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Working Mechanism of Peroxiredoxins (Prxs) and Sulphiredoxin1 (Srx1) in *Arabidopsis thaliana*

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Plants generate reactive oxygen species (ROS) as a by-product of normal aerobic metabolism or when exposed to a variety of stress conditions, which can cause widespread damage to biological macromolecules. To protect themselves from oxidative stress, plant cells are equipped with a wide range of antioxidant proteins. However, the detailed reaction mechanisms of these are still unknown. Peroxiredoxins (Prxs) are ubiquitous thiol-containing antioxidants that reduce hydrogen peroxide with an N-terminal cysteine. The active-site cysteine of peroxiredoxins is selectively oxidized to cysteine sulfinic acid during catalysis, which leads to inactivation of peroxidase activity. This oxidation was thought to be irreversible. Recently identified small protein sulphiredoxin (Srx1), which is conserved in higher eukaryotes, reduces cysteine–sulphinic acid in yeast peroxiredoxin. Srx1 is highly induced by H_2O_2-treatment and the deletion of its gene causes decreased yeast tolerance to H_2O_2, which suggest its involvement in the metabolism of oxidants. Moreover, Srx1 is required for heat shock and oxidative stress induced functional, as well as conformational switch of yeast cytosolic peroxiredoxins. This change enhances protein stability and peroxidase activity, indicating that Srx1 plays a crucial role in peroxiredoxin stability and its regulation mechanism. Thus, the understanding of the molecular basis of Srx1 and its regulation is critical for revealing the mechanism of peroxiredoxin action. We postulate here that Srx1 is involved in dealing with oxidative stress via controlling peroxiredoxin recycling in *Arabidopsis*. This review article thus will be describing the functions of Prxs and Srx in *Arabidopsis thaliana*. There will be a special focus on the possible role of Srx1 in interacting with and reducing hyperoxidized Cys-sulphenic acid of Prxs.

Key words : Peroxiredoxin, sulphiredoxin, reactive oxygen species (ROS), reduction, oxidation

Introduction

Reactive oxygen species (ROS) are incessantly generated as by-products of normal cellular metabolism in plants and removed by antioxidant mechanisms. ROS include free radicals, which can be described as atoms or molecules containing one or more unpaired electrons, such as superoxide anions and hydroxyl radicals. Other substances such as atoms or molecules with a full complement of electrons in an unstable or reactive state, like singlet oxygen and hydrogen peroxide, are also called ROS [12,30]. It is believed that ROS are necessary for plants, because they play a role in signal transduction [11,26,32,34] and response to pathogen attack [12,44]. However, biotic or abiotic stress may promote ROS generation and break the redox balance of the cell resulting in adverse effects to macromolecules [5,19,33]. To control the concentration of ROS and protect themselves from oxidative damage, cells are armed with a large variety of antioxidant enzymes that include catalase, superoxide dismutase, ascorbate- and glutathione-dependent peroxidases, and the more recently described peroxiredoxin (Prx) family [4,15].

Superoxide dismutase (SOD) converts two superoxide anions into a molecule of hydrogen peroxide and one of oxygen. Then, hydrogen peroxide is reduced by peroxiredoxins (Prxs). Prxs are ubiquitous thioredoxin- or glutaredoxin-dependent peroxidases that reduce hydrogen peroxide and alkyl hydroperoxide to water and alcohol, respectively [7,21]. Members of the Prx family have been identified in a wide variety of organisms ranging from archae and eubacteria to eukaryotes, including vertebrates and plants. There are at least six Prx isoforms in mammals, five in yeast, and eight, at least, out of the 10 genes present in the genome of *Arabidopsis* are expressed [15,17,35]. All Prx proteins contain a conserved Cys in their N-terminal part and some of them possess a second conserved Cys residue. The Prx enzymes containing two conserved Cys residues are

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Table 1. The ten peroxiredoxin genes in the *Arabidopsis thaliana* genome. The length of protein indicates the amino acid number of preprotein. Isoelectric point was calculated without signal sequence. aa means amino acid

Group		MATBD entry	Location	Length (aa)/molecular mass (kDa)	Isoelectric point
1-Cys Prx		At1g48130	Nucleus	216/24.1	6.14
2-Cys Prx	A	At3g11630	Chloroplast	266/29.1	4.91
	B	At5g06290	Chloroplast	271/29.6	4.71
Prx Q		At3g26060	Chloroplast ?	216/23.7	5.53
Type II Prx	A	At1g65990	Pseudogene ?	553/62.7	6.04
	B	At1g65980	Cytosol	162/17.4	5.17
	C	At1g65970	Cytosol	162/17.4	5.33
	D	At1g60740	Pseudogene ?	174/19.2	6.10
	E	At3g52960	Chloroplast	234/24.7	5.03
	F	At3g06050	Mitochondria	199/21.2	6.29

thus called 2-Cys Prx, in comparison with a small number of Prx proteins termed 1-Cys Prx, which contain only one conserved cysteine residue in the N-terminal domain [8,38]. In 2-Cys Prx enzymes, the N-terminal conserved cysteine is oxidized into cysteine-sulfenic acid (Cys-SOH) in the process of H_2O_2 reduction, which then reacts with C-terminal Cys-SH of the other subunit to produce an intermolecular disulfide [7,9,36]. This intermediate is known to be reduced and recycled by thioredoxin. However, some of sulphenic acids are selectively further oxidized into sulphinic acid (Cys-SO_2H), which was thought to be irreversible.

Prxs and the mechanism of peroxide detoxification in *Arabidopsis thaliana*

Peroxiredoxins (Prxs) are ubiquitous proteins found in organisms ranging from bacteria to humans which catalyze the reduction of hydrogen peroxide. In mammalian cells, they typically constitute 0.1 to 0.8% of the total soluble protein of the cell. In addition to the report that Prxs are involved in the regulation of the amount of peroxide and peroxide-mediated signaling cascades [2,18], a large number of Prxs are associated with diverse cellular functions, such as cell proliferation, differentiation, immune response, growth control, tumor promotion, apoptotic process, and numerous unidentified functions [20,33].

In the genome of *Arabidopsis thaliana* there are ten open reading frames with sequence similarity to peroxiredoxins and they can be classified into four distinct subgroups (Table 1). They are: 1-Cys Prx, 2-Cys Prx, Prx Q and type II Prx. All of the identified yeast Prxs are perfectly fit into these four groups based on phylogenetic tree analysis (Fig. 1). A phylogenetic distance analysis shows that 2-Cys Prx, Prx Q, and 1-Cys Prx are closely related proteins, whereas the group of type II Prx is likely to have evolved independently [22]. In contrast to other peroxidases, Prx enzymes do not have redox cofactors such as metal or prosthetic groups and four Prx subgroups have different reaction mechanisms (Fig. 2).

1-Cys Prx

In *Arabidopsis* genome, only one 1-Cys Prx gene located

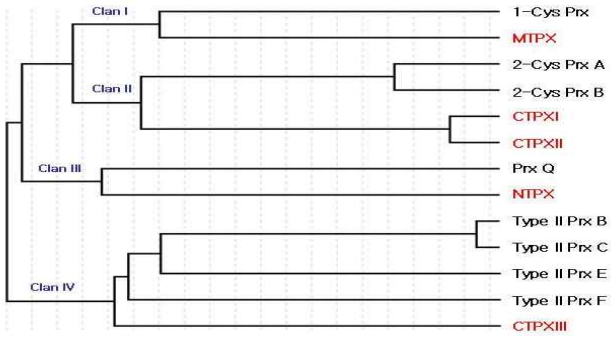

Fig. 1. Phylogenetic tree analysis of *A. thaliana* and *S. cerevisae* Prx proteins. Clans in blue indicate four groups of Prxs. Prxs of *S. cerevisae* are shown in red. Abbreviations and gene accession number; MTPX: mitochondrial TPX, P34227; CTPXI: cytoplasmic TPXI, P34760; CTPXII: cytoplasmic TPXII, Q04120; CTPXIII: cytoplasmic TPXIII, P38013; NTPX: nuclear TPX, CAA21907; 1-Cys Prx: At1g48130; 2-Cys Prx A: At3g11630; 2-Cys Prx B: At5g06290; Prx Q: At3g26060; Type II Prx B: At1g65980; Type II Prx C: At1g65970; Type II Prx E: At3g52960; Type II Prx F: At3g06050.

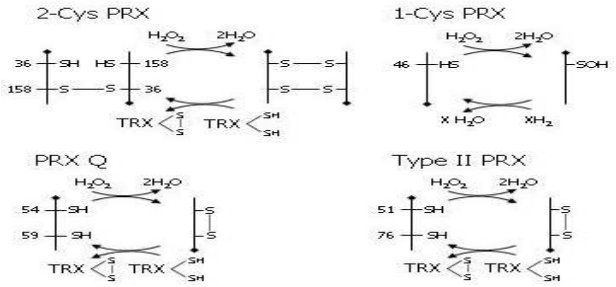

Fig. 2. Proposed mechanism of H_2O_2 reduction and Prx regeneration for the four groups of peroxiredoxins found in the *Arabidopsis thaliana*. The proteins are represented by pins with the knob being the N-terminus. Positions of the catalytic cysteins are given in numbers [23].

on chromosome 1 has been identified [41,42]. Predicted molecular weight and isoelectric point of this protein are 24.1 kDa and 6.14, respectively. 1-Cys Prxs are seed-restricted enzymes that are mainly localized in the nucleus of the embryo and the aleurone layer and known to function to protect the embryo and the aleurone cells from oxidative stress during development and desiccation of the seed [42]. It has been reported that 1-Cys Peroxiredoxin is a bifunctional enzyme with glutathione peroxidase and phospholipase A_2 activities [10]. Comparing amino acid sequences with those of yeast mTPX (mitochondria thiol peroxiredoxin), *Arabidopsis* 1-Cys Prx shows high sequence similarity, especially around the motif of 1-Cys Prx including functional Cys residue located at 46. Mitochondria are the place where high level of ROS is generated in yeast system and mTPX is involved in the process of ROS scavenging. Though both proteins are involved in hydrogen peroxide scavenging with high amino acid sequence homology, they are found in different places. *Arabidopsis* 1-Cys Prx works in the nucleus of embryo and the aleurone layer of seeds. In contrast, mTPX is targeting into mitochondria.

1-Cys Prx mediated peroxide reduction mechanism is unclear. As mentioned, 1-Cys Prx contains only one catalytic Cys residue positioned at 46 and that is the site of oxidation during catalytic cycle. However, regeneration of the oxidized Prx remains to be studied. In contrast to 2-Cys Prx, in which N-terminal cysteine is oxidized to Cys-SOH and then immediately reacts with the second conserved C-terminal cysteine of the second subunit of the enzyme to form an intermolecular disulfide, the Cys-SOH of 1-Cys Prx does not form a disulfide. Neither thioredoxin, which reduces the disulfide of 2-Cys Prx, nor glutathione, which reduces the Cys-SeOH of oxidized glutathione peroxidase, are able to reduce the Cys-SOH intermediate of the oxidized protein [25].

2-Cys Prx

The two 2-Cys Prxs, 2-Cys Prx A and 2-Cys Prx B, are highly similar at the amino acid sequences, molecular weight (29.1 and 29.6 kDa, respectively), and exon-intron structure. Two Cys residues involved in peroxide detoxification are unchanged. Mature protein is encoded by six exons and contains signal sequences to chloroplast which is absent in non-plant species [1]. In plants, the chloroplast is particularly prone to oxidative damage by photosynthetic oxygen production and activation. Partial suppression of 2-Cys protein causes impairment of photosynthesis and increased oxidative damage of chloroplast proteins during early plant development [15]. The 2-Cys prx has a broad substrate specificity with activity toward hydrogen peroxides and complex alkyl hydroproxides and mediates peroxide detoxification in the plastids during the dark phase [28].

Two 2-Cys Prxs of *Arabidopsis* shows very high sequence identity (51.6%) in coding region with yeast cytosolic peroxiredoxin, Tsa1. It has been reported that sulphinic acid (Cys-SO_2H) of Tsa1 can be reduced into suphenic acid (Cys-SOH) and recycled [3] with the aid of Srx1. 2-Cys Prxs and Tsa1 have almost same molecular weight and maintain exactly same distance between two active site Cys residues. Moreover, the region spanning the first functional Cys residues is completely conserved (12 amino acids). These facts suggest 2-Cys Prxs are the strongest candidates for Srx1 protein substrate if Srx1 function of reducing sulphinic acid of peroxiredoxin into suphenic acid is conserved in plants.

The 2-Cys Prx functions as homodimer and two catalytic Cys residues are located at 36 and 158. These two Cys residues are crucial for peroxide reduction. In the first, N-terminal Cys-residue of one subunit is attacked by peroxide and oxidized. The resulting sulfenic acid intermediate (Cys-SOH) then interacts with a second conserved C-terminal Cys from the other subunit. This interaction forms an intermolecular disulfide bond in the homodimer [28]. In animal homologues of 2-Cys Prx, catalytic cycle accompanies large conformational changes including partial unwinding of the active site helix in the vicinity of the N-terminal Cys [20,39]. Also it has been reported that the active site is largely hydrophobic with the more C-terminally located Cys being exposed in the oxidized 2-Cys Prx form to allow for reduction by electron donors such as thioredoxin (Trx).

Prx Q

The Arabidopsis genome contains one Prx Q gene. The deduced amino acid sequence of this antioxidant enzyme consists of 216 residues (23.7 kDa) with a putative transit peptide of 57 residues which locates the protein into the chloroplast. Arabidopsis Prx Q contains two putative active site cysteines in position 54 and 59, which are essential for thioredoxin-dependent peroxidase activity [27]. Synthesis of Prx Q transcript is promoted by high light and oxidants, and its disappearance is enhanced by reductants such as ascorbate [24].

One of two conserved catalytic Cys residues, Cys 54, is located at the position corresponding to Cys 36 in 2-Cys Prx. But the second Cys of Prx Q is only 5 amino acids distant from the first Cys-residue. Prx Q acts as a monomer with an intramolecular disulfide bridge between two conserved cysteines [27].

Type II Prx

The *Arabidopsis* genome contains 6 putative peroxiredoxin genes on chromosomes 1 and 3 (Type II Prx A through Type II Prx F). This divergent group of Prx is most recently identified. They are different in molecular weight, isoelectric points and predicted subcellular localization. Three of them, type II Prx B, type II Prx C and type II Prx D, have no targeting sequence and probably remain in the cytosol. Type II Prx E is targeted into the chloplast and type II Prx F has a targeting address into mitochondria [22,24,29]. They also accumulate in different plant organs and show different mRNA accumulation patterns on various external stimuli [6]. Recently it has been reported that The mitochondrial type II Prx F is essential for redox homeostasis and root growth of *Arabidopsis thaliana* under stress [16]. However, this large and diverse group shows well conserved active site, PGAFTPTCSMSHVPG, neighboring the N-terminal Cys. In addition, two catalytic Cys residues are separated by 24 amino acids in all genes except type II Prx D [14]. Type II Prx A and D could be pseudogenes. Type II Prx A wasn't detected in RT-PCR experiment using several primer sets and Type II Prx D contains internal stop codon which makes this truncated protein product [6]. Type II Prxs do not show significant sequence similarity with any of yeast prxs.

Type II Prx does not form dimers upon oxidation. Like Prx Q, Type II Prx forms an intramolecular disulfide bond during the catalytic cycle. But different to Prx Q, in which the two essential catalytic Cys residues are spaced by only 5 amino acids, two catalytic Cys residues are separated by an intervening sequence of 24 amino acids. They are positioned at 51 and 76 [40]. A major conformational change is necessary to form the disulfide bridge between the catalytic Cys residues upon oxidation [13,43].

Cysteine and sulphiredoxin1 (Srx1)

The cysteines are sulphur-containing amino acids bearing a thiol group (-SH), which makes them highly vulnerable to oxidation. This property of the cysteines is biologically useful: for example their oxidation to form disulphide bridges (-SS-) is essential for maintaining the structure of secreted proteins; cysteines form the catalytic center of several important enzymes [21]. Reversible redox reactions of cysteines thus occur perpetually during normal cell metabolism. However, their ready oxidation puts them at risk of becoming overoxidized [20,39]. The first product of the oxidation of a thiol (-SH) is a sulphenic acid (-SOH), which is generally unstable and forms a disulphide bridge by condensation with a neighboring thiol group, when one is close enough. Some of them are converted into sulphinic acid (-SO_2H) by a second oxidation step. The –SOH and –SS- forms can be converted back to thiol by biological reducing agents thioredoxin or glutathion, but - SO_2H formation had been until recently considered irreversible and protein-damaging [7,31,37].

Members of Prx contain conserved H_2O_2 sensitive Cys residues. In mammalian system, the sulfur atoms of Cys^{51} and Cys^{172} are relatively far apart (≈13 Å) and the formation of an intermolecular disulfide between these residues is a slow process [20,39]. Thus, the Cys^{51}–SOH intermediate is occasionally oxidized to Cys–SO_2H before it forms a disulfide. Cys–SO_2H in Cys residue is not susceptible to reduction by thioredoxin and thus Prx enzymes with a Cys–SO_2H group are catalytically inactive and irreversible into active form of Prx.

Within last couple of years, different research groups have independently shown that sulfinic acids of Cys can be reduced to thiols *in vivo*. An enzyme with sulfinic acid reductase activity, called sulfiredoxin (Srx1), has been isolated from yeast [3] and mammalian [45]. Srx1 was initially identified in yeast, *Saccharomyces cerevisiae* by its high H_2O_2 induced expression and the deletion of its gene compromises yeast tolerance to H_2O_2 and t-BOOH [1], which strongly suggests its involvement in the metabolism of this oxidant. Srx1

interacts with yeast Prx, Tsa1, and reduces cysteine-sulphinic acid in this protein.

NCBI (National Center for Biotechnology Information) BLAST searches revealed that Srx1 is pretty well conserved protein family in lower and higher eukaryotes. All of these proteins share the active-site cysteine (Cys 84) and a high degree of identity in the region spanning this residue (Fig. 3). All these information suggests that Srx1 function is conserved in plant and involved in the processes of ROS scavenging via the action of plant prxs. In contrast to yeast and mammalian Srx1 proteins which likely remains in the cytosol, probable destination of putative plant Srx1, however, is chloroplast where 2-Cys prx A and B are localized.

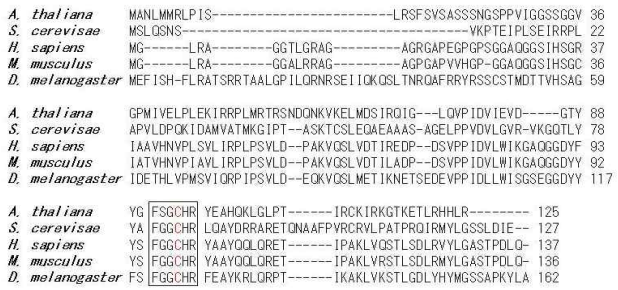

Fig. 3. Sequence alignment (http://www.ebi.ac.uk/clustalw/) of Srx1 with four representative Srx1 family members. Red C and square indicate Cys residue at 84 and conserved span, respectively. Gene bank accession numbers; *A. thaliana*: AAO42977; *S. cerevisae*: YKL086W; *H. sapiens*: AAH47707; *M. musculus*:AAH11325; *D. melanogaster*:AAF48773.

Concluding remarks and future perspectives

Identification of Srx1 and reversibility of sulfinic acid formation opened the door to a range of yet unexplored redox cycles, cell signaling processes and reduction mechanisms. As described, results supporting the involvement of Srx1 in the process of Prxs recycling resulting in increased tolerance against H_2O_2 and heat shock are accumulating in yeast studies. Putative plant Srx1 shares active-site cysteine (Cys 84) and shows high degree of identity in the region spanning this residue with those of other organisms. Taken together, it is possible to expect that the functions of Srx1 are conserved in plants and make them more resistant against unbalanced ROS shock. Though, all Prxs expressed in plant are possible partners to interact and work with Srx1, 2-Cys Prxs are the strongest candidates for Srx1 protein substrate if Srx1 function of reducing Cys-sulphinic acid of peroxiredoxin into Cys-suphenic acid is conserved in plants. 2-Cys Prx A and B show similar molecular weight and maintain distance between two catalytic Cys residues compared with yeast Prx Tsa1 which interacts with yeast Srx1. Also both of them form intermolecular disulfide bridge. Further more, plant Srx1 and 2-Cys Prxs are likely targeted into plant specific subcellular location chloroplast. Recently reported results showing that Srx1 mediated Cys-sulphinic reduction is 2-Cys specific process in yeast further reinforce this hypothesis [46]. Especially this notion supports plant specific functions of 2-Cys Prx that mediate detoxification of peroxides in the redox-hirachy of photosynthetic electron flux reinforced by Srx1 mediated activity.

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초록 : 애기장대 peroxiredoxins (Prxs)과 sulphiredoxin1 (Srx1)의 작용기작

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식물체는 대사과정의 부산물로서 또는 생물학적으로 피해를 줄 수 있는 다양한 종류의 외부 스트레스에 직면했을 활성산소(Reactive Oxygen Species, ROS)를 생산한다. 이러한 oxidative 스트레스로부터 자신들을 보호하기 위하여 식물세포들은 다양한 종류의 항산화 단백질들을 보유하고 있다. 하지만 이들의 작용기작은 여전히 자세히 밝혀지지 않았다. Peroxiredoxins (Prxs) 은 식물체에 광범위하게 존재하는 thiol-을 함유한 항산화 단백질로 N-말단에 존재하는 cysteine 잔기를 이용하여 hydrogen peroxide를 환원한다. 이러한 과정에서 peroxiredoxins의 활성부위인 cysteine 잔기는 선택적으로 cysteine sulfinic acid로 산화됨으로써 peroxidase activity의 불활성화를 일으킨다. 이러한 산화과정은 비가역적으로 일어난다. 최근 발견된 진핵생물들에 잘 보존된 sulphiredoxin (Srx1)이라 불리는 단백질은 cysteine-sulphinic acid를 환원시키는 기능을 지닌다. 본 논문에서는 애기장대에 존재하는 Prxs와 Srx의 기능에 대하여 서술할 예정이다.