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Enzymatic Properties of a Recombinant Phospholipid Hydroperoxide Glutathione Peroxidase from *Momordica charantia* and Its Complementation Function in Yeast

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Abstract—The entire encoding region for *Momordica charantia* phospholipid hydroperoxide glutathione peroxidase (McPHGPx) was cloned into pET-28a(+) vector and expressed in *Escherichia coli* BL21(DE3). The purified recombinant McPHGPx displayed GSH-dependent peroxidase activity towards phospholipid hydroperoxide, H_2O_2, and *tert*-butyl hydroperoxide and had the highest affinity with and catalytic efficiency for phospholipid hydroperoxide. The optimum temperature of the enzyme activity ranged from 40 to 50°C, thus it is a thermostable enzyme compared to other PHGPx enzymes. Furthermore, *McPHGPx* expression in *Saccharomyces cerevisiae* PHGPx-deletion mutant rescued the susceptibilities to the oxidation-sensitive polyunsaturated fatty acid (linolenic acid), indicating its PHGPx complementation function in yeast. These results have well documented that McPHGPx functions as a PHGPx *in vitro* and *in vivo* and will be beneficial for further functional studies on plant PHGPx enzymes.

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Key words: phospholipid hydroperoxide glutathione peroxidase, *Momordica charantia*, enzymatic properties, functional complementation, antioxidant enzyme

In aerobic biological systems including higher plants, the generation of reactive oxygen species (ROS) such as superoxide radical ($^{\cdot}O^{2-}$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2) is an inevitable consequence of aerobic metabolic processes such as respiration and photosynthesis in mitochondria, chloroplasts, and peroxisomes [1]. Furthermore, biotic and abiotic stresses also lead to the accumulation of ROS, resulting in changes in cellular redox homeostasis [1]. When accumulated at high concentration, ROS can directly damage a large variety of biomolecules (such as lipids, proteins, and nucleic acids) that are essential for the activity and integrity of the cells [1]. On the other hand, plants have also evolved various antioxidative defense mechanisms for efficiently scavenging excessive ROS molecules. The major ROS-scavenging enzymes include ascorbate peroxidases (APx), catalase (CAT), superoxide dismutase (SOD), peroxidase (Px), glutathione peroxidases (GPx), and phospholipid hydroperoxide glutathione peroxidase (PHGPx) [2]. Together with nonenzymatic antioxidants such as ascorbic acid and glutathione, these enzymes provide plant cells with highly efficient machinery for detoxifying excessive ROS [2]. Among these enzymes, we have paid special attention to PHGPx.

PHGPx (EC 1.11.1.12) is a unique intracellular antioxidant enzyme that directly reduces phospholipid hydroperoxides produced in cell membranes and has been considered the main line of enzymatic defense against oxidative biomembrane damage in mammalian cells [3-6]. Although the biochemical function of PHGPx

Abbreviations: BHT, butylated hydroxytoluene; GPx, glutathione peroxidase; IPTG, isopropyl β-D-thiogalactopyranoside; LA, linolenic acid; (Mc)PHGPx, (*Momordica charantia*) phospholipid hydroperoxide glutathione peroxidase; MDA, malondialdehyde; PCOOH, phosphatidylcholine hydroperoxide; ROS, reactive oxygen species; SD/Trp$^-$, synthetic complete medium without tryptophan; TBA, thiobarbituric acid; *t*-BHP, *tert*-butyl hydroperoxide.

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has not been studied extensively in plants, an increasing number of *PHGPx* genes have been characterized in diverse plant species including *Nicotiana sylvestris* [7], *Citrus sinensis* [8, 9], *Arabidopsis thaliana* [10], *Oryza sativa* [11], *Momordica charantia* [12], *Setaria italica* [13], *Raphanus sativus* [14, 15], *Helianthus annuus* [16]. Furthermore, *PHGPx* gene expression levels have been recorded to increase in plant tissues in response to pathogen infections [7, 16], high salinity [8, 12, 17], heavy metals [10, 11], and extreme temperatures [18], suggesting their important roles in the defense responses of plants to various biotic and abiotic stresses. It is well known that ROS accumulation resulting from biotic and abiotic stresses can cause severe cellular damage, and ROS are thus tightly controlled and detoxified by complex enzymatic and nonenzymatic mechanisms [1, 2]. Recently, the expression of the tomato *PHGPx* gene (*LePHGPx*) was shown to inhibit cell death induced by Bax and oxidative stress in yeast and plants [18]. Moreover, the radish *PHGPx* gene (*RsPHGPx*) harbored in a yeast *PHGPx*-deleted mutant was observed to efficiently rescue the growth of a recombinant cell exposed to linolenic acid (LA), indicating a similar role to the yeast *PHGPx3* (*ScPHGPx3*) in biomembrane protection against phospholipid peroxidation [14]. These results suggest that plant *PHGPx* genes expressed under various stresses may function as cytoprotectors against oxidative damage and play an important role in scavenging ROS, such as lipid hydroperoxides.

Bitter melon (*Momordica charantia* L.) is a common vegetable in Asian countries and has long been recognized as having medicinal value in traditional Chinese medicine. The most noteworthy health benefit of bitter melon is antioxidant activity, as oxidative stress is intricately linked with age-related diseases [19, 20]. Therefore, it is important to characterize its antioxidant enzymes such as PHGPx. In a previous work, we cloned a cDNA for *PHGPx* from *M. charantia*, named *McPHGPx* (GenBank Accession No. AF346906), and characterized its tissue expression pattern [12]. In the present study, *Escherichia coli* BL21(DE3) and the pET-28a(+) vector were used to produce recombinant McPHGPx as a histidine-tagged protein. The product was soluble and could be purified to homogeneity with high yield. Furthermore, the enzymatic properties were investigated, and the role of *McPHGPx* in complementing the yeast *PHGPx*-deletion mutant was also characterized.

MATERIALS AND METHODS

Strains, plasmids, and chemicals. The cDNA of *McPHGPx* was previously cloned in our laboratory [12]. Host cell *E. coli* BL21(DE3) strain and expression vector pET-28a(+) were purchased from Novagen (USA). *Saccharomyces cerevisiae* wild-type YPH250 and the derivative *gpx1/2/3*Δ triple mutant were kindly provided by Prof. Y. Inoue of Kyoto University, Kyoto, Japan [21]. Restriction endonucleases, T4 DNA ligase, and pGEM-T Easy were from Promega (USA). The plasmid extraction kit, acrylamide, bis-acrylamide, and SDS were also from Promega. Oligonucleotides were synthesized by Sangon (China). All chemicals were of analytical grade and were purchased from Sigma Chemicals (USA) unless stated otherwise.

Protein expression and purification. The cloned cDNA of *McPHGPx* encoding a protein with 167 amino acids was directly used as a template for PCR amplification, using the gene-specific primers (5′-GCGGATCC-ACCATGGCTGAATCCCC-3′ and 5′-CCGCTCGAG-TCAAGCAGATCCCAATAG-3′). The forward primer contained an engineered *Bam*HI site (underlined) and the reverse primer incorporated an engineered *Xho*I site (underlined) for cloning into the pET-28a(+) vector to yield the fusion open reading frame with a six-histidine tag at the N-terminus of McPHGPx (His_6-McPHGPx). To improve the production of soluble recombinant McPHGPx protein, low-temperature cultivation was employed. With the induction of 1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) at 20°C, the fusion protein was expressed in *E. coli* BL21(DE3) and then purified with nickel affinity chromatography as described previously [15]. The peak protein fractions were pooled and desalted through an ultrafiltration column. The protein concentration was determined using a BCA™ protein assay kit (Pierce, USA). The molecular mass of the purified protein was estimated by SDS-PAGE (12.5% polyacrylamide gel) according to the method of Laemmli [22].

Enzyme activity and kinetic analysis. The GSH-dependent glutathione peroxidase activity of the recombinant McPHGPx towards hydroperoxides was measured as described previously with standard reaction buffer (50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 15 mM GSH, 0.25 mM NADPH, 3 U/ml glutathione reductase, and 0.12% Triton X-100) [15]. When assayed, the above standard mixture plus McPHGPx protein (10 μg/ml) was preincubated for 5 min at the selected temperature to achieve a steady base line. Then the reaction was started by the addition of hydroperoxide solution as substrate. A millimolar absorption coefficient 6.22 $mM^{-1} \cdot cm^{-1}$ for NADPH was also used. To evaluate the effects of temperature and pH on McPHGPx activity, the enzyme activity towards phosphatidylcholine hydroperoxide (PCOOH) was assayed either at different temperatures (10-60°C) or in 50 mM buffer of various pH values from 3 to 11 (pH 3, glycine; pH 4-6, sodium phosphate buffer; pH 7-10, Tris-HCl buffer; pH 11, Na_2HPO_4/NaOH buffer).

To examine the substrate preference of McPHGPx, the enzyme activity for various concentrations of three substrates including H_2O_2, *tert*-butyl hydroperoxide (*t*-BHP), and PCOOH at the optimum temperature and pH

was assayed as described above. Enzyme activities were plotted against hydroperoxide concentrations and used to calculate the apparent kinetic V_{max} and K_m for McPHGPx with the Michaelis–Menten equation $v = V_{max}[S]/(K_m + [S])$ using SigmaPlot software (SPSS, USA). Data represent the mean of three independent experiments.

Functional complementation test in yeast. The coding region of *McPHGPx* was amplified using gene-specific primers as described above and inserted into the *Bam*HI/*Xho*I sites of the YEpGAP112 vector to generate a *McPHGPx* gene expression vector YEp-McPHGPx. Because of the vital importance of the *Saccharomyces cerevisiae* PHGPx3 gene (*ScPHGPx3*) in the protection of yeast cells from oxidative damage [21, 23], it was taken as a positive control for comparison with *McPHGPx*. The PCR-amplified coding region of *ScPHGPx3* was similarly cloned into YEpGAP to yield YEp-ScPHGPx3. The generated YEp-McPHGPx and YEp-ScPHGPx3 as well as the empty vector YEpGAP112 were separately transformed into the yeast mutant or into the wild-type strain. All the transformants were grown in synthetic complete medium without tryptophan (SD/Trp$^-$) to maintain the selection for plasmids. Cells were cultured at 30°C to logarithmic phase (A_{600} = 1.0-1.5). The yeast cells of different strains cultured in the liquid SD/Trp$^-$ medium were harvested in the logarithmic phase. For the spotting assay, all the yeast cultures were adjusted to the same concentration with sterilized 0.85% NaCl solution for each strain. Four microliters of serial 1 : 10 dilutions were spotted onto the SD/Trp$^-$ agar plates supplemented without (control) or with H_2O_2, *t*-BHP, or LA. The preparation of the SD/Trp$^-$ plates with various peroxides was performed as described previously [14, 23]. Growth was examined after incubation for 3-4 days at 30°C.

Linolenic acid treatment and malondialdehyde (MDA) assay. Cells were treated with LA as previously reported [24] with some modifications. Log-phase yeast cells were harvested, washed, and resuspended with sterile 0.2% dextrose in 100 mM sodium phosphate buffer (pH 6.2) to a density of 10^7 cells/ml. The suspensions were then treated with 1 or 2 mM LA (prepared in ethanol) at 30°C with shaking (160 rpm) under atmospheric oxygen. Samples were taken prior to the addition of LA and after 4, 8, 12, or 18 h of incubation. Parallel assays were carried out by an additional 0.1 mM butylated hydroxytoluene (BHT, dissolved in ethanol) treatment prior to the addition of LA. These samples were taken after 12 h of LA treatment.

The lipid peroxidation level in the yeast cells was expressed in MDA content. The level of cellular MDA in yeast cells was determined by the thiobarbituric acid (TBA) assay as previously reported with some modifications [24]. The reaction mixture (500 µl) was constituted as follows: 100 µl of cell lysate, 250 µl of 1% TBA (prepared in 1% sulfuric acid), and 150 µl of 1% BHT in methanol. The mixture was incubated at 100°C for 15 min and then cooled to room temperature. Levels of MDA were determined by the absorbance at 532 nm corrected by subtracting the nonspecific turbidity at 600 nm. Standard concentrations of MDA were prepared by incubating malonaldehyde bis (dimethyl acetal; Sigma) in 1% sulfuric acid (MDA $\varepsilon_{245} = 1.37 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ in 1% sulfuric acid).

RESULTS

Expression and purification of recombinant McPHGPx. The coding region of the *McPHGPx* gene was amplified by PCR and inserted into the *Bam*HI/*Xho*I sites of vector pET28a(+). The recombinant plasmid with the correct reading frame was confirmed by DNA sequencing. After IPTG induction, there was an obvious extra band around the molecular weight of 22.5 kDa (Fig. 1, lane *2*). His_6-McPHGPx fusion protein (rMcPHGPx) was purified by Ni^{2+} affinity chromatography. The purified fusion protein migrated as one intense band of about 22.5 kDa on a 12.5% SDS-PAGE gel (Fig. 1, lane *3*), suggesting that one-step Ni-NTA affinity chromatography did produce highly pure rMcPHGPx protein. The size of the fusion protein matched well with its theoretical molecular weight. The yield was about 14.8 mg/liter of culture. These results indicated that the protein purity is suitable for analysis of the enzymatic properties of this protein.

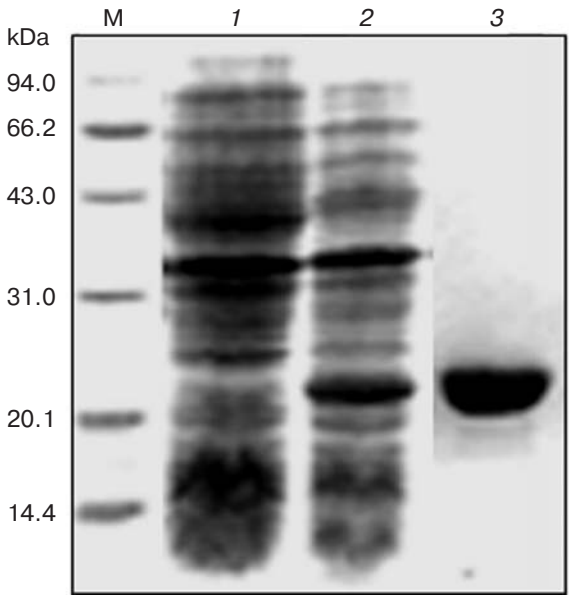

Fig. 1. SDS-PAGE analysis of the recombinant His-McPHGPx protein from *E. coli*. Lanes: *1*, *2*) total bacterial proteins from BL21(DE3) cells without and with IPTG induction, respectively; *3*) the purified His-McPHGPx through nickel affinity chromatography; M, the protein marker weights in kDa.

Catalytic and kinetic properties of recombinant McPHGPx. The catalytic activity of purified rMcPHGPx was measured using PCOOH as a substrate and showed a linear decrease in A_{340} (absorbance of NADPH) following the addition of PCOOH, whereas no changes in the absorbance were detected when either GSH or the protein sample was omitted from the reaction mixture (Fig. 2a), indicating remarkably GSH-dependent PHGPx activity of the purified rMcPHGPx.

The effects of pH and temperature on the activity of the purified rMcPHGPx are shown in Figs. 2b and 2c, respectively. The activity of the enzyme was the highest in the pH range from 9.0 to 10.0 and decreased significantly under conditions below pH 8.0 and above pH 10.5 (Fig. 2b). The optimum pH was in the alkaline range, which was in agreement with the mammalian PHGPx [25] and previously reported plant PHGPx enzymes [15, 18, 26, 27]. In the temperature range from 30 to 50°C, maximum activity was observed (Fig. 2c). The enzyme was significantly less active at temperatures lower than 20°C and was totally inactive when the reaction was carried out at 60°C. The optimum temperature for the enzyme activity was much higher than that of other plant PHGPx enzymes [15, 18, 26, 27].

To investigate the substrate preference of the purified rMcPHGPx, the peroxidase activities towards different concentrations of H_2O_2, *t*-BHP, and PCOOH were assayed at 40°C and pH 9.0. The kinetic curves of reaction velocity (catalytic activity) versus substrate concentration for each substrate are illustrated in Figs. 2d-2f. The apparent kinetic constants were calculated using these curves (the table). It can be seen that rMcPHGPx was active on all of substrates including H_2O_2, *t*-BHP, and PCOOH, indicating a similar broadness of substrate

McPHGPx activity with different hydroperoxide substrates

Substrate	Apparent V_{max}, μmol/min per mg protein	Apparent K_m, μM	V_{max}/K_m
H_2O_2	12.6 ± 0.24	638.3 ± 37.31	$1.97 \cdot 10^{-2}$
t-BHP	6.91 ± 0.13	2932 ± 131.2	$2.38 \cdot 10^{-3}$
PCOOH	0.26 ± 0.02	11.13 ± 2.21	$2.31 \cdot 10^{-2}$

Note: Activity for McPHGPx was measured with reaction buffer (pH 9.0) at 15 mM GSH concentration, 40°C. Apparent V_{max} and apparent K_m were calculated by SigmaPlot 7.0 software from the spots of activity versus varying concentration of substrates as illustrated in Fig. 2, and the values represent the mean ± S.D. of three separate experiments.

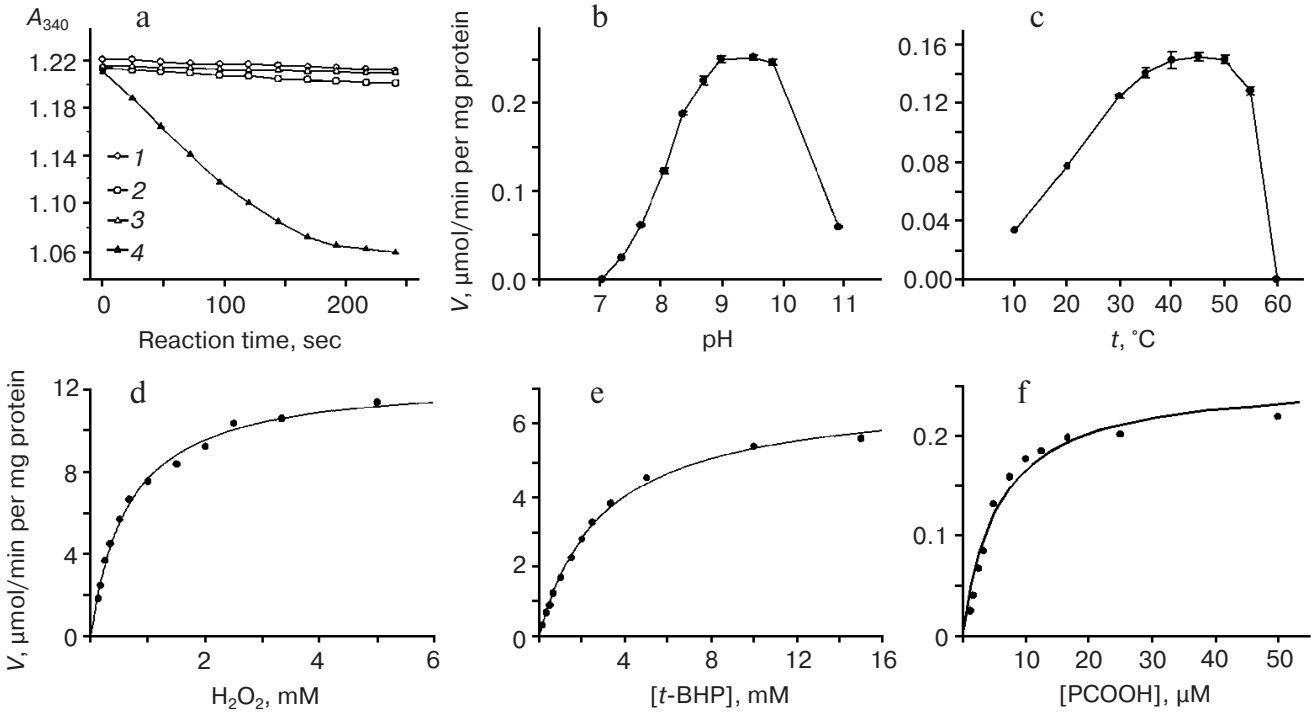

Fig. 2. Catalytic and kinetic properties of recombinant McPHGPx. a) GSH-dependent enzymatic activity of rMcPHGPx towards PCOOH: *1*) without GSH; *2*) without PCOOH; *3*) without McPHGPx; *4*) all components of the reaction are present. b, c) Profiles of rMcPHGPx activity with PCOOH substrate versus pH (b) and temperature (c). d-f) Profiles of rMcPHGPx activity versus different concentrations of hydroperoxide substrates: H_2O_2 (d), *t*-BHP (e), and PCOOH (f). All the activities were measured in velocity (*V*, Y axis) using the assay described in "Materials and Methods".

specificity to animal PHGPx [28]. Among the three hydroperoxide substrates, McPHGPx exhibited the highest affinity toward PCOOH, as indicated by the K_m values, and the largest catalytic efficiency, as indicated by the V_{max}/K_m values. The K_m and V_{max} values of rMcPHGPx for PCOOH were 11.13 μM and 0.26 μmol/min per mg protein, respectively. These results suggest that rMcPHGPx is a catalytic PHGPx with PCOOH as its optimal substrate *in vitro*.

McPHGPx activity in the *gpx1/2/3*Δ yeast mutant. *Saccharomyces cerevisiae* was reported to contain three PHGPx proteins (ScPHGPx1, ScPHGPx2, and ScPHGPx3), of which ScPHGPx3 has stronger activity in yeast cellular protection against hydroperoxides [21, 23, 29]. In this work, the *McPHGPx* transformed ScPHGPx-deletion yeast mutants (*gpx1/2/3*Δ) were sensitive to H_2O_2 and to *t*-BHP and the growth was almost fully abolished in the presence of 2 mM H_2O_2 (Fig. 3a) or 0.5 mM *t*-BHP (Fig. 3b). On the other hand, the expression of *McPHGPx* could rescue the growth of the *gpx1/2/3*Δ mutant on the plate supplemented with LA (Fig. 3c), showing that the growth was somehow better than those of *gpx1/2/3*Δ mutant cells. The result indicates that McPHGPx is required for growth in the case of LA stress.

To examine membrane phospholipid peroxidation, the levels of lipid peroxidation product MDA in yeast strains were measured. As shown by the solid lines in Fig. 4a (with 1 mM LA treatment), the MDA accumulation was gradually increasing from 4 to 18 h after treatments,

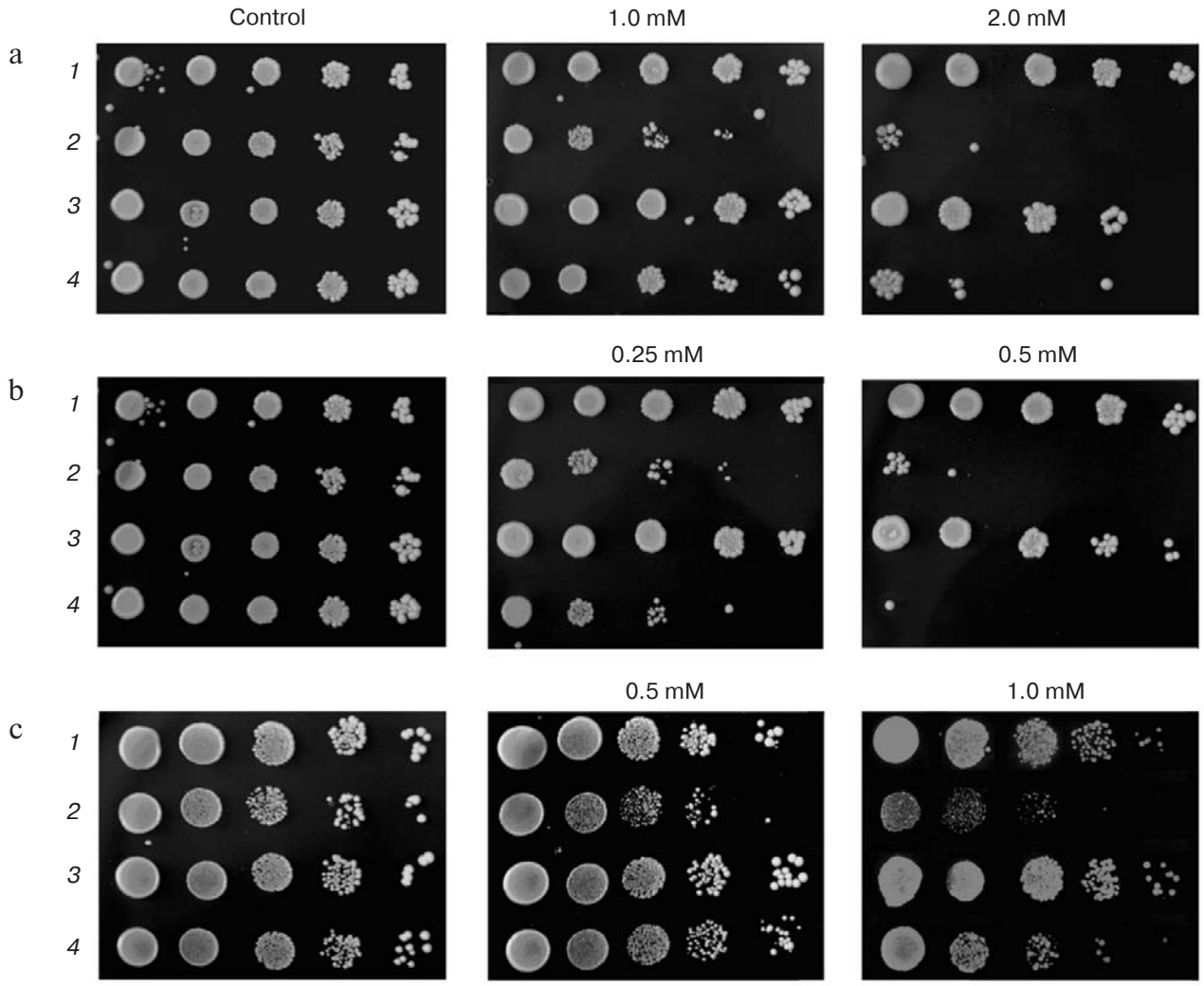

Fig. 3. Sensitivity of different yeast strains to hydroperoxides toxicity: H_2O_2 (a), *t*-BHP (b), and LA (c). Four *S. cerevisiae* strains were used: *1*) wild-type strain; *2*) PHGPx-deficient mutant (*gpx1/2/3*Δ); *3*) *gpx1/2/3*Δ mutant expressing yeast *ScPHGPx3* (*gpx1/2/3*Δ + *ScPHGPx3*); *4*) *gpx1/2/3*Δ mutant expressing bitter melon *McPHGPx* (*gpx1/2/3*Δ + *McPHGPx*). As controls, the wild-type strain and *gpx1/2/3*Δ were transformed with the empty YEpGAP112 vector (wild-type and *gpx1/2/3*Δ, respectively). Each strain was spotted equally on SD/Trp$^-$ agar plates without (control) or with different hydroperoxides (see "Materials and Methods"). Typical results from one of three independent experiments are shown.

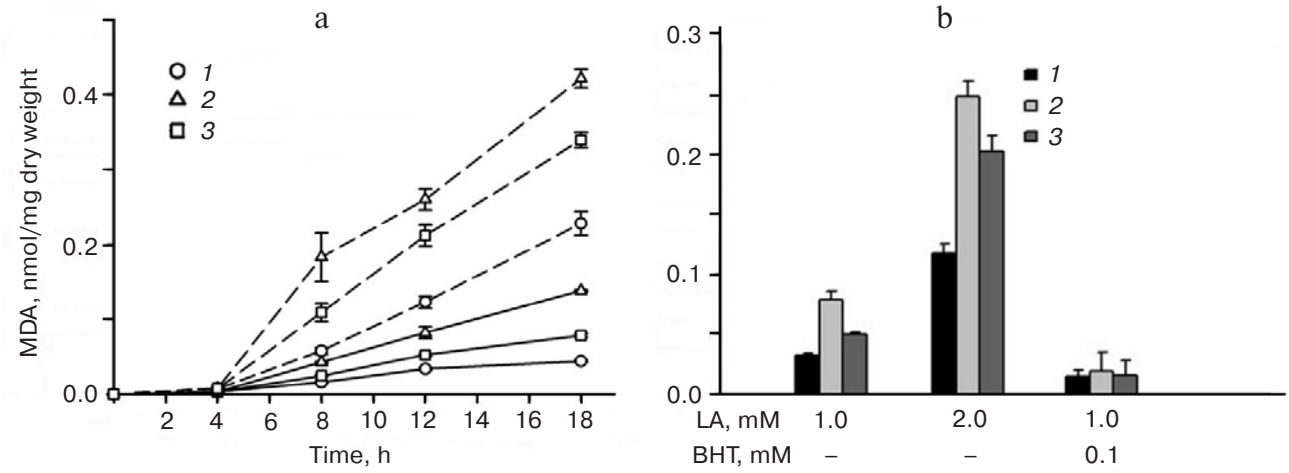

Fig. 4. MDA production in yeast strains treated with LA (a) and with additional inhibitor BHT (b). a) Cell lysates from three yeast strains including the wild-type (*1*), *gpx1/2/3*Δ mutant (*2*), and *McPHGPx*-transformed *gpx1/2/3*Δ mutant (*3*) were prepared for MDA production assay at various time points of LA treatments (X axis). Different concentrations of LA were applied as indicated by the solid lines for 1 mM and the dashed lines for 2 mM. b) MDA levels in three strains in the presence of different concentrations of LA and presence or absence of BHT. All the assays were performed after 12 h of LA treatment. Values refer to the mean ± S.D. of three independent experiments.

and the increasing rates are 1.5-fold between the *McPHGPx*-transformed triple-deletion mutant and the wild-type, and 2.6-fold between the triple-deletion mutant and the wild-type at 18 h after treatment. A similar increasing was also obtained when treated with 2 mM LA (Fig. 4a, dashed lines), besides significantly higher increasing values (about 3.2-fold) between the triple-deletion mutant and the wild-type at 18 h after treatment. The data presented above indicated that MDA accumulation after LA treatment was significantly lower in the *McPHGPx*-transformed mutant cells compared with that in the triple-deletion mutant cells, suggesting that McPHGPx may play an important role in protection of yeast cell membranes from autooxidation with LA. Moreover, when 0.1 mM BHT, an inhibitor of lipid peroxidation, was added into the yeast cultures prior to LA treatment, the levels of MDA were reduced to the background at 12 h after treatment with 1 mM LA (Fig. 4b), indicating that MDA accumulation was indeed induced by LA treatment.

DISCUSSION

Although many studies have been reported for isolations of plant *PHGPx* genes and dynamic changes of their transcriptional levels, only several plant PHGPx enzymes have been characterized on the protein level, including a native protein from citrus (CitPHGPx) [9] and four recombinant proteins encoded by tomato (LePHGPx) [26], sunflower (HaPHGPx) [26], radish (RsPHGPx) [15], and rice (OsPHGPx) [27]. Comparing the enzymatic activity among these plant PHGPx enzymes, the activity (V_{max}) of McPHGPx to PCOOH (0.26 ± 0.02) was about 4-6 times higher than that of CitPHGPx (0.040) [9, 15] or OsPHGPx (0.064 ± 0.003) [27], but only one fifteenth of that of RsPHGPx (3.96 ± 0.19) [15]. It is reasonable to assume that there might be some subtle distinctions among their structures, which lead to the different enzymatic activity. In terms of substrate specificity of plant PHGPx enzymes, the related studies had been carried out only in RsPHGPx [15] and OsPHGPx [27]. The kinetic characterization of McPHGPx displayed obvious similarity in substrate preference with other reported plant PHGPx enzymes: all of them could use H_2O_2, organic peroxides (*t*-BHP), and phospholipid hydroperoxides as substrates, but they prefer the phospholipid hydroperoxides and their enzymatic efficiency (V_{max}/K_m) increased in the order: *t*-BHP < H_2O_2 < PCOOH ([15, 27], the table).

It is noteworthy that the optimum temperature for the catalysis of McPHGPx ranged from 40 to 50°C (Fig. 2c) and was more than 10°C higher than that of other animal and plant PHGPx enzymes [15, 27], indicating that the enzyme is a thermostable protein. This finding provokes our interest in understanding its structural feature and also provides an application possibility for commercial use. However, it is questioned whether a thermostable enzyme plays an important functional role in bitter melon plants. With the recent report that transient expression of tomato PHGPx gene *LePHGPx* protected tobacco leaves from heat stress and suppressed apoptotic-like features via the reduced generation of oxidative stress [18], it is speculated that this thermostable activity might account for the efficient machineries to detoxify excessive ROS when the plants are under heat stress.

The yeast complementation test and MDA assay revealed that McPHGPx might function as an antioxi-

dant to protect cell membranes against hydroperoxide damage. McPHGPx is able to restore the growth of the *gpx1/2/3*Δ mutant cells in the presence of LA, and the result is consistent with the complementation effect of yeast *ScPHGPx3* and radish *RsPHGPx* in the same case [14, 23, 29], suggesting that McPHGPx might detoxify the lipid peroxides resulting from the autooxidation of exogenous LA. By contrast, the expression of *McPHGPx* failed to complement the *t*-BHP-sensitive phenotype of the mutant (Fig. 3b) and had no efficient complement effect on the H_2O_2-sensitive phenotype (Fig. 3a). Either the cell death under these treatments was controlled by other than phospholipids peroxidation factors, or the unspecific phospholipid peroxidation under the high concentrations of *t*-BHP or H_2O_2 used in these models could not be overcome by *McPHGPx*. On the other hand, MDA assay displayed again that expression of *McPHGPx* could reduce the accumulation of LA-induced MDA in yeast cells (Fig. 4), indicating that McPHGPx was capable of reducing the membrane phospholipid peroxidation and therefore protecting cells from the oxidative stresses. It is worth noting the coincidence of the *in vivo* specificity of this enzyme with the enzyme specificity *in vitro*.

In this paper, we characterized the enzymatic properties of McPHGPx and its function in oxidative stress resistance in yeast cells. These results collectively document that the *McPHGPx* from *M. charantia* encodes an active thermostable PHGPx enzyme and can complement the yeast PHGPx genes to produce an antioxidant protein for efficiently scavenging excessive phospholipid hydroperoxides. Therefore, it is reasonable to believe that McPHGPx might play an important role in antioxidant function in this traditional Chinese medicine plant and will be helpful for further physiological functional studies on plant PHGPx enzymes.

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