Journal of Biochemistry and Molecular Biology, Vol. 31, No. 4, July 1998, pp. 377–383

JOURNAL OF
Biochemistry
and
Molecular Biology

Thioltransferase (Glutaredoxin) from Chinese Cabbage: Purification and Properties

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Received 23 March 1998
Revised 20 April 1998

Thioltransferase, also known as glutaredoxin, was purified from Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*) by a combination of ion-exchange chromatography and gel filtration. Its purity was confirmed by SDS-polyacrylamide gel electrophoresis and its molecular weight was estimated to be about 12,000 which is comparable with those of most known thioltransferases. The enzyme utilizes 2-hydroxyethyl disulfide, S-sulfocysteine, α-chymotrypsin, insulin, and trypsin as substrates in the presence of reduced glutathione. The enzyme has K_m values of 0.03–0.97 mM for these substrates. It appeared to contain dehydroascorbate reductase activity. The pH optimum of the enzyme was 8.5, when 2-hydroxyethyl disulfide was used as a substrate. It was greatly activated by reduced glutathione. Its activity was not significantly lost when stored at high temperature, indicating its thermostable character. It may play an important role in thiol-disulfide exchange in plant cells.

Keywords: Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*), Glutaredoxin, Purification, Thioltransferase.

Introduction

Thioltransferase (TTase), also known as glutaredoxin (Grx), is essentially a thiol-disulfide oxidoreductase. TTase reductively cleaves a variety of disulfides including protein disulfides and low-molecular-mass disulfides in the presence of reduced glutathione (GSH) (Holmgren, 1976; Axelsson *et al.*, 1978; Hatakeyama *et al.*, 1985a). TTase either controls the ratio of cellular thiol and disulfide or regulates various enzyme activities. In fact, enzyme activities of pyruvate kinase (Axelsson and Mannervik, 1983), papain (Holmgren, 1979a), and iodothyronine 5′-deiodinase (Goswami and Rosenberg, 1985) have been reported to be affected by TTase. Determination of amino acids sequences of various TTases demonstrated an active site sequence of Cys-Pro-Tyr(Phe)-Cys which is conserved from *E. coli* to mammalians (Wells *et al.*, 1993). TTase (or Grx) seems to play an important role in DNA synthesis since it can act as a hydrogen donor for ribonucleotide reductase (Holmgren, 1976; 1979b; Kren *et al.*, 1988). It was originally identified as an alternative hydrogen donor for the reduction of ribonucleotide in an *E. coli* mutant deficient in thioredoxin. TTase can also be efficiently reduced *in vitro* by dithiols such as dithiothreitol or dihydrolipoamide (Holmgren, 1979c; Tsang, 1981; Hopper *et al.*, 1989). TTase (or Grx), together with glutathione and glutathione reductase, has been shown to participate in reduction of methionine sulfoxide (Fuchs, 1977), and sulfate (Mark and Richardson, 1976; Tsang, 1981; Kren *et al.*, 1988) in *E. coli.*

Glutaredoxin (or TTase) has been purified from wild-type *E. coli* B cells (Holmgren, 1979a) and characterized as a small (M_r = 11,600) acidic protein, containing a single disulfide bond, which could be reduced to a dithiol by GSH, glutathione reductase, and NADPH. Since then, TTase (or Grx) have already been identified and isolated in a number of species, including bacteriophage T4 (Lemaster, 1986), yeast (Gan *et al.*, 1990), vaccinia virus (Johnson *et al.*, 1991), and *Cryptococcus neoformans* (Sa *et al.*, 1997). The first mammalian TTase was isolated from calf thymus as a hydrogen donor for the corresponding ribonucleotide reductase (Luthman *et al.*, 1979; Luthman and Holmgren, 1982). Other mammalian TTases have been isolated from calf liver (Gan and Wells, 1986; Klintrot *et al.*, 1984; Papayannopoulos *et al.*, 1989), rabbit liver and

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bone marrow (Hatakeyama *et al.*, 1985b; Hopper *et al.*, 1989), pig liver (Gan and Wells, 1987b), and human placenta and erythrocytes (Mieyal *et al.*, 1991; Papov *et al.*, 1994; Chrestensen *et al.*, 1995). The primary structures of the TTase from calf, rabbit, and pig have been determined and there is a high degree of similarity among these three proteins (Klintrot *et al.*, 1984; Gan and Wells, 1987a; Hopper *et al.*, 1989; Papayannopoulos *et al.*, 1989). These structures showed about 30% sequence similarity to *E. coli* TTase with an identical active site.

The first plant TTase has been identified and purified to homogeneity from spinach leaves (Morell *et al.*, 1995). Spinach TTase showed a significant cross-reactivity with antibodies raised against *E. coli* glutaredoxin (or TTase) (Morell *et al.*, 1995). Sha *et al.* (1997) also identified the TTase gene in rice, and rice TTase was also purified from rice (Minakuchi *et al.*, 1994). Recently, TTase was purified and characterized from kale (Sa *et al.*, 1998). However, the enzymatic characteristics of plant TTase have been studied less extensively. In this paper, we report the purification and characterization of TTase from Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*).

Materials and Methods

Plant material Fresh Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*) was purchased from a local market at Chuncheon, Korea. It was washed very cleanly prior to the purification process.

Chemicals Bovine serum albumin (BSA), reduced glutathione (GSH), glutathione reductase (yeast), NADPH, Tris, silver nitrate, acrylamide, N,N′-methylenebisacrylamide, SDS, Coomassie Brilliant Blue R-250, N,N,N′,N′-tetramethylethylenediamine (TEMED), ammonium persulfate, EDTA, Sephadex G-50, Sephadex G-75, DEAE-Sephadex A-50, DEAE-Sephadex A-25, and Q-Sepharose were products of Sigma Chemical Co. (St. Louis, USA). Dehydroascorbic acid and 2-hydroxyethyl disulfide (HED) were from Aldrich Chemical Co. (Milwaukee, USA). DEAE-cellulose was a product of Whatman International Ltd. (Maidstone, England). Molecular weight standards for SDS-PAGE were obtained from Bio-Rad Laboratories (Richmond, USA). All other chemicals and reagents were of the highest grade commercially available.

Enzyme assay Since thioltransferase (or glutaredoxin) contained transhydrogenase activity, its activity was measured spectrophotometrically at 340 nm by the use of glutathione reductase as a coupling enzyme (Höög *et al.*, 1986). The reaction was proceeded in 0.4 ml of two cuvettes each containing 100 μg/ml of BSA, 1 mM GSH, 6 μg/ml yeast glutathione reductase, 0.4 mM NADPH, and 0.1 M Tris-HCl, pH 8.8, 2 mM EDTA. To each cuvette was added 40 μl of 15 mM 2-hydroxyethyl disulfide dissolved in 0.02 M Tris-HCl, pH 8.8. The absorbance at 340 nm was recorded for several minutes to ensure that both cuvettes were balanced with respect to the nonenzymatic spontaneous reaction between GSH and 2-hydroxyethyl disulfide. Enzyme was added to the sample cuvette and distilled water to the control cuvette. The change in absorbance resulting from the oxidation of NADPH was then recorded with time and its activity was expressed as ΔA_{340}/min. The K_m values of the enzyme for several substrates were determined by the method of Lineweaver and Burk in the standard assay system containing one of the disulfides in place of 2-hydroxyethyl disulfide.

To test whether the purified thioltransferase contains dehydroascorbate reductase activity, dehydroascorbic acid was used as a substrate. Dehydroascorbate reductase activity was measured by means of the spectrophotometric assay described by Stahl *et al.* (1983), which is based on the change in absorbance at 265.5 nm, as dehydroascorbic acid is reduced to ascorbic acid. The reaction mixture consisted of 0.1 M Tris-HCl, pH 8.8, 2 mM EDTA, 1 mM glutathione, 0.8 mM dehydroascorbic acid, and purified Chinese cabbage thioltransferase in a total volume of 0.5 ml. The reaction was initiated by adding dehydroascorbic acid and it was linear for up to 2 min at 20°C.

Protein determination Protein concentration was determined according to the procedure of Lowry *et al.* (1951) and Bradford (1976) using bovine serum albumin (BSA) as a standard. The protein content in fractions obtained during the chromatographic process was determined by measuring the absorbance at 280 nm.

Purification of thioltransferase All purification procedures were carried out at 4°C.

Step 1. Preparation of crude extract: Fresh Chinese cabbage (500 g) was ground up and disrupted by using a glass bead-beater in a total volume of 600 ml buffer A solution (0.02 M Tris-HCl, pH 8.8, 2 mM EDTA). After centrifugation for 90 min at 4500 rpm, the supernatant (Fraction I) was obtained for DEAE-cellulose ion-exchange chromatography.

Step 2. DEAE-cellulose chromatography: Fraction I was applied to a column (2.5 × 17 cm) of DEAE-cellulose pre-equilibrated with buffer A. The column was eluted with a linear gradient of 0–0.5 M NaCl in buffer A (total volume, 800 ml). The flow rate was 1.44 ml/min. The fractions containing thioltransferase activity (133 ml) were pooled and concentrated to 10 ml by centrifugation with Amicon Centriprep 3000 (Beverly, USA) (Fraction II).

Step 3. Sephadex G-75 gel filtration: Fraction II was applied to a Sephadex G-75 column (2.5 × 70 cm), which was eluted with buffer A. The flow rate was 0.27 ml/min. The fractions (96 ml) containing thioltransferase activity were pooled (Fraction III).

Step 4. Q-Sepharose chromatography: Fraction III was applied to a Q-Sepharose column (2.5 × 8 cm). The column was eluted with buffer A containing a linear gradient of 0–0.4 M NaCl (total volume, 400 ml). The flow rate was 1.2 ml/min. The fractions containing thioltransferase activity were pooled, and concentrated by Amicon Centriprep 3000 (Fraction IV).

Step 5. Sephadex G-50 chromatography: Fraction IV was applied to a Sephadex G-50 column (1.6 × 50 cm) which was eluted with buffer A. The flow rate was 0.36 ml/min. The fractions containing thioltransferase activity were pooled (Fraction V).

Step 6. DEAE-Sephadex A-50 chromatography: Fraction V was applied to a DEAE-Sephadex A-50 chromatography column (2.5 × 7 cm). The column was eluted with a linear gradient

(0–0.3 M NaCl) in buffer A (total volume, 200 ml). The flow rate was 0.34 ml/min. The active fractions were pooled (Fraction VI).

Step 7. DEAE-Sephadex A-25 chromatography: Fraction VI was dialyzed with Spectrum membrane tubing (MWCO: 6000–8000). After then, Fraction VI was applied to a DEAE-Sephadex A-25 chromatography column (2.5 × 7 cm). The column was eluted with a linear gradient (0–0.5 M NaCl) in buffer A (total volume, 100 ml). The flow rate was 0.2 ml/min. The active fractions were pooled (Fraction VII).

Step 8. Second Sephadex G-50 filtration: Fraction VII was concentrated by Amicon Centriprep 3000 and applied to a second Sephadex G-50 column (2.8 × 80 cm), which was eluted with buffer A. The flow rate was 0.3 ml/min. The fractions containing thioltransferase activity were pooled (Fraction VIII).

SDS-polyacrylamide gel electrophoresis SDS-polyacrylamide gel electrophoresis with 10–20% precasting gel was run according to the procedure described by Laemmli (1970). The gels were stained with silver nitrate.

Results and Discussion

Thioltransferase (or glutaredoxin) was purified from various organisms and identified to contain multiple biological functions. One of the important functions has been known to be the reduction of disulfide compounds including some disulfide proteins. By the use of this action as a tool in an enzyme assay, thioltransferase was purified and characterized from Chinese cabbage in this report.

Purification of thioltransferase from Chinese cabbage A thioltransferase was purified to homogeneity from Chinese cabbage according to the procedure described in Materials and Methods. It was purified by column chromatography in the order of DEAE-cellulose ion-exchange chromatography, gel filtration on Sephadex G-75, Q-Sepharose ion-exchange chromatography, gel filtration on Sephadex G-50, DEAE-Sephadex A-50 chromatography, DEAE-Sephadex A-25 chromatography, and gel filtration on a second Sephadex G-50. Table 1 summarizes the results of purification of thioltransferase from Chinese cabbage. After the final step, the second Sephadex G-50 gel filtration, the enzyme has been purified more than 1200-fold in an overall yield of 5.3% (Table 1).

On the profile of the second Sephadex G-50 gel filtration, the unique activity peak appeared to coincide with a protein peak. Its purity was tested on SDS-polyacrylamide gel electrophoresis (Fig. 1), and it showed a single protein band, indicating that Fraction VII is in a homogeneous state. Then, it could be confirmed that thioltransferase was successfully purified from Chinese cabbage.

To verify the identity of the purified enzyme from Chinese cabbage, the individual component was deleted in a complete assay (data not shown). It indicated that thioltransferase from Chinese cabbage absolutely requires glutathione and glutathione reductase for its action. It was also shown that the HED reduction activity was proportional to enzyme concentration (data not shown).

Molecular weight estimation The molecular weight of Chinese cabbage thioltransferase was estimated to be about

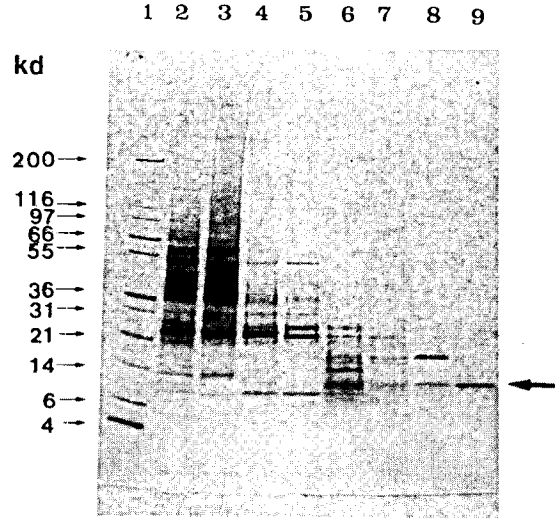

Fig. 1. Electrophoretic pattern on SDS-polyacrylamide gel. Electrophoresis was performed in 4–20% SDS-PAGE. Proteins were stained with silver nitrate. Lane 1, protein marker; lane 2, crude extracts; lane 3, DEAE-cellulose chromatography; lane 4, Sephadex G-75 gel filtration; lane 5, Q-Sepharose chromatography; lane 6, Sephadex G-50 chromatography; lane 7, DEAE-Sephadex A-50 chromatography; lane 8, DEAE-Sephadex A-25 chromatography; lane 9, second Sephadex G-50 chromatography.

Table 1. Purification of thioltransferase from Chinese cabbage.

Steps	Total protein (mg)	Total activity (ΔA_{340}/min)	Specific acitivty (ΔA_{340}/min/mg)	Yield (%)	Purification (-fold)
Crude extract	1003.3	495.0	0.49	100	1.00
DEAE-cellulose	594.5	305.9	0.51	62	1.04
Sephadex G-75	51.1	160.8	3.15	32	6.43
Q-Sepharose	21.0	114.1	5.40	23	11.02
Sephadex G-50	2.6	84.7	32.57	17	66.47
DEAE-Sephadex A-50	0.9	77.7	86.33	14	176.18
DEAE-Sephadex A-25	0.20	50.2	239.05	10	487.86
2nd Sephadex G-50	0.04	26.4	596.20	5.3	1216.73

12,000 on SDS-PAGE (Fig. 1). Thioltransferases are generally heat-stable proteins of approximately 12 kDa containing a GSH-dependent redox-active disulfide bond (Wells *et al.*, 1993). Plant thioltransferases from spinach and rice were found to have typical molecular sizes (MW 11,000).

Substrate specificity and kinetic properties We examined the kinetics of dependence on various substrates under standard conditions (Table 2). Kinetic parameters were obtained by Lineweaver-Burk plots. Chinese cabbage thioltransferase is able to reduce a variety of disulfides such as 2-hydroxyethyl disulfide, S-sulfocysteine, α-chymotrypsin, insulin, and trypsin. It showed K_m values of between 0.03 mM and 0.97 mM against those disulfide substrates. Catalytic efficiency was highest in the case of 2-hydroxyethyl disulfide. However, bovine serum albumin and ribonuclease A are not substrates for Chinese cabbage thioltransferase. It indicates that the purified enzyme is very specific on protein disulfides.

Dehydroascorbate reductase activity Mammalian thioltransferase has significant dehydroascorbate reductase activity, as reported by Wells *et al.* (1990). The specific dehydroascorbate reductase activity of the purified Chinese cabbage thioltransferase was about 93.9 ($\Delta A_{265.5}$/min/mg) in the presence of 0.8 mM dehydroascorbic acid. Figure 2 shows the kinetic behavior of the activity at various concentrations of dehydroascorbic acid. Chinese cabbage thioltransferase was found to have the K_m value of 0.79 mM for dehydroascorbic acid.

pH optimum The activity of Chinese cabbage thioltransferase as a function of pH was determined using the standard assay mixture. The maximal activity of the Chinese cabbage thioltransferase was observed at about pH 8.5 (Fig. 3), when 2-hydroxyethyl disulfide was used as

Table 2. Kinetic parameters of thioltransferase purified from Chinese cabbage against various substrates.

Substrate	V_{max} (ΔA_{340}/min)	K_m (mM)	Catalytic efficiency (V_{max}/K_m)
HED	0.101	0.05	2.02
S-Sulfocysteine	0.086	0.22	0.39
α-chymotrypsin	0.023	0.03	0.77
Insulin	0.315	0.97	0.32
Trypsin	0.100	0.18	0.56

The reaction mixture contained 100 μg/ml of bovine serum albumin, 1 mM GSH, 6 μg/ml yeast glutathione reductase, 0.4 mM NADPH, and 0.1 M Tris-HCl, 2 mM EDTA buffer (pH 8.8), and various amounts of disulfide in a total volume of 400 μl, and change in the absorbance at 340 nm was measured. The K_m and V_{max} values were obtained from Lineweaver-Burk plots.

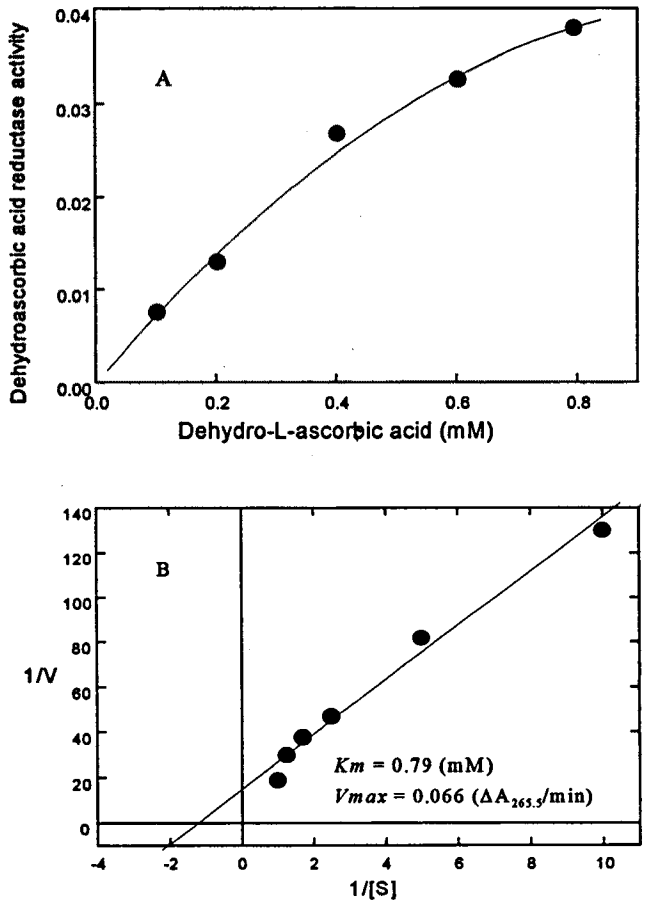

Fig. 2. Kinetics of Chinese cabbage thioltransferase activity on dehydroascorbic acid. A. The standard assay of dehydroascorbic activity is described in Materials and Methods. The dehydroascorbate reductase activity was detected at 265.5 nm resulting from appearance of ascorbic acid. B. A Lineweaver-Burk plot ($1/V$ versus $1/[S]$).

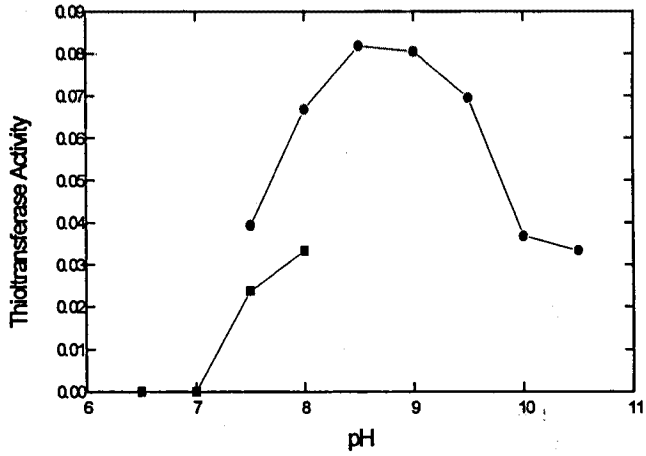

Fig. 3. Effects of pH on the activity of thioltransferase from Chinese cabbage. The initial velocities of thioltransferase activity were monitored in the standard assay system with buffer replaced by 0.1 M sodium phosphate (pH 6.5~8.0) (—■—) and 0.1 M Tris-HCl (pH 7.5~10.5) (—●—)

a substrate. Most of known thioltransferases have basic pH optima and the optimal pH values of pig, bovine, and rat thioltransferases are about pH 8.5, 8.5, and 9.0, respectively (Hatakeyama *et al.*, 1984; Gan and Wells, 1986; 1987a).

Effect of thiol compounds Since various thiol compounds were found to activate several thioltransferases, the effect of a few thiol compounds was tested on the thioltransferase from Chinese cabbage (Table 3). An enzyme activation of about 1.6-fold was observed in the presence of 0.5 mM reduced GSH. However, other thiol compounds tested did not significantly activate Chinese cabbage thioltransferase. The enzyme was found to have the K_m value of 0.41 mM for reduced glutathione (Fig. 4)

Heat stability Thioltransferase is generally believed to be heat-stable (Wells *et al.*, 1993). In this regard, the thermal stability of the purified thioltransferase was examined. The purified enzyme was incubated in a water bath with a temperature range from 40°C to 95°C for 30 min. After rapid cooling in an ice bath, the heat-treated samples were added to a standard assay to measure the HED reduction activity. Figure 5 shows that no significant loss of activity was observed at temperatures up to 95°C for 30 min. It indicated the thermostable nature of the enzyme.

In the present article, we described the purification and some properties of thioltransferase from Chinese cabbage. The precise physiological role of plant thioltransferase remains to be elucidated, but it is postulated to be related with sulfohydryl homeostasis and growth.

Acknowledgments This work was supported in part by the Basic Science Research Institute Program (BSRI-97-4439), the Ministry of Education, and the Korea Science and Engineering Foundation (grant no. 971-0501-003-2).

Table 3. Effects on Chinese cabbage thioltransferase activity by thiol compounds.

Thiol compounds	Relative activity (%)
None	100
Reduced glutathione	164
L-Cysteine	100
β-Mercaptoethanol	109
Dithiothreitol	118

The purified thioltransferase was pre-incubated at 30°C for 30 min containing 0.5 mM thiol compounds. The enzymatic activity of the incubation mixtures was measured in the standard assay and expressed as relative activity.

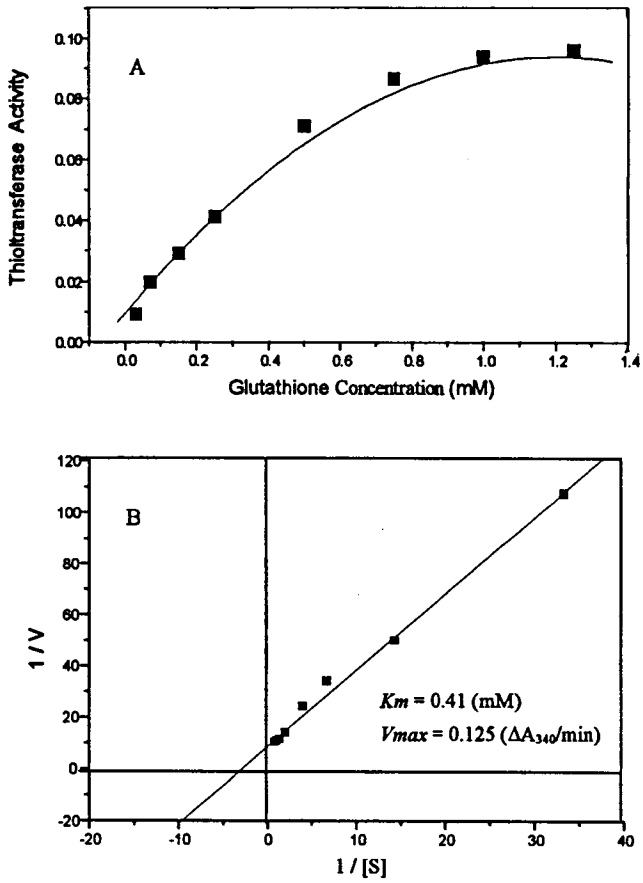

Fig. 4. Effects of GSH concentrations on Chinese cabbage thioltransferase activity. A. The thioltransferase activity (ΔA_{340}/min) was assayed by HED reduction assay with various GSH concentrations, and corrected for nonenzymatic cleavage by the use of control experiments. About 2.4 μg of enzyme was used per assay. B. A Lineweaver-Burk plot ($1/V$ versus $1/[S]$).

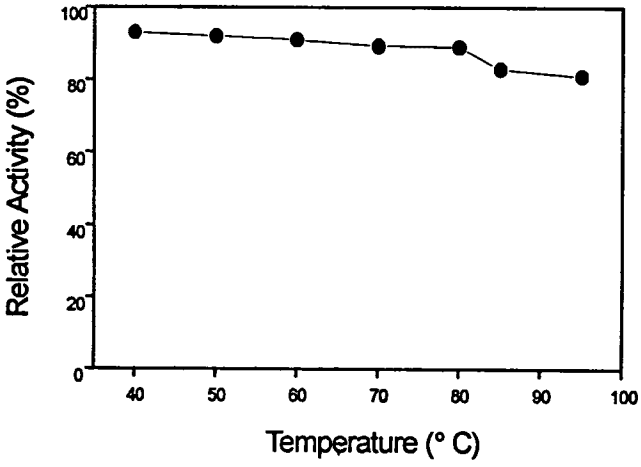

Fig. 5. Heat stability of the thioltransferase purified from Chinese cabbage. The thioltransferase was incubated in a water bath with a temperature range from 40°C to 95°C for 30 min. After rapid cooling in an icebox, the heat-treated sample was added to an assay mixture to measure thioltransferase activity.

References

Axelsson, K., Eriksson S. and Mannervik, B. (1978) Purification and characterization of cytoplasmic thioltransferase (glutathione:disulfide oxidoreductase) from rat liver. *Biochemistry* **17**, 2978–2984.

Axelsson, K. and Mannervik, B. (1983) An essential role of cytosolic thioltransferase in protection of pyruvate kinase from rabbit liver against oxidative inactivation. *FEBS Lett.* **152**, 114–118.

Bradford, M. M. (1976) A rapid and sensitive method for the quantity of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Chrestensen, C. A., Eckman, C. B., Starke, D. W. and Mieyal, J. J. (1995) Cloning, expression and characterization of human thioltransferase (glutaredoxin) in *E. coli*. *FEBS Lett.* **374**, 25–28.

Fuchs, J. A. (1977) Isolation of an *Escherichia coli* mutant deficient in thioredoxin reductase. *J. Bacteriol.* **129**, 967–972.

Gan, Z.-R. and Wells, W. W. (1986) Purification and properties of thioltransferase. *J. Biol. Chem.* **261**, 996–1001.

Gan, Z.-R. and Wells, W. W. (1987a) Preparation of homogeneous pig thioltransferase by a thiol:disulfide mediated pI shift. *Anal. Biochem.* **162**, 265–273.

Gan, Z.-R. and Wells, W. W. (1987b) The primary structure of pig liver thioltransferase. *J. Biol. Chem.* **262**, 6699–6703.

Goswami, A. and Rosenberg, I. N. (1985) Purification and characterization of a cytosolic protein enhancing GSH-dependent microsomal iodothyronine 5′-monodeiodination. *J. Biol. Chem.* **260**, 6012–6019.

Hatakeyama, M., Lee, C., Chon, C., Hayashi, M. and Mizoguchi, T. (1985a) Purification and some properties of rabbit liver cytosol thioltransferase. *J. Biochem.* **97**, 893–897.

Hatakeyama, M., Lee, C., Chon, C., Hayashi, M. and Mizoguchi, T. (1985b) Release of thioltransferase from rabbit's polymorphonuclear leucocytes by immune complex *in vitro* and inhibition of the enzyme by chloramphenicol. *Biochem. Biophys. Res. Commun.* **127**, 458–463.

Holmgren, A. (1976) Hydrogen donor system for *Escherichia coli* ribonuleotide-diphosphate reductase dependent upon glutathione. *Proc. Natl. Acad. Sci. USA* **73**, 2275–2279.

Holmgren, A. (1979a) Glutathione-dependent synthesis of deoxyribonucleotides: Purification and characterization of glutaredoxin from *Escherichia coli*. *J. Biol. Chem.* **254**, 3664–3671.

Holmgren, A. (1979b) Glutathione-dependent synthesis of deoxyribonucleotides. Characterization of the enzymatic mechanism of *Escherichia coli* glutaredoxin. *J. Biol. Chem.* **254**, 3672–3678.

Holmgren, A. (1979c) Thioredoxin catalyzes the reaction of insulin disulfides by dithiothreitol and dihydrolipoamide. *J. Biol. Chem.* **254**, 9627–9632.

Höög, J. O., von Bahr-Lindström, H., Jörnvall, H. and Holmgren, A. (1986) Cloning and expression of the glutaredoxin (*grx*) gene of *Escherichia coli*. *Gene* **43**, 13–21.

Hopper, S., Johnson, P. S., Vath, J. E. and Biemann, K. (1989) Glutaredoxin from rabbit bone marrow. *J. Biol. Chem.* **264**, 20438–20447.

Johnson, G. P., Goebel, S. J., Perkus, M. E., Davis, S. W., Winslow, J. P. and Paoletti, E. (1991) Vaccinia virus encodes a protein with similarity to glutaredoxins. *Virology* **181**, 378–381.

Klintrot, I.-M., Höög, I.-O., Jörnvall, H., Holmgren, A. and Luthman, M. (1984) The primary structure of calf thymus glutaredoxin. Homology with the corresponding *Escherichia coli* protein but elongation at both ends and with additional half-cystine/cysteine pair. *Eur. J. Biochem.* **144**, 417–423.

Kren, B., Parsell, D. and Fuchs, J. A. (1988) Isolation and characterization of an *Escherichia coli* K-12 mutant deficient in glutaredoxin. *J. Bacteriol.* **170**, 308–315.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680–685.

Lemaster, D. M. (1986) Nucleotide sequence and protein overproduction of bacteriophage T4 thioredoxin. *J. Virol.* **59**, 759–760.

Lowry, O. H., Rogebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Luthman, M., Ericksson, S., Holmgren, A. and Thelander, L. (1979) Glutathione-dependent hydrogen donor system for calf thymus ribonucleoside-diphosphate reductase. *Proc. Natl. Acad. Sci. USA* **76**, 2158–2162.

Luthman, M. and Holmgren, A. (1982) Glutaredoxin from calf thymus: Purification to homogeneity. *J. Biol. Chem.* **257**, 6686–6690.

Mark, D. F. and Richardson, C. C. (1976) *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA* **73**, 780–784.

Mieyal, J. J., Starke, D. W., Garvina, S. A., Dothey, C. and Chung, J. S. (1991) Thioltransferase in human red blood cells: Purification and properties. *Biochemistry* **30**, 6088–6097.

Minakuchi, K., Yabushita, T., Masumura, T., Ichihara, K. and Tanaka, K. (1994) Cloning and sequence analysis of a cDNA encoding rice glutaredoxin. *FEBS Lett.* **337**, 157–160.

Morell S., Follmann, H. and Höberlein, I. (1995) Identification and localization of the first glutaredoxin in leaves of a higher plant. *FEBS Lett.* **369**,149–152.

Papayannopoulos, I. A., Gan, Z.-R., Wells, W. W. and Biemann, K. (1989) A revised sequence of calf thymus glutaredoxin. *Biochem. Biophys. Res. Commun.* **159**, 1448–1454.

Papov, V. V., Gravina, S. A., Mieyal, J. J. and Biemann, K. (1994) The primary structure and properties of thioltransferase (glutaredoxin) from human red blood cells. *Protein Sci.* **3**, 428–434.

Sa, J.-H., Kim, K. and Lim, C.-J. (1997) Purification and characterization of glutaredoxin from *Cryptococcus neoformans*. *Mol. Cells* **7**, 655–660.

Sa, J.-H., Yong, M.-Y., Song, B.-L. and Lim, C.-J. (1998) Characterization of thioltransferase from kale. *J. Biochem. Mol. Biol.* **31**, 20–24.

Sha, S., Minakuchi, K., Higaki, N., Sato, K., Ohtsuki, K., Kurata, A., Yoshikawa, H., Kotaru, M., Masumura, T., Ichihara, K. and Tanaka, K. (1997) Purification and characterization of glutaredoxin (thioltransferase) from rice (*Oryza sativa* L.). *J. Biochem.* (Tokyo) **121**, 842–848.

Stahl, R. L., Liebes, L. F., Farber, C. M. and Silber, R. (1983) A spectrophotometric assay for dehydroascorbate reductase. *Anal. Biochem.* **131**, 341–344.

Tsang, M. L.-S. (1981) Assimilatory sulfate reduction in *Escherichia coli*: Identification of the alternate cofactor for adenosine 3′-phosphate 5′-phosphate reductase as glutaredoxin. *J. Bacteriol.* **146**, 1059–1066.

Wells, W. W., Yang, Y., Deits, T. L. and Gan, Z.-R. (1993) Thioltransferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **66**, 149–201.

Wells, W. W., Xu, D. P., Yang, Y. and Rocque, P. A. (1990) Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dihydroascorbate reductase activity. *J. Biol. Chem.* **265**, 15361–15364.