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Short communication

Regulation of Thioltransferase Activity from *Schizosaccharomyces pombe*

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Thioltransferase (TTase), also known as glutaredoxin (Grx), is an enzyme catalyzing the reduction of a variety of disulfide compounds and acting as a cofactor for various enzymes such as ribonucleotide reductase. The *Schizosaccharomyces pombe* cells, exponentially grown in rich medium at 30°C, were shifted to 20°C and 35°C. The yeast cells, shifted to 35°C, showed higher TTase activity than the cells continuously grown at 30°C, whereas the yeast cells, shifted to 20°C, gave lower TTase activity. The *S. pombe* cells, exponentially grown in minimal medium and shifted from 30°C to 35°C and 40°C, produced higher TTase activity. When the *S. pombe* cells were initially incubated in rich and minimal media at three different temperatures (25°C, 30°C and 35°C), they showed higher TTase activity at higher temperature. These results suggest that the TTase activity of *S. pombe* is regulated by temperature.

Keywords: Fission yeast, Glutaredoxin, *Schizosaccharomyces pombe*, Temperature, Thioltransferase.

Introduction

Thioltransferase (TTase) is a small and ubiquitous glutathione-dependent disulfide oxidoreductase. TTase participates in a pathway that couples the oxidation of NADPH to the reduction of ribonucleotide (Holmgren, 1979), sulfate (Tsang, 1981), methionine sulfoxide (Fuchs, 1977), and arsenate (Shi *et al.*, 1999). Some TTases are able to reduce non-disulfide substrates such as dehydroascorbate (Wells *et al.*, 1990) and alloxan (Washburn and Wells, 1997). The OxyR transcription factor, which is activated through the formation of a disulfide bond, is deactivated by TTase (Zheng *et al.*, 1998). TTase was implicated to be involved in the regulation and maintenance of protease activity in HIV-1 infected cells (Davis *et al.*, 1997). The three-dimensional structure of a mammalian TTase contains an α/β structure with a four-stranded mixed β-sheet in the corn, flanked on either side by helices (Katti *et al.*, 1995).

TTases from mammalian, plant, and microbial cells contain the active site seqeunce of -Cys-Pro-Phe(Tyr)-Cys-. Recently, TTases were isolated and characterized from various sources such as rice (Sha *et al.*, 1997), kale (Sa *et al.*, 1998a), Chinese cabbage (Cho *et al.*, 1998a; Cho *et al.*, 1999), *Arabidopsis thaliana* (Cho *et al.*, 1998b; Kim *et al.*, 1999a), and *Schizosaccharomyces pombe* (Kim *et al.*, 1998; Kim *et al.*, 1999b). Some TTases were found to contain glutathione S-transferase activity (Kim *et al.*, 1999b; Cho *et al.*, 2000a).

In this communication we describe the temperature-dependent regulation of TTase activity from the fission yeast *S. pombe*, which resembles higher eukaryotes in some regulatory mechanisms (Cho *et al.*, 2000b).

Materials and Methods

Chemicals Bovine serum albumin (BSA), reduced glutathione (GSH), glutathione reductase (yeast), NADPH, Tris, EDTA, glucose, adenine, L-leucine, and uracil were obtained from Sigma Chemical Co. (St. Louis, USA). 2-Hydroxyethyl 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 medium The wild-type *S. pombe* was grown in YEALU medium and minimal 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. Minimal medium contains 3 g KH phthalate, 4.16 g NaH_2PO4, 5 g NH_4Cl, 20 g glucose, 20 ml salt stock (50×), 0.1 ml minerals stock (10,000×), 1 ml vitamins stock (1000×). Salt stock (50×) contains 5.2 mM $MgCl_2 \cdot 6H_2O$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 13.4 mM KCl, and 0.28 mM Na_2SO_4. Minerals stock (10,000×) contains 8.1 μM H_3BO_3, 2.37 μM $MnSO_4$, 1.39 μM $ZnSO_4 \cdot 7H_2O$, 0.74 μM

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$FeCl_3 \cdot 6H_2O$, 0.25 μM $MoO_4 \cdot 2H_2O$, 0.6 μM KI, 0.16 μM $CuSO_4 \cdot 5H_2O$, and 4.76 μM cirtric acid. Vitamins stock (1,000×) contains 81.2 μM nicotinic acid, 55.5 μM inositol, 40.8 μM biotin, and 4.2 μM pantothenic acid.

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.

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.*, 1998b; Park *et al.*, 1999; Cho *et al.*, 2000c). 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 μl/ml yeast glutathione reductase, 50 μl of 4 mM NADPH (ε_{340} = 6220 $M^{-1}cm^{-1}$), 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 mmoles/min/mg protein.

Protein determination The protein content in cell extract was determined by the Bradford method (1976), using bovine serum albumin as a standard.

Results and Discussion

Thioltransferase was purified from various organisms and identified to have multiple biological functions. However, its precise regulation in cells remains to be elucidated. The yeast *Saccharomyces cerevisiae* contains two TTase genes, which are required for protection against reactive oxygen species (Luikenhuis *et al.*, 1998). In v-jun-transformed chicken embryo fibroblasts, the expression of TTase mRNA could be induced, indicating that TTase is a direct target of v-Jun (Goller *et al.*, 1998). Recently, TTase has been found to play a central role in protection against protein damage caused by menadione and hydrogen peroxide in *S. cerevisiae* (Rodriguez-Manzaneque *et al.*, 1999). Treatment with oxidative stresses causes a variation in the activities of various enzymes (Lamb *et al.*, 1998). High temperature is known to be one kind of oxidative stresses. Here we describe the temperature-dependent regulation of *S. pombe* thioltransferase.

Effect of temperature shift It is probable that temperature is one of the regulating factors in thioltransferase activity. In

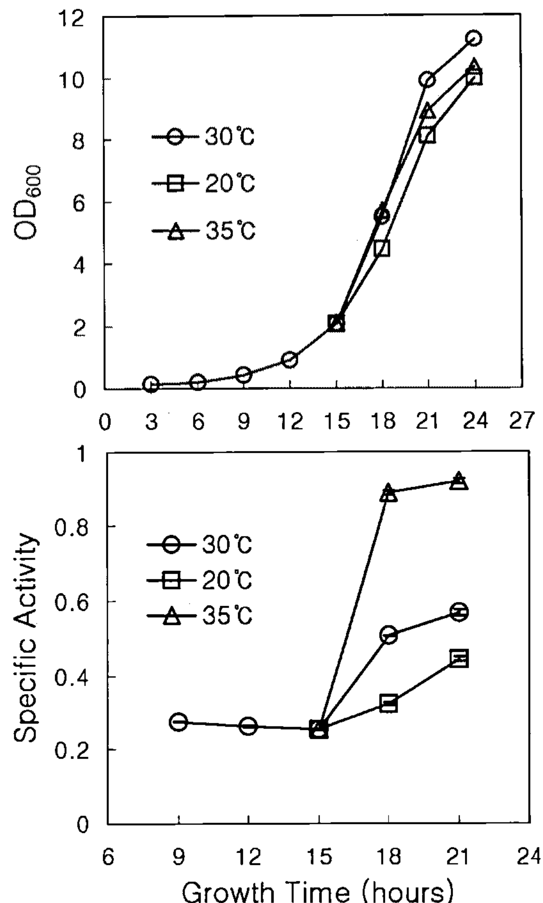

Fig. 1. Effect of temperature shift to 20°C and 35°C on thioltransferase activity of wild-type *S. pombe* cells grown in rich medium (YEALU) at 30°C. A. Growth Curves. B. Thioltransferase activity. The yeast culture, grown in rich medium, was split in the early exponential phase. The two culture flasks were transferred to 20°C and 35°C, whereas the one culture flask was left at 30°C. Thioltransferase activities were assayed as described in 'Materials and Methods'. Specific activities of thioltransferase were represented as μmoles/min/mg protein.

the temperature-shift experiment, the wild-type *S. pombe* cells were grown in rich and minimal media. The *S. pombe* culture, grown in rich medium at 30°C, was split at the early exponential phase and subjected to different incubation temperatures such as 20°C and 35°C (Fig. 1). After the temperature-shift, the growth curves of shifted cultures appeared to be almost same with that of *S. pombe* cells grown at 30°C (Fig. 1A). This indicates that the incubation temperature such as 20°C and 35°C donít give a detectable effect on the growth of *S. pombe* cells. However, thioltransferase activity was changed by the temperature shifts (Fig. 1B). At 3 hours after temperature-shifts to 20°C and 35°C, thioltransferase activity became 0.63- and 1.77-fold, respectively, compared with that of untreated *S. pombe* cells (Fig. 1B). This indicates that the *S. pombe* thioltransferase activity may be regulated by temperature and it can be

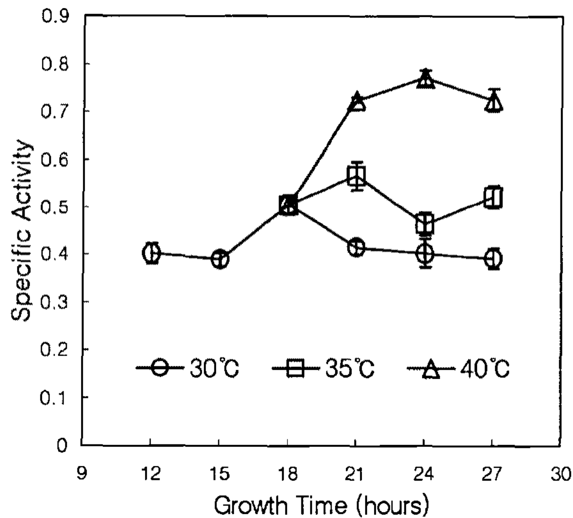

Fig. 2. Effect of temperature shift to 35°C and 40°C on thioltransferase activity of wild-type *S. pombe* cells grown in minimal medium at 30°C. The yeast culture, grown in minimal medium, was split in the early exponential phase. The two culture flasks were transferred to 35°C and 40°C, whereas the one culture flask was left at 30°C. Thioltransferase activities were assayed as described in 'Materials and Methods'. Specific activities of thioltransferase were represented as µmoles/min/mg protein.

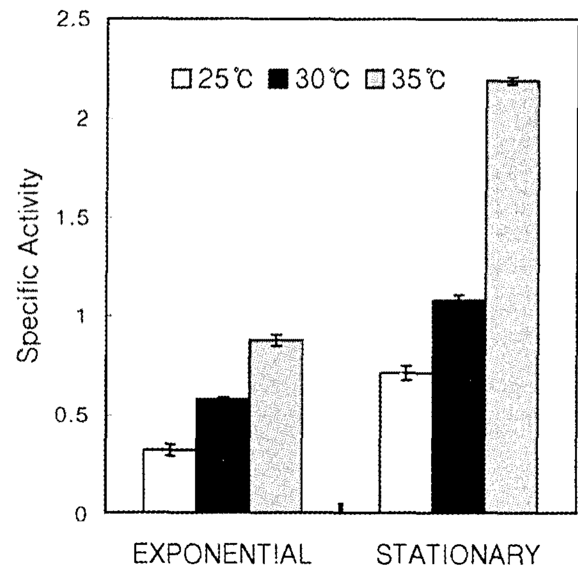

Fig. 3. Temperature-dependent variation in thioltransferase activity of wild-type *S. pombe* cells. The yeast cells were grown in rich medium (YEALU) at three different temperatures (25°C, 30°C and 35°C), and harvested at exponential and stationary phases. Thioltransferase activities were assayed as described in 'Materials and Methods'. Specific activities of thioltransferase were represented as µmoles/min/mg protein.

induced by high temperature. The similar experiment was done with the *S. pombe* cells grown in minimal medium (Fig. 2). This work was considered to be necessary, since types of medium would affect temperature-dependence. The *S. pombe* cells, grown in minimal medium at 30°C, were split and subjected to high temperatures such as 35°C and 40°C. After the temperature-shifts, the growth rates at 35°C were not changed but the growth rate at 40°C was lower than the control (data not shown). Thioltransferase activity was increased to 1.73- and 2.03-fold, respectively, when the yeast cells were harvested at 3 hours after the temperature-shift to 35°C and 40°C (Fig. 2). This suggests that the thioltransferase activity of *S. pombe* cells is induced by temperature. The shifting experiments strongly propose that the thioltransferase activity of *S. pombe* is subject to temperature control. It could be at the transcriptional level, but it needs to be further examined on the RNA level.

Effect of incubation temperature Using the shifting experiments, the possibility of temperature regulation was observed on the thioltransferase activity of *S. pombe*. In this part, temperature regulation of thioltransferase activity was examined using *S. pombe* cells, originally grown at various temperatures. The yeast cells were grown in rich medium at three different incubation temperatures (25°C, 30°C and 35°C). The *S. pombe* cells, grown at higher temperature, were found to contain higher thioltransferase activity (Fig. 3). Thioltransferase activity at the stationary phase was significantly higher than that at the exponential phase (Fig. 3).

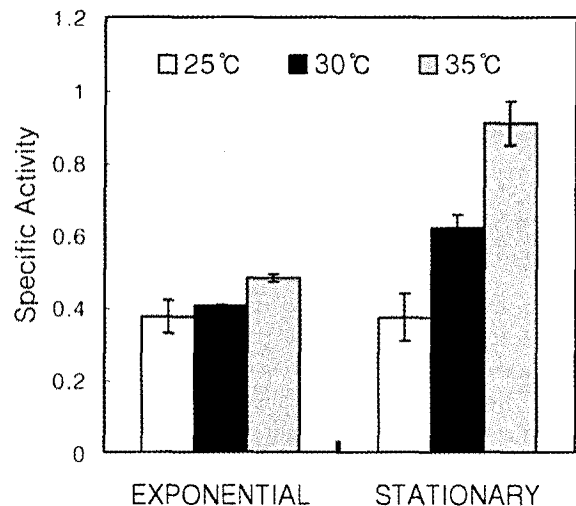

Fig. 4. Temperature-dependent variation in thioltransferase activity of wild-type *S. pombe* cells. The yeast cells were grown in minimal medium at three different temperatures (25°C, 30°C and 35°C), and harvested at exponential and stationary phases. Thioltransferase activities were assayed as described in 'Materials and Methods'. Specific activities of thioltransferase were represented as µmoles/min/mg protein.

The same type of experiment was done with the *S. pombe* cells grown in minimal medium (Fig. 4). It resulted in the similar results. Through these experiments, it is confirmed that the thioltransferase activity of *S. pombe* is regulated by temperature.

Temperature control of *S. pombe* thioltransferase activity might be linked with stress response, since thioltransferase is believed to be involved in the defense mechanism against oxidative stress. Its temperature regulation probably happens at the transcriptional level. It requires the detailed study on the gene level. The precise physiological significance of temperature regulation of *S. pombe* thioltransferase activity remains to be elucidated.

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