Sweet potato storage root thioredoxin *h2* with both dehydroascorbate reductase and monodehydroascorbate reductase activities

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ABSTRACT. Recombinant thioredoxin *h* (Trx *h*2) overproduced in *E. coli* (M15) was purified by Ni^{2+}-chelate affinity chromatography as previously reported (Huang et al., 2004a). The molecular mass of Trx *h*2 was ca. 14 kDa determined by SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis). It had antioxidant activity (Huang et al., 2004b) and it reduced dehydroascorbate (DHA) in the presence of glutathione to regenerate ascorbate (AsA). However, without glutathione, Trx *h*2 had very low DHA reductase activity. AsA was oxidized by AsA oxidase to generate monodehydroascorbate (MDA) free radicals. MDA was also reduced by Trx *h*2 to AsA in the presence of NADH mimicking the MDA reductase catalyzed reaction. These data suggest that Trx *h*2 has both DHA reductase and MDA reductase activities.

Keywords: Dehydroascorbate reductase; Monodehydroascorbate reductase; Sweet potato storage roots; Thioredoxin *h*.

INTRODUCTION

Ascorbate (AsA) plays an important role in protecting plant cells against the action of reactive oxygen species (Dalton et al., 1986; Hou et al., 2000). In plants, peroxide-scavenging is accomplished through the AsA-glutathione pathway, a coupled series of redox reactions involving four enzymes: AsA-specific peroxidase (EC 1.11.1.11), monodehydroascorbate (MDA) reductase (EC 1.6.5.4), dehydroascorbate (DHA) reductase (EC 1.8.5.1), and glutathione reductase (EC 1.6.4.2) (Dalton et al., 1993; Leonardis et al., 1995). This pathway has been studied mainly in chloroplasts, in which the possible reactive oxygen species produced by photosystem I during photosynthesis might cause serious damage. However, the AsA-glutathione pathway has also been found in cytosol (Borraccino et al., 1986; Elia et al., 1992), mitochondria (Lunde et al., 2006), and peroxisomes (Jimenez et al., 1997). When AsA functions as an antioxidant in cells, it is oxidized to MDA free radical, and MDA reductase catalyzes the reduction of MDA back to AsA with NAD(P)H (Hossain et al., 1984). MDA was a sensitive endogenous index of oxidative stress in leaf tissues (Heber et al., 1996).

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Thioredoxins, the ubiquitous small proteins with a redox active disulfide bridge, are important regulatory elements in a number of cellular processes (Buchanan, 1991). They all contain a distinct active site, WCGPC, which is able to reduce the disulfide bridges of target proteins. Initially described as hydrogen carriers in ribonucleotide reduction in *E. coli*, they were found to serve as electron donors in a variety of cellular redox reaction (Holmgren, 1985). From genome sequencing data, a significant diversity of thioredoxin genes containing five different multigenic families (f, m, x, o and h) was observed (Mestres-Ortega and Meyer, 1999; Meyer et al., 2002; Balmer and Buchanan, 2002). The ferredoxin-thioredoxin system (thioredoxins f and m) has been proven to regulate several enzymatic activities associated with photosynthetic CO_2 assimilation in chloroplasts. Thioredoxin x contains a transit peptide similar to those required for chloroplast and mitochondria targeting; however, its function is not clearly defined (Mestres-Ortega and Meyer, 1999). A new type of plant mitochondrial thioredoxin o was also shown to regulate the activities of several mitochondrial proteins by disulfide bond reduction (Laloi et al., 2001).

Thioredoxin *h* is generally assumed to be cytosolic, which was supported by the absence of a transit peptide in the genes cloned for the isoforms from tobacco (Marty and

Meyer, 1991; Brugidou et al., 1993), *Arabidopsis* (Rivera-Madrid et al., 1993; 1995), *Triticum aestivum* (Gautier et al., 1998), germinating wheat seeds (Serrato et al., 2001) and barley seed proteome (Kenji et al., 2003). Moreover, the existence of several forms of thioredoxin *h* detected in spinach leaves (Florencio et al., 1988), and wheat flour (Johnson et al., 1987) supports the view that higher plants possess multiple and divergent thioredoxin genes (Rivera-Madrid et al., 1995). In this study, we present evidence to show that the recombination protein, thioredoxin *h*2 exhibits both DHA reductase and MDA reductase activities.

MATERIALS AND METHODS

Chemicals

Ascorbate, dehydroascorbate, electrophoresis grade acrylamide and Bis (*N*, *N′*-methylenediacrylamide), TEMED (*N*, *N*, *N′*, *N′*-tetramethylethylenediamine) and APS (ammonium persulfate) were from E. Merck Inc. (Germany). Other chemicals and solvents were purchased from Sigma Chemical Company (St. Louis, MO). The low molecular weight kits for electrophoresis were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden).

Expression of thioredoxin *h2* in *E. coli*

Thioredoxin *h*2 (Gene Bank accession number: AY344228; Trx *h*2) was expressed in *E. coli*. The coding sequence was amplified from Trx *h*2 cDNA using an oligonucleotide (5′-GAG AGG ATC CAA TGG GAG GGG CT-3′) with a *BamH*I site (underlined) at the putative initial Met redisue, and an oligonucleotide (5′-ATT TGA AGC TTG ATT GAT GCT-3′) with a *Hind*III site at the 3′ end. The PCR fragment was subcloned in pGEM T-easy vector, and the plasmid was then digested with *BamH*I and *Hind*III and subcloned in pQE32 expression vector (QIAexpress expression system, Qiagen). The resulting plasmid, termed pQE-Trx *h*2, was introduced into *E. coli* (M15). Cultures of the transformed *E. coli* (M15) overexpressed a protein of the expected molecular mass, which was purified by affinity chromatography in Ni-NTA columns (Qiagen), according to Huang et al (Huang et al., 2007).

DHA reductase activity assay

The DHA reductase activity of Trx *h*2 was assayed according to the method of Trümper et al. (Trümper et al., 1994) with some modifications. Ten milligrams DHA were dissolved in 5.0 mL of 100 mM phosphate buffer with two pH values (pH 6 and 7). The reaction was carried out at 30 °C by adding 100 µL Trx *h*2 solution (100 µg protein) to 0.9 mL DHA solution with or without 4 mM glutathione. The increase of absorbance at 265 nm was recorded for 5 min. The non-enzymatic reduction of DHA in phosphate buffer was measured in a separate cuvette at the same time. A standard curve was plotted using 0.1-50 nmol AsA (Jung et al., 2002; Washburn and Wells, 1999).

MDA reductase activity assay

The MDA reductase activity of Trx *h*2 was assayed according to Hossain et al. (Hossain et al., 1984) by following the decrease in absorbance at 340 nm due to NADH oxidation. MDA free radicals were generated by AsA oxidase (EC 1.10.3.3) in the assay system (Yamazaki and Pette, 1961). The reaction mixtures contained 50 mM phosphate buffer (pH 6 and 7, respectively), 0.33 mM NADH, 3 mM AsA, AsA oxidase (0.9 U), and 200 µL Trx *h*2 solution (200 µg protein) in a final volume of 1 mL. Trx *h*2 solution was replaced with glutathione for controls.

Protein stainings of thioredoxin *h2* in 15% SDS-PAGE gels

Trx *h*2 were examined by protein staining in 15% SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) gels (Huang et al., 2004c; 2007). Twenty microliter samples were mixed with 25 µL sample buffer containing 60 mM Tris buffer (pH 6.8), 2% SDS, 25% glycerol and 0.1% bromophenol blue, with 2-mercaptoethanol (2-ME) in a final concentration of 14.4 mM, and heated at 100°C for 5 min for protein staining. Coomassie brilliant blue G-250 was used for protein staining (Hou et al., 2002). The protein concentration of the supernatant was determined by the Bradford dye-binding assay (Bio-Rad, Hercules, CA).

MDA reductase activity staining in 15% SDS-PAGE gels

Trx *h*2 were examined for MDA reductase by activity stainings in 15% SDS-PAGE gels. Diaphorase activity staining for MDA reductase activity of Trx *h*2 was according to the methods of Kaplan and Beutler (Kaplan and Beutler, 1967) in a 15% SDS-PAGE gel. After electrophoresis, the gel was washed with 25% isopropanol in 10 mM Tris buffer (pH 7.9) twice to remove SDS before activity staining.

Statistical Analysis. Means of triplicate were calculated. Student's *t* test was used for comparison between two treatments. A difference was considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of pH (6 and 7) on dehydroascorbate reductase activity of thioredoxin *h2*

To express sweet potato Trx *h*2 in *E. coli*, the coding sequence of Trx *h*2 was subcloned in a pQE-32 expression vector so that sweet potato thioredoxin *h* was produced with a 6x His-tag at the N-terminus. SDS-PAGE analysis of crude extracts from transformed *E. coli* (M15) showed a high level of a polypeptide with the expected molecular mass (ca. 14 kDa). The expressed protein was purified from crude extracts by Ni^{2+}-chelate affinity chromatography, which yielded highly purified His-tagged thiredoxin *h2* (Huang et al., 2004b).

The purified Trx *h*2 samples were used to examine DHA reductase activity. Figure 1 shows AsA regeneration (ΔA 265 nm) from DHA at both pH 6 and 7 with (A) or without (B) glutathione. Figure 1A shows that Trx *h*2 exhibited DHA reductase activity and was able to reduce DHA back to AsA. The specific activities of DHA reductase for Trx *h*2 in the presence of glutathione were 7.17 and 35.91 nmol AsA produced/min/mg protein at pH 6 and 7, respectively. However, in the absence of glutathione, very low DHA reductase activities of Trx *h*2 were found (Figure 1B): only 0.01 and 0.68 nmol AsA were produced/min/mg protein at pH 6 and 7, respectively. Trx *h*2 acts as a GSH-dependent DHA reductase (Figure 2), and the rate of reduction was closely proportional to the concentration of GSH. There was a significant increase in DHA activity treated with 1, 2, 3 and 4 mM GSH at pH 7 ($p < 0.05$). It was reported that thioredoxin m and thioredoxin f from spinach chloroplast and thioredoxin from *E. coli* exhibit very low DHA reductase activities without glutathione (Kobrehel et al., 1992).

Effect of pH (6 and 7) on monodehydroascorbate reductase activity of thioredoxin *h*.

MDA was reduced to AsA in coupling with NADH oxidation (ΔA340 nm) at pH 6 and 7 when Trx *h*2 was used as MDA reductase. Trx *h*2 exhibited MDA reductase activity at pH 6 and 7 (Figure 3), with higher activity at pH 6 than pH 7 in our assay system. Trx *h*2 acts as a GSH-dependent MDA reductase (Figure 3), and the rate of reduction was closely proportional to the concentration of GSH.

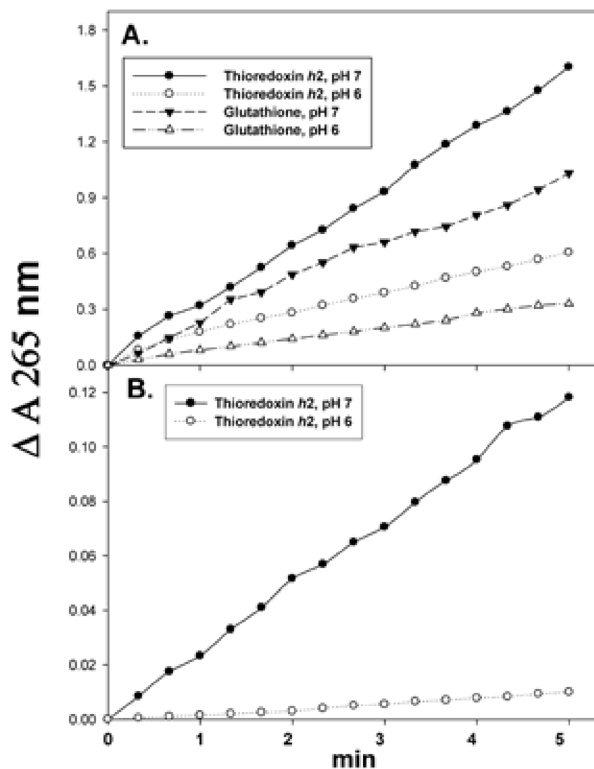

Figure 1. Effect of pH (6 and 7) on dehydroascorbate reductase activity. Purified recombinant protein of thioredoxin *h2* was with (A) or without (B) 4 mM glutathione in the reaction mixtures. The reaction was carried out at 30°C by adding 100 µL thioredoxin *h2* solution (100 µg protein, 100 mM phosphate buffer, pH 7 and 6) to 0.9 mL DHA solution with or without 4 mM glutathione. Glutathione was used as a control. The increase of absorbance at 265 nm was recorded for 5 min.

Protein and diaphorase activity stainings in 15% SDS-PAGE gels for detection of monodehydroascorbate reductase activity of thioredoxin *h*2.

MDA reductase activity staining of Trx *h*2 was done for

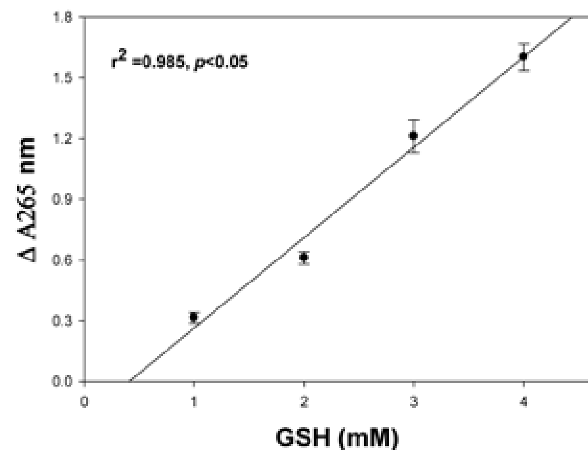

Figure 2. Dependence of dehydroascorbate reductase activity of thioredoxin *h2* on GSH concentration. The reaction was carried out at 30°C by adding 100 µL thioredoxin *h2* solution (100 µg protein, 100 mM phosphate buffer, pH 7) to 0.9 mL DHA solution with different concentrations of glutathione. The increase of absorbance at 265 nm was recorded for 5 min.

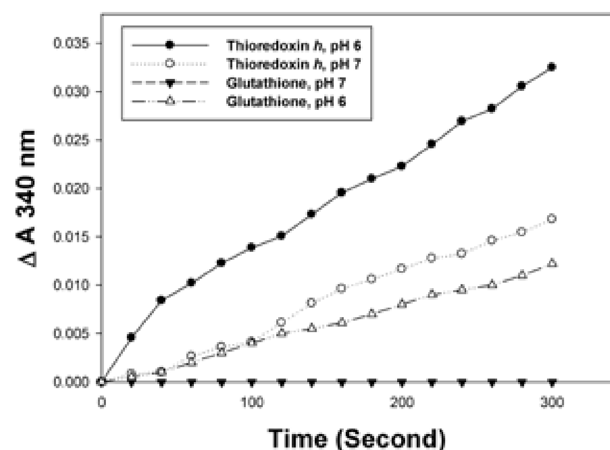

Figure 3. Effect of pH (6 and 7) on monodehydroascorbate reductase activity of thioredoxin *h2*. The reaction mixtures contained 50 mM phosphate buffer (pH 6 and 7), 0.33 mM NADH, 3 mM AsA, AsA oxidase (0.9 U), and 200 µL thioredoxin *h2* solution (200 µg protein) in a final volume of 1 mL. Thioredoxin *h2* solution was replaced with glutathione for controls.

diaphorase activity (Kaplan and Beutler, 1967) on SDS-PAGE gels (Figure 4). Comparing Figure 4A (protein staining) with Figure 4B of Trx *h*2, one can see that the diaphorase activity staining for MDA reductase activity came from 14 kDa Trx *h*2. MDA reductase and DHA reductase were shown to contain free thiol groups in their catalytic sites (Borraccino et al., 1989). When AsA is the sole hydrogen donor, the AsA peroxidase, guaiacol peroxidase, and AsA oxidase can produce MDA (Kaplan and Beutler, 1967). Nonenzymatic oxidations of AsA also produce MDA when cells were under oxidative stress (Hossain et al., 1984). Dimerization of heat shock protein 25 via S-S bond formation can occur in cells in response to various oxidative stresses (Zavialov et al., 1998).

DISCUSSION

This is the first report showing that Trx *h*2 displays both DHA reductase and MDA reductase activities with some unique characteristics.

In many physiological studies DHA reductase is regarded as one of the chloroplast enzymes involved in the protection against oxidative stress. A specific DHA reductase is frequently demanded as part of the enzymatic equipment to avoid oxidative stress. In plant extracts, a glutathione-dependent DHA reductase activity which will recycle DHA to ascorbate has been observed (Hossain et al., 1984). An increase of DHA reductase activity and an accumulation of DHA have been frequently implied as biochemical indicators of oxidative stress in plant metabolism (Wise, 1995; Hung et al., 2005) but a characterization of DHA reductase has remained elusive because of rapid loss of enzyme activity (Hou et al., 1997; 1999).

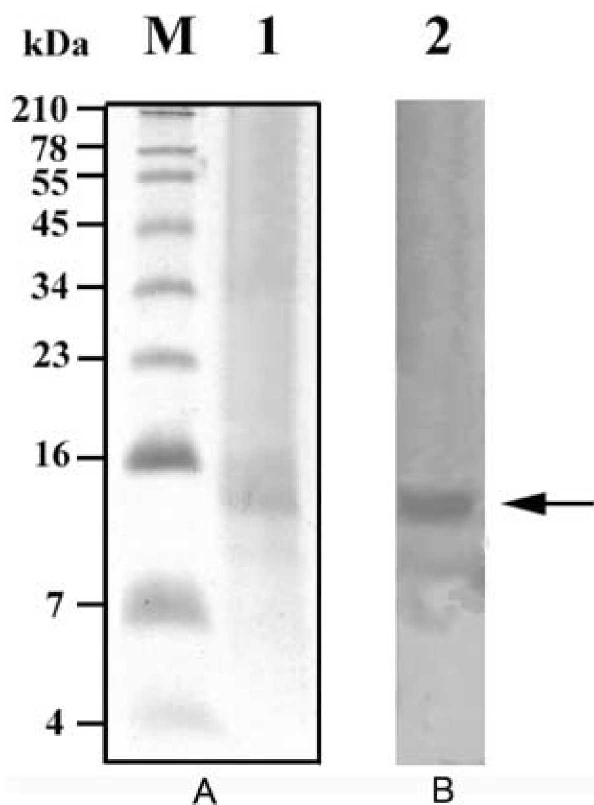

Figure 4. Protein (A) and diaphorase activity (B) stainings in 15% SDS-PAGE gels for detection of monodehydroascorbate reductase activity of thioredoxin *h2*. The experiments were done twice and a representative one is shown. ‘M’ represents the molecular weight marker and 10 µg protein was loaded in each well.

The thioredoxin system is vital for chloroplast metabolism because redox control of at least 12 different enzymes is achieved by the reductive cleavage of regulatory disulfide bridges in these target enzymes (Buchanan, 1991). Trx *h*2 thiol-disulfide interchanges were found during DHA reduction to regenerate AsA. Thionin was reported to have intermolecular disulfide linkages with other proteins (Pinerio et al., 1995). Thiol groups are central to most redox-sensitive processes in the cell, and their redox state controls cellular processes such as growth, differentiation, and apoptosis. Intracellular thiol homeostasis is maintained by the thioredoxin systems, which utilize reducing equivalents from NADPH to reduce both protein and low molecular weight disulfides.

MDA reductase purified from potato was shown to contain thiol groups in its catalytic sites (Leonardis et al., 1995). Fernando et al. (1992) found that thioredoxin can act as a radical scavenger and facilitate the regeneration of oxidatively damaged proteins and Trx *h*2 might contribute to its antioxidant activities against hydroxyl and peroxyl radicals (Huang et al., 2004b). When AsA is the sole hydrogen donor, the AsA oxidase can produce MDA (Yamazaki and Pette, 1961). Nonenzymatic oxidations of AsA also produce MDA when cells suffer from oxidative stress (Heber et al., 1996). Taking the above results into consideration, we constructed a reduction scheme of both DHA and MDA to AsA catalyzed by the Trx *h*2 of sweet potato roots. DHA and MDA can be reduced to regenerate AsA by Trx *h*2 in order to prevent oxidative damage to cytosols of sweet potato storage roots.

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LITERATURE CITED

Balmer, Y. and B.B. Buchanan. 2002.Yet another plant thioredoxin. Trends Plant Sci. **7:** 191-193.

Borraccino, G., S. Dipierro, and O. Arrigoni. 1989. Interaction of ascorbate free radical reductase with sulphhydryl reagents, Phytochemistry **28:** 715-717.

Borraccino, G., S. Dipierro, and O. Arrigoni. 1986. Purification and properties of ascorbate free-radical reductase from potato tubers. Planta **167:** 521-526.

Brugidou, C., I. Marty, Y. Chartier, and Y. Meyer. 1993. The

Nicotiana tabacum genome encodes two cytoplasmic thioredoxin genes which are differently expressed. Mol. Gen. Genet. **238:** 285-293.

Buchanan, B.B. 1991. Regulation of CO_2 assimilation in oxygenic photosynthesis: The ferredoxin/thioredoxin system. Perspective on its discovery, present status and future development. Arch. Biochem. Biophys. **288:** 1-9.

Dalton, D.A., L.M. Baird, L. Langeberg, C.Y. Taugher, W.R. Anyan, C.P. Vance, and G. Sarath. 1993. Subcellular location of oxygen defense enzymes in soybean (*Glycine max* [L.] Merr.) root nodules. Plant Physiol. **102:** 481-489.

Dalton, D.A., S.A. Russell, F.J. Hanus, G.A. Pascoe, and H.J. Evans. 1986. Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. Proc. Natl. Acad. Sci. USA **83:** 3811-3815.

Elia, M.R., G. Borraccino, and S. Dipierro. 1992. Soluble ascorbate peroxidase from potato tubers. Plant Sci. **85:** 17-21.

Fernando, M.R., H. Nanri, S. Yoshitake, K. Nagatakuno, and S. Minakami. 1992. Thioredoxin regenerates proteins inactivated by oxidative stress in endothelial cell. Eur. J. Biochem. **209:** 917-922.

Florencio, F.J., B.C. Yee, T.C. Johnson, and B.B. Buchanan. 1988. An NADP/thioredoxin system in leaves: purification and characterization of NADP-thioredoxin reductase and thioredoxin *h* from spinach. Arch. Biochem. Biophys. **26:** 496-507.

Gautier, M.F., V. Lullien-Pellerin, F. Lamotte-Guery, A. de Guirao, and P. Joudrier. 1998. Characterization of wheat thioredoxin *h* cDNA and production of an active *Triticum aestivum* protein in *Escherichia coli*. Eur. J. Biochem. **252:** 314-324.

Heber, U., C. Miyake, J. Mano, C. Ohno, and K. Asada. 1996. Monodehydroascorbate radical detected by electron paramagnetic resonance spectrometry is a sensitive probe of oxidative stress in intact leaves. Plant Cell Physiol. **37:** 1066-1072.

Holmgren, A. 1985. Thioredoxin. Annu. Rev. Biochem. **54:** 237-271.

Hossain, M.A., Y. Nakano, and K. Asada. 1984. Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol. **25:** 385-395.

Hou, W.C., D.J. Huang, and Y.H. Lin. 2002. An aspartic type protease degrades trypsin inhibitors, the major root storage proteins of sweet potato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). Bot. Bull. Acad. Sin. **43:** 271-276.

Hou, W.C., H.J. Chen, and Y.H. Lin. 2000. Dioscorins from different *dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. Bot. Bull. Acad. Sin. **41:** 191-196.

Hou, W.C. and Y.H. Lin. 1997. Dehydroascorbate reductase and monodehydroascorbate reductase activities of trypsin inhibitors, the major sweet potato (*Ipomoea batatas* [L.] Lam.) root storage protein. Plant Sci. **128:** 151-158.

Hou, W.C., H.J. Chen, and Y.H. Lin. 1999. Dioscorins, the major tuber storage proteins of yam (*Dioscorea batatas* Decne), with dehydroascorbate reductase and monodehydroascorbate reductase activities. Plant Sci. **149:** 151-156.

Huang, D.J., H.J. Chen, W.C. Hou, and Y.H. Lin. 2004a. Isolation and characterization of thioredoxin *h* cDNA from sweet potato (*Ipomoea batatas* [L.] Lam. ‘Tainong 57’) storage roots. Plant Sci. **166:** 515-523.

Huang, D.J., H.J. Chen, W.C. Hou, C.D. Lin, and Y.H. Lin. 2004b. Active recombinant thioredoxin h protein with antioxidant activities from sweet potato (*Ipomoea batatas* [L.] Lam. ‘Tainong 57’) storage roots. J. Agri. Food Chem. **52:** 4720-4724.

Huang, D.J., H.J. Chen, W.C. Hou, T.E. Chen, and Y.H. Lin. 2004c. In vitro reduction of trypsin inhibitor by purified NADPH/ Thioredoxin system from sprouts of sweet potato (*Ipomoea batatas* (L) Lam.) storage roots. Plant Sci. **166:** 435-441.

Huang, G.J., H.J. Chen, Y.S. Chang, M.J. Sheu, and Y.H. Lin. 2007. Recombinant Sporamin and its synthesized peptides with Antioxidant Activities in vitro. Bot. Stud. **48:** 133-140.

Hung, S.H., C.W. Yu, and C.H. Lin. 2005. Hydrogen peroxide functions as a stress signal in plants. Bot. Bull. Acad. Sin. **46:** 1-10.

Isabelle, B. and B.B. Buchanan. 1997. Thioredoxin-linked plant and animal process: the new generation. Bot. Bull. Acad. Sin. **38:** 1-11.

Jimenez, A., J.A. Hernandez, L.A. del Rio, and F. Sevilla. 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. **114:** 275-284.

Johnson, T.C., K. Wada, and B.B. Buchanan. 1987. Reduction of purothionin by the wheat seed thioredoxin system. Plant Physiol. **85:** 446-451.

Jung, C.H., M.P. Washburn, and W.W. Wells. 2002. Ebselen has dehydroascorbate reductase and thioltransferase-like activities. Biochem Biophys Res. Commun. **291(3):** 550-553.

Kaplan, J.C. and E. Beutler. 1967. Electrophoresis of red cell NADH- and NADPH-diaphorases in normal subjects and patients with congenital methemoglobinemia. Biochem. Biophys. Res. Commun. **29:** 605-610.

Kenji, M., C. Finnie, O. Østergarrrd, and B. Svensson. 2003. Identification, cloning and characterization of two thioredoxin *h* isoforms, HvTrxh1 and HvTrxh2, from the barley seed proteome. Eur. J. Biochem. **270:** 2633-2643.

Kobrehel, K., J.H. Wong, A. Balogh, F. Kiss, B.C Yee, and B.B. Buchanan. 1992. Specific reduction of wheat storage proteins by thioredoxin *h*. Plant Physiol. **99:** 919-924.

Laloi, C., N. Rayapuram, Y. Chartier, J.M. Grienenberger, G. Bonnard, and Y. Meyer. 2001. Identification and characterization of mitochondrial thioredoxin system in plants. Proc. Natl. Acad. Sci. USA **98:** 14144-14149.

Leonardis, S.D., G.D. Lorenzo, G. Borraccino, and S. Dipierro. 1995. A specific ascorbate free radical reductase isozyme participates in the regeneration of ascorbate for scavenging toxic oxygen species in potato tuber mitochondria. Plant Physiol. **109:** 847-851.

Lunde, C., U. Baumann , N.J. Shirley, D.P. Drew, and G.B. Fincher. 2006. Gene structure and expression pattern analysis of three monodehydroascorbate reductase (Mdhar) genes in Physcomitrella patens: implications for the evolution of the MDHAR family in plants. Plant Mol. Biol. **60(2):** 259-275.

Marty, I. and Y. Meyer. 1991. Nucleotide sequence of a cDNA encoding a tobacco thioredoxin. Plant Mol. Biol. **17:** 143-147.

Mestres-Ortega, D. and Y. Meyer. 1999. The Arabidopsis thaliana genome encodes at least four thioredoxins m and a new prokaryotic-like thioredoxin. Gene **240:** 307-316.

Meyer, Y., F. Vignols, and J.P. Reichheld. 2002. Classification of plant thioredoxins by sequence similarity and intron position. Methods Enzymol. **347:** 394-402.

Pinerio, M., I. Diaz, P. Rodriguez-Palenzuela, E. Titarenko, and F. Garcia-Olmedo. 1995. Selective disulphide linkage of plant thionins with other proteins. FEBS Lett. **369:** 239-242.

Rivera-Madrid, R., D. Mestres, P. Marinho, J.P. Jacquot, P. Decottignies, M. Miginiac-Maslow, and Y. Meyer. 1995. Evidence for five divergent thioredoxin h sequences in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **92:** 5620-5624.

Rivera-Madrid, R., P. Marinho, C. Brugidou, Y. Chartier, and Y. Meyer. 1993. Nucleotide sequence of a cDNA clone encoding an *Arabidopsis thaliana* thioredoxin *h*. Plant Physiol. **102:** 327-328.

Serrato, A.J., J.L. Crespo, F.J. Florencio, and J.C. Francisco. 2001. Characterization of two thioredoxins *h* with predominant localization in the nucleus of aleurone and scutellum cells of germinating wheat seeds. Plant Mol. Biol. **46:** 361-371.

Trümper, S., H. Follmann, and I. Haberlein. 1994. A novel dehydroascorbate reductase from spinach chloroplasts homologous to plant trypsin inhibitor. FEBS Lett. **352:** 159-162.

Washburn, M.P. and W.W. Wells. 1999. Identification of the dehydroascorbic acid reductase and thioltransferase (Glutaredoxin) activities of bovine erythrocyte glutathione peroxidase. Biochem Biophys Res Commun. **257(2):** 567-571.

Wise, R.R. 1995. Chilling-enhanced photooxidation: The production, action and study of reactive oxygen species produced during chilling in the light. Photosynthesis Res. **45:** 79-97.

Yamazaki, I. and L.H. Pette. 1961. Mechanism of free radical formation and disappearance during the ascorbic acid oxidase and peroxidase reactions. Biochem. Biophys. Acta **50:** 62-69.

Zavialov, A.V., M. Gaestel, T. Korpela, and V.P. Zav'yalov. 1998. Thiol:disulfide exchange between small heat shock protein 25 and glutathione. Biochem. Biophys. Acta **1388:** 123-132.

甘藷塊根中硫氧化還原蛋白 *h2* 具有去氫抗壞血酸還原酶和單去氫抗壞血酸還原酶的活性

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在大腸桿菌 (M15) 中大量表現重組蛋白質硫氧化還原蛋白 *h2* (Trx *h2*)，利用鎳離子螯合之親和性管柱純化。Trx *h2* 經 SDS-PAGE 分析其分子量約為 1.4 kDa 。Trx *h2* 在含有穀胱甘肽時，去氫抗壞血酸 (dehydroascorbate, DHA) 含量會降低而生成抗壞血酸 (ascorbate, AsA)。但是，在不含有穀胱甘肽時，Trx *h2* 只有非常低 DHA reductase 活性。AsA 經由 AsA 氧化酶氧化生成單去氫抗壞血酸 (monodehydroascorbate, MDA) 自由基。MDA 也可經由 Trx *h2* 而降低了 AsA 生成，在 NADH 存在時模仿 MDA reductase 催化反應。這結果建議，Trx *h2* 同時具有去氫抗壞血酸還原酶和單去氫抗壞血酸還原酶的活性。

關鍵詞：甘藷塊根；硫氧化還原蛋白 *h2*；去氫抗壞血酸還原酶；單去氫抗壞血酸還原酶。