40] Thompson, J.D., Higgins, D.G. and Gilson, T.J. (1994) Nucleic Acids Res. 22, 4673–4680.
[41] Emanuelson, O., Nielsen, H., Brunak, S. and von Heijne, G. (2000) J. Mol. Biol. 300, 1005–1016.