Fish and Shellfish Immunology 89 (2019) 91–97

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

The biological role of peroxiredoxins in innate immune responses of aquatic invertebrates

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ARTICLE INFO

Keywords:
Antioxidants
Innate immunity
Microbial infection
Peroxiredoxins
Oxidative stress

ABSTRACT

Peroxiredoxins (Prxs) are a widespread and greatly transcribed family of antioxidant proteins, which rapidly detoxify peroxynitrite, hydrogen peroxide and organic hydroperoxides. The Prxs family members also modulate various physiological functions, including cell growth, differentiation, embryonic development, immune response, apoptosis, lipid metabolism, and cellular homeostasis. In mammals, the physiological functions of Prxs have extensively been studied; however, the knowledge is scanty in their counterpart, aquatic invertebrates. In recent years, substantial progress has been made in our knowledge of Prxs physiological functions in aquatic invertebrates, which has raised interest in defining the contribution of immune responses and removal of reactive oxygen species. In this review, we describe the recent knowledge on the Prxs physiological function in immune responses and DNA protection activity in aquatic invertebrates.

1. Introduction

Living organisms have to deal with the risk of oxidative stress in an oxygen rich environment. Oxidative stress displays a biochemical state characterized by an excessive existence of reactive metabolites and free radicals potentially detrimental for the living organisms [1,2]. Free radicals are highly reactive chemical species, usually with a very short half-life, comprising a molecule or an atom containing one or more unpaired electrons. These unpaired electrons are responsible to give remarkable reactivity to the radical, making it able to interact to other radicals or subtract an electron from other nearby molecules. Reactive oxygen species are the most important class of free radicals that are generated by living organisms: raised levels result from an imbalance between the formation of oxidants and their removal by the antioxidant system protecting living organism. The superoxide anion radical is one of the more common reactive oxygen species. Its metabolites, such as hydrogen peroxide and hydroxyl radical are highly reactive [3]. Reactive nitrogen species can also damage cells and their components, when they act together with reactive oxygen species.

In animals, the production of ATP by aerobic respiration in mitochondria continuously generates reactive oxygen species and reactive nitrogen species, including the by-products of oxidative phosphorylation. At low to moderate concentrations, reactive oxygen species exert an important positive biological role in various physiological processes, such as cell signaling and defense against pathogens [4], although at higher concentrations, the reactive oxygen species are able to react with different components of a cell, including proteins, lipids, and nucleic acids causing damage of DNA that escapes the repair system of DNA. For this reason, their concentration needs to be precisely regulated. Living organisms are equipped with various enzymatic (peroxiredoxin, ascorbate peroxidase, catalase, superoxide dismutase and glutathione peroxidase) or nonenzymatic (glutathione, ascorbate, carotenoid and tocopherol) or antioxidant systems to control concentration level of reactive oxygen species and reactive nitrogen species [5].

The family of Peroxiredoxins (Prxs) have received a great deal of attention in recent years as a new and expanding family of antioxidant molecules. The Prxs play antioxidant function in cells by peroxidase activity, thereby peroxynitrite, hydrogen peroxide and a wide range of different organic hydroperoxides are detoxified and reduced to protect cells against reactive oxygen species [6–9]. The members of Prxs family are broadly distributed among living organisms and having been identified and characterized both in prokaryotes and eukaryotes. Recently, multiple Prxs have been identified and molecularly characterized from various organisms, particularly in aquatic invertebrates [10–12]. In mammals, to date six isoforms of Prx (Prx1–Prx6) have been reported, which are categorized into three subgroups (1-Cys, atypical 2-Cys and 2-Cys) based on the position and number of cysteine residues participating in catalysis [6,12]. Prx1–Prx4 constitute the first

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https://doi.org/10.1016/j.fsi.2019.03.062
Received 14 January 2019; Received in revised form 22 March 2019; Accepted 26 March 2019
Available online 29 March 2019

subgroup, 2-Cys subgroup, and Prx5 comprising two additional cysteine residues belongs to the second subgroup, atypical 2-Cys. Prx6 is categorized into 1-Cys Prx family for its one highly conserved cysteine residue. Several Prx6 and other members have also been identified from invertebrate and vertebrate species [13–16]. However, the information is sparse on the Prxs family members in aquatic invertebrates. Here, our main focus is to review recent knowledge on the physiological functions of Prxs in aquatic invertebrates. In particular, this review describes the classification and molecular mechanism of Prxs, highlights the involvement of these proteins in immune responses and also describes the potential contribution of Prxs in protection of cellular components from oxidative stress.

2. Discovery and classification of Prxs

In the late 1980s, Prx was first discovered in yeast as an enzyme with 25 kDa molecular weight, which provides protection to cellular components against reactive sulfur species (e.g. RSOOH, $RSSR^{-}\bullet$, or RS•) rather than reactive oxygen species (e.g. ROOH, H_2O_2 or $O_2^{-}\bullet$) [10,17]. The purified Prx (thiol specific antioxidant) was lack of redox co-factor like flavin, heme or metal ion. This redox co-factor was considered an essential component of other reactive oxygen species removing enzymes. So far, several members of this family have been identified and molecularly characterized in different species of invertebrates and vertebrates, and are reported to be diverse in nature, which made its classification challenging for researchers. Thus, different classification systems were given at different times.

The “peroxidatic” Cys (C_P) residue is highly conserved in all of the reported Prx proteins. On the basis of position or absence of the “resolving” Cys (C_R). Prxs are categorized into typical 2-Cys, atypical 2-Cys and 1-Cys subfamilies. The typical 2-Cys proteins are homo-dimeric and comprise two conserved cysteine (C_P and C_R) residues per subunit. The C_P-SOH reacts with the C_R-SH of other subunit to form an inter-subunit disulfide. In atypical 2-Cys (PrxV), the C_P–SOH reacts with the C_R–SH of the same subunit to form an intra-subunit disulfide. Hence, atypical 2-Cys is also a 2-Cys Prx, but is different from the members of 2-Cys Prx, which on oxidation form an intermolecular disulfide. The absence of a C_R, C_P–SOH of 1-Cys Prx cannot be resolved within the Prx molecules and instead forms a disulfide with C_R-SH provided by other proteins or small thiol molecules [18]. In mammals six isoforms of Prx (Prx1 to Prx6) have been identified and are categorized into typical 2-Cys (Prx1 to Prx4), atypical 2-Cys (Prx5), and 1-Cys (Prx6) subfamilies. Based on their biological functions, these members express at different levels in different compartments of cell [19]. Likely, aquatic invertebrates also have the same number of Prx isoforms and are classified in the same subfamilies like mammals (typical 2-Cys, atypical 2-Cys and 1-Cys subfamilies) [20,21].

The classification based on the location of C_R is mechanistically informative. However, the number of Prx homologs/orthologs have been increased and different species may have different number of Prx isoforms, further they are ubiquitously produced in different tissues of animals with multiple isoforms. For instance, nine Prx homologs/orthologs have been described in *Arabidopsis thaliana*, six in *Homo sapiens*, five in *Saccharomyces cerevisiae* and three in *Escherichia coli*. Hence, a new system of classification (Evolutionary classification) has been suggested based on bioinformatic analysis of 29 crystal structures and more than 3500 sequences of Prxs that had been determined during the last decade [22]. This classification system divides Prx enzymes into Prx1, Prx5, Prx6, Tpx, PrxQ, and AhpE subfamilies. The structural and biochemical characteristics and phylogenetic distributions of each of these Prx subgroups are summarized in the review by Poole and Nelson [23]. Members of the Prx1 subgroup are those referred to as “typical” 2-Cys Prxs that contain Prx1 to Prx6 of mammals, AhpC of bacteria, and thiol-specific antioxidant of yeast. Mammalian Prx5 and Prx6 belong to the Prx5 and Prx6 subfamilies, respectively. While, mammalian cells do not express members of the Tpx and PrxQ subfamilies.

3. Structural features of Prxs

Multiple sequence alignment and prediction of secondary structure disclosed that all of the Prx family members share a common thioredoxin fold that contains a C-terminal ββα motif and an N-terminal βαβ motif, linked by a central loop region consisting another α-helix [12]. Prxs comprise many additions to the basic thioredoxin fold, specifically an N-terminal extension, an insertion between the second β-strand and α-helix and in some cases, a C-terminal extension, e.g. in the subfamily Prx1/AhpC. Structural information from all subfamilies of Prx have shown that they share a common core organization containing five α-helices and seven β-strands.

All Prxs function as dimers or higher order oligomers except monomeric BCP family members. Two different types of subunit interfaces interact to form the basic dimeric subunit and then mediate dimer dimer assembly into toroidal structures [24]. Dimeric Prx4 crystal structures showed that B-type dimers are organized in a ‘head-to-tail’ fashion in which the β8-strands of adjacent subunits interact in an anti-parallel manner to generate a stable 14-stranded β-sheet involving the 7 central β-strands of each subunit [25]. The C-terminal region also extends across the two-fold axis, further stabilizing the dimeric interface. In contrast, Prx5 forms a dimer via an A-type (alternative) interface, utilizing hydrophobic interactions and hydrogen bonding [26].

The members of Prxs 1–6 family and some members of the family Prx6, five or six B-type dimers can additionally associate through their A-type interfaces to generate dodecameric ordecameric toroids with a large central cavity. Higher order complexes have also been observed. These involve ring stacking, ring catenation or even larger oligomeric assemblies [27–29]. The A-type interface is less extensive and mainly involves residues from the α3 and α6-helices, the CP loop, and the loops surrounding the α4-helix. In this group of Prxs, decamers and dodecamers are stabilized in the reduced state and exhibit a pronounced tendency to revert to dimers upon oxidation. This redox dependence in the oligomeric state is accounted for by the associated conformational changes in the CP loop, which forms an integral part of the dimer-dimer interface (Fig. 1).

4. Immune system of aquatic invertebrates

In both aquatic and terrestrial invertebrates, the mechanical barriers (e.g. skin) are the first obstacle to detain microbial pathogens [30,31]. However, when mechanical barriers are disrupted or damaged the invading microbial pathogens get entry into the tissue/body of an animal. The exposure to pathogens instantly stimulates the proteolytic pathways, allowing diminution or elimination of microbial pathogens invading the animal [32,33]. In invertebrates, the effector mechanisms for immune responses include coagulation cascade, which avoids the loss of hemolymph, and induce oxidative metabolites and generation of melanin by activating the prophenoloxidase system [34–36]. The activation of prophenoloxidase system stimulates other crucial innate immune processes for quick immune responses, such as encapsulation, nodule formation and phagocytosis [37–39]. The activation of these innate immune processes appears to be mediated by the particular recognition of glycosylated pathogen associated molecular patterns through aquatic invertebrate proteins (Fig. 2).

5. Effect of biotic stress on the production of Prxs

In water bodies, microbial communities (e.g. Viral, bacterial and fungal) are the most common cause of diseases in aquatic invertebrates. They consume host nutrients in order to rapidly grow and proliferate in the body of host. The microbial pathogens utilize different strategies (Bacterial sheds peptidoglycans or secretion of proteases) to interrupt the host's cellular and biochemical processes [40,41]. Over the course of their evolution, aquatic invertebrates have developed innate immune

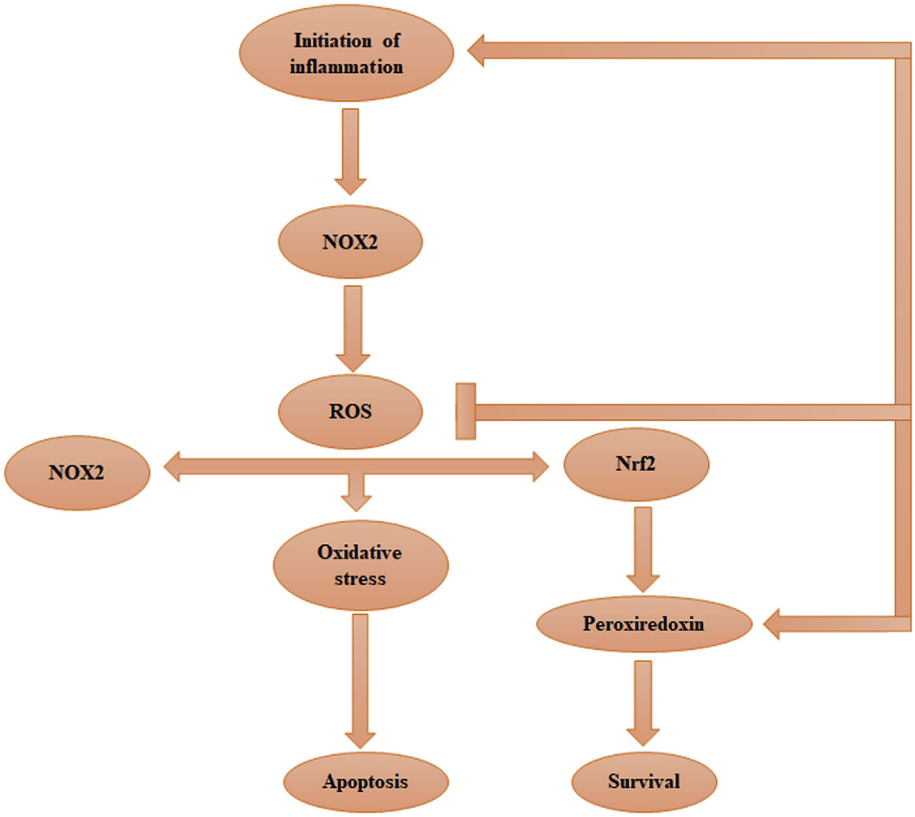

Fig. 1. Initiation process of inflammation and its impact on cell survival and apoptosis, as well as stimulation of associated innate immune defense system.

system to defend themselves against microbial infections, however they lack adaptive immune system [20,42]. The innate immune system contains the recognition of invading microbial pathogens and subsequent synthesis of effectors to eliminate them [33]. Under normal physiological conditions, Prxs are transcribed in cells at variable levels depending on type of tissue. However, transcription of these genes is rapidly induced following exposure to different microbial pathogens. Most of the Prxs family members are greatly induced in response to bacterial, viral and fungal pathogens in aquatic invertebrates [42,43] (Table 1).

In general, there is no simple relationship between a microbial pathogen and the transcription patterns of Prxs. Microbial pathogens stimulate expression of Prxs tend to vary with respect to tissue being studied [20,21,42]. For example, Gram negative bacteria and their cell membrane components (LPS) induce expression of Prx1, Prx4 and Prx5 and 2-Cys Prx, particularly in hemocytes and hepatopancreas of *Sepiella*

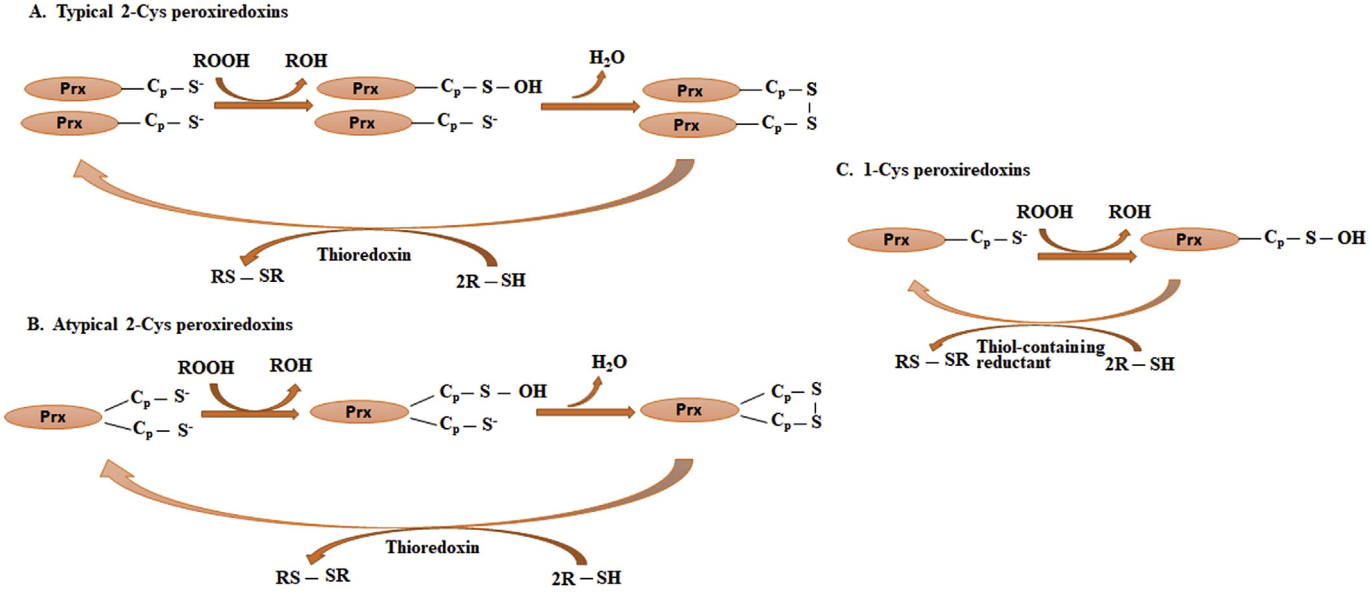

Fig. 2. Biological mechanism of the peroxiredoxins. (A): the main cyctein residue (C_p) reacts with the residue C_r on the second subunit of the dimer. (B): in atypical 2-cys peroxiredoxins, the oxidized Cp reacts with the Cr residue located in the same molecule. (C) in 1-cys peroxiredoxins, the Cp residue generates sulfenic acid and is regenerated directly through donation of an electron to the thiol from in response of ascorbate.

Table 1
Summary of the peroxiredoxins stimulated by biotic and abiotic stress in aquatic invertebrates.

Biotic/abiotic stress	Species name	Tissue/organ	Expression	Gene name	Class	Reference
Bacterial exposure						
Vibrio parahemolyticus	*Penaeus monodon*	Hepatopancreas, gills	↑			[46]
	Marsupenaeus japonicus	Heart, lymphoid tissue	↓	Prx	2-Cys	[51]
Vibrio anguillarum	*Exopalaemon carinicauda*	Hepatopancreas	↑	Prx5		[55]
Vibrio alginolyticus	*Sepiella maindroni*	Liver, hemocytes	↑	Prx1		[47]
	Portunus trituberculatus	Haemocytes, hepatopancreas	↑	Prx		[49]
	Scylla paramamosain	Hepatopancreas	↓	Prx5-1, Prx5-2		[48,56]
		Hepatopancreas	↑	Prx3, Prx4 and Prx6		[48]
Listonella anguillarum	*Eriocheir sinensis*	Hemocytes	↓	Prx6		[45]
Aeromonas hydrophila	*Cristaria plicata*	Hemocytes, hepatopancreas	↑	Prx	2-Cys	[52]
Lipopolysaccharide	*Procambarus clarkii*	Hemocytes, gills, hepatopancreas	↑	Prx4		[50]
	Procambarus clarkii	Hemocytes, gills, hepatopancreas	↑	Prx5		[42]
Peptidoglycan	*Cristaria plicata*	Hemocytes, hepatopancreas	↑	Prx	2-Cys	[52]
Viral exposure						
White spot syndrome virus	*Exopalaemon carinicauda*	Hepatopancreas	↑	Prx5		[55]
	Fenneropenaeus chinensis	Hemocytes, hepatopancreas	↑	Prx, Prx4		[44,78]
	Marsupenaeus japonicus	Gonads, gills, hemocytes	↑	Prx4	2-Cys	[54]
Hypodermal and hematopoietic necrosis virus	*Macrobrachium rosenbergii*	Gills	↑	Prx		[53]
Poly I:C	*Procambarus clarkii*	Hemocytes, gills, hepatopancreas	↑	Prx4		[20]
	Procambarus clarkii	Hemocytes, gills, hepatopancreas	↑	Prx5		[42]
	Scylla paramamosain	Hepatopancreas	↑	Prx5		[56]
Abiotic stress						
Hypoosmotic stress	*Eurypanopeus depressus*	Gills	↑	Prx	2-Cys	[64]
Pollution level	*Crassostrea gigas*	Gills, digestive gland	↑	Prx6		[62]
Thermal stress	*Laternula elliptica*	Gills, digestive gland	↑	Prx5, Prx6		[65]
Copper, and cadmium	*Penaeus monodon*	Gills, hepatopancreas	↑	Prx5	2-Cys	[21]

maindroni, *P. clarkii, Eriocheir sinensis* and other aquatic invertebrates [20,44–47]. Hemocytes and hepatopancreas play a crucial biological role in the innate immune system of invertebrates. Further, it is considered to be homologous to the mammalian liver and pancreas, and it has been shown to modulate major events of immunity and metabolism in aquatic invertebrates [42,48]. Furthermore, the expression patterns of Prxs vary with the type of tissue, and also on the exposure time of pathogen [42–50]. Gram positive bacteria such as *Streptococcus agalactiae* can induce the production of Prx5 in animals [21]. Peptidoglycan, an essential component of both the Gram positive and Gram-negative bacteria, has also a suppressive biological role on 2-Cys Prx production in heart, hemocytes and lymphoids of kuruma shrimp, *Marsupenaeus japonicus* [51]. Whereas it induced 2-Cys Prx production in *Cristaria plicata* [52]. It seems the Prxs family members are highly sensitive to bacterial infection in aquatic invertebrates, and they may have different expression levels after bacterial infection in different species and in different tissues of invertebrates. Additionally, the Prxs are also highly sensitive to viral infections, such as the infectious hypodermal and hematopoietic necrosis virus (IHHNV) induced the synthesis of Prxs in gills of freshwater prawn, *Macrobrachium rosenbergii* [53]. In kruma shrimp, and *Exopalaemon carinicauda* white spotted syndrome virus (WSSV) enhanced the production of Prx 4 and Prx5 in different tissues [54,55]. The production of Prx4 and Prx5 in crayfish has also been reported to increase after viral wall components (Poly I:C) exposure [42,50]. A recent study compared the expression profiles of different Prxs in *Scylla paramamosain*, which showed all of the Prxs (Prx1/2, Prx3, Prx4, Prx5-1, Prx5-2 and Prx6) are highly responsive to viral infection in this species [48,56].

Collectively, microbial infection may enhance or suppress production of Prxs in aquatic invertebrates suggesting the involvement of these genes in the stimulation of host immune responses, particularly against bacterial and viral infections. Moreover, their variable level of production against infectious agents indicates that they may have different biological functions in response to microbial pathogen infection. The main challenge for researchers is to examine the mechanisms underlying the production of Prxs in aquatic invertebrates.

6. Effect of abiotic stress on the production of Prxs

The unwise application of pesticides, rapid industrial revolution, mining operations and so on are the major polluting and water quality damaging factors in aquatic bodies [57–59]. The continuous discharge and accumulation of these pollutants in the aquatic bodies are considered to be a major threat for the survival of aquatic vertebrates and invertebrates [60,61]. Under these drastic conditions, aquatic invertebrates undergo through various physiological and behavioral modifications to maintain homeostasis. The abiotic factors, including contaminants, temperature, heavy metals and hypo-osmotic stress have been shown to affect the normal physiology of animal and influence their health, and these animals make immunological modifications to maintain normal homeostasis (Table 1) [62–64].

Elevated level of pollutants in water bodies led to the over-expression of various immune related genes including Prxs. David and his co-workers [62] suggested that Prx6 can be used as a physiological and a genetic marker to monitor level of stress (level of pollutants) in disturbed aquatic ecosystems. The authors noted variable production of Prx6 in Pacific oyster, *Crassostrea gigas* in different stress conditions, and suggested that the variable production of this gene can relate to different levels of pollutants in aquatic bodies. A recent study showed that different types of heavy metals and their levels of concentration can differentially influence the production of Prx5 in Penaeus *monodon*. In addition, the level and type of heavy metals also have different impact on various cellular processes in different tissues of an animal. The higher level of copper can reduce the production of Prx5 in gills. Whereas, cadmium and zinc levels decrease the production of this gene in both hepatopancreas and gills. This study further described that pH and salinity levels in the immediate environment of animals can also influence the production of Prx5 [21]. In contrast, another study showed that Prx5 production is increased at higher levels of cadmium in *Daphnia magna* [63]. Many studies suggested that hypo-osmotic conditions can also stimulate the production of Prxs (Prx1) in various tissues (e.g. gill hypodermis and hepatopancreas) of aquatic invertebrates [62,64]. Park and his colleagues [65] found greater production of Prx5 and Prx6 at higher temperature in *Laternula elliptica*.

The xenobiotic stress responsive genes such Prxs can help the

aquatic invertebrates for physiological and genetic adaptation in the contaminated water bodies. Additionally, the Prxs production levels in aquatic invertebrates can also be used as a key indicator of environmental pollution and monitoring the production of these genes in response to environmental stress conditions has become an important technique in toxicological studies. Researchers suggested that there might be a correlation between environmental pollutants and antioxidant (enzymatic and non-enzymatic) activities in aquatic invertebrates [66]. So far, only few Prxs were studied in aquatic invertebrates, but their mechanism of production under different pollutants need to be explored. Further, for more generalization, more studies are required to understand the relationship between Prxs and pollutants.

7. Antioxidant potential of Prxs

Oxidative stress arises when the rate of reactive oxygen species production exceeds that of their removal [67,68]. The detrimental impacts of oxidative stress include oxidation of DNA, steroid components, proteins and peroxidation of lipids in cell membranes. This oxidation process generates unstable lipid hydro-peroxides, the products of which, on decomposing, are highly reactive, threatening the cell integrity. In addition, these products can break down into free radicals that can perpetuate the destructive cycle of lipid peroxidation chain reactions. Biological oxidation is a primitive process and, in the face of the certain consequences of O_2 toxicity, evolution has provided suitable defensive strategies. When, more complex aerobic forms of life developed on the earth, the expansion and diversification of antioxidant defense systems also evolved for adapting new environmental conditions. The first line of defense is the utilization of antioxidant molecules such as carotenoids, uric acid, vitamin E, vitamin C and glutathione. The diverse antioxidant substances inhibit the cascade of oxidant reactions, inactivating and intercepting with the reactive inter-mediates of oxygen, closing the cycle of lipid-peroxidation. Antioxidant proteins are critical in the effort to counter-act oxygen toxicity, when the supply of other antioxidant compounds is scarce or depleted [69,70]. The antioxidant substances together with the enzymes constitute what are called primary antioxidants [71].

Recently, the family of Prxs have received considerable deal of attention owing to their biological function in controlling H_2O_2 levels, an intracellular signaling molecule common to various cytokine induced signaling pathways [72–74]. Unquestionably, the peroxidase activity of Prx proteins against H_2O_2, peroxynitrite and organic hydroperoxides is greatly important to protect different cellular components from oxidative damage [18,22]. Prx proteins possess peroxidase activity against organic hydroperoxides, peroxynitrite and H_2O_2. It has been shown that various cellular components are protected from oxidative damage by the antioxidant activity of Prxs. In mammalian cells, Prxs act as a barrier against oxidative stress, and that the ratio of active to inactive proteins might play a role in whether cells are susceptible to cytokine-induced apoptosis [75].

The importance of Prxs family prompted the researchers to investigate the biological functions of these proteins in aquatic invertebrates, which are usually exposed to various oxidants. So far, several studies on aquatic invertebrates and vertebrates have shown that Prx play a crucial biological role in DNA protection against oxidative stress [20,50,76]. Most of the studies used mixed function oxidase (MFO) system, auto-oxidation of thiols like DTT in the presence of metals like iron produces reactive oxygen species including, •OH, O_2• and H_2O_2. The •OH radical has ability to react with biological molecules, wreaking indiscriminate but extensive intracellular damage. The reactive oxygen species like •OH radical are produced nearby the nucleic acids and can add hydrogen atoms to DNA bases or abstract hydrogen atoms from the sugar moiety, producing changes like modified bases, DNA strand breaks or abasic sites. The single strand breaks result in the linearization of supercoiled plasmid DNA [(covalently closed circular DNA (CCC DNA)] to open circular plasmid DNA (OC DNA/nicked DNA). The percentage of conversion of CCC DNA to OC DNA suggests the amount of DNA damages caused by reactive oxygen species and the inhibition of conversion of the CCC DNA to OC DNA, reflects the antioxidant activity of the protein [20]. Many members of Prxs family have been described in aquatic invertebrates, which protect DNA damage against oxidative stress. For example, Prx4, Prx5, Prx6 in red swamp crayfish have been reported to protect DNA against oxidative stress [42,46]. Likewise, the DNA protective activity of Prxs have also been described in many other aquatic invertebrate species e.g. *Oplegnathus fasciatus* [76]. Another study examined the comparative antioxidant potential of Prx1 and Prx2 in disk abalone (*Haliotis discus discus*) using in vitro (using metal catalyzed assay) and in vivo experimental techniques. The authors suggested that recombinant pMALAb Prx2 protein has great antioxidant potential at medium to low level of H_2O_2 concentration [77]. A recent study also reported similar findings for Prx of *Cristaria plicata*. The authors observed particular range of H_2O_2 concentration (0.4 and 0.8 mmoL/L) has no obvious effect on the growth of DE3-pET-32-CpPrx containing cells. These findings indicate that Prxs are highly effective antioxidant player against reactive oxygen species. Additionally, higher concentration levels of reactive oxygen species, usually inhibit the growth of cells, which might be due to the blocking of cellular respiration [78]. Furthermore, the antioxidant potential of Prxs have also been reported in many other species e.g. *Fenneropenaeus chinensis* [44,79].

Overall, Prxs are good candidates to remove the reactive oxygen species, and play a crucial biological in the protection of cellular components from free radicals, which are produced as a byproduct of cellular metabolism. The future studies should focus to investigate the molecular mechanism of Prxs underlying the potential antioxidant activity in aquatic invertebrates.

8. Contribution of Prxs in the regulation of signaling pathways

The family of Prxs is crucially important to regulate multiple physiological functions by controlling the activation and inhibition of different biochemical pathways in animals. However, functional and biochemical studies on Prxs are still at very basic level in aquatic invertebrates. The Prx proteins as a regulator of different signaling pathways have largely been studied in mammals. Min and his coworkers [80] described that Prx6 can induce the activation of nuclear factor-kappa B (NF-κB) in HEK293T cells by Prx6 interacting with the C-terminal TRAF-C domain of TRAF6, which translocate Prx6 into the mitochondria. Where, it competitively interacts with ECSIT to TRAF6 by its C-terminal TRAF-C domain, leading to the interruption of TRAF6-ECSIT interaction. The inhabitation of TRAF6-ECSIT complex induce the activation of NF-κB induced through TLR4. Overexpression of Prdx6 led to the inhibition of NF-κB induced by TLR4, whereas Prdx6 KD THP-1 cells displayed enhanced production of pro-inflammatory cytokines including interleukin-6 and -1β, and the up-regulation of NF-κB-dependent genes induced by TLR4 stimulation. Taken together, the data demonstrate that Prdx6 interrupts the formation of TRAF6-ECSIT complex induced by TLR4 stimulation, leading to suppression of bactericidal activity because of inhibited mitochondrial reactive oxygen species production in mitochondria and the inhibition of NF-κB activation in the cytoplasm [80].

In aquatic invertebrates, a recent study reported the involvement of Prx6 in Toll signaling pathway of red swamp crayfish. This study suggested that suppression of *PcPrx6* by double stranded RNA significantly enhance the activity of toll signaling pathway, the authors suggested that Prx6 is an important negative regulator of Toll signaling pathway [72]. Overall, knowledge is scarce on the involvement of Prxs in different cellular signaling pathways in aquatic invertebrates. Therefore, future studies should focus to investigate the biological function of Prxs in modulation of different signaling pathways.

9. Conclusion and future perspectives

Peroxiredoxins form an important superfamily of peroxidases, constitutively occurs throughout evolution in in prokaryotes (bacteria, archaea) and eukaryotes (invertebrates and vertebrates) [81]. In invertebrates, Prxs are ubiquitously transcribed in approximately all tissues where they can act as peroxynitrite and peroxide scavenging enzymes in collaboration with glutathione peroxidases and catalase. With regard to their biological function, Prxs have been described to protect cellular components against harmful oxidation of different macromolecules induced by pathophysiological or physiological production of reactive oxygen species and reactive nitrogen species. This cytoprotective role could be considered as an ancestral biological role of Prxs endowed primarily by archaeal and bacterial Prxs. But, as the function of peroxides, and particularly hydrogen peroxide, as signaling molecules is an emerging field, and because Prx activity is controlled through post translational modifications, now it is recognized that Prxs may regulate peroxide signaling implicated in vital functions of cell. Therefore, during the recent years, several lines of evidence pointed that members of Prxs family may act as key players in innate immunity. Indeed, it was reported that Prxs may act as cytoprotective proteins against reactive oxygen species/reactive nitrogen species generated during different biological processes. In this context, and considering the multiple potential biological functions of Prxs, challenges for the coming years will be to determine where (in which cells, in which subcellular compartment or extracellularly), and how (as ligand for membrane, or as redox relay, or as peroxide reductase, or intracellular receptors) Prxs act in innate immune system.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful for funding support from the National Key Research and Development Program of China (No. 2016YFC1302204 and 2017YFC1308600 to H. Cui), the National Natural Science Foundation of China (No. 81672502 to H. Cui).

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