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보 문

Participation of protein disulfide isomerase 2 in the tolerance against mercury toxicity in *Schizosaccharomyces pombe*

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수은 독성에 대한 *Schizosaccharomyces pombe* 단백질2황화물이성질화효소 2의 저항성

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ABSTRACT: The present work was undertaken to address the role of protein disulfide isomerase 2 (Pdi2) in the mercury-tolerance of *Schizosaccharomyces pombe*, using the Pdi2-overexpressing recombinant plasmid pYPDI2 and the corresponding vector plasmid pRS316. When exposed to mercuric chloride, the *PDI2* overepxression cells grew significantly better than the vector control cells. They revealed the lower levels of intracellular reactive oxygen species (ROS) and nitric oxide (NO), when incubated with mercuric chloride for 6 h, than the vector control cells. The *PDI2* overepxression cells contained the higher levels of total glutathione (GSH) and superoxide dismutase (SOD) activity than the vector control cells, after 6 h of incubation in mercuric chloride. However, the *PDI2* overepxression cells contained similar levels of glutathione peroxidase (GPx) activities, compared to those of the vector control cells. Taken together, the *S. pombe* Pdi2 promotes the tolerance against mercury toxicity through up-regulating total GSH and SOD and subsequently attenuating ROS and NO elevations.

Key words: *Schizosaccharomyces pombe*, glutathione, mercury, protein disulfide isomerase, reactive oxygen species, superoxide dismutase

Single-cell microbes, like yeasts, are generally very sensitive to fluctuations in their living environments. The toxicity of heavy metals to microbes has attracted considerable research attention, particularly as a result of the continuing anthropogenic mobilization of metals in the environment. The exposure of microbes to environmental heavy metals, such as mercury and cadmium, requires appropriate defense programs to protect themselves. Heavy metal detoxification is based on three different classes of peptides or proteins, such as glutathione (GSH), a diverse family of cysteine-rich low molecular weight proteins, the metallothioneins, and heavy metal binding peptides termed phytochelatins from GSH (Vatamaniuk *et al.*, 2001). In the environment, mercury exists in the elemental form, as inorganic monovalent and divalent salts, and organomercurials such as methyl mercury. After the addition of a lethal concentration of mercuric chloride, a very poisonous form of mercury, to growing yeast cells, mercury was found to be associated with the cell wall and cytoplasmic membrane, and little or no mercury was present in the cytoplasm (Whittaker *et al.*, 1990). Toxicity of inorganic or organic mercury mostly occurs via interactions, in its ionic form, with sulfhydryl-containing molecules such as GSH, cysteine, metallothionein, homocysteine, *N*-acetylcysteine, *S*-adenosyl-methionine and albumin, and imidazole groups of macromolecules whereby

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resulting in their inactivation (Genestra, 2007; Jan *et al.*, 2011). Mercury, as a well-known pro-oxidant, exerts oxidative stress through the production of hydrogen peroxide, GSH depletion, and reactivity with membrane-bound protein thiols, and the resultant generation of reactive oxygen species (ROS) capable of damaging lipids, proteins, and DNA (Lund *et al.*, 1991; Jan *et al.*, 2011).

Protein disulfide isomerase (PDI) is essential in the folding of nascent proteins and the formation of disulfide bonds. PDI is a multifunctional enzyme in the lumen of the endoplasmic reticulum (ER), which catalyzes two types of reactions, oxidation of free sulfhydryls into disulfides (oxidase activity) and rearrangement of incorrectly formed disulfides (isomerase activity) (Gilbert, 1997). The oxidase activity is displayed by PDI in the oxidized state, while the isomerase activity is exhibited by PDI in the reduced state. The classical PDI is a highly flexible molecule consisting of four domains (a–b–b'–a'); the a and a' catalytic domains contain CXXC motifs, whereas the b and b' domains possess no catalytic activity (Wilkins and Gilbert, 2004; Tian *et al.*, 2008). Disturbances in protein folding cause the accumulation of the unfolded proteins leading to ER stress and activating signaling cascades, designated the unfolded protein response (UPR), which restores folding capacity (Lu and Christopher, 2008).

Through the previous works (Kim *et al.*, 2006; Lee *et al.*, 2010), the two isoforms of PDIs were cloned and basically characterized from the fission yeast *Schizosaccharomyces pombe*. The first PDI, later named Pdi1, which is very homologous to *Arabidopsis thaliana* PDI, is involved in the yeast growth, and its expression is up-regulated by oxidative stress, such as superoxide anion and hydrogen peroxide, and nitrogen starvation in a Pap1-independent manner (Kim *et al.*, 2006). Pdi2, a second *S. pombe* PDI, plays a protective role against nitrosative and nutritional stresses, and its expression is positively regulated by nitric oxide (NO) and nitrogen starvation in a Pap1-dependent manner (Lee *et al.*, 2010). In the present work using the Pdi2-overexpressing yeast cells, we demonstrate that Pdi2 participates in the tolerant response against mercury toxicity in *S. pombe*, possibly via specifically up-regulating total GSH and superoxide dismutase (SOD), but not glutathione peroxidase (GPx).

Materials and Methods

Chemicals

Bradford reagent, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), sodium nitrite, Griess reagent, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione reductase (GR), reduced glutathione (GSH), NADPH, cytochrome c, catalase, xanthine, xanthine oxidase, and *t*-butyl hydroperoxide were obtained from Sigma-Aldrich Chemical Co.. Yeast extract, peptone and agar were obtained from Amersham Life Science. All other chemicals used were of the highest grade commercially available.

Strain and growth conditions

S. pombe KP1 (*h*$^+$ *leu1-32 ura4-294*), used in the present work, was a derivative of *S. pombe* heterothallic haploid strain 975*h*$^+$. The Pdi2-overexpressing recombinant plasmid pYPDI2 was previously constructed using a yeast-*E. coli* shuttle plasmid vector pRS316 (Lee *et al.*, 2010). Yeast cells were grown in YEPD medium (pH 6.5) which contains 1% yeast extract, 2% peptone, and 1% glucose. Yeast cells were incubated with shaking at 30°C and their growth was monitored by measuring the absorbance at 600 nm.

Preparation of cellular extract

The desired number of the yeast cells, obtained by centrifugation, was re-suspended in 20 mM Tris buffer (pH 8.0) with 2 mM EDTA and disrupted using glass beads and ultrasonication. The cellular extracts, taken after centrifugation, were used for total GSH, SOD, and GSH peroxidase activities, and protein determinations. Protein content in cellular extracts was determined by Bradford protein assay (1976) with bovine serum albumin as a reference protein.

Quantitation of intracellular ROS

To quantitate intracellular ROS, the redox-sensitive fluorescent probe DCFH-DA was used as previously described (Royall and Ischiropoulos, 1993). When DCFH-DA enters cells, its diacetate group is cleaved by an esterase, leaving a non-fluorescent molecule, which is amenable to oxidization to fluorescent dichlorofluorescein (DCF) in the presence of ROS (Kiani-Esfahani *et al.*, 2012). Yeast cells were incubated with 5 μM

DCFH-DA for 30 min at 30°C, and the treated cells were analyzed immediately using a microplate fluorometer.

Determination of nitrite

Accumulated nitrite (NO_2^-), as an index of NO, in conditioned medium was determined using a colorimetric assay based on the Griess reaction (Sherman *et al.*, 1993). Conditioned medium (100 μl) were reacted with 100 μl Griess reagent (6 mg/ml) at room temperature for 10 min, and nitrite concentration was determined by measuring the absorbance at 540 nm. The standard curve was constructed using the known concentrations (0–160 μM) of sodium nitrite

Quantitation of total GSH

As previously described (Nakagawa *et al.*, 1990), the total GSH content in cellular extracts was quantitated using an enzymatic recycling assay based on GR. The reaction mixture (200 μl) contained 175 mM KH_2PO_4, 6.3 mM EDTA, 0.21 mM NADPH, 0.6 mM DTNB, 0.5 units/ml GR, and cellular extract at 25°C. The absorbance at 412 nm was monitored using a microplate reader. Total GSH content was expressed as μg/mg protein.

Determination of total SOD activity

As previously described (Lee *et al.*, 2002), the total SOD activity in cellular extracts was spectrophotometrically determined as reduced cytochrome c with xanthine/xanthine oxidase system. The reaction mixture (200 μl) contained 50 mM phosphate buffer (pH 7.4), 0.01 units/ml xanthine oxidase, 0.1 mM EDTA, 1 μM catalase, 0.05 mM xanthine, 20 μM cytochrome c and cellular extract. A change in absorbance was monitored at 550 nm. Total SOD activity was represented as Δ_{550}/min/mg protein.

Determination of GPx activity

As previously described (Lee *et al.*, 2002), GPx activity in cellular extracts was spectrophotometrically determined. The reaction mixture (1.2 ml) contained 0.07 M phosphate buffer (pH 7.0), 0.24 units GR, 0.8 mM GSH, 0.13 mM NADPH, and cellular extract. The hydroperoxide-independent consumption of NADPH was preceded for 10 min. The overall reaction was started by adding 100 μl of prewarmed *t*-butyl hydroperoxide solution (1.5 mM). The decrease in absorbance at 340 nm was monitored. GPx activity was expressed as Δ_{340}/min/mg protein.

Statistical analysis

The results are reported as mean ± standard deviation (SD). Statistical comparisons between experimental groups were performed using unpaired Student's *t*-test. A *P* value less than 0.05 was considered to be statistically significant.

Results

Growth enhancement

Participation of the *S. pombe* Pdi2 in the yeast growth under mercury stress was examined using medium-shift experiments. The same numbers of the *S. pombe* cells harboring pRS316 or pYPDI2, grown to the early exponential phase, were transferred to fresh rich medium with 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride. The yeast growths, determined by measuring absorbance at 600 nm, at 3 and 6 h after the shifts were compared (Fig. 1). Even in the absence of mercuric chloride, the *S. pombe* cells harboring pYPDI2 displayed a slight tendency to grow better after the shift than the vector control cells (Fig. 1A). In the presence of mercuric chloride, the *S. pombe* cells harboring pYPDI2 grew significantly better than the vector control cells (Fig. 1B, C, and D). Although the growth of the vector control cells tended to be arrested at 100 μM of mercuric chloride, the *S. pombe* cells harboring pYPDI2 could reasonably grow albeit a little delayed (Fig. 1D). When the estimated growth rates after the shifts were compared, the enhanced growth of the *S. pombe* cells harboring pYPDI2 in the presence of mercuric chloride was also confirmed, compared with those of the corresponding vector control cells (Fig. 2). In the presence of 25 μM, 50 μM, and 100 μM of mercuric chloride, the growth rates of the *S. pombe* cells harboring pYPDI2 were 1.4-, 1.8-, and 2.4-fold higher than those of the corresponding vector control cells, respectively (Fig. 2). Taken together, Pdi2 is positively involved in the growth of the *S. pombe* cells under mercury stress.

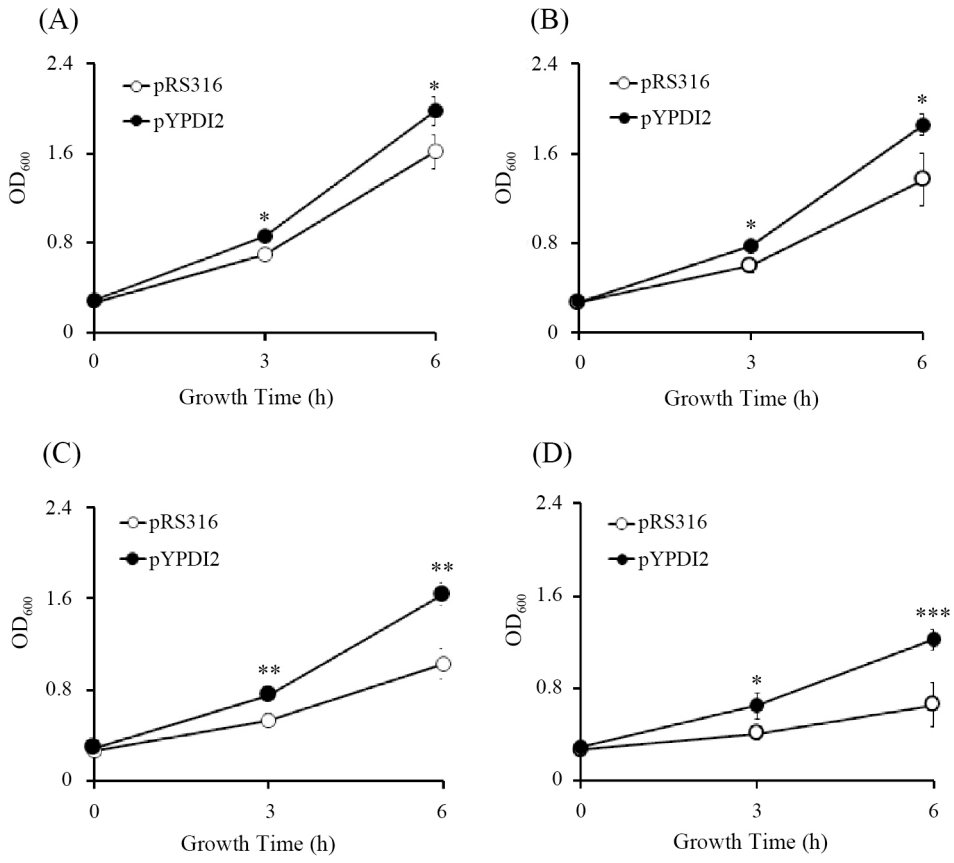

Fig. 1. Enhancing effect of Pdi2 on the *S. pombe* growth under mercury stress. The *S. pombe* cells harboring pRS316 or pYPDI2, grown to early exponential phase at 30°C in rich growth medium, were transferred to 0 μM (Control, A), 25 μM (B), 50 μM (C), and 100 μM (D) of mercuric chloride. The yeast growth was monitored by absorbance (OD_{600}) at 3 and 6 h following the shifts. Each point shows the mean ± SD of the three independent experiments. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ versus the corresponding pRS316-containing cells.

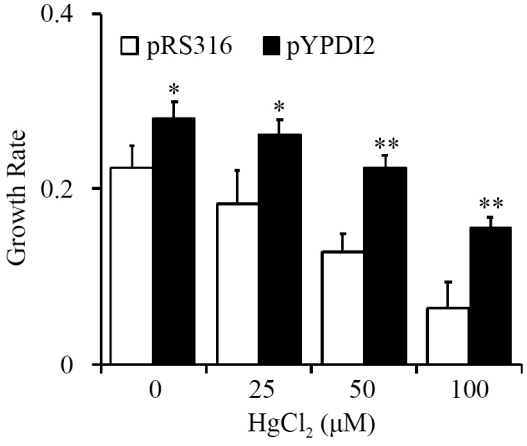

Fig. 2. Enhancing effect of Pdi2 on the growth rates of the *S. pombe* cells under mercury stress. The *S. pombe* cells harboring pRS316 or pYPDI2, grown to early exponential phase at 30°C in rich growth medium, were transferred to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride. The growth rates were obtained as ΔOD_{600}/h. Each point shows the mean ± SD of the three independent experiments. *, $P<0.05$; **, $P<0.01$ versus the corresponding pRS316-containing cells.

ROS attenuation

The ROS levels were determined in the *S. pombe* cells harboring pRS316 or pYPDI2, when the same numbers of the exponentially grown yeast cells were transferred and grown under 0 μM, 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. As the mercury concentration went up, the intracellular ROS levels of the vector control cells also became higher (Fig. 3). The vector control cells contained the 1.4-, 1.8-, and 3.9-fold higher ROS levels at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively, than the non-treated cells, proving that the yeast cells undergo enhanced oxidative stress when grown under mercury stress (Fig. 3). The ROS levels of the *S. pombe* cells harboring pYPDI2 declined to 76.1%, 72.8%, and 67.0% of those from the corresponding vector control cells, at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively (Fig. 3). In brief, Pdi2 plays a scavenging role on mercury-induced ROS

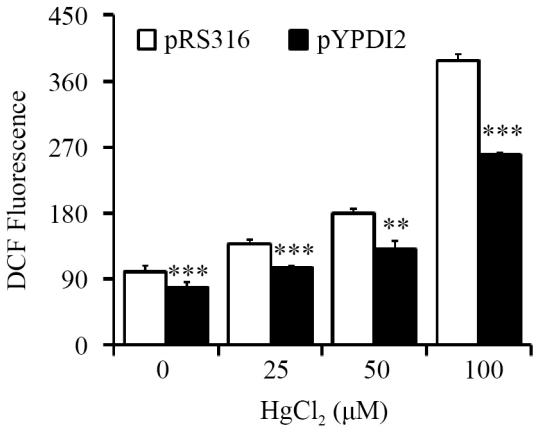

Fig. 3. Effect of Pdi2 on the reactive oxygen species (ROS) levels of the *S. pombe* cells under mercury stress. The yeast cells harboring pRS316 or pYPDI2 in the early exponential phase at 30°C were subjected to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. The ROS levels were relatively represented as DCF fluorescence, an arbitrary unit. Each point shows the mean ± SD of the three independent experiments. **, $P<0.01$; ***, $P<0.001$ versus the corresponding pRS316-containing cells.

in *S. pombe* cells.

NO attenuation

As shown in Fig. 4, the vector control cells was found to contain the 1.3-, 1.4-, and 1.7-fold higher contents of nitrite, an index of NO, at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively, compared with that of the non-treated cells. This finding confirms that NO elevates in *S. pombe* cells under mercury stress. The *S. pombe* cells harboring pYPDI2 had the nitrite levels declined to 60.8%, 60.0%, and 66.4% of those of the corresponding vector control cells at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively (Fig. 4). Even in the absence of mercuric chloride, the nitrite levels were significantly lower in the *S. pombe* cells harboring pYPDI2 than in the vector control cells (Fig. 4). Taken together, Pdi2 plays an diminishing role on mercury-induced NO in *S. pombe* cells.

Total GSH

The total GSH contents in the vector control cells became gradually lower as the mercury concentration went up (Fig. 5). As shown in Fig. 5, the total GSH contents in the vector control cells dropped to 78.0%, 49.0%, and 15.0%, respectively, at 25 μM, 50 μM, and 100 μM of mercuric chloride, compared to those of the non-treated cells. The total GSH contents in *S. pombe* cells harboring pYPDI2 were 1.2-, 1.4-, and 3.2-fold higher at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively, than those in the corresponding vector control cells (Fig. 5). Collectively, Pdi2 is involved in maintaining the high levels of total GSH in the *S. pombe* cells under mercury stress.

SOD and GPx

In addition to GSH, antioxidant enzymes such as superoxide

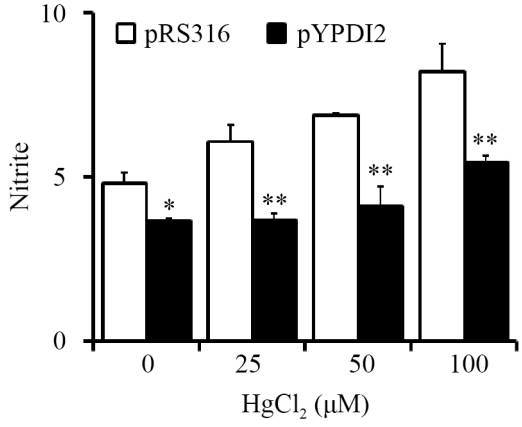

Fig. 4. Effect of Pdi2 on the nitric oxide (NO) levels of *S. pombe* cells under mercury stress. The yeast cells harboring pRS316 or pYPDI2 in the early exponential phase were subjected to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. The levels of nitrite, an index of NO, in supernatant fractions, were expressed in μM. Each point shows the mean ± SD of the three independent experiments. *, $P<0.05$; **, $P<0.01$ versus corresponding pRS316-containing cells.

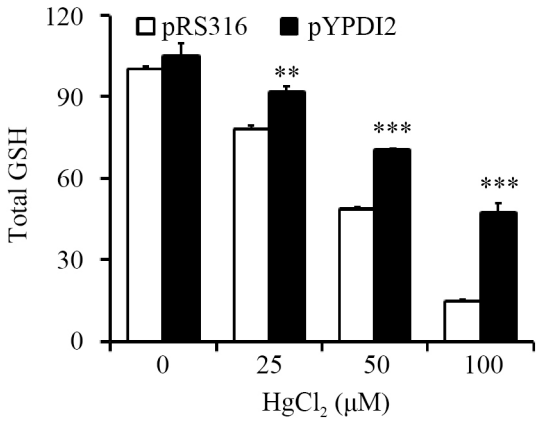

Fig. 5. Effect of Pdi2 on the total glutathione (GSH) contents in the *S. pombe* cells under mercury stress. The yeast cells harboring pRS316 or pYPDI2 in the early exponential phase were subjected to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. The total GSH levels were calculated as μg/mg protein, and expressed in relative percentages. **, $P<0.01$; ***, $P<0.001$ versus the corresponding pRS316-containing cells.

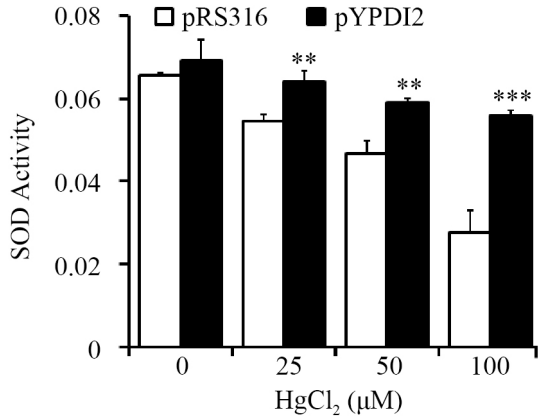

Fig. 6. Effect of Pdi2 on total superoxide dismutase (SOD) activities in the *S. pombe* cells under mercury stress. The yeast cells harboring pRS316 or pYPDI2 in the early exponential phase were subjected to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. Total SOD activity was represented as Δ*550*/min/mg protein. **, $P<0.01$; ***, $P<0.001$ versus the corresponding pRS316-containing cells.

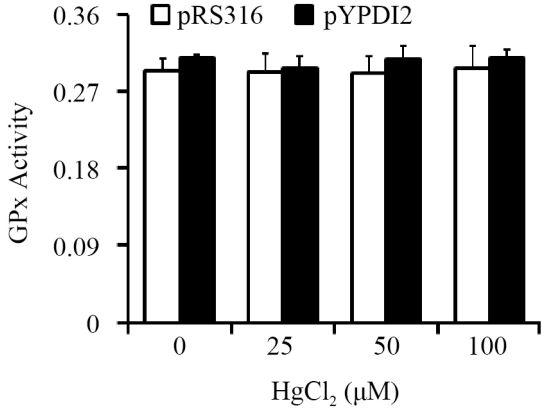

Fig. 7. Effect of Pdi2 on glutathione peroxidase (GPx) activities in the *S. pombe* cells under mercury stress. The yeast cells harboring pRS316 or pYPDI2 in the early exponential phase were subjected to 0 μM (Control), 25 μM, 50 μM, and 100 μM of mercuric chloride for 6 h. GPx activity was represented as Δ*340*/min/mg protein.

dismutase, catalase, peroxidase, and peroxiredoxin are the principal components responsible for the cellular defense against diverse stresses. As shown in Fig. 6, the total SOD activities in the vector control cells were reduced to 83.2%, 71.1%, and 42.1% of those of the non-treated cells, at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively. This suggests that the total SOD activities are concentration-dependently decreased in the *S. pombe* cells under mercury. Although total SOD activities in the *S. pombe* cells harboring pYPDI2 also became gradually lower at the higher concentrations of mercuric chloride, their levels were maintained to be significantly higher than those in the vector control cells. The total SOD activity levels in the *S. pombe* cells harboring pYPDI2 were 1.2-, 1.3-, and 2.0-fold higher at 25 μM, 50 μM, and 100 μM of mercuric chloride, respectively, than those in the corresponding vector control cells (Fig. 6). Taken together, Pdi2 is capable of enhancing total SOD activities in *S. pombe* under mercury stress.

As shown in Fig. 7, the GPx activities of the *S. pombe* cells harboring pYPDI2 were found to be similar to those of the vector control cells. This implies that Pdi2 is unable to modulate GPx activity in *S. pombe* under mercury stress.

Discussion

Various living cells and organisms, which are exposed to aerobic conditions, are constantly challenged by ROS that can arise from incomplete reduction of molecular oxygen and are potentially damaging to all cellular macromolecules including DNA, proteins, and membrane lipids. They defend themselves against ROS damage through the catalytic action of enzymes such as peroxidases, SOD, and catalases which, acting alone or in sequence, reduce ROS to water or alcohols. However, when ROS are excessively generated beyond the cellular defense capabilities, the cells are subjected to oxidative stress. Serious oxidative stress makes the cells undergo the abnormal states and ultimately the cellular death. Avoiding oxidative stress is very important for the cells to maintain their healthy conditions. Strengthening the cellular defense capabilities, for example the enhancement of antioxidant components, including GSH, GPx, and SOD, can be desirable conferring resistance to oxidative stress by removing ROS directly or indirectly. This work demonstrates that Pdi2 is capable of specifically upregulating GSH and SOD, but not GPx, in *S. pombe* cells under mercury stress.

Toxicities of heavy metals, including mercury and cadmium, have been partly occurring from their abilities to produce ROS. For example, the oxidative stress produced by mercury exposure is similar to that produced by treatment with hydrogen peroxide, consistent with the finding that Yap1 is also involved in the response of yeast towards mercury (Westwater *et al*., 2002). Exposure to the excessive amounts of heavy metals

would make living cells undergo oxidative stress. Since the single-celled organisms are in directly contact with the changes in environments, they could experience oxidative stress much easily. Several findings on the defense against mercury toxicity in microbes have been already reported. Low concentrations (5 μg/ml) of mercuric ion enhances the activities of antioxidant defense enzymes including SOD, GPx, GR, and mercuric reductase in some rumen bacteria such as *Streptococcus bovis* and *Selenomonas ruminantium*, which prevent the toxic effect of mercury on those bacteria (Lenártová *et al.*, 1998). Mitigation of endogenous mercury depends as a part on the presence of antioxidants such as GSH − most abundant intracellular non-protein thiol that plays a central role in the maintenance of cellular redox status by quenching free radicals generated during oxidative stress (Jan *et al.*, 2011). Expression of the gene encoding γ-glutamylcysteine synthetase is regulated by mercury and cadmium, but not other redox-active metals, including copper and iron, and its mercury-mediated regulation is not by the same mechanism used by cadmium (Westwater *et al*., 2002). *S. cerevisiae* is capable of inducing an adaptive stress response towards mercury and there is some overlap between mercury and hydrogen peroxide-adaptive stress responses (Westwater *et al.*, 2002). Ycf1 plays a protective role against mercuric ion in *S. cerevisiae*, especially pronounced when yeast cells are grown in rich medium or in minimal medium supplemented with GSH (Gueldry *et al.*, 2003). In the present work, we demonstrate that Pdi2 participates in scavenging ROS and NO excessively generated under mercury-induced oxidative stress. Although how mercury can induce NO production remains uncertain, it may indirectly result from the ROS elevation caused by mercury. The previously identified proof (Kig and Temizkan, 2009) on the existence of NOS-like activity in *S. pombe* may further support the NO induction under mercury-induced oxidative stress. The results, obtained in the present work, demonstrate that the *S. pombe* Pdi2's diminishing capabilities on ROS and NO induced by mercury are possibly based upon its enhancing activity on total GSH and SOD.

Although upregulation of PDIs under diverse stresses has been reported in several cell types, their participation in the stress response has rarely been reported so far. In *Saccharomyces cerevisiae*, PDI is induced by the deletion of calnexin, a 90 kDa integral protein of the ER, under conditions of heat stress, leading the growth rate of the calnexin-disrupted yeast being the same as that of the wild-type yeast under those conditions (Zhang *et al.*, 2008). PDI is up-regulated not only by hypoxia in glia *in vitro*, but also by transient forebrain ischemic stress in rats *in vivo*, which plays an important role in resistance to ischemic damage (Tanaka *et al.*, 2000). It was supported by the finding that, in proteomic studies, PDI was up-regulated by hypoxia (Sørensen *et al.*, 2009). In the filamentous fungus *Trichoderma reesei*, PDI is induced by ER stress and regulated by carbon sources, being lowest in glucose-containing media and highest on carbon sources inducing the genes encoding extracellular enzyme (Saloheimo *et al.*, 1999). The *Aspergillus niger* gene encoding PDI is also induced by ER stress (Jeenes *et al.*, 1997). Both the *S. pombe* Pdi1 and Pdi2 were previously verified to participate in the control of cellular growth and response to nutritional and oxidative stresses (Kim *et al.*, 2006; Lee *et al.*, 2010). This work additionally demonstrates that the *S. pombe* Pdi2 plays a defensive role against mercury stress. Although the *S. pombe* Pdi2 is estimated to scavenge ROS and NO by enhancing total GSH and SOD activity, its action mechanism(s) remain to be elucidated, which requires further detailed approaches. However, although oxidized PDI enhances ROS production, reduced PDI suppresses the production of ROS, suggesting that PDI plays a crucial role in the regulation of ROS (De *et al.*, 2011). This finding may indicate that the *S. pombe* Pdi2 in the reduced form may diminish ROS production triggered by mercury, known as an oxidative stress-inducing agent.

In conclusion, the Pdi2-overexpressing *S. pombe* cells were able to grow much better than the vector control cells in the presence of mercury. This growth enhancement by Pdi2 may be based upon its scavenging property on ROS and NO enhanced under mercury-induced oxidative stress, and the ROS- and NO-scavenging properties of Pdi2 are sequentially due to its up-regulating power on total GSH and SOD. In brief, the *S. pombe* Pdi2 participates in the response to mercury-induced oxidative stress.

적 요

본 연구는 *Schizosaccharomyces pombe*의 단백질2황화물

이성질화효소 2 (Pdi2)가 수은 독성에 대한 저항성에 관여하는지를 입증하기 위하여, Pdi2 과잉 발현 재조합 플라즈미드 pYPDI2와 대응되는 벡터플라즈미드인 pRS316을 사용하여 수행되었다. 염화제2수은(25 μM, 50 μM, 200 μM)에 노출되었을 때, pYPDI2 포함 *S. pombe* 세포는 벡터 포함 대조 세포보다 훨씬 더 잘 성장하였다. pYPDI2 포함 *S. pombe* 세포는, 6시간 동안 염화제2수은과 같이 배양하였을 때, 벡터 포함 대조 세포보다 낮은 세포 내 활성산소종과 일산화질소 수준을 나타내었다. 반면, 같은 배양 조건에서, pYPDI2 포함 *S. pombe* 세포는 벡터 포함 대조 세포보다 높은 수준의 총 글루타치온 및 수퍼옥시드 디스뮤타아제을 나타내었다. 그러나, pYPDI2 포함 *S. pombe* 세포는 벡터 포함 대조 세포와 비슷한 수준의 글루타치온과산화효소 활성을 보여 주었다. 종합하면, *S. pombe* Pdi2는 총 글루타치온과 수퍼옥시드 디스뮤타아제 활성을 증가시켜, 그에 따라 활성산소종과 일산화질소의 상승을 억제함으로써 수은 독성에 대한 저항성에 관여한다.

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