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Cytosolic malate dehydrogenase (cytMDH) was captured by thioredoxin affinity chromatography as a possible target protein of cytosolic thioredoxin (Yamazaki, D., Motohashi, K., Kasama, T., Hara, Y., and Hisabori, T. (2004) Plant Cell Physiol. 45, 18–27). To further dissect this interaction, we aimed to determine whether cytMDH can interact with the cytosolic thioredoxin and whether its activity is redox-regulated. We obtained the active recombinant cytMDH that could be oxidized and rendered inactive. Inactivation was reversed by incubation with low concentrations of dithiothreitol in the presence of recombinant thioredoxin affinity chromatography. Moreover, we found this bond to be efficiently reduced by the reduced form of thioredoxin-h1. These results demonstrate that the oxidized form cytMDH dimer is a preferable target protein of the reduced form thioredoxin-h1 as suggested by thioredoxin affinity chromatography.

Malate dehydrogenase (MDH)3 catalyzes the reversible reaction of oxaloacetate to malate utilizing the NAD+/NADH or NADP+/NADPH cofactor system and is known to occur ubiquitously in mammals, plants, and most prokaryotes. NAD-dependent MDHs (EC 1.1.1.37) generally exist as a homodimer with a subunit of 32–37 kDa (1, 2), whereas NADP-dependent MDH (EC 1.1.1.82) possesses a larger subunit of ~42 kDa (3, 4). In plants, MDH isoforms have been reported in the cytosol and in organelles and have been found to carry out important roles in a number of metabolic pathways (5). In general, MDH proteins are grouped into five classes according to their location within the cell; (i) cytosolic NAD-dependent MDH (cytMDH); (ii) mitochondrial NAD-dependent MDH, which is part of the tricarboxylic acid cycle; (iii) glyoxisomal and peroxisomal NAD-dependent MDHs, which are involved in photorespiration; (iv) chloroplast NADP-dependent MDH, which is required for the transfer of reducing equivalents from chloroplast stroma to cytosol; and (v) chloroplast NAD-dependent MDH (6).

Among the classes listed, chloroplast NADP-dependent MDH is a thiol enzyme that has been subject of intense study (4, 7–12) and that uses NADP+ for catalysis. Chloroplast NADP-dependent MDH possesses both N- and C-terminal extensions, both of which harbor a redox-sensitive cysteine pair (4, 12–14). Central to the regulation of chloroplast MDH, ferredoxin-thioredoxin reductase sources electrons from photosynthesis to reduce chloroplast thioredoxins (Trx) (8). Trxs are an important class of regulatory proteins that modulate the activity of a wide variety of thiol-containing chloroplast enzymes by the reduction of specific disulfide bonds (15). In the light, excess NADPH (produced by photosynthesis but surplus to CO2 fixation requirements) induces the conversion of oxaloacetate to malate, resulting in the regeneration of the electron acceptor NADP+. In chloroplasts, the activated form of MDH is responsible for this reaction. The resulting malate is translocated to the cytosol via the dicarboxylate transporter (16), where cytosolic MDH (cytMDH) converts malate back to oxaloacetate, with NAD+ acting as an oxidant to form NADH. This mechanism allows chloroplast reducing equivalents to be transferred to the cytosol, and the transport system described above is known as the “malate valve” (6). NADH accumulated in the cytosol is then used in the mitochondria for respiratory ATP generation or may alternatively be used for cytosolic nitrate reduction. Hence cytMDH functions as a key player in the transfer of reducing equivalents from the chloroplast to other destinations in plant cells.

In the cytosol, a number of Trx-h isoforms have been reported, although the function of each of these Trx-h isoforms has not been determined very well (17–19). Cytosolic Trx-h...
isofoms are thought to be reduced by cytosolic NADPH Trx reductase, which in turn uses NADPH as a reducing equivalent (20). The NADPH/NADP⁺ ratio in the cytosol does not change substantially during light/dark transitions and is maintained between 1 and 1.2 in pea leaves (21), suggesting that the reduction levels of cytosolic Trx-isoforms must also be maintained at a certain level irrespective of light/dark conditions. Therefore, determination of the target proteins of Trx is of significant importance in our understanding of the physiological roles of Trx-isoforms as transducer of reducing equivalents.

Recent enrichment of genome data bases has allowed the screening and molecular characterization of possible target proteins of Trx using a proteome approach (22–28). We recently used Trx affinity chromatography (29) in an attempt to capture the potential cytosolic target proteins of *Arabidopsis thaliana* Trx-isoforms and identified a number of proteins including anti-oxidative stress-related proteins, proteins involved in biosynthesis and degradation of proteins, and several metabolic enzymes (30). Within these captured enzymes, we sought to verify and characterize Trx-dependent regulation of cytMDH in this study.

**EXPERIMENTAL PROCEDURES**

**Materials**—The Bradford protein assay system was from Bio-Rad. DTT was from Sigma. All of the other chemicals were of the highest grade commercially available.

**Preparation of Recombinant Trx-h1**—Recombinant Trx-h1 was expressed in *Escherichia coli* using an expression plasmid and the Trx-h1 gene sequence from *A. thaliana* (30), purified as described (31), and stored at –80 °C. The Trx concentration was determined by the BCA assay (Pierce).

**Preparation of the cDNA for cytMDH**—Total RNA was isolated from 14-day-old *A. thaliana* as described in (32) and used as a template for RT-PCR. The cDNA for cytMDH was synthesized by RT-PCR using RNA LA PCR kit (Takara, Kyoto, Japan) with the following oligonucleotide primers; 5′-GGAAATCCATATGGCGAAAGAACCAGTTCTG-3′ (NdeI) and 5′-CCGCTCGAGTTAAGAGAGGCATGAGTACG-3′ (XhoI). The restriction sites for the enzyme shown in parentheses are underlined. The amplified DNA was ligated into the NdeI and XhoI sites of the pET23a expression vector (Novagen, Madison, WI), and the sequences were confirmed by DNA sequencing (PRISM 310; Applied Biosystems).

**Expression and Purification of cytMDH**—The resulting expression vector was transformed into *E. coli* strain BL21(DE3), and transformed cells were cultured at 37 °C until A₆₀₀ = 0.7. Expression was induced by the addition of 1 mM isopropyl-1-thio-d-galactopyranoside, followed by further culture at 21 °C for 16 h. For purification of the recombinant cytMDH, all of the procedures were carried out at 4 °C. The supernatant was then applied to a DEAE-Toyopearl 650M column (Tosoh) and eluted with 30–0% inverse ammonium sulfate gradient. The obtained fraction containing the desired protein was collected and ultrafiltered to remove ammonium sulfate using Amicon Ultra-15 (cut-off molecular weight, 10,000; Millipore). The protein concentration was determined by Bradford assay using bovine serum albumin as standard.

**Oxidation and Reduction of the Recombinant cytMDH**—Because the recombinant cytMDH was obtained in the reduced form (cytMDHred), after purification, the protein was oxidized by incubation with 50 μM CuCl₂ for 1 h at 30 °C. Oxidized cytMDH (cytMDHox) was dialyzed against 25 mM Tris-HCl (pH 7.5) to remove CuCl₂ prior to the assay. For *in vitro* reduction, 5 μM cytMDHox was incubated for 1 h at 30 °C with DTT in the presence or absence of Trx-h1. Following treatment, the redox state of cytMDH was confirmed by nonreducing SDS-PAGE using 10% (w/v) polyacrylamide gel.

**Preparation of the Cys Mutant of cytMDH**—The Cys mutants of cytMDH were prepared by using the mega-primer method (33) using KOD DNA polymerase (Toyobo, Osaka, Japan) and the suitable oligonucleotide primers. Expression and purification of the mutant proteins were performed as above. A C125A mutant was prepared instead of the C125S mutant because the latter was found to be insoluble.

**MDH Activity**—cytMDH activity was measured at 30 °C in a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM oxaloacetic acid, and 0.1 mM NADH. The activity was monitored as a decrease in absorbance at 340 nm because of NADH oxidation. ε₃₄₀ for NADH of 6.25 mM⁻¹ was used for the calculation of the amounts of the catalyzed NADH. In this study, MDH activity is shown as the oxidation of NADH/min/mg of protein.

**Peptide Mapping Analysis**—To determine the disulfide partners cysteines, cytMDHox was digested with sequencing grade modified trypsin (Promega) for 20 h at 37 °C in 25 mM (NH₄)HCO₃ (pH 7.0) with a protein-to-protein ratio of 90:1 (w/w). The proteolytic peptides obtained were analyzed directly by reversed-phase HPLC or reduced before analysis. For reduction of the fragments obtained from cytMDHox, the mixture of proteolytic peptides was incubated with 10 mM DTT for 1 h at 37 °C. The peptides were then separated by reversed-phase HPLC column (Cosmosil 5C₁₈ AR-300, 4.6 × 150 mm; Nacalai tesque, Kyoto, Japan) at a flow rate of 0.5 ml/min with solvent A (0.1% (v/v) trifluoroacetic acid) and solvent B (90% (v/v) acetonitrile and 10% (v/v) trifluoroacetic acid) using the gradient elution (2% solvent B at 0–5 min, 2–50% solvent B at 5–50 min, 50–80% solvent B at 50–65 min, and 80% solvent B at 65–75 min). The peptide fragments were monitored at 220 nm. N-terminal amino acid sequences of the separated fragments were analyzed by N-terminal peptide sequencing (PPSQ-21; Shimadzu, Kyoto, Japan), and their molecular masses were determined by using matrix-assisted laser desorption ionization time-of-flight mass spectrometer (Axima-CFR Plus; Shimadzu).

**Gel Filtration Analysis**—Reduced or oxidized cytMDH was loaded onto a gel filtration column TSK-G2000SWxl (Tosoh)
that had been previously equilibrated with buffer containing 25 mM Tris-HCl (pH 7.5) and 100 mM KCl.

**Sedimentation Analysis**—Sedimentation velocity experiments were carried out with an ultracentrifuge, Optima XLI (Beckman-Coulter), using a four-hole An60Ti rotor at 20 °C. The measurements were carried out in 25 mM Tris-HCl buffer (pH 7.5) and 150 mM KCl and monitored by measuring the absorbance at 280 nm. The partial specific volume was calculated based on the amino acid composition using the software SEDNTERP, which was kindly provided by J. Philo. The results were analyzed by using the program SEDFIT (34), and the distribution function of sedimentation coefficients, c(s), was calculated.

**RESULTS**

**Reactivation of the Oxidized Form of Recombinant cytMDH by Reduction**—Measurement of the activity of purified recombinant cytMDH in the presence of 0.1 mM NADH and 1 mM oxaloacetic acid showed comparable rates (1.5 mmol/min/mg of protein) to cytosolic MDHs from other organisms and organelles (35, 36). Therefore, measurements of redox-induced changes in cytMDH activity were carried out as described above in all of the subsequent experiments. We first examined whether the enzyme activity of cytMDH is affected by redox conditions. Although recombinant cytMDH expressed in *E. coli* was purified as a reduced form, the protein could be oxidized by incubation with low concentrations of CuCl₂ (Fig. 1), resulting in cytMDH inactivation (Fig. 1A). Upon reduction of oxidized cytMDH by incubation with 1 mM DTT, enzyme activity was restored, indicating that cytMDH could be reversibly activated and inactivated by a change in redox state.

**Oxidized cytMDH Forms an Intermolecular Disulfide Bond**—The reported crystal structures of MDH proteins of various
organisms suggest that this protein forms a homodimer (37–42). To investigate the formation of the intermolecular disulfide bond within the dimer under oxidizing conditions, we used nonreducing SDS-PAGE (Fig. 1B). The reduced form of cytMDH migrated to the monomeric position (35.6 kDa), whereas oxidized cytMDH appeared as a 71-kDa homodimeric band, implying that cytMDH was able to form an intermolecular disulfide bond(s) under oxidizing conditions.

Oxidized cytMDH Is Reduced by Trx-h1—Interaction between oxidized cytMDH and the reduced form of Trx-h1 was initially identified by Trx affinity chromatography (30). To confirm this interaction, oxidized cytMDH was incubated with various concentrations of DTT in the presence or the absence of Trx-h1. CytMDH activity was found to be gradually restored by increasing concentrations of DTT (Fig. 2A), and, most significantly, recovery was greatly accelerated by the addition of Trx-h1. Efficiency of the reduction of intermolecular disulfide bond(s) was also increased by Trx-h1 (Fig. 2B and C). The linear correlation observed between cytMDH activity and its reduction level (Fig. 2 inset) strongly suggests that cytMDH activity is regulated by formation of an intermolecular disulfide bond. Enhancement of the reduction by Trx-h1 was observed in the presence of 0.5 μM Trx-h1 and 10 μM DTT (Fig. 2D), suggesting that the reduced form of Trx-h1 can efficiently reduce cytMDHox.

Identification of the Critical Cysteines of cytMDH for Dimer Formation Using Cys Mutants—A. thaliana cytMDH contains six cysteine residues, all of which are conserved in plant cytosolic MDH isoforms (supplemental Fig. S1). To determine the regulatory Trx-targeted cysteine pairs responsible for dimer formation, we prepared six cytMDH Cys mutants (C79S, C125A, C155S, C252S, C292S, and C330S) and investigated their redox sensitivity. The C330S mutation alone was found to affect both the redox sensitivity of MDH activity and intermolecular disulfide bond formation (Fig. 3). These results imply that the oxidized cytMDH dimer is formed by disulfide formation between Cys330 on the monomeric protein.

Peptide Mapping Analysis—Peptide mapping analysis (31) was used to characterize both the intra- and intermolecular disulfide bonds formed in the protein molecule under oxidizing conditions. To achieve this, cytMDHox was digested by trypsin, and the resulting peptide fragments were either directly separated by reversed-phase HPLC (Fig. 4A) or separated following incubation with 10 mM DTT for reduction (Fig. 4B). Two redox-responsive peptide fragments were identified in the oxidized form peptides (Fig. 4A, TO-1 and TO-2), which were not observed in the reduced form fragments (Fig. 4B). In contrast, a novel peptide fragment appeared when the peptide fragments were reduced in advance (Fig. 4B, TR-1). These redox-specific peptide fragments were analyzed by N-terminal sequencing and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Table 1). The TO-1 fragment was found to have a mass of 1739.9 and the sequence DLAYS derived from the C-terminal region of cytMDH. This fragment mass corresponds to that of a dimer of the C terminus peptide fragment (325DLAYSCLSC322), which was formed by the Cys330–Cys330 disulfide bond. TO-2 was also observed only in the oxidizing peptide fragments, and it contained two peptide fragments, one including Cys252 and another one probably including Cys292, respectively. Although this result suggests the existence of a Cys252–Cys292 disulfide bond, the finding that the two C252S and C292S mutants remained inactive in the oxidized form implies that the suggested disulfide bond does not play a regulatory role in the cytMDH protein (Fig. 3B). An additional peak corresponding to TR-1 was specifically found in reduced peptide fragments and was found to contain no cysteine residues. So far, we have been unable to explain the occurrence of the TR-1 peptide in the reduced form fragments.

Molecular Size of the Oxidized cytMDH—Finally, the molecular sizes of the reduced and the oxidized cytMDH were determined. The oxidized form cytMDH gave two molecular species in the gel filtration chromatography in the presence of 0.1 M KCl, whereas the elution time for the smaller molecule was almost the same as that for the reduced form, which was eluted as a single peak (Fig. 5A). Both fractions of cytMDHox were composed of cytMDH disulfide dimer and monomer molecules, although the monomer content of the O2 fraction (the first half of the second peak fraction) was found to be higher than in the O3 fraction (Fig. 5A, inset). In addition, the weak MDH activity of the cytMDHox preparation was recovered in these three fractions (40% in O1, 50% in O2, and 10% in O3), implying that both peaks contained the reduced form dimer molecule, but the presumed tetramer molecule eluted in the O1 fraction may be composed of a heterogeneous mixture of cytMDHox dimer and cytMDHred dimer. The deduced molecular weight of the reduced form cytMDH was 53,000, and that for the smallest peak of the oxidized form cytMDH was 43,000. These values were smaller than the theoretically expected values for dimer (71,200) and larger than that for monomer (35,600), suggesting that the molecular shapes of these cytMDH dimers were
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The sequences of each of the peptide fractions obtained by the protein sequencer are underlined.

TABLE 1

Peptide mapping analysis of cytMDHox using N-terminal sequencer and matrix-assisted laser desorption ionization time-of-flight mass spectrometry

| Peptide fraction | Peptide fragment | Peptide mass |
|------------------|------------------|--------------|
| TO-1             | 225DLAYSCLS      | Not detected | 871.4 |
|                  |                  | 1739.9       | 1739.8 |
|                   |                  | Not detected | 3974.9 |
| TO-2             | 215DWVLGTPEGTVSFPVTCR |          | 1589.0 |
|                  |                  | 1588.8       | 1588.8 |
| TR-1             | 28GIMLGADQV      | 2883.8       | 2883.5 |

* The numbers denote the amino acid positions in the sequence of the mature protein. The underlined portion indicates amino acid sequences determined by N-terminal sequencing.
* The observed mass (m/z) was estimated by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.
* The calculated monoisotopic mass of a single-charged molecule ([M + H]^+) was based on the assigned peptide sequence.
* The calculated mass of the homodimer formed by disulfide bond.

very different from those of the globular proteins or that these cytMDH proteins have some nonspecific weak affinity to the gel.

To determine the molecular species in the oxidized and the reduced fractions, we carried out sedimentation velocity measurements. The c(s) analysis of the moving boundaries of the oxidized form cytMDH showed again two molecular species, whose s values were 4.85 S (the major peak) and 7.00 S (Fig. 5B). The c(s) analysis for the reduced form cytMDH gave only one peak with the s value of 4.64 S, which is almost identical to the s value for the major peak of the oxidized form. Using these values, the conversion of c(s) to c(M) gave the molecular weight of 76,200, which is close to the value for dimer for the reduced form, and that of 69,500 for the major peak fraction of the oxidized form.

**DISCUSSION**

Nine genes encoding cytosolic Trx-isoforms have been reported in the A. thaliana genome (43), at least five of which have been found to be expressed (17); the physiological role of each Trx isoform is yet to be determined. Trx affinity chromatography was used in an attempt to uncover the target proteins of the Trx-isoforms, leading to the identification of several potential metabolic enzyme targets (29, 30). Among the captured proteins, cytMDH was found to be of particular interest, given that redox regulation of its chloroplastic counterpart has been the subject of intense study. Moreover, in contrast to the cytosolic form that, until now, was thought to be a redox-insensitive enzyme, the chloroplast MDH isoform is characterized by two unique N- and C-terminal extensions containing redox-sensitive cysteines. The work presented here elegantly demonstrates that, although lacking these N- and C-terminal extensions, cytMDH is also a redox-sensitive enzyme.

As shown in Figs. 1 and 2, we were able to confirm that recombinant cytMDH forms an intermolecular disulfide bond...
under oxidizing conditions. As a target of the reduced form of Trx-h1, this disulfide bond was found to be responsible for the regulation of the enzyme activity of recombinant cytMDH. The MDH activity in the cytosol obtained from the Arabidopsis cell cultures was about 7.6 μmol/min/mg of protein, and ~15% of this activity was suppressed upon previous incubation of the cell cultures with H₂O₂ (data not shown). Assuming that (i) the suppressed activity is caused by oxidation of cytMDH and (ii) the specific activity of cytMDH in vivo is equivalent to that of the recombinant cytMDH, the amount of cytMDH protein was roughly estimated to be 0.1% of the total cytosolic protein. Although Trx-h is known to be a ubiquitous and constitutively expressed protein, and several reports on Trx-dependent reduction of plant MDH activity in the cytosol obtained from the plant cytosol. According to measurements carried out using a specific antibody, TrxA1 in the cyanobacterium Synechocystis sp. PCC6803, the major Trx in this bacterium, represents 1% (w/w) of the total soluble proteins. In view of the known cellular expression levels of Trxs, the experimental conditions used for reduction of recombinant cytMDH in this study (Trx concentration range of 0.5–3 μM being similar or slightly lower than that used for the recent study of plant Trx target enzymes (46–50)) are considered appropriate to evoke the physiological stoichiometry of cytMDH and Trx.

In addition to the functional characterization of cytMDH, we were able to determine that Cys₃⁵⁰ is the redox-responsive cysteine residue required to form the intermolecular disulfide bond under oxidizing conditions (Fig. 3). Interestingly, the two Cys₃³⁹ residues have been shown to be located at opposite ends of the dimeric contact point in previously reported crystal structures of dimeric MDH proteins from other organisms (40, 51). This notion raises the issue of whether recombinant cytMDH is able to assume the previously reported dimeric structure under oxidizing conditions. Three-dimensional structural information on the oxidized form of cytMDH will allow us to further elucidate the nature of the newly reported disulfide bond-linked dimer described here. Peptide mapping analysis revealed one additional intramolecular disulfide bond between Cys₃⁵² and Cys²⁹² (Table 1) that is not involved in conferring redox sensitivity to the enzyme activity of cytMDH. Moreover, based on the reported crystal structures of the cytMDH of A. thaliana is likely to be somewhat different from the previously reported structures of MDH. During analysis of dimer formation by nonreducing SDS-PAGE, we noted two monomeric bands on the gel (Figs. 1B and 2B). However, this was not due to the suggested intramolecular disulfide bond because both C252S and C292S mutants also showed this heterogeneity under the reducing conditions (Fig. 3A). Interestingly, the mutant C330S did not show the duplex bands (Fig. 3A). We found that the lower band of these two monomeric bands disappeared when the reduced protein was treated with N-ethylmaleimide prior to nonreducing SDS-PAGE (data not shown). We therefore concluded that the observed lower band is the result of formation of the unidentified intramolecular disulfide bond between the Cys³³⁰ and the other Cys residue in the cytMDH molecule, which must be easily formed in the denatured state by oxidation during nonreducing SDS-PAGE.

To characterize the oxidized form cytMDH in more detail, we measured the molecular weights using two methods. Both gel filtration chromatography and sedimentation velocity analysis revealed two hydrodynamically distinct species in the oxidized cytMDH fraction (Fig. 5). However, in both cases, the smaller molecular species was the major fraction, and the major peak for the reduced form of cytMDH was unambiguously identified as dimer based on the c(s) analysis. Because the major peak for oxidized form has the close s value of 4.85 S, this must be a dimer species. The presence of multiple species may introduce some significant errors into the molecular weight calculation based on the measured c(s) values, because the c(s) analysis assumes a common value for fₗ₀. It is, however, very likely that the peak with the s value of 7.00 S corresponds to tetramer. Based on the reported crystal structure of cytMDH, a large conformational change is expected to occur upon intermolecular disulfide bond formation between the Cys³³⁰ residues on the monomeric protein. This conformational change may significantly alter the molecular surface charges and the hydrophobicity. Moreover, this hypothesized conformational change may induce additional intermolecular interaction and facilitate the formation of tetramer. The observed slight difference in the elution time between the oxidized and reduced form of dimeric cytMDH may be due to the change in surface properties of the molecule upon oxidation. Three-dimensional structural analysis of the oxidized form cytMDH will therefore provide crucial information on the oxidation of cytMDH.

In plant cytosol, the reducing equivalents transferred from chloroplasts are converted to NADPH by cytMDH. The resulting NADPH is then used as reducing equivalents in the cytosol or transferred into mitochondria by another malate shuttle system where it is used for respiration. In contrast, cytosolic NADPH levels in plant cells are maintained by the oxidative pentose phosphate pathway irrespective of the light/dark transition (21) because cytosolic NADPH is an important reducing equivalent required for amino acid and fatty acid biosynthesis. In addition, the reduction level of cytosolic Trx-h isoforms is likely to be influenced by the cytosolic NADPH/NADP⁺ ratio, because the cytosolic Trx-h protein is efficiently reduced by NADPH-Trx reductase. Reduced Trx-h is likely to be instrumental in maintaining a high ratio of reduced/oxidized cytMDH under physiological conditions.

What is the reason for the highly efficient reduction of oxidized cytMDH by Trx-h? As mentioned above, the physiological importance of cytMDH means that its activity must be highly maintained. Thus Trx-h may constitute a safety mechanism required to minimize unexpected inactivation of cytMDH caused by intermolecular disulfide bond formation. So far, the existence of multiple cytosolic Trx isoforms has been somewhat enigmatic, and little is known about the individual roles of these isoforms. The Trx-targeted metabolic enzymes captured by Trx affinity chromatography (30) are likely to be of signifi-
cant physiological importance. Consequently, oxidation of these enzymes is likely to result in a marked impairment in cell viability. To avoid this eventuality, plant cells are probably equipped with a large and efficient reduction system comprised of multiple Trx-\( h \) isoforms, which serve to reduce redox-sensitive disulfide bonds on these important enzymes. Further investigation into the interaction between Trx-\( h \) isoforms and their target proteins and on the redox states of the metabolic enzymes in the cytosol should allow additional insight into the multiple and varied functions of the cytosolic Trx-\( h \) family.

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