Modulation of Enzymatic Activities of Dual Functional Peroxiredoxin by Gamma Irradiation

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1. Introduction

Recently, enzymes have frequently been used as catalysts in various bio-industrial, commercial, and pharmaceutical applications [1-2], because they are more stable, more efficient, and less toxic than the synthetic catalysts. However, one of their major disadvantages is their low thermostability, which leads the researchers to develop new forms of industrially important enzymes with increased resistance to inactivation and aggregation.

This study describes a strategy for modifying the molecular chaperone activity of peroxiredoxin (Prx) by using gamma irradiation. Prxs are a ubiquitous family of antioxidant enzymes. Upon oxidation of their peroxidatic Cys, the molecules undergo a structural conversion from a low-molecular-weight (LMW) species acting as a peroxidase to a high-molecular-weight (HMW) complex functioning as a chaperone [3].

In the present study, we examined the effect of gamma irradiation on PP1084 with respect to its protein structure and enzymatic function. The use of gamma irradiation as a physical treatment can increase the cohesive strength of the protein by forming cross-links. The aims of the present work were (1) to improve the chaperone activity of PP1084 by gamma irradiation, (2) to identify the "optimal" intensity of gamma irradiation, and (3) to investigate the influence of gamma irradiation on protein hydrophobicity as related to chaperone function. Following PP1084 treatment with 30 kGy gamma irradiation, the PP1084 chaperone activity enhanced by about 3-4-fold compared with nonirradiated PP1084, while the peroxidase activity decreased. Ongoing research efforts are addressing the physical modifications of PP1084 protein by gamma irradiation.

2. Methods and Results

2.1 Bacterial strains, media, and materials

The bacterial Pseudomonas putida KT2440 strain and Escherichia coli strains [DH5α (Promega, Madison, USA) and KRX (Promega)] were grown under aerobic conditions at 30°C and 37°C, respectively, in Luria-Bertani (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 PP1084 gene (An et al., 2011). Yeast Trx and TR were prepared as described previously [4].

2.2 Cloning of PP1084 gene from P. putida KT2440

The PP1084 gene was cloned from P. putida KT2440 genomic DNA by PCR. Briefly, specific PCR reactions were performed in 20-μl mixtures containing 10 ng of genomic DNA, 0.2 μM deoxyribonucleoside triphosphates (dNTPs), 20 pmol of each primer set for PP1084 (XhoI, 5′ -ccgctcgagatgagcgtactc-3′ ; SacI, 5′ -cgagctcttacagcttgccagc-3′), and 1 unit of Taq DNA polymerase (Promega) in a standard PCR buffer. After PCR amplification, the products (615 bp) were collected, purified, and subcloned into the pGEM-T vector (Promega), which was then transformed into DH5α cells. The PP1084 fragment from pGEM-T was transferred to the pRSETa expression vector (Promega) to create pRSETa::PP1084 [5].

2.3. Expression and purification of the recombinant PP1084 protein

KRX cells were transformed with pRSETa::PP1084, 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. Protein expression was induced by adding 20% L-rhamnose. His6-fused Prxs were purified using a native Ni-NTA column (Peptron, Daejeon, Korea) and were eluted with a linear gradient of 200 to 500 mM imidazole in phosphate-buffered saline (PBS) buffer (pH 8.0).

2.4. Gamma irradiation treatment

PP1084 proteins (1 mg/ml) were individually divided into 1-ml aliquots and treated with increasing doses (2-500 kGy) of gamma irradiation at 25°C. Changes in the PP1084 structure and enzymatic dual functions were analyzed.

2.5. PAGE and SEC

Gamma irradiation-induced structural modifications of PP1084 proteins were analyzed by PAGE under reducing, nonreducing, and native conditions. Proteins were stained with Coomassie Brilliant Blue R-250.

PP1084 proteins were analyzed by SEC at 4°C by fast protein liquid chromatography (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.

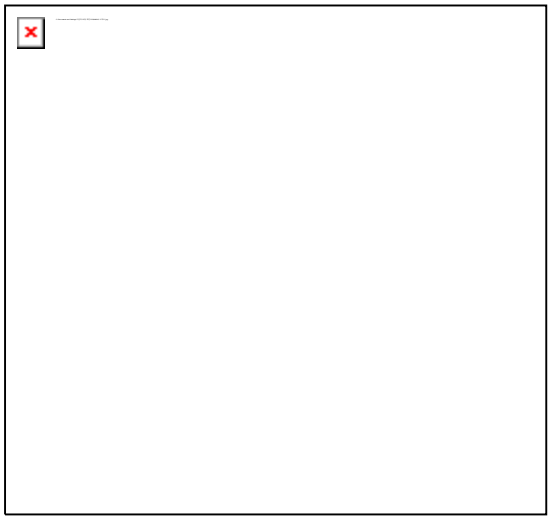

Fig. 1. SEC profiles of non-irradiated and irradiated PP1084 proteins. (A) Separation of the non-irradiated PP1084 protein. PP1084 proteins irradiated with (B) 30, (C) 200, and (D) 500 kGy were separated by FPLC.

Gamma irradiation was closely associated with increased degradation and oligomerization of the oligomeric structures (Fig. 1), and optimized gamma irradiation levels generated new structures (protein bands) by unknown bonds (oligomers or aggregates), whereas over-irradiation (50–500 kGy) promoted structural disruption or protein degradation.

The size-exclusion chromatography (SEC) pattern of non-irradiated PP1084 displayed one major HMW peak and one minor LMW peak, with the majority of PP1084 molecules being found in the first peak (Fig. 1A). However, this major first peak of irradiated PP1084 decreased as the radiation dose increased, and new peaks appeared corresponding to products with masses of 43-232 kDa (Fig. 1B and C). Increasing the gamma irradiation dose enhanced the amount of LWM products. A gamma irradiation dose of 200-500 kGy significantly promoted the degradation of PP1084 oligomers (Fig. 1D). Similar dose-dependent effects of gamma irradiation (i.e., protein oligomerization and degradation) were also observed.

2.6. Peroxidase and chaperone activity assay

To investigate the effects of gamma irradiation on the dual functions of PP1084, peroxidase and chaperone activities were compared under increasing doses of gamma irradiation. Chaperone activity of the irradiated PP1084 was significantly increased (about 3-4 folds) compared to the non-irradiated PP1084 (Fig. 2A), while the peroxidase activity was dramatically decreased to the control (no PP1084 protein) levels (Fig. 2B). In addition, comparing the results of the structural analyses (PAGE analysis and SEC profiling) and enzymatic analyses (peroxidase and chaperone assays), we conclude that gamma irradiation promotes the formation of new oligomeric structures with altered physical characteristics and concomitantly increases PP1084 chaperone activity (Fig. 2A).

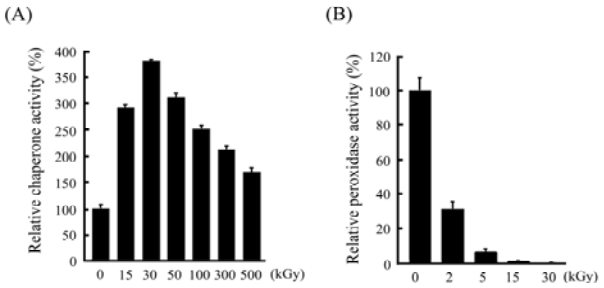

Fig. 2. Effect of gamma irradiation on the enzymatic activities of PP1084. (A) The chaperone activities of recombinant PP1084 proteins were measured at 350 nm by using malate dehydrogenase (MDH) aggregation at 43°C.

3. Conclusions

We have primarily provided a radiomodification guideline for chaperone protein engineering by gamma irradiation. Our results suggest that the radiomodification of chaperone function could be easily adapted for use in bio-engineering systems and industrial applications.

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