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Improvement of Chaperone Activity of 2-Cys Peroxiredoxin Using Gamma Ray

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Chaperone/Dual functions/Dual function engineering/Gamma ray/Peroxiredoxin.

A typical 2-cysteine peroxiredoxin (2-Cys Prx) PaPrx can act alternatively as thioredoxin (Trx)-dependent peroxidase and molecular chaperone in *Pseudomonas aeruginosa* PAO1. In addition, the functional switch of PaPrx is regulated by its structural change which is dependently induced by stress conditions. In the present study, we examined the effect of gamma ray on structural modification related to chaperone activity of PaPrx. The structural change of PaPrx occupied with gamma ray irradiation (2 kGy) based on polyacrylamide gel electrophoresis (PAGE) analysis and the functional change also began. The enhanced chaperone activity was increased about 3–4 folds at 30 kGy gamma irradiation compared with nonirradiated PaPrx, while the peroxidase activity was significantly decreased. We also investigated the influence of the gamma ray on protein hydrophobicity as related to chaperone function. The exposure of hydrophobic domains reached a peak at 30 kGy gamma ray and then decreased dependently with increasing gamma irradiation. Our results suggest that highly enhanced chaperone activity could be adapted for use in bio-engineering systems and industrial applications such as enzyme stabilization during industrial process (inactivation protection), improvement of useful protein productivity (refolding and secretion) and industrial animal cell cultivation (stress protection).

INTRODUCTION

2-Cysteine peroxiredoxins (2-Cys Prxs) found in human, plant and bacteria display diversity in structure and apparent molecular weight (MW), and can act alternatively as peroxidases and molecular chaperones.[1–3] Under oxidative stress condition, Prxs exert their protective antioxidant role in cells through their peroxidase activity, whereby hydrogen peroxide (H_2O_2), peroxynitrite and a wide range of organic hydroperoxide (ROOH) are reduced and detoxified.[4] During the reaction cycle, the peroxidatic Cys residue is oxidized to sulfenic acid, whereas hydrogen peroxide, peroxynitrite, and a broad range of alkyl hydroperoxides are reduced to water, nitrite, or the corresponding alcohol.[5–7] The oxidized peroxidatic Cys residue is regenerated via intra- or intermolecular disulfide formation with resolving Cys and then the disulfide bond is reduced by thioredoxin (Trx), which is then regenerated by thioredoxin reductase (TR) using reducing equivalents from nicotinamide adenine dinucleotide phosphate (NADPH).[4,8] On the other hand, the H_2O_2-catalyzing peroxidase activity of 2-Cys Prxs in the yeast and mammalian cells is completely inactivated by the high concentrations of H_2O_2, producing the hyperoxidized sulfinic acid from of the Prxs.[2,9] Prxs can induce their structures to undergo large conformational changes under external stress condition. For example, the chaperone activity of *Yeast* 2-Cys Prx significantly increased by heat stress and this functional change might promote by its structural change from low molecular weight (LMW) forms to high molecular weight (HMW) complex structures.[2] The hyperoxidaized peroxidatic Cys induceds significant changes in its protein structure from LMW species into HMW complexes and the enzymatic function is also reversibly switch from a peroxidase to a molecular chaperone.[2,10] Thus, Prxs can protect the denaturation or aggregation of intracellular substrate proteins caused by external stresses.[2,10]

Recent works also provided engineering methods for enhanced chaperone activity with structural oligomerization. Firstly, the phosphorylated status of hPrxI protein (T90D-

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hPrxI) using site-directed mutagenesis showed higher chaperone activity than WT-hPrx.[11] Secondly, the additional Cys substitution of *Pseudomonas* 2-Cys Prx remarkably enhanced chaperone activity and is accompanied HMW complex formation (unpublished data). Thirdly, the chemical modification of α-crystallin and heat shock protein 27 (Hsp27) by methylglyoxal enhanced their chaperone activities.[12] Although, these methods suggest that the structural changes caused an increase of chaperone activity may be an important mechanism of functional switching of Prxs, these modification methods are required high cost, long times and complex procedures for the enhanced chaperone activity. In this study, we applied gamma irradiation method for enhanced chaperone activity of 2-Cys Prx. Initially, the gamma irradiation processing techniques have evolved so that radiosterilization has become the first choice method for thermosensitive and nondestructive sterilization.[13] In addition, the gamma irradiation of proteins in aqueous solution may promote changes in their structure and may alter their functional property.[14] However, gamma irradiation should not be applied to proteins in aqueous solution because of the greater degradation compared to the solid state.[15,16]

The purpose of the present work is to improve chaperone activity of 2-Cys Prx (PaPrx) by gamma irradiation and to investigate an optimized intensity of gamma irradiation. Consequently, the enhanced chaperone activity was increased about 3–4 folds at 30 kGy compared with nonirradiated PaPrx, while the peroxidase activity was decreased. We also investigated the influence of the gamma irradiation on protein hydrophobicity as related to chaperone function. Finally, our research efforts are addressing the physical modification of PaPrx protein by gamma irradiation.

MATERIALS AND MTEHODS

Bacterial strains, media and materials

The bacterial strains *Pseudomonas aeruginosa* PAO1 and *E. coli* strains [DH5α (Promega, Madison, USA) and KRX (Promega)], were grown aerobically at 30°C and 37°C in LB medium (0.5% sodium chloride, 0.5% yeast extract, and 1% tryptone) (DB, Franklin Lakes, NJ, USA), and were used for the cloning of the *PaPrx* gene.[1] *Yeast* Trx and TR were prepared as described.[17] Protein molecular size standards used in polyacrylamide gel electrophoresis (PAGE) were purchased from ELPIS (Daejeon, Korea). Ampicillin (Amp), Imidazole, L-rhamnose, bovine serum albumin (BSA), hydrogen peroxide (H_2O_2; 30% v/v), and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma (St. Louis, Missouri, USA). 1,1'-bi(4-anilino) naphthalene-5,5'-disulfonic acid (bis-ANS) was from Molecular Probes (Invitrogen corporation, Carlsband, CA, USA).

Cloning of PaPrx gene form P. aeruginosa PAO1

The *PaPrx* gene was cloned from *P. aeruginosa* PAO1 genomic DNA by the polymerase chain reaction (PCR). Briefly, specific PCR reactions were carried out in 20 μl mixtures containing 10 ng of genomic DNA, 0.2 μM deoxyribonucleoside triphosphates (dNTPs), 20 pmol of each primer set for *PaPrx* (*Xho*I, 5'-CCGCTCGAGATGAGCGTACTCGTA-3'; *Hin*dIII, 5'-CCCAAGCTTTTACAG CTTGCTGGC-3'), and 1 unit of Taq DNA polymerase (Promega) in a standard PCR buffer under the following conditions: denaturation for one cycle at 94°C, 60 s, 35 cycles at 94°C, 30 s; 50°C, 45 s; 72°C, 45 s, followed by one cycle at 72°C for 10 min. After PCR amplification, the products (615 bp) were collected and purified and subcloned into the pGEM-T vector (Promega) to produce PaPrx, which were then transformed into DH5α cells. The PaPrx fragment from pGEM-T was transferred to the pRSETa expression vector (Promega) to create *pRSETa::PaPrx*.

Expression and purification of recombinant PaPrx protein

KRX cells were transformed with *pRSETa::PaPrx* and cultured at 30°C overnight in 5 ml of LB medium supplemented with Amp (100 μg/ml) and then transferred to 500 ml of fresh LB medium in a shaking incubator. When the absorbance of the culture at 600 nm reached 0.4, expression was induced by adding 20% L-rhamnose to the medium to obtain a final concentration of 0.2%. After incubation for an additional 8 h, the cells were collected by centrifugation, frozen in liquid nitrogen, and stored at –70°C until used. The His_6-fused Prxs were purified by using a native Ni-NTA column (Peptron, Daejeon, Korea) and eluted with a linear gradient of 200 to 500 mM imidazole in phosphate buffered saline (PBS) buffer (pH 8.0). After dialysis against 50 mM HEPES (pH 8.0), the protein concentration was measured using the Bradford method,[18] with BSA as the standard. The purity of the purified recombinant Prxs was determined to be > 99% based on SDS-PAGE.

Gamma irradiation treatment

To investigate the effect of gamma irradiation, PaPrx proteins (1 mg/ml) were individually divided into 1 ml aliquots. The protein samples were treated with increasing doses (2 to 500 kGy) of gamma irradiation at ambient temperature and then were analyzed their structural changes and enzymatic dual functions by gamma irradiation. Gamma irradiation was carried out at ambient temperature, using a high-level cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, ON, Canada) in the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongeup, Korea). The source strength was approximately 215 kCi with a dose rate of 10 kGy/h. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). The dosimeters were calibrated against an International Standard Set by the International Atomic Energy

Agency (Vienna, Austria).

Polyacrylamide gel electrophoresis (PAGE) and size exclusion chromatography (SEC)

The structural modification of PaPrx by gamma irradiation was analyzed by PAGE under reducing, non-reducing and native conditions. Proteins were stained with Coomassie Brilliant Blue R-250. The SEC analysis of PaPrx proteins were analyzed at 4°C by FPLC (AKTA; Amersham Biosciences, Uppsala, Sweden) using a superdex 200 HR 10/30 column equilibrated at a flow rate of 0.5 ml/min at 25°C with 50 mM HEPES (pH 8.0) buffer containing 100 mM NaCl. The numbers on the chromatogram represent the MWs of the standard proteins: blue dextran (2,000 kDa; Vo), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), bovine serum albumin (67 kDa) and ovalbumin (43 kDa).

Peroxidase activity assay

The thioredoxin-dependent peroxidase activity of purified PaPrx was measured as described previously with minor modifications.[2,19–21] The PaPrx and irradiated proteins were incubated in 50 mM HEPES (pH 8.0) containing 200 μM NADPH, 3 μM Yeast Trx, and 1.5 μM Yeast TR. The reaction mixture was incubated at 30°C for 5 min, followed by the addition of a 10 μl aliquot of H_2O_2 at various concentrations. NADPH oxidation was monitored for the next 6 min by a decrease in absorbance at 340 nm as measured by an EVOLUTION 300 UV-VIS spectrophotometer (Thermoscientific, Worcester, MA, USA).

Molecular chaperone activity assay

The molecular chaperone activity was determined as described previously by assessing the ability of PaPrx protein to inhibit the thermal aggregation of substrate proteins.[10,19,21,22] Briefly, 1 μM of malate dehydrogenase (MDH) was mixed with various concentrations of PaPrx and irradiated proteins in a degassed 50 mM HEPES (pH 8.0) solution. The reaction mixture was incubated at 43°C for 15 min, and the increase in light scattering as a result of the thermal aggregation of substrate proteins was monitored at 360 nm with an EVOLUTION 300 UV-VIS spectrophotometer equipped with a thermostatic cell holder (Thermoscientific).

Fluorescence measurement

Hydrophobic domain exposure of the PaPrx and irradiated proteins were investigated using a SFM25 spectrofluorometer (Kontron, Germany) to examine the binding of 10 μl of 10 mM bis-ANS to 100 μg each of the PaPrx protein and the spectra were accumulated five times. The excitation wavelength was set at 380 nm and emission spectra were monitored from 400 to 600 nm.[22]

Circular dichroism (CD) spectroscopy

The PaPrx and irradiated proteins in 10 mM sodium phosphate buffer (pH 7.4) were used for Far UV-CD spectral analysis with a Jasco J-715 spectropolarimeter (Jasco, Great Dunmow, UK) and the spectra were accumulated five times.[23]

RESULTS

Structural modification of PaPrx subjected to gamma irradiation

We previously isolated the PaPrx from *P. aeruginosa*

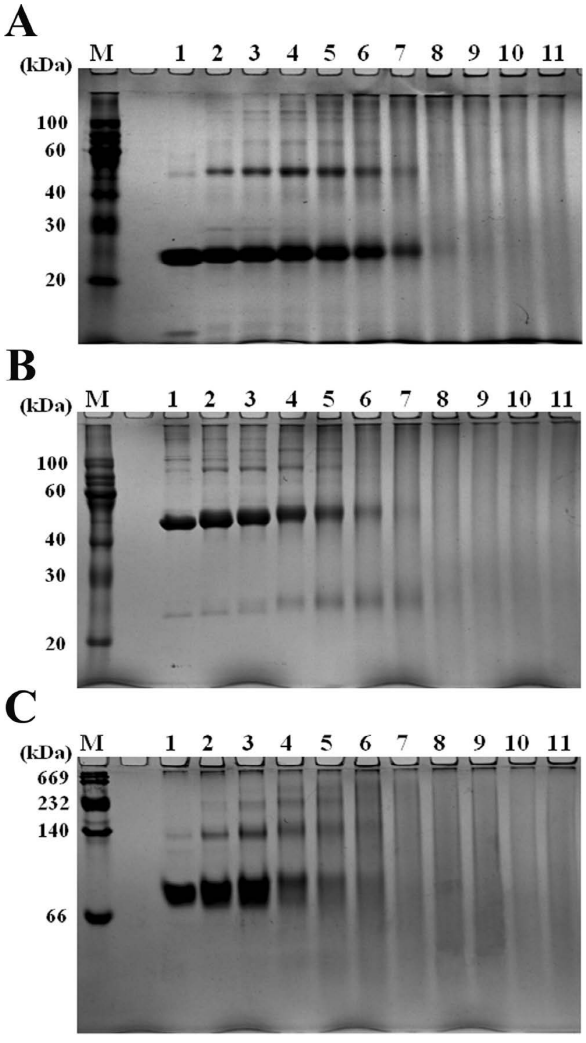

Fig. 1. PAGE patterns of irradiated PaPrx under different conditions. **A,** The irradiated PaPrx proteins were separated by 12% SDS-PAGE under reducing condition, **B,** 12% SDS-PAGE under nonreducing condition and **C,** 10% native PAGE under native condition. The proteins were stained with Coomassie Brilliant Blue R-250. Lanes 1–11 contain the patterns for the irradiated samples of PaPrx at 0, 2, 5, 15, 30, 50, 100, 200, 300, 400 and 500 kGy, respectively. Lane M shows protein molecular weight makers.

PAO1 consists of 200 amino acids that has dual functions as dominant peroxidase and recessive chaperone activities similar to members of the Prx family especially base on the results of enzymatic analysis.[1)] To investigate the gamma ray effect of PaPrx on its protein structure, PaPrx protein was exposed to several doses of gamma ray. In the PAGE analyses, 2–100 kGy of gamma irradiation might significantly promote dimerization between PaPrx subunits and slightly oligomerization, although PaPrx proteins were separated by sodium dodecyl sulfate (SDS)-PAGE under reducing condition (Fig. 1A). However, the degradation of these oligomeric bands and monomeric band by gamma irradiation were occupied with increasing gamma irradiation based on PAGE results (Fig. 1). In addition, the nonreducing-PAGE pattern of the samples in Fig. 1B also showed loss of dimeric PaPrx band intensity and smeared bands were appeared in a region of HMW by gamma irradiation (Fig. 1B). Actually, nonreducing-PAGE could display disulfide bonded structures of PaPrx (Fig. 1B). However, the oligomer structures of irradiated PaPrx (Fig. 1B, lanes 2–5) are increased compared with nonirradiated PaPrx (Fig. 1B, lane 1), therefore increased oligomers might be generated by disulfide and unknown bonds between subunits. The gamma irradiation effects are also observed similar protein oligomerization and degradation on native-PAGE by dose intensity of gamma ray. The oligomer structures of PaPrx on native-PAGE are maintained by various protein interaction factors such as hydrogen bond, ionic bond, disulfide bond and hydrophobic interaction. Among of them, the disulfide bond revealed higher resistance than other protein interaction factors against gamma irradiation (Fig. 1B and C). Consequently, the optimized gamma irradiation could generate new structures (protein bands) by unknown bonds could be oligomers or aggregates, whereas over-irradiation (50–500 kGy) of gamma ray promoted structural disruption or protein degradation with the increase of the radiation dose.

In addition, SEC profile is showed in Fig. 2 and the SEC pattern of nonirradiated PaPrx displayed two major peaks. The minority of the molecules for PaPrx were contained in the first peak and a majority in the second peak (Fig. 2A). However, the major second peak of irradiated PaPrx is significantly decreased as the radiation dose increased and new peaks corresponding to products with masses of 43 to 232 kDa (Fig. 2B and C). The increasing of the gamma irradiation dose enhances the amount of products produced with lower molecular mass. Actually, 500 kGy of gamma irradiation significantly promoted degradation of PaPrx oligomers (Fig. 2D).

Influence of structural modification by gamma irradiation on dual functions

To investigate the influence of gamma irradiation on its

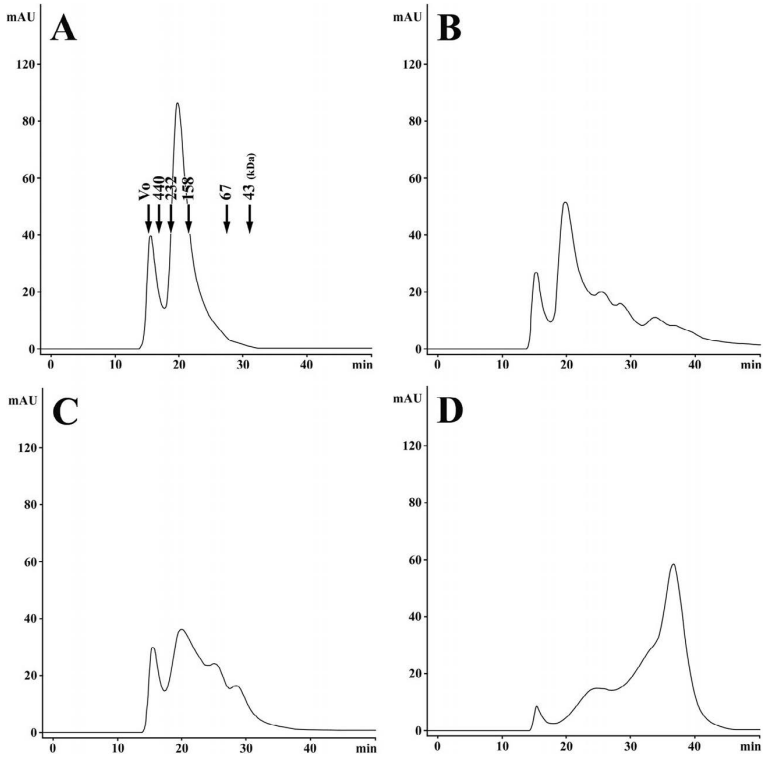

Fig. 2. SEC profiles of nonirradiated and irradiated PaPrx proteins. **A,** The nonirradiated PaPrx protein was separated. **B,** 15, **C,** 30 and **D,** 500 kGy irradiated PaPrx proteins were separated by high performance exclusion chromatography (HPLC).

dual functions, we compared the peroxidase and chaperone activities. The peroxidase activity of irradiated PaPrx was dramatically decreased by up to control (no PaPrx protein) (Fig. 3A), but the chaperone activity was drastically increased in the irradiated PaPrx compared with nonirradiated PaPrx. The chaperone activity of PaPrx was increased about 3–4 folds by gamma irradiation, showing that the dual enzymatic activities of irradiated PaPrx were significantly altered to resemble those of nonirradiated PpPrx. These results, taken together with the structural assays presented in Fig. 1 and 2, strongly suggest that gamma irradiation of PaPrx is responsible for the structural changes that result in switching of the major function of PaPrx from a peroxidase to a chaperone (Fig. 3). When we compared the results of the structural analyses (PAGE analysis and SEC profiling), and the enzymatic analyses (peroxidase and chaperone assays), we concluded that the gamma irradiation resulted in a promotion in the formation of new complex structures with changed physical character and a concomitant increase in chaperone activity (Fig. 3B).

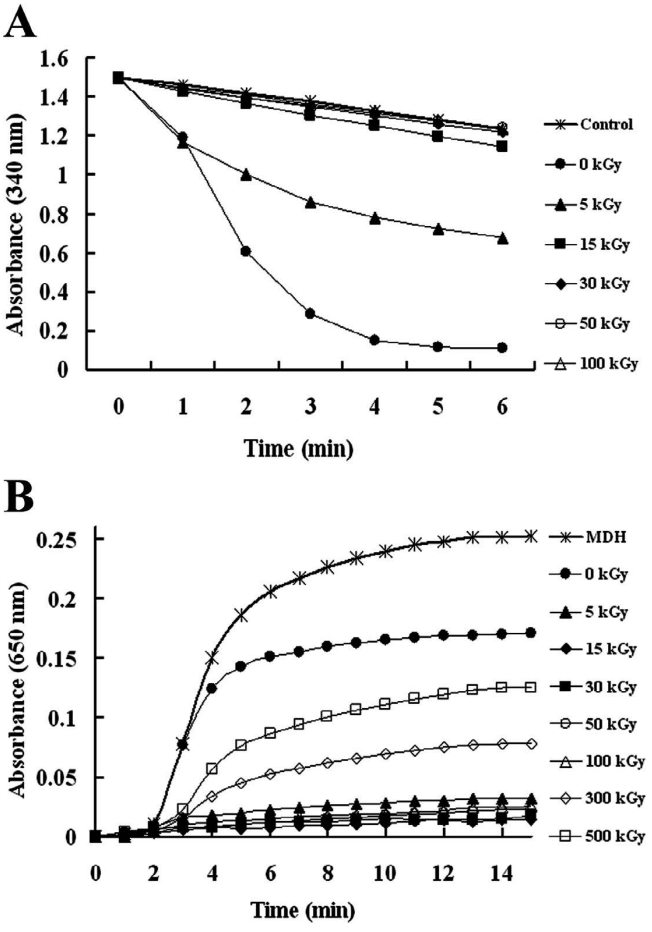

Fig. 3. The comparison of peroxidase and chaperone activities between nonirradiated and irradiated PaPrx. **A,** The peroxidase activities of nonirradiated and irradiated PaPrx were measured with the yeast Trx system. Peroxidase activities were measured at 10 μM concentration. **B,** The chaperone activities of nonirradiated and irradiated PaPrx were measured using the aggregation of MDH at 43°C at 1 vs 5 molar ratio. The data shown are the means of at least three independent experiments.

Influence of gamma irradiation on hydrophobicity

In the chaperone function, to protect target substrates against stress-induced aggregation, chaperones bind to non-native states of substrate proteins through hydrophobic interactions.[24,25] Therefore, we compared the extent of the hydrophobicity between nonirradiated and irradiated PaPrx by measuring the binding of the fluorophore, bis-ANS, which has been widely used as a probe in the detection of hydrophobic regions on the surface of proteins.[22,26] The fluorescence levels of bis-ANS, bound to irradiated PaPrx was significantly higher than that of non-irradiated PaPrx (Fig. 4). In addition, the exposure of hydrophobic domains reached a peak at 30 kGy gamma irradiation and then decreased dependently with increasing gamma irradiation. This result suggests that the gamma irradiation on PaPrx greatly increased the exposure of hydrophobic domains, which resulted in generation of the new PaPrx complexes and provided binding sites for the partially denatured substrate proteins. However, by more than 50 kGy gamma irradiation was partially reduced the exposure of hydrophobic domains, thus exhibited decreased chaperone activity compared with 30 kGy irradiated PaPrx (Fig. 3), although gamma irradiated PaPrx exhibited highly improved chaperone activity compared with nonirradiated PaPrx.

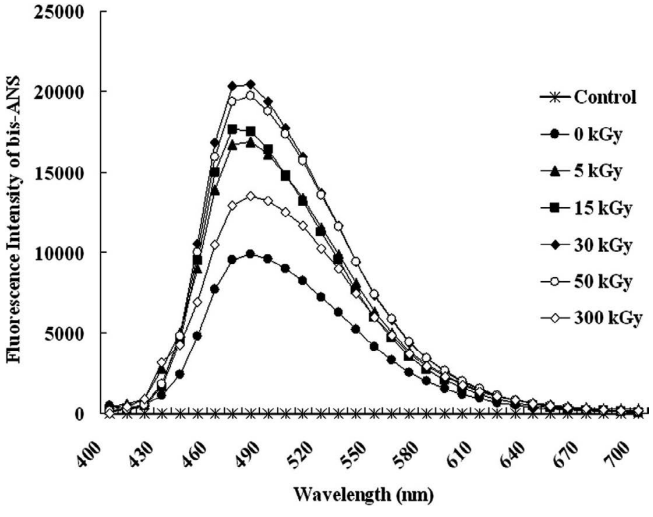

Fig. 4. The hydrophobicity change of PaPrx by gamma irradiation. Fluorescence spectra of bis-ANS bound to 100 μg/ml each irradiated PaPrx protein. The controls were measured in the absence of PaPrx protein. The data shown are the means of at least three independent experiments.

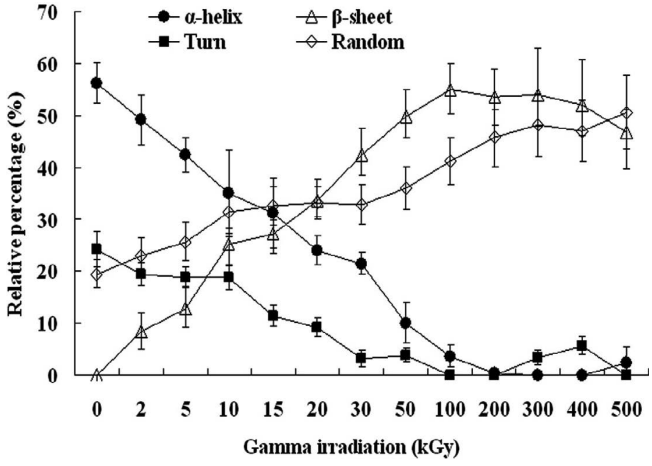

Fig. 5. The secondary structure change of PaPrx by gamma irradiation. The comparison of the secondary structure index values (%) was based on the far UV-CD spectra results of PaPrx under different gamma irradiation doses. The data shown are the means of at least three independent experiments.

Influence of gamma irradiation on secondary structure

To determine why the structural and functional alterations of the PaPrx depended on the gamma irradiation, far-UV CD spectra were used to estimate and compare the secondary structural components between nonirradiated and irradiated PaPrx (Fig. 5). Changes in secondary structural elements due to the exposure of gamma ray were as follows: the β-sheet content increased from 0% to 55%, random coil content increased from 19.5% to 50.7%, α-helical content decreased from 56.3% to 0%, and the turn content decreased from 24.2% to 0%. In particular, the β-sheet content markedly increased when gamma irradiated, whereas the α-helix and turn contents markedly decreased. Consequently, these changes in secondary structural elements by gamma irradiation enhanced chaperone activity compared with nonirradiated PaPrx.

DISCUSSION

The 2-Cys Prx proteins are members of a ubiquitous family of peroxidases that exhibit dual functions as peroxidases and molecular chaperones.[2,25,27] Most 2-Cys Prxs have been unknown regarding the physiological relevance of the association, or dissociation, of these proteins by gamma irradiation, although previous reports have demonstrated that oligomerization of 2-Cys Prxs could enhance their chaperone activity. Some report indicated that oligomerization of 2-Cys Prxs is dependent on redox state,[28] heat stress[2] and the phosphorylation of Thr^{90}.[29] Although, the improved chaperone activity of 2-Cys Prxs was caused by their structural oligomerization, the mechanism responsible for the increased activity is still unclear.[2,29,30]

The subject of present study was not to establish a ready-to-use radiomodification protocol but to make a fundamental study that may help to design such protocol. In the present study, we might optimize radiomodification protocol based on structural and functional changes of PaPrx by gamma irradiation. Firstly, we analyzed structural changes by gamma irradiation (Fig. 1 and 2). At low gamma irradiation doses (2 to 30 kGy) could generate new dimeric structures which are linked by dityrosine and unknown bonds under reducing condition (Fig. 1A).[13] In contrast, at high gamma irradiation doses (50 to 500 kGy) promoted structural disruption or protein degradation with the increase of the radiation dose (Fig. 2). Secondly, we determined influence of gamma irradiation on enzymatic dual functions, the irradiated PaPrx showed 3–4 folds higher chaperone activity than nonirradiated PaPrx (Fig. 3B). While, the peroxidase activity was significantly decreased (Fig. 3A). An absorbed dose of 10 kGy corresponds to a temperature rise of 2.4°C in a food having the heat capacity of water.[31] In our irradiation facility system, we cannot measure a correct absorbed temperature to samples because we do not check the heat absorbed and heat losses during gamma irradiation. However, the protein sample is little affected by irradiation doses of less than 50 kGy, whereas the samples might be significantly exposed to heat stress by irradiation doses of more than 100 kGy. Therefore, we think that the changes of dual functions of PaPrx protein by gamma irradiation may be associated with heat stress. Consequently, the structural changes of gamma irradiated PaPrx could enhance chaperone activity and these results implied that the physical properties of PaPrx might be changed by gamma irradiation as well as heat stress. Thirdly, we determined changes of protein physical property factors such as hydrophobicity and secondary structure. Our results revealed that the secondary structure elements and hydrophobicity were significantly transformed by gamma irradiation and provided a structural explanation for the increase in chaperone activity. Actually, the influence of hydrophobicity as related to chaperone function. The gamma irradiated PaPrx greatly increased the exposure of hydrophobic domains (Fig. 4) and significantly increased exposure of β-sheet and random coil elements on the protein surface. While, exposure of α-helix and turn elements was decreased (Fig. 5).

In conclusion, we have primarily provided a radiomodification guideline for conserved 2-Cys Prx protein engineering by gamma irradiation. Our results suggest that radiomodification of chaperone function could be easily adapted for use in bio-engineering systems and industry and that it may be possible to develop organisms that are more resistant to extreme environments.

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REFERENCES

1. An B-C, *et al* (2010) A new antioxidant with dual functions as a peroxidase and chaperone in *Pseudomonas aeruginosa*. Mol cells **29**: 145–151.
2. Jang H-H, *et al* (2004) Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. Cell **117**: 625–635.
3. Schröder E, *et al* (2000) Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes at 1.7 Å resolution. Structure **8**: 605–615.
4. Hofmann B, Hecht HJ and Flohé L (2002) Peroxiredoxins. Biol Chem **383**: 347–364.
5. Bryk R, Griffin P and Nathan C (2000) Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature **407**: 211–215.
6. Hillas PJ, *et al* (2000) The AhpC and AhpD antioxidant defense system of *Mycobacterium tuberculosis*. J Biol Chem **275**: 18801–18809.
7. Nogoceke E, *et al* (1997) A unique cascade of oxidoreductases catalyses trypanothione-dependent peroxide metabolism in *Crithidia fasciculata*. Biol Chem **378**: 827–836.
8. Dietz KJ (2003) Plant peroxiredoxins. Annu Rev Plant Biol **54**: 93–107.
9. Yang K-S, *et al* (2002) Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid. J Biol Chem **277**: 38029–38036.
10. Chuang M-H, *et al* (2006) The antioxidant protein alkylhydroperoxide reductase of *Helicobacter pylori* switches from a peroxide reductase to a molecular chaperone function. Proc Natl Acad Sci USA **103**: 2552–2557.
11. Chang T-S, *et al* (2002) Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. J Biol Chem **277**: 25370–25376.
12. Nagaraj RH, *et al* (2003) Enhancement of chaperone function of alpha-crystallin by methylglyoxal modification. Biochemistry **42**: 10746–10755.
13. Terryn H, *et al* (2007) Irradiation of human insulin in aqueous solution, first step towards radiosterilization. Int J Pharm 343: 4–11.
14. Tilquin BJ (1991) Radiation sterilization of drugs. Pharm Belg **46**: 396–398.
15. Boess C and Bögl KW (1996) Influence of radiation treatment on pharmaceuticals-a review: alkaloids, morphine derivatives, and antibiotics. Drug Dev Ind Pharm **22**: 495–529.
16. Jacobs GP (1995) A review of the effects of gamma radiation on pharmaceutical materials. J Biomed Appl **10**: 59–96.
17. Chae H-Z, *et al* (1994) Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. Proc Natl Acad Sci USA **91**: 7017–7021.
18. Bradford MM (1976) The role of cysteine residues as redox-sensitive regulatory switches. Anal Biochem **72**: 248–254.
19. Cheong N-E, *et al* (1999) Molecular cloning, expression, and functional characterization of a 2 Cys-peroxiredoxin in Chinese cabbage. Plant Mol Biol **40**: 825–834.
20. Jeong W-J, Cha M-K and Kim I-H (2000) A new member of human Tsa/AhpC as thioredoxin-dependent thiol peroxidase. BMB Rep **33**: 234–241.
21. Lee G-J, *et al* (1997) A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J **16**: 659–671.
22. Sharma KK, *et al* (1998) Interaction of 1,1'-bi(4-anilino)naphthalene-5,5'-disulfonic acid with alpha-crystallin. J Biol Chem **273**: 8965–8970.
23. Ito H, *et al* (2001) Phosphorylation-induced change of the oligomerization state of alpha B-crystallin. J Biol Chem **276**: 5346–5352.
24. Ganea E (2001) Chaperone-like activity of alpha-crystallin and other small heat shock proteins. Curr Prot Pept Sci **2**: 205–225.
25. Jang H-H, *et al* (2006a) Structural and functional regulation of eukaryotic 2-Cys peroxiredoxins including the plant ones in cellular defense signaling mechanisms against oxidative stress. Physiol Plant **126**: 549–559.
26. Shi L, Palleros DR and Fink AL (1994) Protein conformational changes induced by 1,1'-bis(4-anilino-5-naphthalenesulfonic acid): preferential binding to the molten globule of DnaK. Biochemistry **33**: 7536–7546.
27. Wood ZA, *et al* (2003) Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci **28**: 32–40.
28. Schröder E, Willis AC and Ponting CP (1998) Porcine natural-killer-enhancing factor-B: oligomerisation and identification as a calpain substrate *in vitro*. Biochim Biophys Acta **1383**: 279–291.
29. Jang H-H, *et al* (2006b) Phosphorylation and concomitant structural changes in human 2-Cys peroxiredoxin isotype I differently regulate its peroxidase and molecular chaperone functions. FEBS Lett **580**: 351–355.
30. Moon J-C, *et al* (2005) Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H_2O_2-induced cell death. J Biol Chem **280**: 28775–28784.
31. WHO (1981) Wholesomeness of irradiation food: Report of a Joint FAO/IAEA/WHO Expert Committee. In: *Aspects of radiation chemistry*. World Health Organization Technical Report Series, No. 659. pp. 13–15. World Health Organization, Geneva.

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