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Plant Physiology and Biochemistry ■■ (2004) ■■■-■■■

Plant Physiology and Biochemistry

www.elsevier.com/locate/plaphy

Review

The thioredoxin *h* system of higher plants

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Received 28 January 2004; accepted 1 March 2004

Abstract

In plants, thioredoxins *h* are encoded by a multigenic family of genes (eight in *Arabidopsis thaliana*, at least five in *Populus* sp.). The multiplicity of these isoforms raises the question of their specificity. This review focuses on thioredoxins *h* in two plant models: Arabidopsis and poplar. Thioredoxins *h* can be divided into three different subgroups according to the analysis of their primary structure. This paper describes the biochemical properties of each subgroup. Recent data in the field indicate that subgroup members differ by their subcellular localization as well as their reduction pathways suggesting specific functions for each subgroup. The development of proteomic tools has also increased considerably the number of potential thioredoxin targets, showing the importance of thioredoxins *h* in plants.

Keywords: Thioredoxin; Glutaredoxin; Thiol reduction

1. Introduction

Thioredoxins are small proteins, which are involved in the cell redox regulation. These ubiquitous proteins are present in all organisms from prokaryotes to higher eukaryotes. In recent years, the number of thioredoxin family members has increased substantially. Based on their amino acid sequences, two distinct families of thioredoxins can be distinguished. Family I include proteins that contain one distinct thioredoxin domain whereas family II is composed of fusion proteins with one or more thioredoxin domains coupled to additional domains. The thioredoxin family I is particularly important in plants in comparison to other organisms since for example at least 20 genes have been detected in the fully sequenced genome of *Arabidopsis thaliana* [39]. In contrast, only two mammalian, three yeast and two *Escherichia coli* thioredoxins have been reported [18,34,42,51,57].

In higher plants, based on the analysis of their primary structure, members of the thioredoxin family I could be divided in six major groups: the thioredoxins *f, h, m, o, x* and *y*. Thioredoxins *m, x* and *y* are related to prokaryotic thioredoxins whereas thioredoxins *f, h* and *o* are specific of eukaryotic organisms. The thioredoxins *f, m, x* and *y* are localized in chloroplasts, whereas the thioredoxins *o* are found in mitochondria. The chloroplastic thioredoxins are encoded in the nucleus and reduced by a ferredoxin–thioredoxin reductase (FTR). The FTR/thioredoxin system is only found in photosynthetic eukaryotes and cyanobacteria. Furthermore, the *A. thaliana* genome sequencing has also revealed the presence of a fusion protein containing a NADPH thioredoxin reductase (NTR) domain and a thioredoxin domain [48]. Recent papers have described the plant chloroplastic systems [5,14,25], but the thioredoxin *h* organization and function is more complicated. This paper will thus summarize the current state of knowledge regarding the thioredoxin *h*, focusing particularly on recent data obtained in *A. thaliana* and poplar (*Populus trichocarpa*), two models of herbaceous and woody plants.

2. Multiplicity of thioredoxins *h* in plants

The thioredoxins *h* can be divided in three different subgroups as shown in Fig. 1. This phylogenetic tree has been constructed with Clustalw (http://clustalw.genome.ad.jp) using the available *A. thaliana* and *P. trichocarpa* thioredoxins

Abbreviations: HED, 2-hydroxyethyl disulfide; FTR, ferredoxin-thioredoxin reductase; GR, glutathione reductase; GRX, glutaredoxin; GSH, glutathione; GSSG, oxidized glutathione; TRX, thioredoxin; NTR, NADPH thioredoxin reductase.

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doi:10.1016/j.plaphy.2004.03.002

PLAPHY-00000182

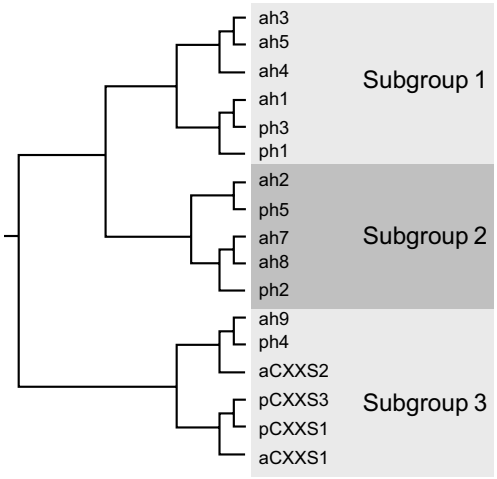

Fig. 1. Phylogenic tree of *P. trichocarpa cv*. Trichobel and *A. thaliana* thioredoxins *h*. Accession number (Genbank) and codes: *P. trichocarpa* cv. *Trichobel* (p): ph1 (AF483625); ph2 (AF483266); ph3 (BU822062); ph4 (BU835000); ph5 (BU869308); pCXXS1 (CA823821); pCXXS3 (BU874060); *A. thaliana* (a) ah1 (P29448); ah2 (S58123); ah3 (S58118); ah4 (S58119); ah5 (S58120); ah7 (AAD39316); ah8 (AAG52561); ah9 (AAG51342), aCXXS1 (AF144390); aCXXS2 (ATU35639).

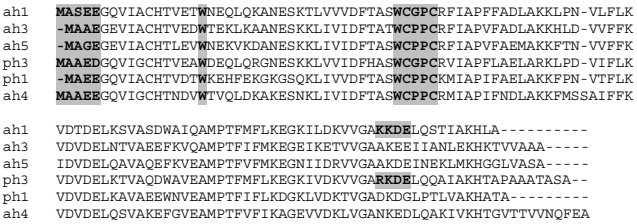

Fig. 2. Amino acid comparison of *P. trichocarpa cv*. Trichobel and *A. thaliana* thioredoxins *h* belonging to the subgroup 1. Each thioredoxin is named as in Fig. 1. The catalytic site (WCGPC), characteristic tryptophan (W) and potential structural motif involved in cell-to-cell transfer are in bold and shaded.

h sequences. The thioredoxins *h* share with other groups of thioredoxins the presence of the conserved catalytic site WC[G/P]PC except the isoforms labeled CXXS, which will be described later in the text. The catalytic site WCGPC is common to the majority of thioredoxins *h*, to the mitochondrial thioredoxins *o* and to the chloroplastic isotypes, whereas the WCPPC active site is only present in some isoforms of subgroup 1. All thioredoxins *h* characterized so far display at least one specific characteristic that allows their identification: the presence of a conserved Trp residue (W16 in poplar thioredoxin *h*1) that gives them an increased extinction coefficient at 290 nm [35,52]. The high number of the presumably cytosolic thioredoxin *h* isoforms has raised the issue of their substrate specificity. Indeed, five isoforms from *A. thaliana* have been produced as recombinant proteins in *E. coli* and had their activities tested with different substrates [44]. All the tested recombinant proteins exhibited nearly the same activity with cytosolic NADPH thioredoxin reductase. Yeast complementation studies have provided in vivo evidence for different target specificities of the *A. thaliana* thioredoxin *h* isoforms [41]. Further studies have also shown that several thioredoxins *h* differ by their level of expression and cell-type expression suggesting that they have specific functions [43].

3. Thioredoxin *h* subgroup 1

Among thioredoxins *h*, members of the first subgroup have been the most extensively studied. The reduction of this subgroup thioredoxin *h* isoforms by NADPH is mediated by NADPH-thioredoxin reductase (NTR). These three components are referred to as the redox NTR/Trx system (NTS). The NTR is a homodimeric flavoprotein, each subunit containing two domains able to bind NADPH and FAD [15]. In *A. thaliana*, four thioredoxins *h*, AtTRX*h*1, AtTRX*h*3 AtTRX*h*4 and AtTRX*h*5 are members of this subgroup whereas only two isoforms have been detected in poplar so far (Fig. 2). The strong amino acid identity between AtTRX*h*3 and AtTRX*h*5 could be explained by a duplication event [43]. Several isoforms harbor the unusual catalytic site WCPPC. It has been reported that the nature of amino acids between the two cysteines may determine the redox potential of the protein [13]. Using yeast complementation experiments, Brehelin and co-workers have demonstrated that changing the active site of AtTRX*h*3 from WCPPC to WCGPC modifies its activity in vivo [10]. While the wild-type AtTRX*h*3 does not confer growth on sulfate to yeast deficient strains, the mutant (WCGPC) restores partial growth. Mutagenesis experiments have also been performed on PtTRX*h*1 (WCPPC) and PtTRX*h*3 (WCGPC) in order to produce the mutants PtTRX*h*1WCGPC and PtTRX*h*3WCPPC. In both cases, the mutations strongly alter activities in vitro and even the folding of the mutant protein derived from PtTRX*h*3. These data suggest that the amino acid immediately after the N-terminal active site cysteine plays a major role in the activity and specificity of thioredoxin *h* [6,21].

Subgroup 1 members are presumed to be cytosolic. The expression pattern of *A. thaliana* thioredoxins *h* genes has been recently reported, demonstrating that these isoforms differ by their cell-type and specificity of expression [43]. Interestingly, members of this subgroup are abundantly found in the phloem sieve tubes in *A. thaliana* as well as in other plants [1,23,43,46], suggesting that these proteins could play a role in the redox regulation of components of the vascular tissue. Thioredoxin has been shown to be synthesized in the companion cell prior to transfer through plasmodesmata, to the enucleate sieve-tube elements [23]. Several structural motifs are critical for this cell-to-cell movement, particularly the N-terminal following sequence MAAEE and an RKDD motif situated in the C-terminal area of the rice sequence [24]. Both motifs are present together in PtTRX*h*3 (Fig. 2).

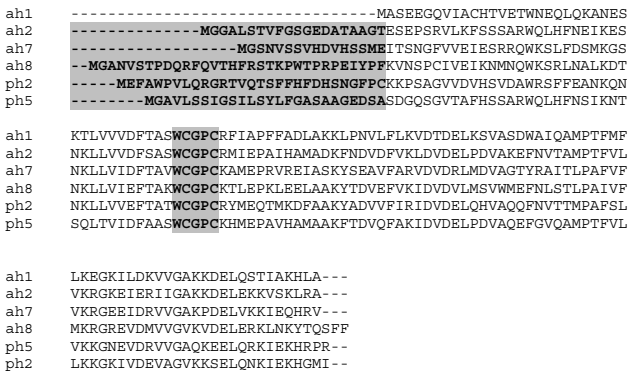

Fig. 3. Amino acid comparison of *P. trichocarpa cv*. Trichobel and *A. thaliana* thioredoxins *h* belonging to subgroup 2. Each thioredoxin is named as in Fig. 1. Catalytic site (WCGPC) and N-terminal extension are bold and shaded. ah1 is shown for comparison.

4. Thioredoxin *h* subgroup 2

The main characteristic of these thioredoxins is the presence of a N-terminal extension, while the WCGPC active site is conserved in this subgroup. Three *A. thaliana* (AtTRX*h*2, *h*7 and *h*8) and two poplar (PtTRX*h*2 and *h*5) thioredoxins *h* isoforms are members of the subgroup 2 (Fig. 3). Genes encoding *AtTrxh7* and *AtTrxh8* are the result of a duplication event [43]. Poplar thioredoxin PtTRX*h*2, related to AtTRX*h*7 and AtTRX*h*8, has been recently characterized [20]. The recombinant protein produced in *E. coli* was subjected to cleavage at its N-terminus suggesting the presence of a putative cleavage site in the N-terminal part of the protein. Using a GFP fusion, this thioredoxin isoform has been shown to be associated with mitochondria (Gelhaye, unpublished). This mitochondrial localization is in agreement with previous papers demonstrating the presence of thioredoxins and particularly thioredoxins *h* in plant mitochondria [4,32,37]. Laloi and co-workers have demonstrated the presence of a complete thioredoxin system in *A. thaliana* mitochondria, involving a NADPH thioredoxin reductase (*AtNtrA*) and at least one specific thioredoxin *o* (*AtTrxo1*) [33]. A homolog of AtTRX*o*1 has also been detected in poplar EST databases (GenBank BU834909). AtNTRA is strongly homologous to the cytosolic NTR (AtNTRB) and is able to reduce either thioredoxin *o* [33] or some thioredoxin *h* isoforms (Gelhaye, pers. commun.). The observation that at least one thioredoxin *h* isoform is localized in mitochondria increases the thioredoxin system complexity in this organelle and raises the question of the specificity of these mitochondrial thioredoxins. The data about thioredoxins *o* are scarce and prevent further comparison with mitochondrials thioredoxin *h*.

Nearly 125,000 poplar ESTs sequence, obtained from various tissues, are present in the available databases (10/2003). About 40% of thioredoxin *h* encoding sequences (209 thioredoxins *h* sequences detected) are similar to *PtTrxh2*, suggesting a major physiological role for this isoform in poplar, particularly in leaf (more than 80% of the total detected ESTs). In contrast, *AtTrxh7* and *AtTrxh8*, the *A. thaliana* orthologs seem to be weakly expressed since only one EST was found in the database for *AtTrxh8* and no EST has been detected for *AtTrxh7* [43]. Semi-quantitative PCR experiments showed that the *AtTrxh8* mRNA is not detected in plant but in calli whereas *AtTrxh7* mRNAs are present in roots and reproductive tissues [43]. This large difference between the expression of these particular thioredoxin *h* isoforms in *Populus* and *A. thaliana* is particularly interesting and requires further investigations.

In *A. thaliana*, as well as in poplar, other different isoforms are present in this thioredoxin subgroup 2, namely AtTRX*h*2 and PtTRX*h*5. Only AtTRX*h*2 has been studied and biochemically characterized so far [10,41,43,44]. Orthologs of these isoforms have been detected in various plants, and notably TRX*h*1 and TRX*h*2 from soybean belong to this subgroup. These isoforms exhibit a hydrophobic N-terminal extension suggesting that they could be membrane-bound [49]. A sequence analysis of AtTRX*h*2 and PtTRX*h*5 plant orthologs has been performed using different localization prediction programs. Several members of this subgroup 2 are predicted to have a subcellular localization, whereas members of other groups are predicted to be cytosolic. Nevertheless, the subcellular localization of these N-terminus extended thioredoxins remains to be elucidated.

In yeast, complementation experiments performed with *AtTrxh1* to *AtTrh5* have shown that only *AtTrxh2* was able to restore growth on both Met and Met sulfoxide [41]. While higher levels of *AtTrxh2* gene expression have been detected in flowers than in other plant organs, *AtTrxh2* mRNAs have been detected in almost all organs [43]. In pea, TRX*h*4, the ortholog of AtTRX*h*2, is present in axes of dried and imbibed seeds but not in germinating seeds or in seedlings [40].

5. Thioredoxin *h* subgroup 3

This thioredoxin *h* subgroup has been first reported by Juttner and co-workers, in *Lolium perenne, Hordeum bulbosum, Phalaris coerulescens* and *Secale cereale* [29]. The usual active site WCGPC is detected in several members of this subgroup, particularly in PtTRX*h*4 and AtTRX*h*9. These thioredoxins exhibit an N-terminal extension when compared to subgroup 1, this extension containing a conserved cysteine in the fourth position (Fig. 4). PtTRX*h*4 has been recently characterized [19]. As reported for different plant orthologs [29], either *A. thaliana* or *E. coli* NTR reduce very poorly the recombinant PtTRX*h*4 produced in *E. coli*. At the same time, these proteins are active in the non-specific insulin test. Complementary experiments showed that poplar as well as three *E. coli* glutaredoxins (Grxs) reduce PtTRX*h*4, demonstrating the presence of a new pathway of thioredoxin *h* reduction in plant cells [19]. Grxs are small ubiquitous oxidoreductases of the thioredoxin superfamily [56]. The size of these proteins is generally around 10–15 kDa with an active site of the form CxxC or CxxS required for their redox properties [17]. Grx are maintained reduced with the help of NADPH, glutathione reductase (GR) and the tripeptide glu-

Fig. 4. Amino acid comparison of *P. trichocarpa cv*. Trichobel and *A. thaliana* thioredoxins *h* belonging to the subgroup 3. Each thioredoxin is named as in Fig. 1. (A) Thioredoxins harboring the usual catalytic site WCGPC. The catalytic site and third conserved cystein (C) are bold and shaded. ah1 is shown for comparison. (B) Thioredoxins harboring the unusual catalytic site WCXXS. The catalytic site and third partially conserved cysteine are bold and shaded.

tathione (GSH). As most characterized thioredoxins have a redox potential of approximately –290 mV while Grxs are more electropositive (ca. –200 mV), the reduction of a Trx-like molecule by Grx is supposed to be an unfavorable reaction. In fact, the catalytic mechanism of this isoform seems to differ notably from the traditional dithiol–disulfide exchange. PtTRX*h*4 and its other plant orthologs exhibit a N-terminal extension containing a conserved cysteinyl residue in fourth position. Mutagenesis experiments demonstrated that this residue is involved in the catalytic process as well as both cysteines present in the WCGPC active site (Gelhaye, unpublished). Despite its unusual reduction pathway, PtTRX*h*4 is able to reduce the known thioredoxin *h* targets as peroxiredoxins or methionine sulfoxide reductases [19]. Analysis of EST databases shows that PtTRX*h*4 is expressed in almost all plant tissues whereas no EST was found for *AtTrxh9*. In *Lolium perenne* and *Phalaris coerulescens*, *PtTrxh4* orthologs are mostly expressed in the mature pollen and stigma and at a much lower level in leaves and roots [29].

Several members of the subgroup 3, harboring the unusual WCXXS active site, are found in *A. thaliana* and in poplar. In *A. thaliana*, the presence of two isoforms (AtCXXS1 and AtCXXS2) has been reported, AtCXXS2 exhibiting an N-terminal extension compared to AtCXXS1. In poplar, two isoforms related to *Atcxxs1* have been detected in the EST databases, *Ptcxxs1* and *Ptcxxs3*. PtCXXS3 is inactive using the insulin test and is not reduced by AtNTR (A and B) [19]. PtCXXS3 is active in the glutathione : HED transhydrogenase assay, a test largely used to assess Grx activity. In this assay, Grx catalyzes the reduction of a mixed disulfide between glutathione and HED [22]. PtCXXS3 represents the first described example in plants of this kind of protein, i.e. a Trx-like protein with a Grx-like activity. PtCXXS3 could exhibit deglutathionylation activity using a monothiol mechanism as suggested previously for some Grx isoforms [56]. In this mechanism, the thiolate of Grx initiates a nucleophilic attack on the mixed disulfide of a protein thiol with GSH. A new disulfide between Grx and GSH is formed, which could then be reduced by GSH, leading to the formation of oxidized glutathione (GSSG) and to the release of the reduced Grx.

6. Functions of thioredoxin *h*

Thioredoxins *h* are involved in multiple processes, the best documented function of Trx *h* being its implication in reserve breakdown that sustains early seedling growth of germinating cereal seeds [60]. Among the known target proteins of thioredoxin *h* in seeds are storage proteins such as hordeins in barley [61] and glutenins and gliadins in wheat [38], which are insolubilized in disulfide-bound complexes during maturation and drying. Upon germination, these proteins are reduced to the sulfhydryl state. Analysis of Trx*h* expression in wheat has shown its involvement in the transfer of compounds from the plant to the developing seed [47].

Furthermore, thioredoxins *h* could be involved in the cellular protection against oxidative stress, in particular during seed dessication and germination [47]. In this case, thioredoxins *h* are found predominantly in the nucleus of both aleurone and scutellum cells. Furthermore, thioredoxins *h* are electron donors to several enzymes involved in the protection against oxidative stress such as peroxiredoxin, methionine sulfoxide reductase, and glutathione reductase [19,28,45,55]. Peroxiredoxins are abundant low-efficiency peroxidases [16]. In addition to their role as antioxidant, plant peroxiredoxins as well as thioredoxins *h* could be involved in modulating redox-dependent signaling cascades [16] as demonstrated in mammalian cells.

Thioredoxins *h* could also be involved in different mechanisms such as self-incompatibility [9,11], or carbon and nitrogen metabolism (for reviews see [5,36,58]). In addition, the development of proteomic tools led to the identification of many thioredoxins *h* potential targets listed in Table 1 [36,38,58,61].

The detection of numerous target proteins in mitochondria has also been recently performed using proteomic approaches [3]. At least 50 potential Trx-linked proteins have been identified as functional in 12 processes: photorespiration, citric acid cycle and associated reactions, lipid metabolism, electron transport, ATP synthesis/transformation, membrane transport, translation, protein assembly/folding, nitrogen metabolism, sulfur metabolism, hormone synthesis and stress reactions. Since at least two different thioredoxin classes are probably present in plant mitochondria (*h* and o), these data raise the question of the specificity of each isoform in this organelle.

Table 1
Potential thioredoxin *h* target protein detected using proteomic or other approaches

Proteomic approaches
Barley Trxh/Barley seed [36]
α-Amylase inhibitor BDAI-I
α-Amylase/trypsin inhibitor
Trx*h* barley (group 1)
Trypsin inhibitor
Non-specific lipid transfer protein
Endochitinase 1
Endochitinase 2
Homologue of wheat chitinase 3
Cyclophilin
Cu–Zn superoxide dismutase
Glyoxalase-like protein
Embryo-specific protein
Chlamy Trxh/peanut seed [61]
Peanut allergens
Dessication related protein
Seed maturation related protein
Chlamy Trxh/Barley seed [38]
Embryo globulin 1
Peroxiredoxin
ClassII heat-shock protein
Seed maturation protein
60S acidic ribosomal protein P^3
E. coli Trxh/mature wheat seeds [58]
Protein disulfide isomerase
Fructose 6-P,1-phosphotransferase
Alpha-amylase subtilisn inhibitor
Avenin precursor
LMW glutenin subunit
Glyceraldehyde 3-P dehydrogenase
ADP-glucose pyrophosphorylase
Triose phosphate isomerase
Enolase
1-cys peroxiredoxin
Seed globulin
Cyclophilin 2a
Peroxidase
Aldolase
Malate dehydrogenase
Pyruvate, Pi dikinase
Alanine aminotransferase
Alpha-amylase inhibitor
Glyoxalase
Proteasome regulating subunit
Serpin
GSH dependent dehydroascorbate reductase

Other approaches
Gliadin [30]
Glutenin [30]
α-Amylase/trypsin inhibitor [31]
Castor seed 2S protein [26]
Pullulanase inhibitor [59]
2S Albumin [50]
Adenyl sulphate reductase [8]
Homeodomain transcription factor [54]
Glutathione peroxidase [28]
Methionine sulfoxide peroxidase [21]
Thiocalsin [7]
Peroxiredoxin [45]

7. Interactions between GSH/Grx and Trx systems

In non-photosynthetic organisms, data showing interconnections of GSH/Grx systems have been accumulating recently. The human thioredoxin activity is regulated by glutathionylation [12]. Furthermore, one human glutaredoxin is reduced directly by thioredoxin reductase [27]. It was also recently reported that GSH is probably not the physiological reducing agent of yeast Grx5 whereas *E. coli* Trx reduces this protein efficiently [53]. In plants, data on this topic are scarce, except those showing the direct reduction of one thioredoxin *h* subgroup 3 members by glutaredoxin (see above, [19]). Furthermore, at least two poplar thioredoxins *h* (PtTRX*h*2, PtTRX*h*4) can be glutathionylated in vitro, without alteration of their redox activities (Gelhaye, unpublished results). Since a large number of both glutaredoxin and thioredoxin isoforms are present in plants, it is possible that other interactions could occur between these redox regulatory systems.

8. Conclusion

The *h* class of thioredoxins represents a large multigenic family, which could be divided in three different subgroups. Recent data suggest that the specificity of thioredoxin *h* is probably linked to tissue and subcellular localizations and specificity of reduction pathway. Indeed, members of subgroup 3, at least in poplar, are reduced by the GSH/Grx system whereas the subgroup 1 and 2 members are reduced by NTRs. All subgroup 2 members exhibit an N-terminal extension and *PtTrxh2* has been shown to be associated to mitochondria. The subcellular localization of these thioredoxin isoforms remains unclear and has to be studied.

In this context, the development of proteomic tools leading to the identification of many thioredoxins potential targets [2,3,38,61] as well as their biochemical characterization will give new insights on the function(s) of each thioredoxin *h* isoform.

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