JBC ARTICLE

Biochemical insight into redox regulation of plastidial 3-phosphoglycerate dehydrogenase from *Arabidopsis thaliana*

Received for publication, May 7, 2020, and in revised form, August 22, 2020 Published, Papers in Press, August 25, 2020, DOI 10.1074/jbc.RA120.014263

Keisuke Yoshida[1,*], **Kinuka Ohtaka**[2,3], **Masami Yokota Hirai**[2], **and Toru Hisabori**[1,*]

From the [1]*Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan, the* [2]*RIKEN Center for Sustainable Resource Science, Yokohama, Japan, and the* [3]*Department of Chemical and Biological Sciences, Faculty of Science, Japan Women's University, Tokyo, Japan*

Edited by Joseph M. Jez

Thiol-based redox regulation is a post-translational protein modification for controlling enzyme activity by switching oxidation/reduction states of Cys residues. In plant cells, numerous proteins involved in a wide range of biological systems have been suggested as the target of redox regulation; however, our knowledge on this issue is still incomplete. Here we report that 3-phosphoglycerate dehydrogenase (PGDH) is a novel redox-regulated protein. PGDH catalyzes the first committed step of Ser biosynthetic pathway in plastids. Using an affinity chromatography-based method, we found that PGDH physically interacts with thioredoxin (Trx), a key factor of redox regulation. The *in vitro* studies using recombinant proteins from *Arabidopsis thaliana* showed that a specific PGDH isoform, PGDH1, forms the intramolecular disulfide bond under nonreducing conditions, which lowers PGDH enzyme activity. MS and site-directed mutagenesis analyses allowed us to identify the redox-active Cys pair that is mainly involved in disulfide bond formation in PGDH1; this Cys pair is uniquely found in land plant PGDH. Furthermore, we revealed that some plastidial Trx subtypes support the reductive activation of PGDH1. The present data show previously uncharacterized regulatory mechanisms of PGDH and expand our understanding of the Trx-mediated redox-regulatory network in plants.

To tune cellular physiology, a number of proteins in the cell undergo several post-translational modifications. Thiol-based redox regulation is one of such mechanisms; it controls enzyme activity by switching the oxidation/reduction states of Cys residues (*e.g.* formation/cleavage of disulfide bonds). A small ubiquitous protein thioredoxin (Trx) is largely responsible for the redox regulation. Trx contains the highly conserved amino acid sequence WCGPC at the active site. By using two Cys residues in this motif, Trx catalyzes a dithiol-disulfide exchange reaction with its target proteins, allowing modulation of their enzyme activities. Trx is thus critical for transmitting reducing power to redox-regulated proteins and adjusting cellular functions in response to changes in local redox environments (1, 2).

Trx-mediated redox-regulatory system is ubiquitously found in all kingdoms of life. Among them, the system in plant chloroplasts has attracted much attention due to its unique mode of action related to photosynthesis. Upon illumination, photochemical reactions are triggered in the thylakoid membrane, generating the reducing power. Trx receives a part of reducing power from ferredoxin (Fd) via Fd-Trx reductase (FTR), and in turn transfers it to several redox-regulated proteins. This redox cascade ensures light-responsive coordination of chloroplast functions, which has long been recognized as the molecular basis of the redox-regulatory system in chloroplasts (3, 4).

Another characteristic of the chloroplast system is the emergence of multiple Trx subtypes, categorized into *f*-, *m*-, *x*-, *y*-, and *z*-types (5, 6). They have different midpoint redox potentials and protein surface charges, conferring functional diversity to each of the Trx subtypes (*e.g.* distinct target selectivity) (7–11). In addition, other proteins serving as the mediator of reducing power have been reported to reside in chloroplasts. A most well-known example is the NADPH-Trx reductase C (NTRC); this protein contains both an NADPH-Trx reductase (NTR) domain and a Trx domain in a single polypeptide, and thus works in the redox regulation relying on NADPH (12). Recent studies have proposed specific and important roles of NTRC in redox regulation (13–15). All these data raise a novel hypothesis that the redox-regulatory system in chloroplasts constitutes a complex network, allowing flexible control of chloroplast functions. Uncovering its whole organization is currently a major challenge in the field of redox study (16–22).

To achieve this goal, it is also important to grasp what chloroplast proteins are redox-regulated. Classically, only a limited number of chloroplast proteins have been known as the target of redox regulation (*e.g.* several Calvin-Benson cycle enzymes and ATP synthase CF_1-γ subunit) (3, 4, 23, 24). In the 2000s, several proteomics-based methods for comprehensively identifying Trx-interacting proteins have been developed (25–29), opening the possibility of unprecedented diversity of redox-regulated proteins in chloroplasts. Furthermore, these integral analyses have been applied to other cellular compartments, including other types of plastids (30), mitochondria (31, 32), and cytosol (33). It is now speculated that a broad spectrum of proteins may be under redox regulation in plant cells (34, 35); however, our consistent understanding of this issue is still weak. To overcome this limitation, we need to verify the possibility of redox regulation while paying attention to each of potential target proteins individually. A plastidial enzyme, 3-phosphoglycerate dehydrogenase (PGDH), is one of such targets that remains to be studied at a biochemical level (27, 30, 36).

This article contains supporting information.

* For correspondence: Keisuke Yoshida, yoshida.k.ao@m.titech.ac.jp; Toru Hisabori, thisabor@res.titech.ac.jp.

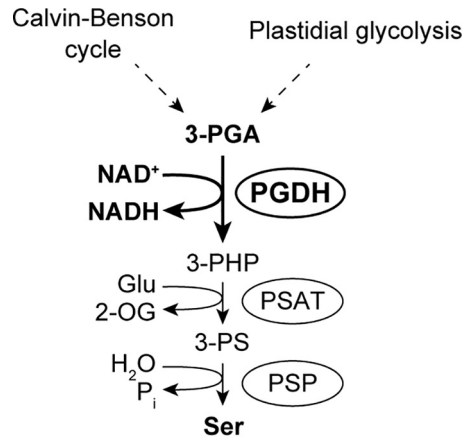


Figure 1. Plastidial Ser biosynthesis mediated by the phosphorylated pathway. The following abbreviations were used: *2-OG*, 2-oxoglutarate; *3-PHP*, 3-phosphohydroxypyruvate; *3-PS*, 3-phosphoserine; *PSAT*, 3-phosphoserine aminotransferase; *PSP*, 3-phosphoserine phosphatase.

Ser is an indispensable amino acid involved in many physiological events in all organisms. In plant cells, there are three different pathways for Ser biosynthesis, including the photorespiratory glycolate pathway (in mitochondria), the glycerate pathway (in the cytosol and peroxisomes), and the phosphorylated pathway (in plastids; Fig. 1) (37, 38). The glycolate pathway has been considered to be of major importance especially under photorespiratory conditions. However, recent reverse-genetic studies indicate that the phosphorylated pathway plays a critical role in supporting plant metabolism and development (39–41). The first step of this pathway, that is the oxidation of 3-phosphoglycerate (3-PGA), is catalyzed by PGDH (Fig. 1). PGDH can be classified into three types based on domain structure; plant PGDH belongs to the type I enzyme composed of four domains, the substrate-binding domain, the nucleotide-binding domain, the allosteric substrate-binding domain, and the Asp kinase-chorismate mutase-TyrA domain (42). Despite its postulated importance in ensuring Ser homeostasis in plants, the regulatory mechanisms of PGDH are largely unclear especially at a post-translational level.

In this study, we have addressed the possibility of PGDH redox regulation using a biochemical procedure. Our data indicate that (i) a specific isoform of *Arabidopsis* PGDH, PGDH1, forms the intramolecular disulfide bond, lowering PGDH enzyme activity; (ii) its disulfide bond is formed between Cys residues uniquely conserved in land plant PGDH; and (iii) some plastid-localized Trx subtypes can reduce and activate PGDH1.

Results

PGDH physically interacts with Trx

We first investigated whether PGDH has a cross-talk with redox-regulatory factors, such as Trx. For this purpose, we applied the affinity chromatography-based screening method (25). One of *Arabidopsis* plastidial Trx isoforms, Trx-*f*1, or *Arabidopsis* NTRC, each of which was prepared in the form of monocysteinic variant (13), was used as bait in this experiment. Chloroplast soluble proteins extracted from spinach leaves were loaded onto a Trx-*f*1- or NTRC-immobilized affinity chromatography column. The proteins associated with Trx-*f*1 or NTRC via the mixed-disulfide bond were eluted by a reducing agent DTT. As shown in Fig. 2*A* (for Trx-*f*1) and Fig. 2*B* (for NTRC), different SDS-PAGE profiles of DTT-eluted proteins were evident between assays using Trx-*f*1 and NTRC, because of their distinct target selectivity (13). Immunoblotting analyses indicated that PGDH was bound to Trx-*f*1, but not to NTRC (Fig. 2, *C* and *D*). After eluted by DTT, the molecular weight of PGDH seems to be slightly varied (Fig. 2*C*), which may reflect the change in PGDH redox state. These results suggest that PGDH may be redox-regulated in a Trx-dependent manner.

Arabidopsis PGDH1, but not PGDH2 and PGDH3, is activated by cleavage of intramolecular disulfide bond

In *Arabidopsis thaliana*, three nuclear genes encoding PGDH (*PGDH1*, At4g34200; *PGDH2*, At1g17745; and *PGDH3*, At3g19480) are identified (38). All PGDH isoforms were confirmed to be targeted to plastids (39, 41). To test whether each PGDH isoform can bind to Trx, affinity chromatography experiments were conducted on *Escherichia coli* that expressed PGDH1–3 individually (Fig. 3, *A*–*C*). In all cases, PGDH was detected in DTT-eluted fractions. These results give an implication that all of PGDH isoforms in *Arabidopsis* are redox-regulated as the target of Trx. However, we cannot conclude the possibility for PGDH redox regulation at this stage, because it is known that affinity chromatography-based methods often traps pseudo-target proteins of Trx during the incubation process (43). To verify this issue, it is necessary to clarify (i) Trx-dependent reduction, (ii) redox-dependent change in the activity, and (*iii*) Cys residues responsible for the redox regulation. Following studies were designed to address these points.

We prepared PGDH1–3 from *Arabidopsis* as the purified recombinant proteins (Fig. S1). We examined whether the redox state of PGDH is variable or not (Fig. 3*D*). Redox shift assays using a thiol-modifying reagent indicated that PGDH1 mainly existed as two different redox states under control (DTT-free) conditions. PGDH1 was converted to a fully reduced state in the presence of DTT. It was thus suggested that PGDH1 can form at least one disulfide bond in the molecule. By contrast, the redox states of PGDH2 and PGDH3 were unaltered by DTT. We then examined the effects of DTT on PGDH enzyme activity (Fig. 3*E*). PGDH1 was largely activated by DTT, whereas PGDH2 and PGDH3 were not. Taken together, these results suggest that PGDH1 is a redox-sensitive protein whose activity is enhanced upon reduction, whereas PGDH2 and PGDH3 are not.

Cys^{86} and Cys^{102} are mainly involved in disulfide bond formation in PGDH1

There are four Cys residues in *Arabidopsis* PGDH1 (Cys^{86}, Cys^{102}, Cys^{148}, and Cys^{534}; Fig. 4*A*, Fig. S2). Among these, only Cys^{102} is not found at the corresponding position of redox-insensitive PGDH2. A three-dimensional structure model of PGDH1 showed that Cys^{102} may be localized in spatial proximity to Cys^{86} and Cys^{148} (Fig. 4*B*, Fig. S3). We thus anticipated that Cys^{102} forms a disulfide bond with either Cys^{86} or Cys^{148}.

To directly evaluate this expectation, we performed peptide mapping analysis based on the MS. PGDH1 protein was in-gel

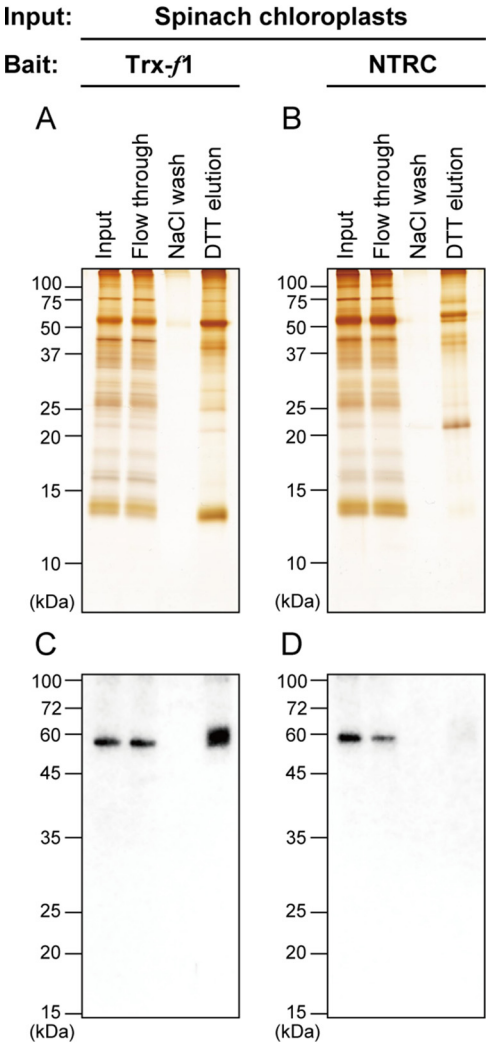


Figure 2. Identification of PGDH as a Trx-interacting protein. *A* and *B*, affinity chromatography-based screening of *Arabidopsis* Trx-*f*1 (*A*) or NTRC (*B*) target candidate proteins in chloroplasts. Protein elution profiles were analyzed by SDS-PAGE, followed by silver staining. *C* and *D*, validation of PGDH binding to Trx-*f*1 (*C*) or NTRC (*D*). PGDH was detected by immunoblotting analysis using a PGDH antibody.

digested using trypsin, and mass spectra of the resulting peptides were compared between control and DTT treatments. As expected, the overall mass spectra were apparently similar between the control and DTT-treated samples (Fig. S4), but a few differences were observed. Mass peaks of 1159.7 and 2567.2 were more dominant in the DTT-treated sample (Fig. 5*A*). Data query using the Mascot search engine indicated that these peaks corresponded to a tryptic peptide of Ile^{99}–Arg^{108} (containing carbamidomethyl Cys^{102}) and Leu^{77}–Lys^{98} (containing carbamidomethyl Cys^{86}) with a peptide tolerance of <50 ppm. By contrast, a mass peak of 3609.9 is specifically found in the control sample (Fig. 5*B*). It corresponded to a conjugated peptide of Leu^{77}–Lys^{98} and Ile^{99}–Arg^{108} linking with a disulfide bond. These results indicate that Cys^{86} and Cys^{102} are involved in disulfide bond formation in PGDH1.

To gain further insight into the regulatory Cys residues in PGDH1, a site-directed mutagenesis analysis was conducted. We generated PGDH1 mutant proteins whose only Cys^{102} (C102S) or both Cys^{86} and Cys^{102} (C86S/C102S) were substituted to Ser (Fig. S1). The C102S mutant still showed DTT-dependent conversion of the redox state, although its conversion range became smaller (Fig. 5*C*). It is likely that, upon Cys^{102} mutation, another Cys (possibly Cys^{148} that lies close to Cys^{102}; Fig. 4*B*, Fig. S3) forms a disulfide bond with Cys^{86}. Such flexibility in disulfide bond formation is also reported in the fructose-1,6-bisphosphatase (44). By contrast, the C86S/C102S mutant completely lacked the ability of DTT-dependent redox shift. All these data suggest that Cys^{86} and Cys^{102} are mainly responsible for PGDH1 redox regulation. Alignment of PGDH from several photosynthetic organisms shows that this Cys pair is largely conserved in land plants, but not in algae or cyanobacteria (Fig. 4*A*, Fig. S2).

Some Trx subtypes in plastids support PGDH1 reductive activation

We finally studied the involvement of Trx and NTRC in PGDH1 redox regulation (Fig. 6, *A*–*D*). Four Trx isoforms (Trx-*f*1, Trx-*m*1, Trx-*x*, and Trx-*y*2) and NTRC from *Arabidopsis* were prepared. All Trx isoforms used here were previously shown to be capable of mediating redox regulation with different target selectivity (10, 11). Low concentrations of DTT (0.1 mM) had little impact on the PGDH1 redox state (Fig. 6*A*) and activity (Fig. 6*B*). When Trx-*f*1, Trx-*m*1, or Trx-*y*2 was added at 0.1 μM, PGDH1 reduction was promoted (Fig. 6*A*). Accordingly, the activity of PGDH1 was also enhanced (Fig. 6*B*). The effects of these Trx isoforms on PGDH1 were more pronounced when each Trx was added at 0.5 μM. By contrast, Trx-*x* hardly affected the PGDH1 redox state and enzyme activity. In the absence of DTT, all Trx isoforms failed to activate PGDH1 (Fig. S5). Experiments with PGDH1 C102S and C86S/C102S mutants confirmed the principal role of Cys^{86} and Cys^{102} for Trx-dependent redox regulation of PGDH1 (Fig. 6, *C* and *D*). NTRC was previously shown to efficiently transmit NADPH-derived reducing power to some proteins (13). However, it was not the case with PGDH1; NTRC was unable to promote PGDH1 reduction largely (Fig. 6*A*). These results suggest that selected types of plastidial Trx are effective in the reductive activation of PGDH1. By contrast, any Trx isoforms affected neither the redox state nor the activity of PGDH2 (Fig. 6*E*) and PGDH3 (Fig. 6*F*).

Discussion

Information on the redox-based regulatory network in plant cells has been increasingly expanding (16–22). Until now, several hundreds of proteins have been proposed to be redox-regulated in plants, based on large-scale proteomic approaches (34, 35). It should be noticed, however, that most of these are still potential, due to the lack of further studies for confirming the validity of redox regulation in detail; this fact largely hampers a consistent understanding of the redox-regulatory system. Using a biochemical procedure, we report here that PGDH is a previously uncharacterized redox-regulated protein.

PGDH has been found as one of the putative Trx-linked proteins by early proteomic studies (27, 30, 36). In accord with this, our sensitive analysis using an antibody indicated that PGDH

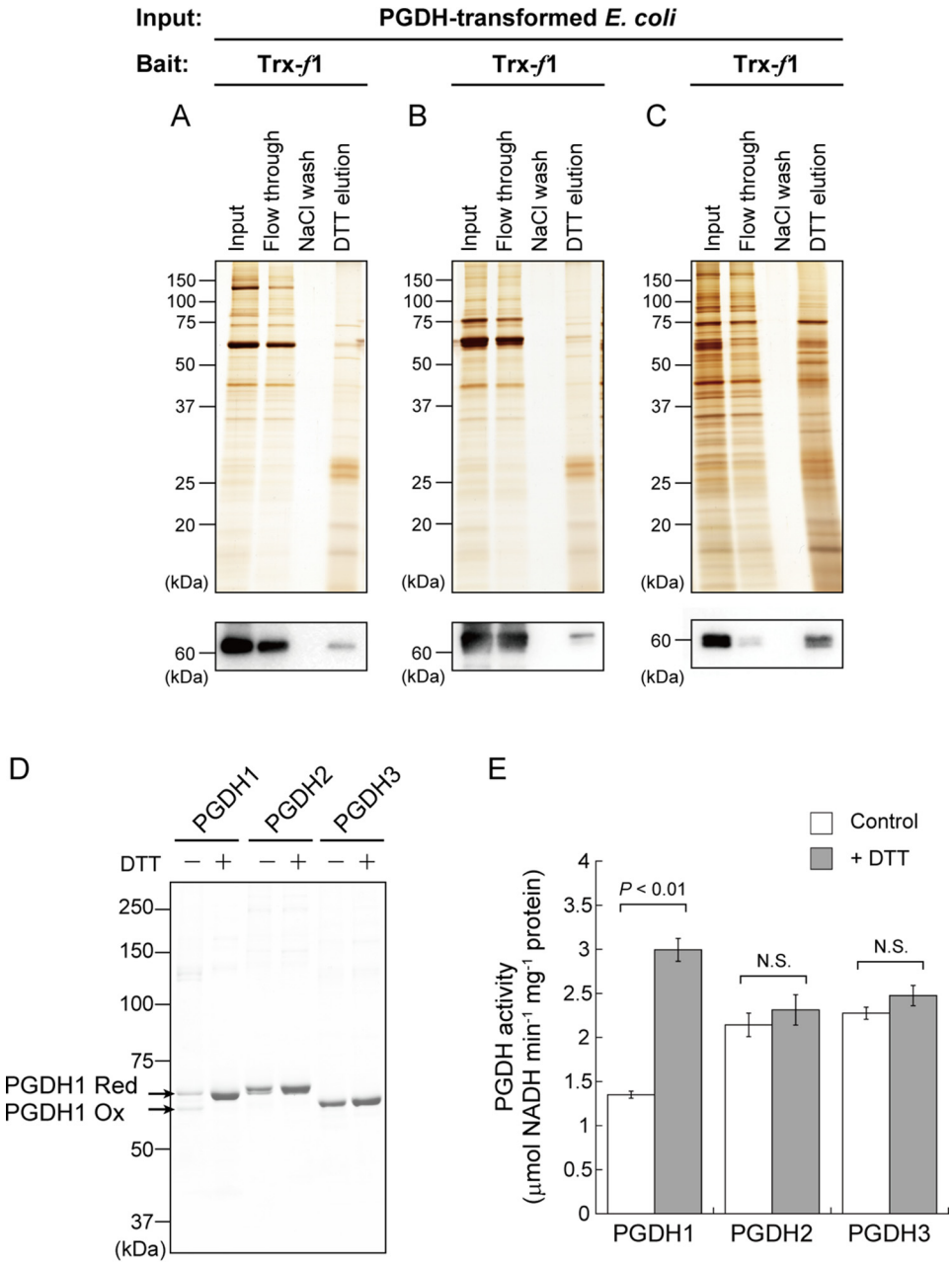


Figure 3. Redox sensitivity of *Arabidopsis* PGDH. *A-C*, affinity chromatography-based test for binding of PGDH1 (*A*), PGDH2 (*B*), or PGDH3 (*C*) to *Arabidopsis* Trx-*f*1. Proteins extracted from each PGDH-transformed *E. coli* cells were loaded as input. Protein elution profiles were analyzed by SDS-PAGE, followed by silver staining. PGDH was detected by immunoblotting analysis using a PGDH antibody. *D,* redox shift assay of PGDH using the thiol-modifying reagent. Each PGDH (1.2 μM) was incubated with or without 10 mM DTT for 15 min. PGDH was then labeled with maleimide-PEG_{11}-biotin and loaded on nonreducing SDS-PAGE. *Ox*, oxidized form; *Red*, reduced form. *E,* enzyme activity measurement of PGDH. Each PGDH (1.2 μM) was incubated with or without 1 mM DTT for 15 min. PGDH activity was then monitored. Data are shown as the mean ± S.D. (n = 4). Statistical analyses were performed using the Student's *t test*. *N.S.*, not significant.

can interact with Trx-*f*1 via the mixed disulfide bond (Fig. 2), firmly supporting a possibility for PGDH redox regulation. Indeed, one isoform of *Arabidopsis* PGDH, PGDH1, showed a clear shift in the redox state, coupled with changes in enzyme activity (Fig. 3, *D* and *E*). The Cys residues mainly responsible for the disulfide bond formation in PGDH1 were then determined (Cys^{86} and Cys^{102}; Fig. 5). Land plants (from liverwort to vascular plants) widely share at least one PGDH isoform containing this Cys pair, whereas algae and cyanobacteria do not (Fig. 4*A*, Fig. S2). PGDH orthologs in nonphotosynthetic organisms also lack this Cys pair (45). PGDH redox regulation is thus considered as a unique strategy acquired during adaptation to terrestrial environments in photosynthetic organisms. Notably, another enzyme using 3-PGA as a substrate, 3-PGA kinase, is reported to be redox-regulated in algae (46, 47) and cyanobacteria (48). Cys residues essential for 3-PGA kinase redox regulation are not conserved in land plants (47). These findings highlight the changing regulatory modes of 3-PGA metabolism across photosynthetic organisms.

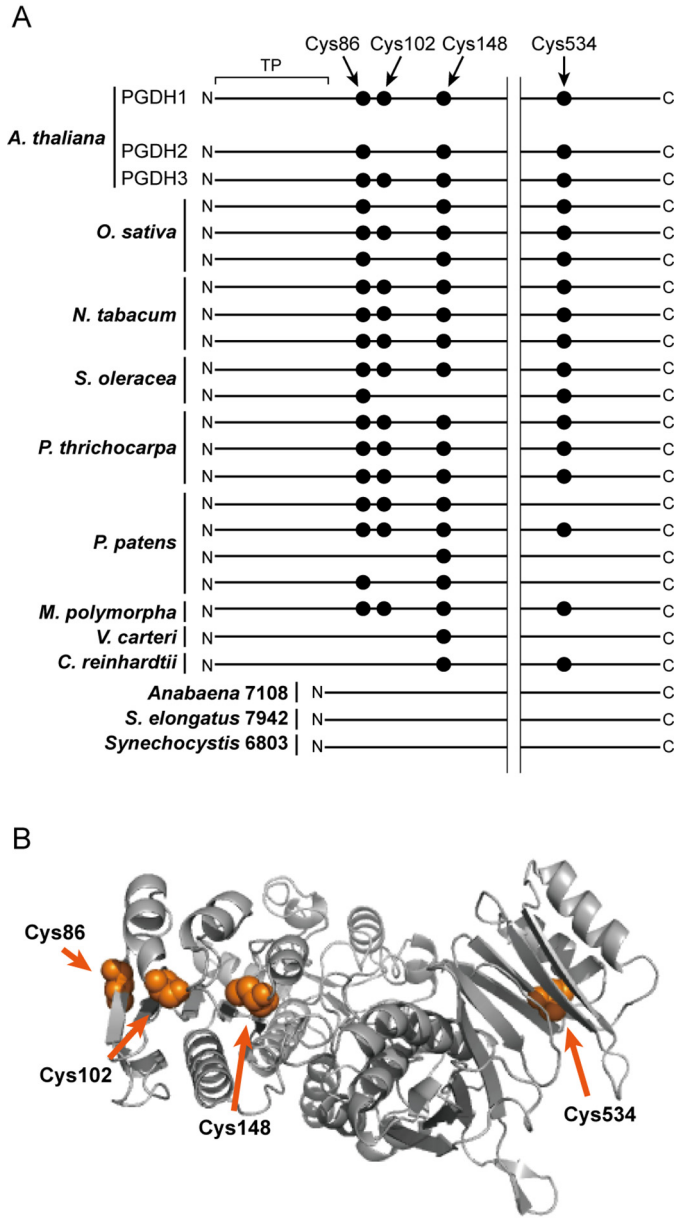


Figure 4. Prediction of redox-active Cys residues in *Arabidopsis* PGDH1. *A,* simplified representation of Cys localization of PGDH1 and their conservation in photosynthetic organisms. There are four Cys residues in the *A. thaliana* PGDH1 mature protein region. The presence or absence of these Cys residues was compared with other PGDH. Other isoforms of *A. thaliana* PGDH (*PGDH2* and *PGDH3*) and PGDH from other photosynthetic organisms, including *Oryza sativa*, *Nicotiana tabacum*, *Spinacia oleracea*, *Populus thrichocarpa*, *Physcomitrella patens*, *Marchantia polymorpha*, *Volvox carteri*, *Chlamydomonas reinhardtii*, *Anabaena* sp. PCC 7108, *Synechococcus elongatus* sp. PCC 7942, and *Synechocystis* sp. PCC 6803, were used for the comparison. The full alignment of PGDH amino acid sequences is shown in Fig. S2. *TP*, transit peptide. *B,* three-dimensional structure model of *Arabidopsis* PGDH1. The model was obtained as a homotetrameric form, but one chain is shown. Four Cys residues are shown by the *orange spheres*.

Although our results suggest that Cys^{86} and Cys^{102} are key to redox regulation of PGDH1, other factors are likely to affect its regulatory property. Despite having this Cys pair, PGDH3 was found to be redox-insensitive (Fig. 3, *D* and *E*, Fig. 6*F*). It seems possible that some amino acids specific to PGDH3 have an inhibitory effect on disulfide bond formation, but future studies are warranted to elucidate the underlying mechanisms in detail. Determining the PGDH1 structures in the oxidized and reduced forms, followed by the comparison of spatial arrangement of amino acids, will be largely helpful for its elucidation.

We demonstrated that Trx-*f*, Trx-*m*, and Trx-*y* assist the reductive activation of PGDH1 (Fig. 6, *A* and *B*). There is little consistent molecular property among these three Trx subtypes. For example, the midpoint redox potential is substantially different (Trx-*f*1, −321 mV; Trx-*m*1, −335 mV; and Trx-*y*2, −295 mV (at pH 7.5)) (10, 11). Recently, we identified the determinant amino acid residues for Trx-*f*–specific functions (49), but they appear to have little impact on PDGH1 redox regulation. Further studies are needed to gain deeper mechanistic insight into divergent or redundant Trx functions. Nevertheless, it is reasonable to consider that, in contrast to other proteins (*e.g.* fructose-1,6-bisphosphatase specifically reduced by Trx-*f* (7, 10)), PGDH1 is redox-regulated with less Trx selectivity.

Our *in vitro* data raise a new question: how is PGDH redox-regulated *in vivo*? In *Arabidopsis*, both PGDH1 and several Trx isoforms were shown to be widely expressed in multiple tissues (39, 41, 50), supporting the idea of their cross-talk *in vivo*. In photosynthetic tissues, redox-sensitive PGDH is thought to be reductively activated in response to light via the Fd/FTR/Trx pathway. The *in vitro* PGDH activity is enhanced under alkaline conditions (45, 51), which may be advantageous for PGDH up-regulation in the light (via an increase in stromal pH). On the other hand, physiological studies have proposed that the PGDH-mediated phosphorylated pathway has only a minor role in Ser biosynthesis when Ser is produced via the photorespiratory glycolate pathway (37, 38). Therefore, the extent to which reductive activation of PGDH is physiologically important in illuminated chloroplasts is unclear. Instead, the phosphorylated pathway becomes more important under conditions where the photorespiratory cycle does not function (*e.g.* under high CO_2 conditions or in nonphotosynthetic tissues). The Trx-based redox-regulatory system is assumed to work also in nonphotosynthetic plastids (30), but its mode of action remains elusive. Further studies should be directed to clarify this point and the linkage to PGDH regulation. In this regard, it is worth noting that PGDH was identified as being Trx-linked in germinating *Medicago truncatula* seeds (36).

In this study, we provide a biochemical insight into PGDH redox regulation. Besides this regulatory way, PGDH activity is allosterically affected by several amino acids (*e.g.* inhibited by Ser in a feedback manner) (39, 45, 51). Furthermore, PGDH is controlled at a transcriptional level. Namely, gene expression of each PGDH isoform is differentially induced or suppressed by light/dark transitions, high CO_2, and salinity stress (39, 41, 52, 53). Taken together, it is likely that PGDH functions are dynamically and sophisticatedly regulated at several levels, determining the rates of the phosphorylated pathway and the resulting Ser biosynthesis. Elucidating its overall regulatory mechanisms and their interplay is of importance as a next step.

Experimental procedures

Preparation of expression plasmids

Total RNA was isolated from *A. thaliana* as described previously (54) and used as a template for RT-PCR. The gene

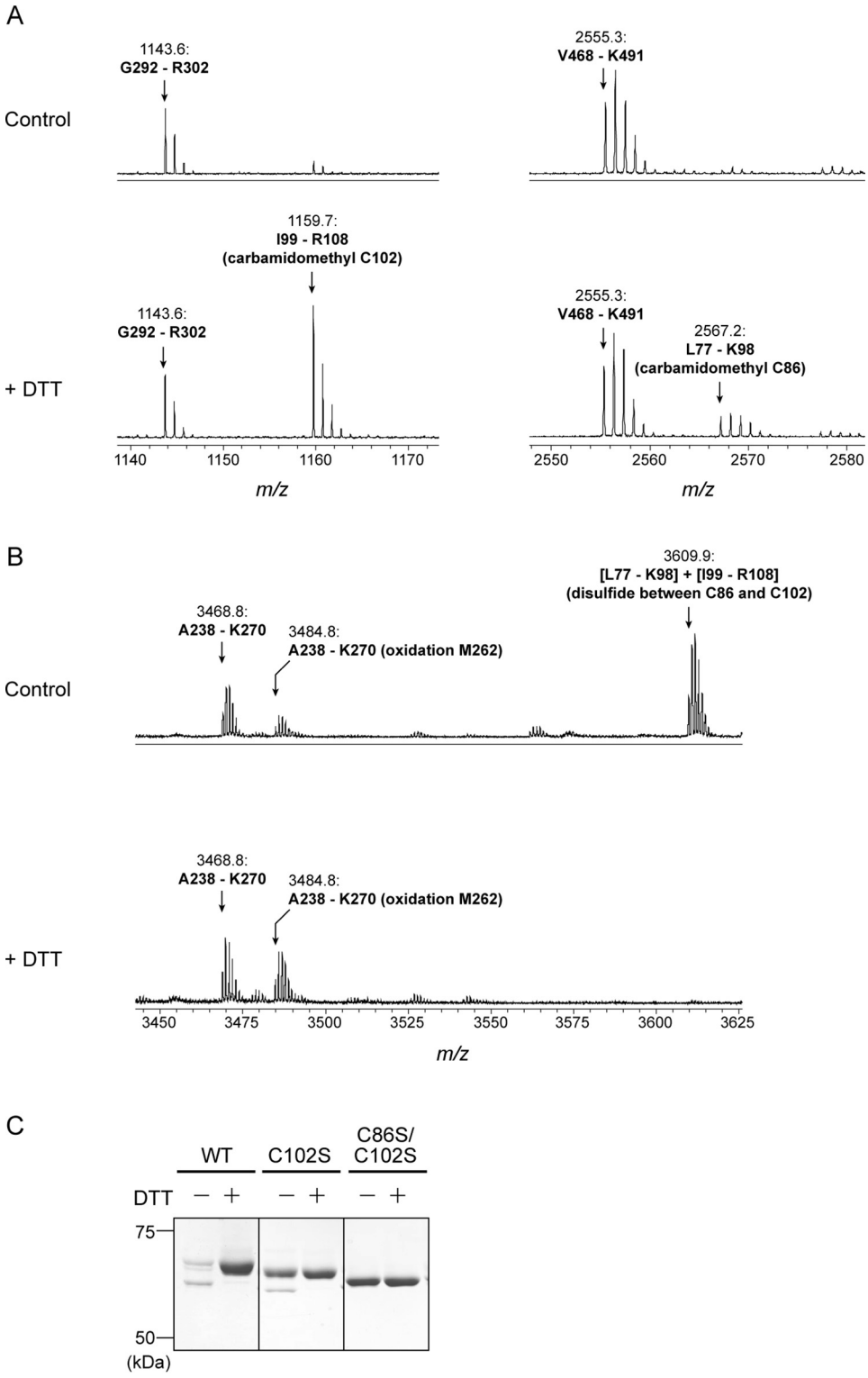
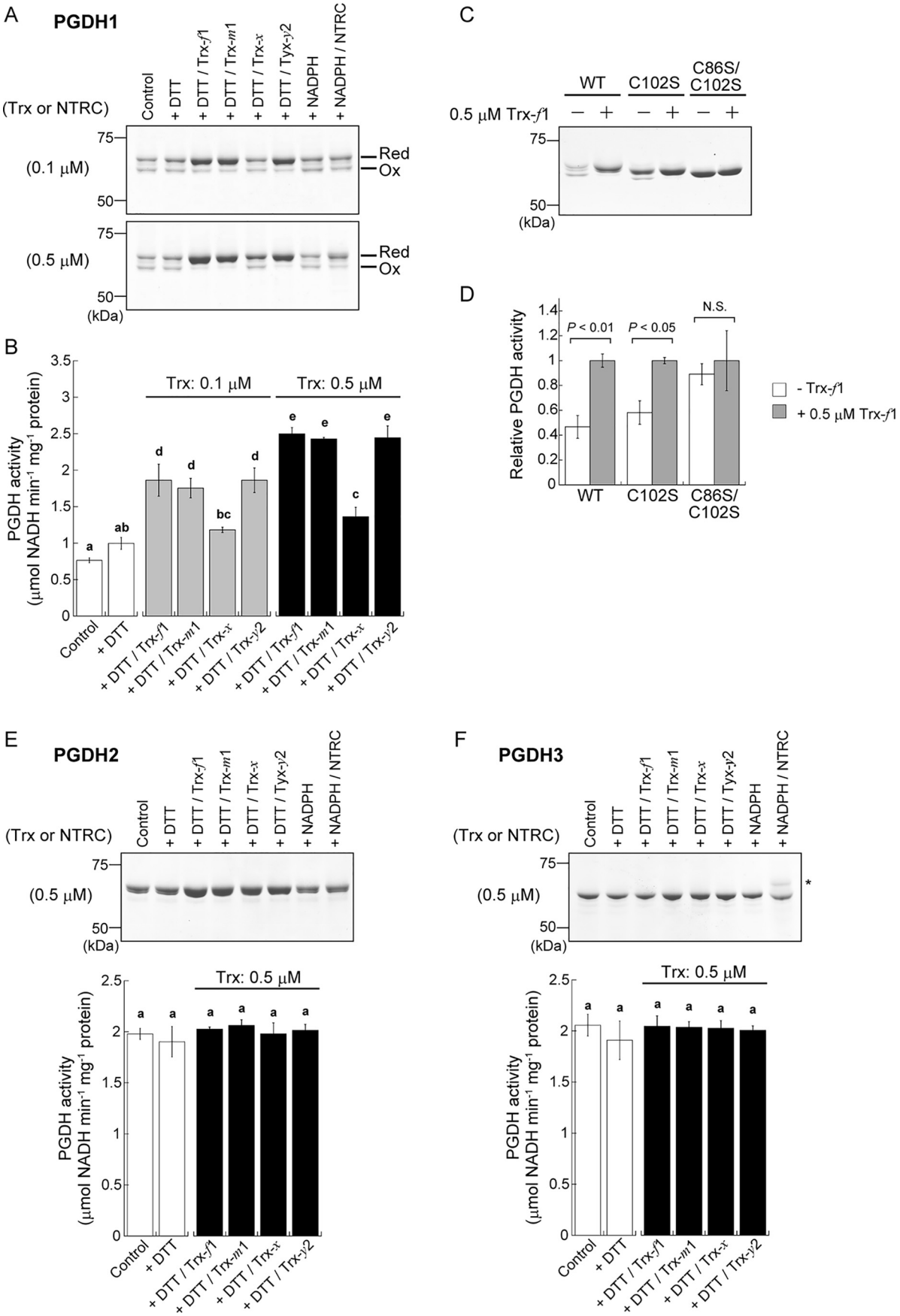


Figure 5. Identification of redox-active Cys residues in *Arabidopsis* PGDH1. *A* and *B,* peptide mapping analysis based on MS. Before the Cys alkylation and following in-gel digestion with trypsin, the protein sample was incubated in the absence (control) or presence of 10 mM DTT. Overall mass spectra are shown in Fig. S4. *C,* redox shift assay of PGDH1 WT and Cys-substituted mutants (C102S and C86S/C102S) using the thiol-modifying reagent. Each PGDH1 was incubated with or without 10 mM DTT for 15 min. PGDH was then labeled with the methyl-PEG_{24}-maleimide and loaded on nonreducing SDS-PAGE.

fragment encoding the predicted mature protein region of PGDH1 (Lys^{61}–Leu^{603}), PGDH2 (Lys^{82}–Leu^{624}), or PGDH3 (Lys^{46}–Leu^{588}) was amplified. The oligonucleotide primers were designed to express objective protein as the His-tagged form at the N-terminal end. The amplified DNA was incorporated into the pET-23d expression vector (Novagen) using the Hot Fusion method (55). The sequences of PGDH expression plasmids were confirmed to be correct by DNA sequencing


A
PGDH1
(Trx or NTRC)
Control
+ DTT
+ DTT / Trx-f1
+ DTT / Trx-m1
+ DTT / Trx-x
+ DTT / Tyx-y2
+ NADPH
+ NADPH / NTRC
(0.1 μM)
(0.5 μM)
Red
Ox
75
50
(kDa)
B
PGDH activity
(μmol NADH min-1 mg-1 protein)
Trx: 0.1 μM
Trx: 0.5 μM
C
WT
C102S
C86S/
C102S
0.5 μM Trx-f1
D
Relative PGDH activity
P < 0.01
P < 0.05
N.S.
- Trx-f1
+ 0.5 μM Trx-f1
E
PGDH2
F
PGDH3
*

(3730xl DNA Analyzer; Applied Biosystems). The other expression plasmids (for Trx isoforms and NTRC) were prepared previously (10, 11, 13).

Site-directed mutagenesis

Point mutations in PGDH1 (Cys^{86} to Ser; Cys^{102} to Ser) were introduced using the PrimeSTAR Mutagenesis Basal Kit (Takara) according to the manufacturer's instructions. The following oligonucleotide primers were used for site-directed mutagenesis: for Cys^{86} to Ser, 5′-GTCGACTCTTCGTATAACATGACTCCT-3′ (forward) and 5′-ATACGAAGAGTCGACATTCGCGACATC-3′ (reverse); for Cys^{102} to Ser, 5′-TCGCTCTCTGACGCCTTGATCGTGAGG-3′ (forward) and 5′-GGCGTCAGAGAGCGAGATCTTAATGTT-3′ (reverse). The mutated codons are underlined.

Protein expression and purification

Each PGDH expression plasmid was transformed into the *E. coli* Rosetta (DE3) pLysS competent cells. The transformed cells were cultured at 37 °C until A_{600} reached 0.2–0.3. For PGDH1 and PGDH2, the expression was induced by the addition of 0.5 mM isopropyl 1-thio-β-D-galactopyranoside, followed by overnight culture at 20 °C. For PGDH3, the expression was slowly induced without adding isopropyl 1-thio-β-D-galactopyranoside, because it rendered most of PGDH3 proteins insoluble. The cells were disrupted by sonication. After centrifugation (125,000 × *g* for 40 min), the resulting supernatant was used to purify the protein. The His-tagged PGDH protein was purified using a nickel-nitrilotriacetic acid affinity column as described previously (56). Other recombinant proteins (Trx isoforms and NTRC) were prepared as described previously (10, 11, 13). The protein concentration of PGDH was determined by Bradford assay with BSA as standard.

Screening for Trx-f1- or NTRC-interacting proteins

The method of Trx affinity chromatography (25) was applied for screening Trx-*f*1- or NTRC-interacting proteins from spinach chloroplasts and PGDH-transformed *E. coli* cells. *Arabidopsis* Trx-*f*1 and NTRC were prepared in the form of monocysteinic variants. NTRC has two redox-active Cys pairs (in NTR domain and Trx domain). Here, the pair in Trx domain was mutated into a single Cys. The detailed procedures are available in Refs. 13 and 32. Binding of the PGDH protein was tested using an antibody raised against *Arabidopsis* PGDH2. This antibody reacted to all of recombinant PGDH1, PGDH2, and PGDH3 proteins, but with different affinities (Fig. S6).

PGDH redox shift assay

For the reducing reaction, PGDH was reacted at 25 °C in a solution containing 50 mM Tris-HCl (pH 7.5) and 50 mM NaCl. Information on protein concentrations, DTT concentrations, or reaction periods are described in each figure.

PGDH redox states were determined by discriminating the thiol status with the use of thiol-modifying reagents described in each figure. These reagents change protein mobility on the SDS-PAGE, allowing the determination of protein redox states with an observable band shift. Following the reducing reaction, proteins were precipitated with 10% (w/v) TCA and then washed with ice-cold acetone. The precipitated proteins were labeled with the thiol-modifying reagents described in each figure for 1 h at room temperature. Proteins were subjected to nonreducing SDS-PAGE and stained with Coomassie Brilliant Blue R-250.

Measurement of PGDH activity

PGDH activity was measured at 25 °C in solution containing 50 mM Tris-HCl (pH 8.8), 600 mM NaCl, 5 mM 3-PGA, 1 mM NAD^+, and 60 nM PGDH. Activity was monitored as an increase in absorbance at 340 nm due to NAD^+ reduction. A molar extinction coefficient for NADH of 6.2 mM^{-1} cm^{-1} was used for calculating the amounts of catalyzed NAD^+.

Peptide mapping analysis using MS

After nonreducing SDS-PAGE, a Coomassie Brilliant Blue-stained protein band of PGDH1 was excised from the gel and fully destained with 50 mM NH_4HCO_3 and 50% (v/v) acetonitrile. For DTT treatment, the gel slice was incubated with 100 mM NH_4HCO_3 and 10 mM DTT at 56 °C for 1 h. The free thiols were then alkylated using iodoacetamide. After being dried completely, the gel slice was incubated with 50 mM NH_4HCO_3 containing 20 ng/μl of trypsin (Promega) at 37 °C overnight. The resulting peptides were then extracted from the gel with 0.1% (v/v) TFA and 50–75% (v/v) acetonitrile. The peptide sample was mixed with the matrix solution (α-cyano-4-hydroxycinnamic acid) on a MALDI plate (MTP 384 target plate ground steel BC, Bruker Daltonics). Mass spectra were obtained using MALDI-TOF MS (UltrafleXtreme; Bruker Daltonics).

Modeling of PGDH1 three-dimensional structure

The three-dimensional structure model of PGDH was constructed using the Swiss-Model software (57). The crystal structure of *Mycobacterium tuberculosis* PGDH (PDB code 3DDN) was used as a template (58).

Figure 6. Involvement of Trx in PGDH redox regulation. *A,* redox shift assay of PGDH1 using the thiol-modifying reagent. PGDH1 (1.2 μM) was incubated in the absence (control) or presence of 0.1 mM DTT or 0.5 mM NADPH for 30 min. Trx-*f*1, Trx-*m*1, Trx-*x*, Trx-*y*2, or NTRC were added at 0.1 or 0.5 μM. PGDH1 was then labeled with the maleimide-PEG_{11}-biotin and loaded on nonreducing SDS-PAGE. *Ox*, oxidized form; *Red*, reduced form. *B,* enzyme activity measurement of PGDH1. PGDH1 was reacted as in *A* and its activity was then monitored. *C,* redox shift assay of PGDH1 WT and Cys-substituted mutants (C102S and C86S/C102S) using the thiol-modifying reagent. Each PGDH1 (1.2 μM) was incubated with or without 0.5 μM Trx in the presence of 0.1 mM DTT for 30 min. PGDH was then labeled with the methyl-PEG_{24}-maleimide and loaded on nonreducing SDS-PAGE. *D,* enzyme activity measurement of PGDH1 WT and Cys-substituted mutants. PGDH1 WT and mutants were reacted in *C* and their activities were then monitored. Each activity is expressed as the relative value. *E* and *F,* redox shift assay and enzyme activity measurement of PGDH2 (*E*) and PGDH3 (*F*). Reaction conditions were same as *A*. Trx or NTRC was added at 0.5 μM. An *asterisk* in *F* indicates a band of NTRC. Data are shown as the mean ± S.D. (n = 3–4). *B*, *E*, and *F,* statistical analyses were performed using the Tukey-Kramer multiple comparison test. *Different letters* denote significant differences at $p < 0.01$. *D,* statistical analyses were performed using the Student's *t* test. *N.S.*, not significant.

Statistical analysis

Statistical analyses were performed with Microsoft Excel software for the Student's *t test*, and SPSS 12.0J software (SPSS Inc.) for the Tukey-Kramer multiple comparison test.

Data availability

All data are contained within the article.

Acknowledgments—We thank the Biomaterial Analysis Division, Tokyo Institute of Technology for supporting DNA sequencing analysis and the Suzukakedai Materials Analysis Division, Tokyo Institute of Technology, for supporting MS analysis. This study was supported by Dynamic Alliance for Open Innovation Bridging Human, Environment and Materials.

Author contributions—K. Y. and K. O. conceptualization; K. Y. investigation; K. Y. writing-original draft; K. Y., K. O., M. Y. H., and T. H. writing-review and editing; M. Y. H. and T. H. supervision.

Funding and additional information—This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grants 19H03241 (to K. Y.) and 16H06556 (to K. Y. and T. H.), Sumitomo Foundation Grant 180881 (to K. Y.), the Yoshinori Ohsumi Fund for Fundamental Research (to K. Y.), and the Tokyo Tech Challenging Research Award (to K. Y.).

Conflict of interest—The authors declare no conflict of interest with the contents of this article.

Abbreviations—The abbreviations used are: Trx, thioredoxin; Fd, ferredoxin; FTR, Fd-Trx reductase; NTRC, NADPH-Trx reductase C; NTR, NADPH-Trx reductase; PGDH, 3-phosphoglycerate dehydrogenase; 3-PGA, 3-phosphoglycerate.

References

1. Holmgren, A. (1985) Thioredoxin. *Annu. Rev. Biochem.* **54,** 237–271 CrossRef Medline
2. Jacquot, J. P., Lancelin, J. M., and Meyer, Y. (1997) Thioredoxins: structure and function in plant cells. *New Phytol.* **136,** 543–570 CrossRef
3. Buchanan, B. B. (1980) Role of light in the regulation of chloroplast enzymes. *Annu. Rev. Plant Physiol.* **31,** 341–374 CrossRef
4. Buchanan, B. B., Schürmann, P., Wolosiuk, R. A., and Jacquot, J. P. (2002) The ferredoxin/thioredoxin system: from discovery to molecular structures and beyond. *Photosynth. Res.* **73,** 215–222 Medline
5. Lemaire, S. D., Michelet, L., Zaffagnini, M., Massot, V., and Issakidis-Bourguet, E. (2007) Thioredoxins in chloroplasts. *Curr. Genet.* **51,** 343–365 CrossRef Medline
6. Serrato, A. J., Fernandez-Trijueque, J., Barajas-Lopez, J. D., Chueca, A., and Sahrawy, M. (2013) Plastid thioredoxins: a "one-for-all" redox-signaling system in plants. *Front. Plant Sci.* **4,** 463 CrossRef Medline
7. Collin, V., Issakidis-Bourguet, E., Marchand, C., Hirasawa, M., Lancelin, J. M., Knaff, D. B., and Miginiac-Maslow, M. (2003) The *Arabidopsis* plastidial thioredoxins: new functions and new insights into specificity. *J. Biol. Chem.* **278,** 23747–23752 CrossRef Medline
8. Michelet, L., Zaffagnini, M., Marchand, C., Collin, V., Decottignies, P., Tsan, P., Lancelin, J. M., Trost, P., Miginiac-Maslow, M., Noctor, G., and Lemaire, S. D. (2005) Glutathionylation of chloroplast thioredoxin f is a redox signaling mechanism in plants. *Proc. Natl. Acad. Sci. U.S.A.* **102,** 16478–16483 CrossRef Medline
9. Toivola, J., Nikkanen, L., Dahlstrom, K. M., Salminen, T. A., Lepisto, A., Vignols, H. F., and Rintamaki, E. (2013) Overexpression of chloroplast NADPH-dependent thioredoxin reductase in *Arabidopsis* enhances leaf growth and elucidates *in vivo* function of reductase and thioredoxin domains. *Front. Plant Sci.* **4,** 389 CrossRef Medline
10. Yoshida, K., Hara, S., and Hisabori, T. (2015) Thioredoxin selectivity for thiol-based redox regulation of target proteins in chloroplasts. *J. Biol. Chem.* **290,** 14278–14288 CrossRef Medline
11. Yoshida, K., and Hisabori, T. (2017) Distinct electron transfer from ferredoxin-thioredoxin reductase to multiple thioredoxin isoforms in chloroplasts. *Biochem. J.* **474,** 1347–1360 CrossRef Medline
12. Serrato, A. J., Pérez-Ruiz, J. M., Spinola, M. C., and Cejudo, F. J. (2004) A novel NADPH thioredoxin reductase, localized in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in *Arabidopsis thaliana*. *J. Biol. Chem.* **279,** 43821–43827 CrossRef Medline
13. Yoshida, K., and Hisabori, T. (2016) Two distinct redox cascades cooperatively regulate chloroplast functions and sustain plant viability. *Proc. Natl. Acad. Sci. U.S.A.* **113,** E3967–E3976 CrossRef Medline
14. Pérez-Ruiz, J. M., Naranjo, B., Ojeda, V., Guinea, M., and Cejudo, F. J. (2017) NTRC-dependent redox balance of 2-Cys peroxiredoxins is needed for optimal function of the photosynthetic apparatus. *Proc. Natl. Acad. Sci. U.S.A.* **114,** 12069–12074 CrossRef Medline
15. Nikkanen, L., Toivola, J., Trotta, A., Diaz, M. G., Tikkanen, M., Aro, E. M., and Rintamäki, E. (2018) Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system. *Plant Direct* **2,** e00093 CrossRef Medline
16. Geigenberger, P., Thormählen, I., Daloso, D. M., and Fernie, A. R. (2017) The unprecedented versatility of the plant thioredoxin system. *Trends Plant Sci* **22,** 249–262 CrossRef Medline
17. Gütle, D. D., Roret, T., Hecker, A., Reski, R., and Jacquot, J. P. (2017) Dithiol disulfide exchange in redox regulation of chloroplast enzymes in response to evolutionary and structural constraints. *Plant Sci.* **255,** 1–11 CrossRef Medline
18. Knuesting, J., and Scheibe, R. (2018) Small molecules govern thiol redox switches. *Trends Plant Sci.* **23,** 769–782 CrossRef Medline
19. Cejudo, F. J., Ojeda, V., Delgado-Requerey, V., Gonzalez, M., and Pérez-Ruiz, J. M. (2019) Chloroplast redox regulatory mechanisms in plant adaptation to light and darkness. *Front. Plant Sci.* **10,** 380 CrossRef Medline
20. Nikkanen, L., and Rintamäki, E. (2019) Chloroplast thioredoxin systems dynamically regulate photosynthesis in plants. *Biochem. J.* **476,** 1159–1172 CrossRef Medline
21. Yoshida, K., Yokochi, Y., and Hisabori, T. (2019) New light on chloroplast redox regulation: molecular mechanism of protein thiol oxidation. *Front. Plant Sci.* **10,** 1534 CrossRef Medline
22. Zaffagnini, M., Fermani, S., Marchand, C. H., Costa, A., Sparla, F., Rouhier, N., Geigenberger, P., Lemaire, S. D., and Trost, P. (2019) Redox homeostasis in photosynthetic organisms: novel and established thiol-based molecular mechanisms. *Antioxid. Redox Signal.* **31,** 155–210 CrossRef Medline
23. Hisabori, T., Sunamura, E., Kim, Y., and Konno, H. (2013) The chloroplast ATP synthase features the characteristic redox regulation machinery. *Antioxid. Redox Signal.* **19,** 1846–1854 CrossRef Medline
24. Michelet, L., Zaffagnini, M., Morisse, S., Sparla, F., Perez-Perez, M. E., Francia, F., Danon, A., Marchand, C. H., Fermani, S., Trost, P., and Lemaire, S. D. (2013) Redox regulation of the Calvin-Benson cycle: something old, something new. *Front. Plant Sci.* **4,** 470 CrossRef Medline
25. Motohashi, K., Kondoh, A., Stumpp, M. T., and Hisabori, T. (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* **98,** 11224–11229 CrossRef Medline
26. Yano, H., Wong, J. H., Lee, Y. M., Cho, M. J., and Buchanan, B. B. (2001) A strategy for the identification of proteins targeted by thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* **98,** 4794–4799 CrossRef Medline
27. Balmer, Y., Koller, A., del Val, G., Manieri, W., Schürmann, P., and Buchanan, B. B. (2003) Proteomics gives insight into the regulatory function of chloroplast thioredoxins. *Proc. Natl. Acad. Sci. U.S.A.* **100,** 370–375 CrossRef Medline
28. Marchand, C., Le Maréchal, P., Meyer, Y., Miginiac-Maslow, M., Issakidis-Bourguet, E., and Decottignies, P. (2004) New targets of *Arabidopsis*

thioredoxins revealed by proteomic analysis. *Proteomics* **4,** 2696–2706 CrossRef Medline
29. Marchand, C., Le Maréchal, P., Meyer, Y., and Decottignies, P. (2006) Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. *Proteomics* **6,** 6528–6537 CrossRef Medline
30. Balmer, Y., Vensel, W. H., Cai, N., Manieri, W., Schürmann, P., Hurkman, W. J., and Buchanan, B. B. (2006) A complete ferredoxin/thioredoxin system regulates fundamental processes in amyloplasts. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 2988–2993 CrossRef Medline
31. Balmer, Y., Vensel, W. H., Tanaka, C. K., Hurkman, W. J., Gelhaye, E., Rouhier, N., Jacquot, J. P., Manieri, W., Schürmann, P., Droux, M., and Buchanan, B. B. (2004) Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **101,** 2642–2647 CrossRef Medline
32. Yoshida, K., Noguchi, K., Motohashi, K., and Hisabori, T. (2013) Systematic exploration of thioredoxin target proteins in plant mitochondria. *Plant Cell Physiol.* **54,** 875–892 CrossRef Medline
33. Yamazaki, D., Motohashi, K., Kasama, T., Hara, Y., and Hisabori, T. (2004) Target proteins of the cytosolic thioredoxins in *Arabidopsis thaliana*. *Plant Cell Physiol.* **45,** 18–27 CrossRef Medline
34. Lindahl, M., and Kieselbach, T. (2009) Disulfide proteomes and interactions with thioredoxin on the track toward understanding redox regulation in chloroplasts and cyanobacteria. *J. Proteomics* **72,** 416–438 CrossRef Medline
35. Montrichard, F., Alkhalfioui, F., Yano, H., Vensel, W. H., Hurkman, W. J., and Buchanan, B. B. (2009) Thioredoxin targets in plants: the first 30 years. *J. Proteomics* **72,** 452–474 CrossRef Medline
36. Alkhalfioui, F., Renard, M., Vensel, W. H., Wong, J., Tanaka, C. K., Hurkman, W. J., Buchanan, B. B., and Montrichard, F. (2007) Thioredoxin-linked proteins are reduced during germination of *Medicago truncatula* seeds. *Plant Physiol.* **144,** 1559–1579 CrossRef Medline
37. Ros, R., Cascales-Miñana, B., Segura, J., Anoman, A. D., Toujani, W., Flores-Tornero, M., Rosa-Tellez, S., and Muñoz-Bertomeu, J. (2013) Serine biosynthesis by photorespiratory and non-photorespiratory pathways: an interesting interplay with unknown regulatory networks. *Plant Biol.* **15,** 707–712 CrossRef Medline
38. Ros, R., Muñoz-Bertomeu, J., and Krueger, S. (2014) Serine in plants: biosynthesis, metabolism, and functions. *Trends Plant Sci.* **19,** 564–569 CrossRef Medline
39. Benstein, R. M., Ludewig, K., Wulfert, S., Wittek, S., Gigolashvili, T., Frerigmann, H., Gierth, M., Flügge, U. I., and Krueger, S. (2013) *Arabidopsis* phosphoglycerate dehydrogenase1 of the phosphoserine pathway is essential for development and required for ammonium assimilation and tryptophan biosynthesis. *Plant Cell* **25,** 5011–5029 CrossRef Medline
40. Cascales-Miñana, B., Muñoz-Bertomeu, J., Flores-Tornero, M., Anoman, A. D., Pertusa, J., Alaiz, M., Osorio, S., Fernie, A. R., Segura, J., and Ros, R. (2013) The phosphorylated pathway of serine biosynthesis is essential both for male gametophyte and embryo development and for root growth in *Arabidopsis*. *Plant Cell* **25,** 2084–2101 CrossRef Medline
41. Toujani, W., Muñoz-Bertomeu, J., Flores-Tornero, M., Rosa-Tellez, S., Anoman, A. D., Alseekh, S., Fernie, A. R., and Ros, R. (2013) Functional characterization of the plastidial 3-phosphoglycerate dehydrogenase family in *Arabidopsis*. *Plant Physiol.* **163,** 1164–1178 CrossRef Medline
42. Grant, G. A. (2018) D-3-Phosphoglycerate dehydrogenase. *Front. Mol. Biosci.* **5,** 110 CrossRef Medline
43. Hisabori, T., Hara, S., Fujii, T., Yamazaki, D., Hosoya-Matsuda, N., and Motohashi, K. (2005) Thioredoxin affinity chromatography: a useful method for further understanding the thioredoxin network. *J. Exp. Bot.* **56,** 1463–1468 CrossRef Medline
44. Jacquot, J. P., Lopez-Jaramillo, J., Miginiac-Maslow, M., Lemaire, S., Cherfils, J., Chueca, A., and Lopez-Gorge, J. (1997) Cysteine-153 is required for redox regulation of pea chloroplast fructose-1,6-bisphosphatase. *FEBS Lett.* **401,** 143–147 CrossRef Medline
45. Okamura, E., and Hirai, M. Y. (2017) Novel regulatory mechanism of serine biosynthesis associated with 3-phosphoglycerate dehydrogenase in *Arabidopsis thaliana*. *Sci. Rep.* **7,** 3533 CrossRef Medline
46. Bosco, M. B., Aleanzi, M. C., and Iglesias, A. A. (2012) Plastidic phosphoglycerate kinase from *Phaeodactylum tricornutum*: on the critical role of cysteine residues for the enzyme function. *Protist* **163,** 188–203 CrossRef Medline
47. Morisse, S., Michelet, L., Bedhomme, M., Marchand, C. H., Calvaresi, M., Trost, P., Fermani, S., Zaffagnini, M., and Lemaire, S. D. (2014) Thioredoxin-dependent redox regulation of chloroplastic phosphoglycerate kinase from *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **289,** 30012–30024 CrossRef Medline
48. Tsukamoto, Y., Fukushima, Y., Hara, S., and Hisabori, T. (2013) Redox control of the activity of phosphoglycerate kinase in *Synechocystis* sp. PCC6803. *Plant Cell Physiol.* **54,** 484–491 CrossRef Medline
49. Yokochi, Y., Sugiura, K., Takemura, K., Yoshida, K., Hara, S., Wakabayashi, K. I., Kitao, A., and Hisabori, T. (2019) Impact of key residues within chloroplast thioredoxin-f on recognition for reduction and oxidation of target proteins. *J. Biol. Chem.* **294,** 17437–17450 CrossRef Medline
50. Belin, C., Bashandy, T., Cela, J., Delorme-Hinoux, V., Riondet, C., and Reichheld, J. P. (2015) A comprehensive study of thiol reduction gene expression under stress conditions in *Arabidopsis thaliana*. *Plant Cell Environ.* **38,** 299–314 CrossRef Medline
51. Akashi, H., Okamura, E., Nishihama, R., Kohchi, T., and Hirai, M. Y. (2018) Identification and biochemical characterization of the serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase in *Marchantia polymorpha*. *Front. Plant Sci.* **9,** 956 CrossRef Medline
52. Ho, C. L., Noji, M., Saito, M., and Saito, K. (1999) Regulation of serine biosynthesis in *Arabidopsis*: crucial role of plastidic 3-phosphoglycerate dehydrogenase in non-photosynthetic tissues. *J. Biol. Chem.* **274,** 397–402 CrossRef Medline
53. Rosa-Telléz, S., Anoman, A. D., Alcántara-Enguidanos, A., Garza-Aguirre, R. A., Alseekh, S., and Ros, R. (2020) PGDH family genes differentially affect *Arabidopsis* tolerance to salt stress. *Plant Sci.* **290,** 110284 CrossRef Medline
54. Yoshida, K., and Noguchi, K. (2009) Differential gene expression profiles of the mitochondrial respiratory components in illuminated *Arabidopsis* leaves. *Plant Cell Physiol.* **50,** 1449–1462 CrossRef Medline
55. Fu, C., Donovan, W. P., Shikapwashya-Hasser, O., Ye, X., and Cole, R. H. (2014) Hot Fusion: an efficient method to clone multiple DNA fragments as well as inverted repeats without ligase. *PLoS ONE* **9,** e115318 CrossRef Medline
56. Yoshida, K., Matsuoka, Y., Hara, S., Konno, H., and Hisabori, T. (2014) Distinct redox behaviors of chloroplast thiol enzymes and their relationships with photosynthetic electron transport in *Arabidopsis thaliana*. *Plant Cell Physiol.* **55,** 1415–1425 CrossRef Medline
57. Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modeling. *Bioinformatics* **22,** 195–201 CrossRef Medline
58. Dey, S., Burton, R. L., Grant, G. A., and Sacchettini, J. C. (2008) Structural analysis of substrate and effector binding in *Mycobacterium tuberculosis* D-3-phosphoglycerate dehydrogenase. *Biochemistry* **47,** 8271–8282 CrossRef Medline