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Molecular and Functional Properties of Three Different Peroxiredoxin Isotypes in Chinese Cabbage

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Peroxiredoxins (Prxs), which are classified into three isotypes in plants, play important roles in protection systems as peroxidases or molecular chaperones. The three Prx isotypes of Chinese cabbage, namely C1C-Prx, C2C-Prx, and C-PrxII, have recently been identified and characterized. The present study compares their molecular properties and biochemical functions to gain insights into their concerted roles in plants. The three *Prx* isotype genes were differentially expressed in tissue- and developmental stage-specific manners. The transcript level of the *C1C-Prx* gene was abundant at the seed stage, but rapidly decreased after imbibitions. In contrast, the *C2C-Prx* transcript was not detected in the seeds, but its expression level increased at germination and was maintained thereafter. The *C-PrxII* transcript level was mild at the seed stage, rapidly increased for 10 days after imbibitions, and gradually disappeared thereafter. In the localization analysis using GFP-fusion proteins, the three isotypes showed different cellular distributions. C1C-Prx was localized in the cytosol and nucleus, whereas C2C-Prx and C-Prx were found mainly in the chloroplast and cytosol, respectively. *In vitro* thiol-dependent antioxidant assays revealed that the relative peroxidase activities of the isotypes were C-PrxII > C2C-Prx > C1C-Prx. C1C-Prx and C2C-Prx, but not C-PrxII, prevented aggregation of malate dehydrogenase as a molecular chaperone. Taken together, these results suggest that the three isotypes of Prx play specific roles in the cells in timely and spatially different manners, but they also cooperate with each other to protect the plant.

INTRODUCTION

In cells, the incomplete reduction of oxygen during respiration or exposure to pathological and environmental stresses generates reactive oxygen species (ROS). All aerobic organisms are equipped with an array of self-protection mechanisms against these toxic molecules (Alvarez et al., 1998; Halliwell and Gutteridge, 1990). Among them, peroxiredoxins (Prxs) are a newly discovered type of peroxidase that can eliminate H_2O_2 and alkyl hydroperoxide using thiol-containing reducing groups (An et al., 2010; Chae et al., 1994). The Prxs exist as large families in diverse organisms ranging from prokaryotes to eukaryotes.

Based on their structures and working mechanisms, Prxs are grouped into three classes: typical 2-Cys Prxs, atypical 2-Cys Prxs, and 1-Cys Prxs (Wood et al., 2003). The 2-Cys Prxs contain two conserved cysteines, called the peroxidatic and resolving Cys residues, respectively (An et al., 2011; Hofmann et al., 2003). The peroxidatic Cys residue of typical 2-Cys Prxs attacks peroxide substrates and is oxidized to its sulfenic acid form (Cys-SOH). This form of the peroxidatic Cys residue is attacked by the resolving Cys residue of the other 2-Cys Prxs, which results in the formation of an interdisulfide bridge. Subsequently, the disulfide is reduced by specific disulfide oxidoreductases (Wood et al., 2003). The atypical 2-Cys Prxs have the same mechanism as typical 2-Cys Prxs, but they are functionally monomeric and result in the formation of intramolecular disulfide bonds (Seo et al., 2000; Wood et al., 2003). In the 1-Cys Prxs, only the peroxidatic Cys residue is conserved, and 1-Cys Prxs do not possess the resolving Cys. The sulfenic acid form of the peroxidatic Cys residue generated during catalysis in the reaction with substrates is regenerated by thiol-containing electron donors (Fisher et al., 1999; Peshenko and Shichi, 2001).

Multiple isotypes of Prx proteins have been isolated from single organisms, including three from *E. coli*, five from *S. cerevisiae*, six from human, and 14 *Prx*-related genes from mouse (Lyu et al., 2003; Park et al., 2000). In plants, most of the known Prx isotypes have been isolated from different sources individually. According to previous studies on Prx proteins in plants, 1-Cys Prx is not involved in seed dormancy, but inhibits germination and confers tolerance to plants under stress conditions (Haslekas et al., 2003; Lee et al., 2000). Most typical 2-Cys Prxs in plants contain specific N-terminal signal peptides

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that target the protein to chloroplasts, where the Prxs play critical roles in scavenging the ROS generated during photosynthesis or photorespiration (Baier and Dietz, 1997; 1999). Partial suppression of the *2-Cys Prx* gene expression results in significant damage to photosynthetic and metabolic processes during development in Arabidopsis plants (Baier and Dietz, 1999). Six atypical 2-Cys Prx genes exist in Arabidopsis. The responsible proteins, PrxII A~F, localize to diverse subcellular compartments (e.g., cytoplasm, mitochondrion, and chloroplast) where they play critical roles in various plant organs (Brehelin et al., 2003).

Despite previous reports about the gene expression of 2-Cys Prxs in diverse organs and their subcellular localization to various cell compartments, the roles and intracellular functions of the plant Prx isotypes are still unclear. To gain insight into the physiological roles of the three individual Prx isotypes in plants, it is important to clone the responsible genes from a single organism and to compare the molecular or biochemical properties under standard experimental conditions. In this study, we compared the molecular and biochemical behaviors of three different Prx isotypes from Chinese cabbage, namely C1C-Prx, C2C-Prx, and C-PrxII.

MATERIALS AND METHODS

Materials

For Northern and Western blot analyses, Chinese cabbage (*Brassica campestris* L. ssp. pekinensis) was grown in a growth chamber with a 16/8 h light/dark cycle at 20°C, with a relative humidity of 70%.

Cloning of the Prx genes from Chinese cabbage

A cDNA expression library (Stratagene, USA) was constructed with the λZAPII vector and mRNA from Chinese cabbage seeds (Kang et al., 2006). The *C1C-Prx* gene was cloned by screening the cDNA library with the rice 1Cys-Prx gene, *R1C-Prx*, as described previously (Kim et al., 2011). The *C2C-Prx* gene was cloned by a PCR amplification using a degenerate primer set corresponding to the conserved sequence motif that appears in most 2Cys-Prx proteins, as described previously (Cheong et al., 1999). The *C-PrxII* gene was obtained in a random sequencing project on flower bud-specific cDNA clones of Chinese cabbage, as described previously (Choi et al., 1999).

Subcellular localization of GFP-fused Prx proteins

Translational fusions of the full-length Prx isotypes to soluble modified green fluorescent protein (smGFP) were generated by cloning the cDNA of the *Prx* genes into the *Xba*I and *Bam*HI sites of psmGFP vectors (Arabidopsis Biological Resource Center, USA). The fusion constructs (*C1C-Prx::smGFP*, *C2C-Prx::smGFP*, *C-PrxII::smGFP*) were introduced into Arabidopsis protoplasts by polyethylene glycol-mediated transformation (Jin et al., 2001). Fluorescence was observed under a fluorescence microscope (Olympus AX70, Japan).

Northern blot analysis

For the Northern blot analysis, plants were grown under normal conditions and various tissues or whole plants in the different developmental stages were obtained. To analyze the responsiveness to stress, seeds for *C1C-Prx* or 10-day-old whole plants for *C2C-Prx* and *C-PrxII* were treated with heat shock at 45°C, 10 mM hydrogen peroxide, and 10 μM methyl viologen for 1 h or not. Total RNA (20 μg) was extracted with the RNeasy Plant Mini Kit (Qiagen, USA). The excised cDNA inserts of full-length *C1C-Prx*, *C2C-Prx*, and *C-PrxII* were labeled with [^{32}P] dATP by the random-priming method, as described previously (Cheong et al., 1999).

Expression of Prx proteins in *E. coli*

The coding regions of the three Prx genes (*C1C-Prx, C2C-Prx*, and *C-PrxII*) were subcloned into the pGEX-2T expression vector by the polymerase chain reaction (PCR) for protein production. The Prx genes were then expressed in *E. coli* BL21 (DE3) and purified as described previously (Cheong et al., 1999). The N-terminal GST portion of the protein was removed with thrombin, and the biochemical properties of the native forms of the Prx proteins were analyzed.

Antioxidant and chaperone activities

The thiol-dependent antioxidant activity of each Prx protein was determined by monitoring the ability of the protein to inhibit the inactivation of glutamine synthetase (GS) in the presence of DTT/Fe3+/O_2 (MCO system). Monitoring was performed with xylenol orange (FOX), as described previously (Chae et al., 1994; Moon et al., 2006). The chaperone activity of each Prx protein was measured by thermally induced aggregation methods with malate dehydrogenase (MDH) as a substrate (Kim et al., 2009). Thermally induced aggregation of MDH was assayed in a DU800 spectrophotometer (Beckman, USA). The turbidity due to substrate aggregation was monitored with a thermostatic cell holder preheated to 45°C.

RESULTS

Grouping the three Chinese cabbage Prx proteins

A comparison of the deduced amino acid sequences of the three Chinese cabbage Prx genes revealed that they had sequence identities of less than 10-30% with each another (Fig. 1A). Despite their overall low homologies, the sequences surrounding their NH_2-terminal Cys residues (Cys^{46} of C1C-Prx, Cys^{124} of C2C-Prx, and Cys^{51} of C-PrxII) were highly homologous to those surrounding the corresponding Cys residues of previously identified Prx enzymes (Seo et al., 2000). The relatedness of the three Chinese cabbage Prx proteins was analyzed by the ClustalW2 program, and the results were placed into a phylogenetic tree constructed from the typical six subfamilies of mammalian Prx proteins (Knoops et al., 1999). Mammalian Prxs can be divided into six subfamilies, Prx I-VI, based on the length of their amino acid sequences, immunological properties, localization in cells, and distinct tissue-specific roles (Seo et al., 2000). In early studies of Prxs, no clear information about their function was available, although they were considered as antioxidant molecules. Later studies reported their diverse cellular functions as antioxidants, endogenous regulators of apoptosis, and intracellular signaling molecules (Ichimiya et al., 1997; Jin et al., 1997; Ross et al., 2000). The three Prx proteins of Chinese cabbage, C1C-Prx, C2C-Prx, and C-PrxII, appeared to be related closely to the mammalian Prx isotypes of groups VI, III, and V, respectively (Fig. 1B) (Seo et al., 2000). Furthermore, when the primary sequences of the three Chinese cabbage Prxs were compared with each another, they showed several distinct specificities. C1C-Prx contained a nuclear localization signal at its C-terminus. C2C-Prx had an N-terminal extension composed of about 65 amino acids, which corresponded to a chloroplast targeting signal sequence. C-PrxII contained a type-1 KAL peroxisomal targeting signal at its C-terminus.

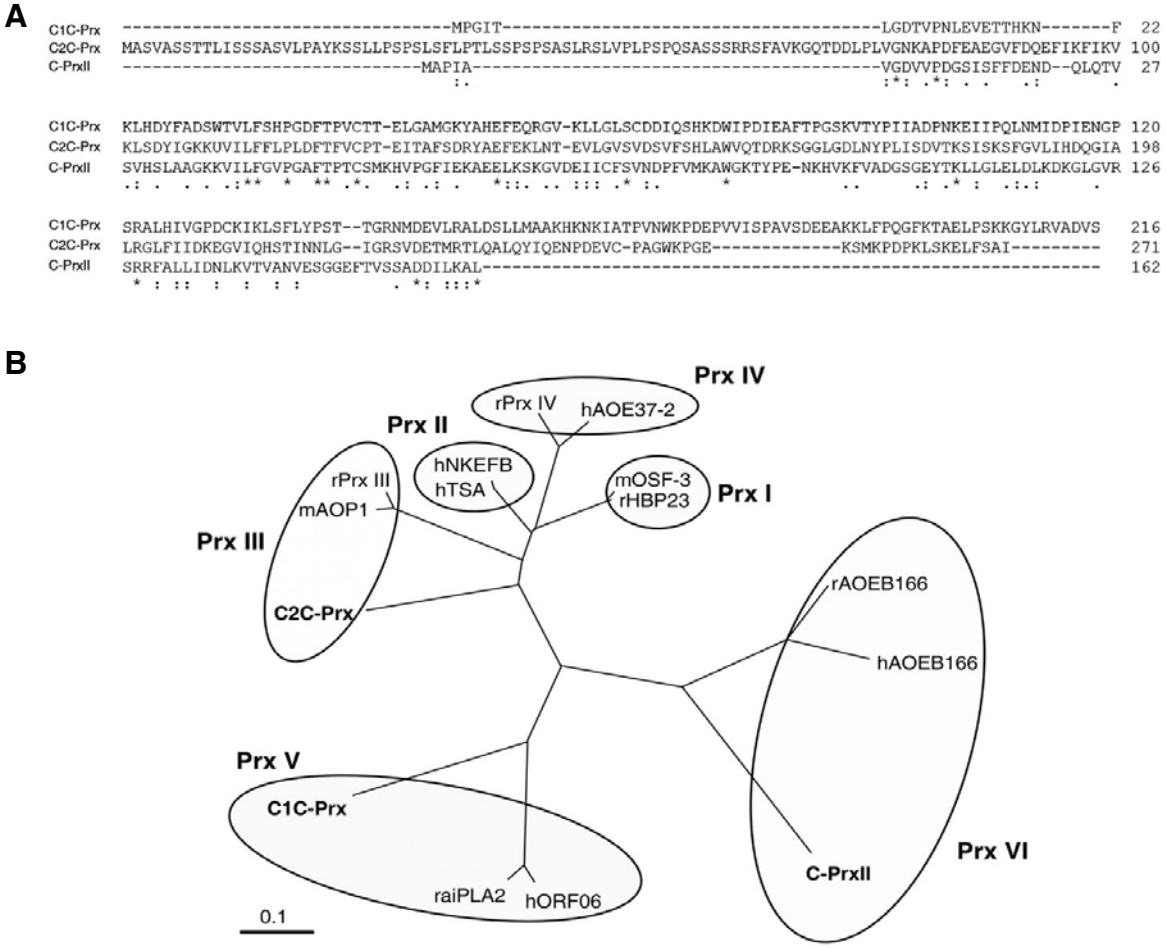

Fig. 1. An amino acid sequence alignment of the three Chinese cabbage *Prx* isotypes (A), and a phylogenetic tree revealing the relationship of the proteins to the six mammalian Prx subfamilies (B). The GenBank accession numbers of *C1C-Prx*, *C2C-Prx*, and *C-PrxII* are AF320690, AF052202, and AF133302, respectively. In (A), dashes within the sequences represent gaps that were introduced to optimize the alignment. The residue numbers are shown on the right side, and the identical amino acids are indicated by stars (*). The colons (:) and dots (.) display columns where there is some conservation of the biochemical character of the side chains. In (B), the deduced amino acid sequences of the well-characterized mammalian Prx proteins were aligned with the ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The tree was calculated by the neighbor-joining distance method and is represented in an unrooted form. Line lengths indicate the relative distances between nodes. Prx members belonging to the same six mammalian subfamilies are grouped together, and their subtypes are indicated around the circles. The three Chinese cabbage Prx isotypes are shown in bold characters.

Expression analysis of the three Chinese cabbage Prx genes

To gain insight into the physiological roles of the three *Prx* genes in Chinese cabbage, we examined their expression by Northern blot analysis. As shown in Fig. 2A, the *C1C-Prx* gene was specifically expressed in seeds, whereas *C2C-Prx* and *C-PrxII* were expressed in various tissues. The transcript level of *C2C-Prx* was relatively high in young leaves, floral leaves, floral stems, flowers, and siliques, while *C-PrxII* was mainly expressed in young roots and flowers. The different spatial expression of the *Prx* isotypes suggest that each *Prx* isotype may have its own specific physiological function in specific tissues of the plant.

We also analyzed the temporal expression of the three *Prx* genes during cabbage plant development (Fig. 2B). The expression level of the *C1C-Prx* gene was high in the seed stage, but its level decreased to trace amounts within 10 days of seed imbibition and was not detected at all after 20 days. Conversely, the *C2C-Prx* transcript was undetectable in seeds, but its expression significantly increased at the initiation of seed germination. The level was maintained up to the green silique stage. The *C-PrxII* transcript was expressed at about half of its maximal level in seeds, but its level rapidly increased until the 10th day after imbibition and then decreased slowly afterwards. The temporal changes of the three Prx protein expressions suggest that each Prx has a specific role in Chinese cabbage development.

To analyze the responsiveness to stress, heat shock, hydrogen peroxide, and methyl viologen were treated to seeds for *C1C-Prx* or 10-day-old whole plants for *C2C-Prx* and *C-PrxII*. The transcript level of *C-PrxII* decreased to half of the initial level after heat shock treatment for 1 h, but *C1C-Prx* and *C2C-Prx* didn't show changes in levels (Fig. 2C). In our conditions the expression levels of all three *Prx* isotypes were not changed by the treatments of the oxidative stress-generating chemicals such as hydrogen peroxide, and methyl viologen.

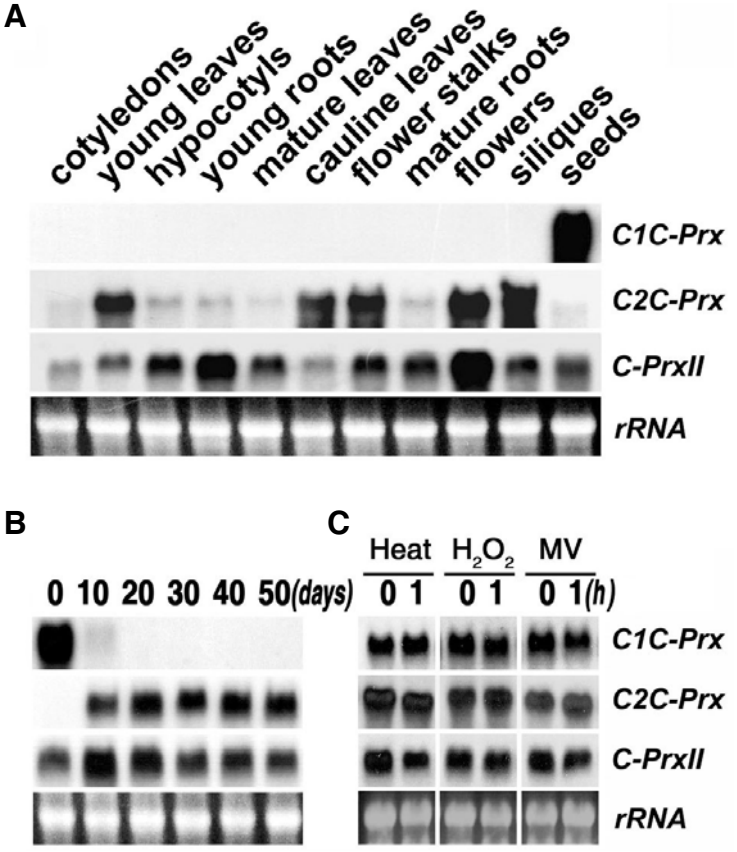

Fig. 2. Expression of the three Chinese cabbage Prx isotypes in the plants. (A) Total RNA was prepared from various tissues of 14-day old Chinese cabbage seedlings, from two-month-old mature plants, or from the seeds. The tissues examined were: cotyledons, young leaves, hypocotyls, young roots, mature leaves, cauline leaves, flower stalk, mature roots, flowers, siliques, and seeds. (B) Total RNA was prepared from Chinese cabbage plants at different developmental stages. The days after seed imbibition are indicated on the panel. (C) Total RNA was prepared from seeds for *C1C-Prx* or 10-day-old whole plants for *C2C-Prx* and *C-PrxII* after treatment of heat shock at 45°C (Heat), 10 mM hydrogen peroxide (H_2O_2), and 10 μM methyl viologen (MV) for 1 h or not. A 20-μg sample of each isolated RNA was subjected to electrophoresis with 2% formaldehyde /agarose gel and transferred to a nylon membrane. The [^{32}P]-labeled cDNA fragments of *C1C-Prx*, *C2C-Prx*, and *C-PrxII* genes were used as probes for the hybridizations of the blots. The rRNA band in the ethidium bromide-stained gel confirms equal RNA loading per lane.

Subcellular localizations of the three Chinese cabbage Prx proteins

To analyze the subcellular localizations of Prx proteins, C-terminal green fluorescent protein (GFP) fusion constructs of the individual proteins were transformed into Arabidopsis protoplasts, and GFP fluorescence was observed *in vivo via* fluorescence microscopy. As shown in Fig. 3, cells expressing the C1C-Prx::GFP fusion protein had fluorescence distributed throughout the cytoplasm and the nucleoplasm. In contrast, the GFP fluorescence of C2C-Prx::GFP matched the red autofluorescence of chloroplasts, indicating its localization to chloroplasts. Although the amino acid sequence of C-PrxII ends with KAL, C-PrxII::GFP did not localize to the peroxisome under the experimental conditions used in this study. Instead, the GFP fluorescence of the fusion protein was mostly dispersed throughout the cytoplasm. These results suggest that the three individual Prx proteins have different roles in different cellular compartments that work in harmony to protect the cells against peroxide-induced oxidative damage.

Comparison of the peroxidase and chaperone activities of Prx isotypes

The peroxidase and antioxidant activities of the three Prx isotypes were compared. Because C1C-Prx was unable to degrade H_2O_2 through the Trx-mediated reduction cascade system, we assayed its ability to protect GS from inactivation by the thiol-containing MCO system. As previously reported, Fe^{3+} catalyzes the reduction of O_2 to H_2O_2 in the presence of an electron donor such as DTT or ascorbate, which is then further converted by the Fenton reaction to hydroxyl radicals. These ROS inflict damage on various bio-macromolecules, including the GS. Prx can prevent such damage by removing H_2O_2 and preventing the Fenton reaction (Wood et al., 2003).

In addition to displaying H_2O_2 reducing peroxidase activity, all three plant Prx isotypes protected GS against inactivation by the thiol-MCO system. However, the amount of protein required for complete GS protection by C1C-Prx was almost 5-fold and 15-fold higher than that required of C2C-Prx and C-PrxII, respectively (Fig. 4A). In contrast, all three Prx isotypes failed to protect GS against inactivation when DTT was replaced with a non-thiol reducing equivalent such as ascorbate (data not shown), indicating that the antioxidant activities of the three Prx isotypes are tightly linked to a thiol-specific reducing group. To define the peroxidase activities of Prx isotypes more clearly, we performed the FOX assay, which measures the colored complex formed between the ferric ion (Fe^{2+}), resulting from H_2O_2 oxidation, and xylenol orange (Fig. 4B). As observed for GS protection, we found that the order of potency of the three Prxs in terms of ROS scavenging activity was C-PrxII > C2C-Prx > C1C-Prx.

C1C-Prx and C2C-Prx can serve as both Trx-dependent peroxidases and molecular chaperones (Kim et al., 2009; 2011). To test whether C-PrxII also exhibits chaperone activity, we performed an unfolding assay using MDH as the model substrate. As shown in Fig. 5A, the addition of C1C-Prx and C2C-Prx prevented thermal aggregation of MDH at 45°C at a molar ratio of Prx to substrate of 3:1. However, addition of C-PrxII and bovine serum albumin (negative control) did not influence the aggregation behavior, even when these proteins were added in high excess over MDH (data not shown). These results suggest

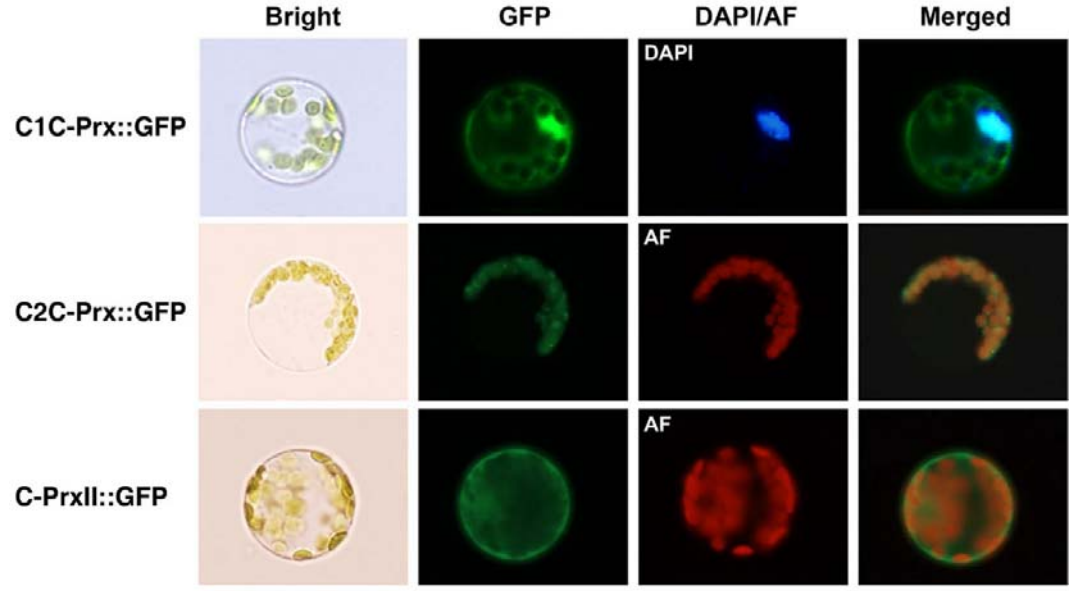

Fig. 3. Subcellular localizations of the three Chinese cabbage Prx isotypes. Arabidopsis protoplasts were transfected with the *GFP* fusion construct of the *C1C-Prx* (top panel), *C2C-Prx* (middle panel), or *C-PrxII* (bottom panel) gene. GFP fluorescence (GFP) *via* fluorescent microscopy and normal images (Bright) are shown. Nuclei were identified by staining with DAPI (DAPI), and chloroplasts were identified by observing the autofluorescence of chlorophylls (AF).

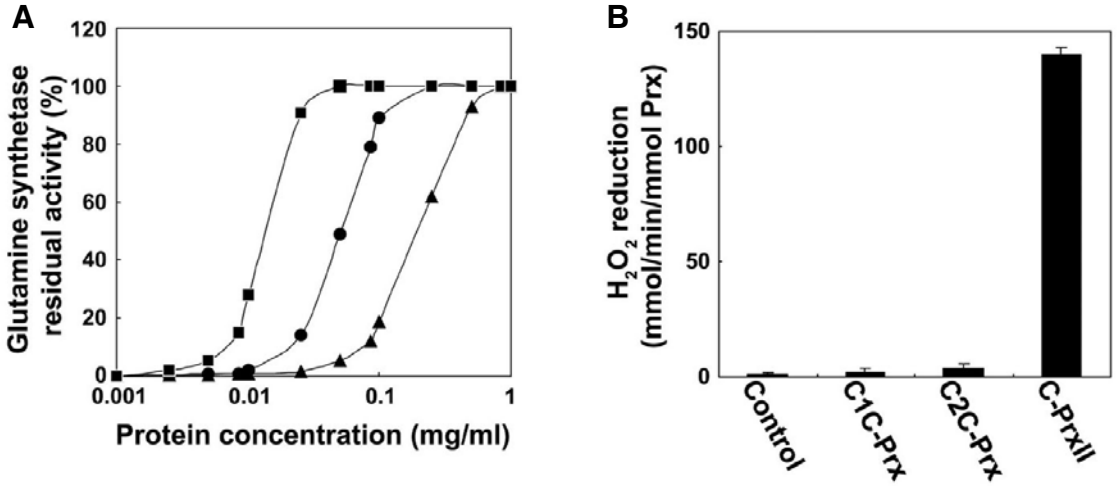

Fig. 4. A comparison of the *in vitro* antioxidant activities of Prx isotypes. (A) Thiol-specific protection of glutamine synthetase (GS) from inactivation in the metal-catalyzed oxidation (MCO) system by various concentrations of C1C-Prx (▲), C2C-Prx (●), and C-PrxII (■)[3]. (B) H_2O_2 reducing activities of C1C-Prx, C2C-Prx, and C-PrxII were measured at 560 nm in a colorimetric reaction with PeroXOquant reagent by using DTT as the electron donor.

that C2C-Prx and C1C-Prx1 play dual functions, as peroxidases and as molecular chaperones.

The functions of C1C-Prx and C2C-Prx were related to their structures and their chaperone activity was increased by oligomeriztion (Kim et al., 2009; 2011). Since only C-PrxII didn't show chaperone activity, the oligomeric states of the three Prx isotypes were compared by native PAGE (Fig. 4B). In the native condition C-PrxII showed at least three bands at around 67 kDa, 200 kDa, and 400 kDa which are estimated as tetramer, dodecamer, and tetracosamer considering its molecular weight of 17 kDa. This means that C-PrxII exists as oligomers in the native condition as C1C-Prx and C2C-Prx do but its oligomeric states don't confer chaperone activity to the protein.

DISCUSSION

In plants, various environmental conditions and metabolic processes can produce large amounts of ROS that may be harmful to plant cells (Alvarez et al., 1998). To overcome the toxicity of ROS and to survive in unfavorable conditions, plant cells are equipped with antioxidant enzymes, including catalase, superoxide dismutase, and peroxiredoxin (Cakmak et al., 1993; Pulido et al., 2009a; Scandalios, 1987). Three genes encoding C1C-Prx, C2C-Prx, and C-PrxII were previously isolated from Chi-

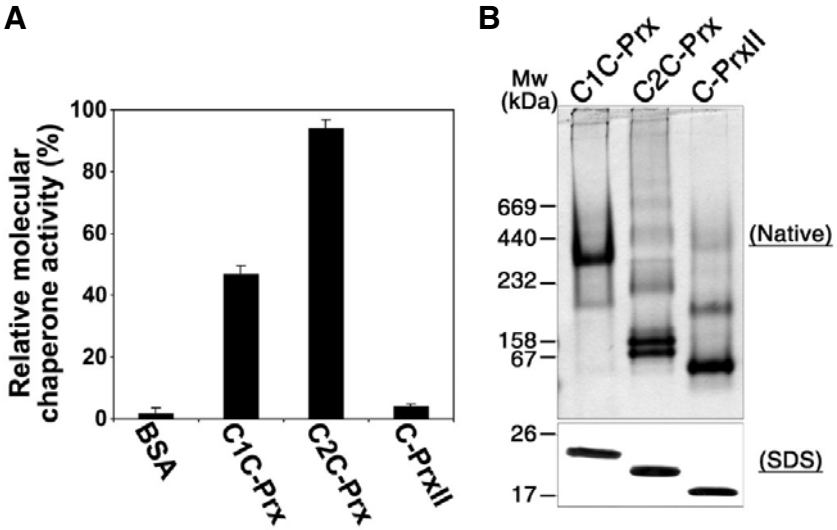

Fig. 5. Analysis of C1C-Prx C2C-Prx, and C-PrxII on the oligomeric properties and on the functions as molecular chaperones. (A) The thermal aggregation of MDH at 45°C was measured in the absence or presence of C1C-Prx, C2C-Prx, C-PrxII, or BSA at a molar ratio of protein to substrate of 3:1. The assay mixture before heating was set to 100% protection from aggregation. (B) The purified C1C-Prx, C2C-Prx, and C-PrxII were separated by 8.5% native PAGE (upper panel) or 13.5% SDS-PAGE (lower panel) gels and subjected to Coomassie staining.

nese cabbage and thereafter were shown to correspond to 1-Cys, 2-Cys, and type II-Prx, respectively. Here, we demonstrate that C1C-Prx, C2C-Prx, and C-PrxII are closely related to the mammalian Prx isotypes of groups VI, II, and V, respectively. The convergence of the plant Prx proteins with those of the mammalian Prx subfamilies suggests that Prx proteins are highly conserved between the two kingdoms, Plantae and Animalia, and that they are indispensable components of aerobic cells.

We analyzed the expression patterns of the three Prx isotype genes in various tissues and at different developmental stages by Northern blot analysis to determine their physiological roles in plants. Interestingly, the *C2C-Prx* gene was highly expressed in young leaves, flower tissues, and siliques; the *C-PrxII* gene was expressed in all organs and, predominantly, in flowers and roots; and the *C1C-Prx* gene was specifically expressed in seeds. The high level of *C1C-Prx* gene expression in seeds rapidly decreased after germination. The *C2C-Prx* transcript was not detected in seeds, but its level gradually increased for 30 days after germination and thereafter remained constant. *C-PrxII* gene was weakly expressed in seeds, but its transcript level rapidly increased to reach a maximum level at 20 days after germination. Afterwards, the level steadily decreased to the low level as much as in seeds. Considering the different special and temporal gene expressions of the three Chinese cabbage *Prx* genes, it is obvious that the individual genes play distinct physiological roles, as previously suggested for mammalian Prx genes on the basis of their diverse physiological roles and cellular functions (Sarafian et al., 1999).

Our analysis of the localizations of the Chinese cabbage Prx isotypes revealed that each isotype has a different distribution pattern in cells. The C1C-Prx::smGFP protein was localized to the cytosol and nucleus, and the C2C-Prx::smGFP protein was localized to the chloroplast. Previous reports have suggested that plant 1-Cys Prxs partially protect nuclear DNA in seed cells under stressed conditions (Pulido et al., 2009b). Comparably, the 2-Cys Prxs have been shown to play important roles in the protection of chloroplasts from damage caused by oxidative stress during photosynthesis (Baier and Dietz, 1997; 1999). C-PrxII contained a type-1 KAL peroxisomal targeting signal at its C-terminus, but the C-PrxII::smGFP protein was unexpectedly expressed in the cytosol (Fig. 3). This result is consistent with the subcellular localizations of AtPrxII-B, AtPrxII-C, and AtPrxII-D in Arabidopsis protoplasts, as revealed by immunoblot analysis (Brehenlin et al., 2003). Interestingly, the amino acid sequence of C-PrxII showed >92% identity to AtPrxII-B~D, whereas it showed about 32-61% identity with mitochondrial type AtPrxII-F and plastidial type AtPrxII-E, respectively (data not shown).

To investigate the biochemical functions of C2C-Prx, C-PrxII, and C1C-Prx *in vitro*, the recombinant proteins were produced in the *E. coli* system. In contrast to C2C-Prx and C-PrxII, recombinant C1C-Prx was unable to use thioredoxin as an electron donor to reduce H_2O_2 (data not shown). However, all three Prx proteins protected GS from inactivation in a metal-catalyzed oxidation system by using dithiols, and the order of protection activity was C-PrxII > C2C-Prx > C1C-Prx. Furthermore, in a chaperone assay, recombinant C2C-Prx and C1C-Prx (but not C-PrxII) prevented the thermal aggregation of MDH. These results suggest that each Prx protein has a specific biochemical activity as either a peroxidase or a molecular chaperone in cells. Furthermore the expression levels of *C1C-Prx* and *C2C-Prx* were not changed by the treatments of heat shock and the oxidative stress-generating chemicals such as hydrogen peroxide and methyl viologen (Fig. 2C). This means that *C1C-Prx* and *C2C-Prx* are not regulated in the transcript levels in response to stress. However based on the previous results (Kim et al., 2009; 2011), C1C-Prx and C2C-Prx proteins can be over-oxidized under oxidative-stressed condition, which derives conformational changes and increase the chaperone activities of the proteins.

The low peroxidase activity and mild chaperone activity of C1C-Prx seem suited to providing relief from free radicals and to regulating gene expression in the nucleus to inhibit seed germination under stress conditions (Haslekas et al., 2003; Lee et al., 2000). The relatively high chaperone activity of C2C-Prx seems suited for the protection of proteins in chloroplasts, where ROS are generated explosively during photosynthesis. The high peroxidase activity of C-PrxII compared to that of other isotypes would suggest its involvement in the direct removal of ROS in the cytosol to maintain cellular homeostasis. Moreover, the capacity of these Prxs to switch between their roles as peroxidases and chaperones shows their functional flexibility, which probably comes into play under different environment conditions; however, C-PrxII does not display this flexibility.

Taken together, the results show that each Prx isoform is expressed in a specific organ at a specific time point during plant development, and that each has a particular function in the cell. However, it is also necessary for them to act in concert to protect plants under unfavorable conditions. Further research is necessary to reveal the physiological roles of the Prx family proteins in plant defense and developmental systems.

ACKNOWLEDGMENTS

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