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Short communication

Catalase, Glutathione S-Transferase and Thioltransferase Respond Differently to Oxidative Stress in *Schizosaccharomyces pombe*

Young-Wook Cho, Eun-Hee Park[†] and Chang-Jin Lim*
Division of Life Sciences, Kangwon National University, Chuncheon 200-701,
[†]College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea

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The logarithmically growing *Schizosaccharomyces pombe* cells were subjected to high heat (40°C), hydrogen peroxide, and heavy metals such as mercuric chloride and cadmium chloride. Then, the stress responses of catalase, glutathione S-transferase and thioltransferase were investigated. The high heat and cadmium chloride enhanced the catalase activity. The glutathione S-transferase activity of *S. pombe* cells was increased after treatments with heavy metals. The thioltransferase activity of *S. pombe* cells was completely abolished by mercuric chloride. Hydrogen peroxide caused no effect on the activities of glutathione S-transferase and thioltransferase. These results suggest that the response of *S. pombe* cells against oxidative stress is very complicated.

Keywords: Catalase, Glutathione S-transferase, Oxidative stress, *Schizosaccharomyces pombe*, Thioltransferase.

Introduction

There has been a great deal of interest in cellular responses to a variety of environmental stresses. The location of the interaction between a stress and a cell may differ considerably depending on the nature of the stress. The exposure of cells to oxidative stress has multiple effects on redox-regulated activities of the cell (Powis *et al.*, 1995). A number of immediate-early genes are transcriptionally activated in cells treated with oxidative stress. These include c-*fos*, c-*jun*, and *egr*-1 (Nose *et al.*, 1991; Amstad *et al.*, 1992; Datta *et al.*, 1993; Rao *et al.*, 1993; Ohba *et al.*, 1994). Levels of thioredoxin in lymphocytes were reported to be increased by hydrogen peroxide (Hayashi *et al.*, 1993). Yap1 and Skn7 are two yeast transcriptional regulators that co-operate in order to activate thioredoxin and thioredoxin reductase in response to redox stress signals (Lee *et al.*, 1999). Activities of other proteins, including some enzymes, are susceptible to induction or repression due to oxidative stress.

All organisms are exposed to reactive oxygen species, such as hydrogen peroxide (H_2O_2), The superoxide anion (O^-_2) and the hydroxyl radical (OH^-) are by-products of oxidative metabolism and the exposure to radical-generating compounds (Yu, 1994). Catalase, which decomposes H_2O_2 to water and O_2, is a widely distributed enzyme and is an important member of the cellular defense system against oxidative stress. Even if it is not strictly essential, the lack or malfunction of catalases may lead to severe defects, such as an increased susceptibility to thermal injury (Leff, 1993), high rates of mutations (Halliwell and Aruoma, 1991) and, in higher organisms, inflammation (Halliwell and Gutteridge, 1990). Expression of the catalase gene in *Schizosaccharomyces pombe* was enhanced in response to oxidative stress (Nakagawa *et al.*, 1998).

Glutathione S-transferases (GSTs), a family of cytosolic multifunctional enzymes, are detoxifying enzymes that are present in all aerobic organisms. They catalyze the conjugation of glutathione with a variety of reactive electrophilic compounds, thereby neutralizing their active electrophilic sites and subsequently making the parent compound more water soluble. In addition to catalytic functions, the GSTs can also bind covalently/non-covalently to a wide number of hydrophobic compounds, such as haem, drugs and carcinogens. The cloned *Arabidopsis thaliana* GST was found to function as a glutathione peroxidase, and may be involved in the removal of reactive organic hydroperoxides, such as the products of lipid peroxidation (Bartling *et al.*, 1993). Thioltransferases, which had been purified from *S. pombe* and *A. thaliana*, were recently reported to contain GST activities (Kim *et al.*, 1999a; Cho *et al.*, 2000). Auxin-inducible and stress-inducible GST genes were isolated from *A. thaliana* and soybean, respectively (van der Kop *et al.*, 1996; Skipsey *et al.*, 1997).

Thioltransferase, also called glutaredoxin, is an enzyme that catalyzes the reduction of low molecular weight disulfides and

*To whom correspondence should be addressed.
Tel: 82-31-250-8514; Fax: 82-31-242-0459.
E-mail: cjlim@cc.kangwon.ac.kr

protein disulfides in the presence of reduced glutathione. Thioltransferase has been isolated and characterized from a number of prokaryotic and eukaryotic species, including kale (Sa *et al.*, 1998b) and Chinese cabbage (Cho *et al.*, 1998; Cho *et al.*, 1999). Thioltransferase was reported to serve as a regenerative system for oxidatively damaged proteins *in vivo* as well as *in vitro* (Yoshitake *et al.*, 1994).

In this article, as a preceding step to study the regulations of several *S. pombe* genes (such as thioltransferase and glutathione S-transferase genes) the variations in the activities of catalase, glutathione S-transferase and thioltransferase were investigated after treatments with hydrogen peroxide, heavy metals, and high temperature.

Materials and Methods

Chemicals Bovine serum albumin (BSA), reduced glutathione (GSH), glutathione reductase (yeast), NADPH, Tris, EDTA, glucose, adenine, L-leucine, uracil, mercuric chloride, cadmium chloride, hydrogen peroxide, and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Chemical Co. (St. Louis, USA). 2-Hydroxy disulfide (HED) was purchased from Aldrich Chemical Co. (Milwaukee, USA). Yeast extract was obtained from United States Biochemicals (Cleveland, USA). All other chemicals and reagents used were of the highest grade commercially available.

Cell growth and treatment A wild-type *S. pombe* was grown in a YEALU medium at 30°C of shaking incubator. YEALU medium contains 30 g glucose, 5 g yeast extract, 75 mg adenine, 75 mg leucine, 75 mg uracil per liter. At the exponential phase (OD_{600} = 1.0) the culture was split and one culture flask was treated with oxidative stress, such as high heat (40°C), mercury (0.3 mM), cadmium (0.3 mM). Both the control flask and the treated flask continued to be shaken at the appropriate temperature.

Cell harvest and disruption According to the time-course of shaking, the yeast cells were harvested by centrifugation. They were frozen and resuspended in 20 mM Tris buffer (pH 8.0)-2 mM EDTA (buffer A) and disrupted by using a glass beater and a sonicator. Supernatant was obtained after centrifugation and used for enzyme assays.

Catalase assay Catalase activity was assayed as previously described (Ueda *et al.*, 1990). In a quartz cuvette (10-mm light path) we added 680 µl of 50 mM potassium phosphate buffer (pH 7.2) and 480 µl of 40 mM hydrogen peroxide. The mixture was then incubated for 2.5 minutes at 30°C. After incubation, the reaction was initiated by the addition of an appropriately diluted cell extract (40 µl). The decrease in absorbance at 240 nm was followed spectrophotometrically. Specific activities of catalase were represented as ΔA_{240}/min/mg protein.

Glutathione S-transferase assay Glutathione S-transferase (GST) was measured by spectrophotometric assay according to the method of Mozer *et al.* (1983). To a 1-ml quartz cuvette (10-mm light path) were added 850 µl of potassium phosphate buffer (pH 6.5), 50 µl of 20 mM glutathione, 50 µl of 20 mM 1-chloro-2,4-dinitrobenzene (dissolved in ethyl alcohol), and 50 µl of crude extract. The cuvette was covered with parafilm and slowly inverted 3-4 times to mix the reaction, and then placed in the sample holder of the spectrophotometer to start reading. The reference cuvette contained everything except the extract being assayed. Specific activities of glutathione S-transferase were represented as ΔA_{340}/min/mg protein.

Thioltransferase assay Thioltransferase catalyzes the reduction of certain disulfides in the presence of glutathione and thus has GSH-disulfide-transhydrogenase. In the present study, 2-hydroxyethyl disulfide was used as a substrate (Sa *et al.*, 1998a; Kim *et al.*, 1999b; Park *et al.*, 1999). Two quartz semimicro cuvettes with 10-mm light path contained 500 µl of mixture at room temperature. To both cuvettes were added 50 µl of 15 mM 2-hydroxyethyl disulfide, 50 µl of 60 µg/ml yeast glutathione reductase, 50 µl of 4 mM NADPH, and 50 µl of 1 M Tris-HCl (pH 8.0)-2 mM EDTA. The absorbance at 340 nm was recorded for 2 minutes to ensure that both cuvettes were balanced with respect to the nonenzymatic spontaneous reaction between glutathione and 2-hydroxyethyl disulfide. Cell extract was added to the sample cuvette and an equal volume of buffer A was added to the reference cuvette. The decrease in absorbance was then recorded for a few minutes. The result was calculated as ΔA_{340}/min/mg protein.

Protein determination The protein content in the cell extract was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Results and Discussion

All living cells are exposed to various environmental stresses. Treatment with oxidative stresses causes a variation in the activities of various enzymes, as reported previously (Lamb *et al.*, 1998). Hydrogen peroxide is a representative chemical frequently used in oxidative experiments. Cadmium was also found to be an inducer of oxidative stress in *Saccharomyces cerevisiae* (Brennan and Schiestl, 1996). In this study, we investigated the time-course variations in the activities of catalase, glutathione S-transferase and thioltransferase after the treatment with oxidative stresses.

Yeast growth For the treatments with hydrogen peroxide, mercuric chloride, and cadmium chloride, they were added to logarithmically growing cells at concentrations of 1.5 mM (hydrogen peroxide), 0.3 mM (mercuric chloride), and 0.3 mM (cadmium chloride). For the high heat treatment, logarithmically growing cells were transferred to 40°C. After the stress treatments, the growth of *S. pombe* cells was retarded (data not shown). Especially the treatment with heavy metals, such as mercury and cadmium, showed significant retardation in the growth of yeast cells.

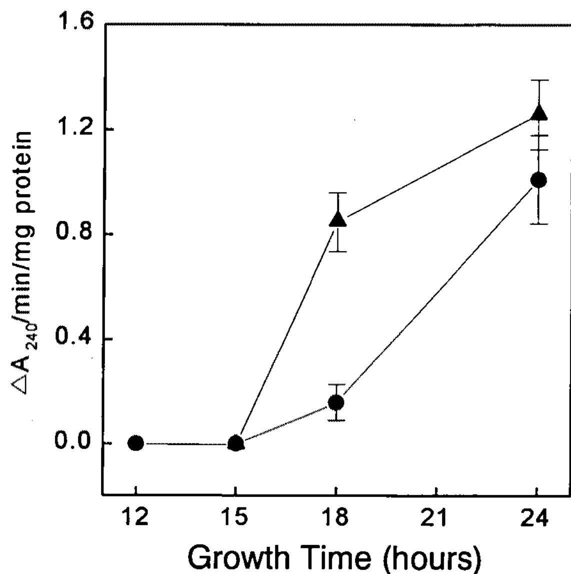

Fig. 1. Effect of temperature shift to 40°C on the catalase activity in *Schizosaccharomyces pombe*. The yeast cells were grown at 30°C (-●-), the culture was split at the exponential phase, and one culture flask was transferred to 40°C (-▲-). Specific activities of catalase were represented as A_{240}/min/mg protein.

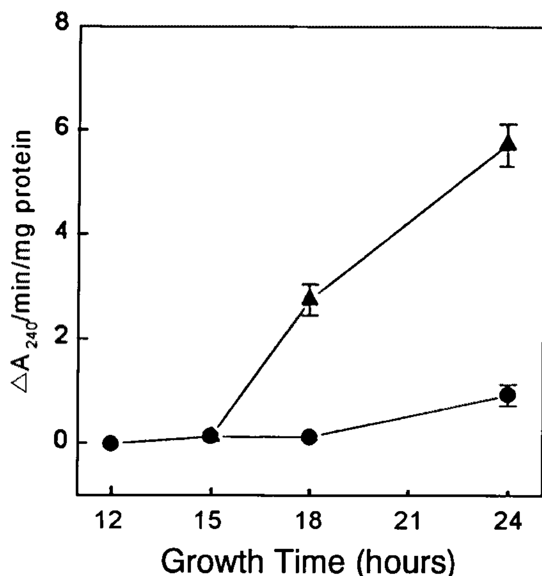

Fig. 2. Effect of cadmium on the catalase activity in *Schizosaccharomyces pombe*. The yeast cells were grown at 30°C (-●-), the culture was split at the exponential phase, and cadmium chloride (final concentration, 0.3 mM) was added into one culture flask (-▲-). Specific activities of catalase were represented as ΔA_{240}/min/mg protein.

Catalase Catalase, which catalyzes the disproportionation of hydrogen peroxide to dioxygen and water, is found in all aerobic microorganisms, plant, and animal cells. Although it was one of the first isolated enzymes, its physiological function, regulation, and *in vivo* importance are still poorly understood. Since reactive oxygen species have the potential to damage protein, lipids, and nucleic acid, aerobic organisms, they require the existence of efficient defense enzymes such as catalases and peroxidases. It was reported that catalase activity was induced in *S. pombe* cells that were exposed to hydrogen peroxide (Mutoh *et al.*, 1995). Induction of catalase activity is regulated at the mRNA level. Also in the present experiments, hydrogen peroxide significantly enhanced the catalase activity in *S. pombe* cells (data not shown). The catalase activity tended to increase in *S. pombe* cells at the stationary phase (Fig. 1). This result may reveal its relative importance in stationary cells. Treatment with high heat (40°C) caused a higher catalase activity compared with the control cells (Fig. 1), although the degree of increase was not that significant. However, the catalase activity was greatly induced after the treatment with cadmium chloride (Fig. 2). A lower concentration (0.01 mM) of cadmium chloride also showed an increasing effect on the catalase activity (data not shown). On the contrary, mercuric chloride did not show an enhancement effect in the catalase activity (data not shown). This may mean that the yeast cells have a different defense against heavy metals, which is interesting.

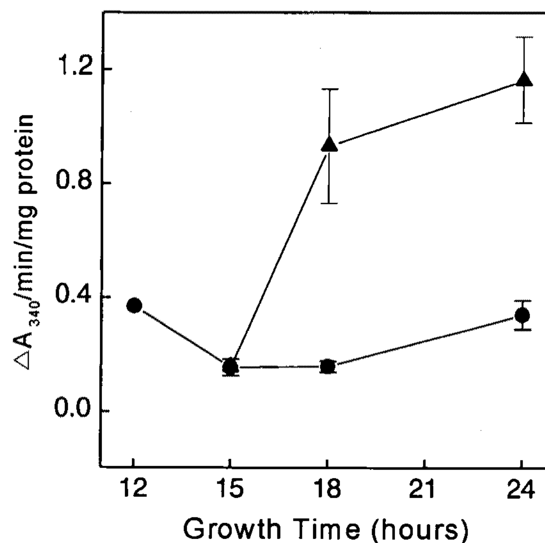

Fig. 3. Effect of mercury on the glutathione S-transferase activity in *Schizosaccharomyces pombe*. The yeast cells were grown at 30°C (-●-), the culture was split at the exponential phase, and mercuric chloride (final concentration, 0.3 mM) was added into one culture flask (-▲-). Specific activities of glutathione S-transferase were represented as ΔA_{340}/min/mg protein.

Glutathione S-transferase Glutathione S-transferases are a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism. Glutathione S-transferase was shown to catalyze essential steps in the biosynthesis of prostaglandins and leukotrienes. In plants, various chemical agents induced glutathione S-transferases

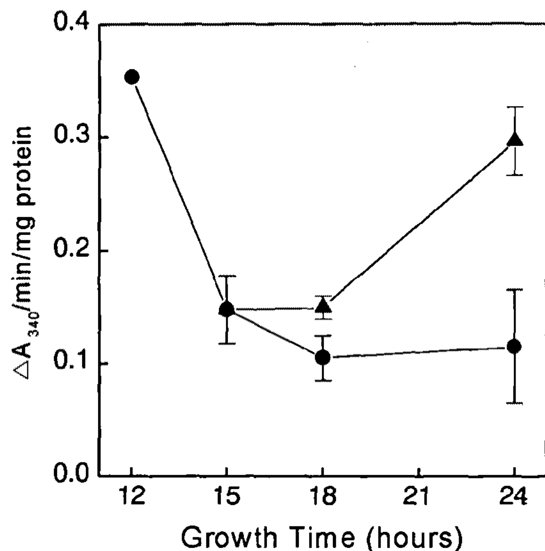

Fig. 4. Effect of cadmium on the glutathione S-transferase activity in *Schizosaccharomyces pombe*. The yeast cells were grown at 30°C (-●-), the culture was split at the exponential phase, and cadmium chloride (final concentration, 0.3 mM) was added into one culture flask (-▲-). Specific activities of glutathione S-transferase were represented as ΔA_{340}/min/mg protein.

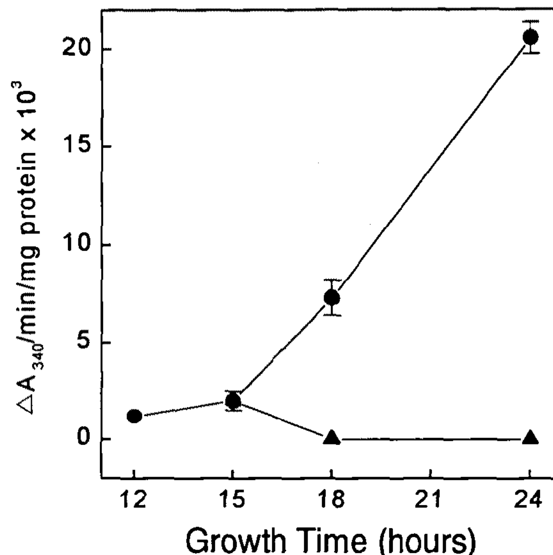

Fig. 5. Effect of mercury on the thioltransferase activity in *Schizosaccharomyces pombe*. The yeast cells were grown at 30°C (-●-), the culture was split at the exponential phase, and mercuric chloride (final concentration, 0.3 mM) was added into one culture flask (-▲-). Specific activities of thioltransferase were represented as ΔA_{340}/min/mg protein.

(Skipsey *et al.*, 1997). However, the physiological role and regulation of glutathione S-transferase are not fully understood in microbial cells. The experimental results obtained from plants suggest that glutathione S-transferase may be closely linked with stress response. The treatment with mercuric chloride greatly induced glutathione S-transferase activity in *S. pombe* cells (Fig. 3). Lower concentrations (0.01 mM) of mercuric chloride and cadmium chloride also gave the induction effect in the activity of glutathione S-transferase (data not shown). This may indicate the involvement of glutathione S-transferase in the stress response of *S. pombe*. Cadmium also induced glutathione S-transferase activity after the treatment, although its effect was less significant when compared with that of mercuric chloride (Fig. 4). The treatments of high heat and hydrogen peroxide did not give variations in the activity of glutathione S-transferase (data not shown). These results indicate that the adaptive responses of *S. pombe* cells against oxidative stresses may not be identical.

Thioltransferase Thioltransferase catalyzes the reversible thiol-disulfide interchange reactions. The enzyme has a major role in maintaining intracellular thiol in the reduced state and functions in this capacity by coupling to glutathione and glutathione reductase. Thioltransferase also has a role in the cellular regulation by catalyzing the reversible modification of proteins by thiol-disulfide interchange. The treatment with mercuric chloride completely inactivated the thioltransferase activity in *S. pombe* cells (Fig. 5). A lower concentration (0.01 mM) of mercuric chloride also gave a similar effect (data not shown). Its physiological significance remains elusive. However, the treatments with high heat, hydrogen peroxide and cadmium chloride had no effect on the activity of thioltransferase activity (data not shown). The inactivation of thioltransferase could also be due to the interaction between the active site and mercuric ions.

In this communication, it was found that catalase and glutathione S-transferase acted in different ways against oxidative stresses. Catalase activity was greatly enhanced by cadmium, whereas glutathione S-transferase activity was significantly increased by mercury. There was a temperature shift to 40°C induced catalase activity, but not glutathione S-transferase activity. The treatment of mercury completely abolished thioltransferase activity. Further approaches will be needed to elucidate the relationships between catalase, glutathione S-transferase and thioltransferase activities and stress response in *S. pombe* cells.

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